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Martin Koenig
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Project 4: Hatchery Trout Evaluations

Subproject 2: Sterile Trout Investigations
Subproject 3: Predator Training

By

Martin Koenig

Idaho Department of Fish and Game
600 South Walnut Street
P.O. Box 25
Boise, ID 83707

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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. We examined the relative growth and survival of triploid and diploid rainbow trout *Oncorhynchus mykiss* stocked in 14 alpine lakes in August 2003. During 2006, 993 trout were sampled, including 763 nontest fish. Of the 230 experimental test fish captured, 49 Hayspur diploids, 19 triploids, and 162 Troutlodge all-female triploids were identified. Catch rates from gill nets of test fish were highly variable between lakes and test groups, with the Troutlodge all-female group having the highest average CPUE of 0.3 fish/hour, followed by Hayspur 2N (0.09 fish/hour) and Hayspur 3N (0.03 fish/hour) fish. On average, gill nets caught 3.4 Troutlodge all-female triploid rainbow trout per hour for every one Hayspur 2N rainbow trout, and 9.9 trout/hr for every one Hayspur 3N caught. Mean total lengths (± 95% confidence intervals, or CIs) of test fish varied between lakes, with Troutlodge all-female averaging 295 ± 6 mm in length, Hayspur 2N averaging 291 ± 10 mm, and Hayspur 3N averaging 281 ± 17 mm. Otolith readings from known-age test fish correctly estimated age 87% of the time, while scale readings were correct 97% of the time. The high relative survival of Troutlodge all-female triploids may have been a result of better survival from adipose fin clips (instead of ventral clips used for the other treatment fish), and a lack of emigration related to spawning behavior. This evaluation suggests that Troutlodge all-female triploid rainbow trout provide better performance in high lakes and should be considered a viable alternative to Hayspur-strain rainbow trout.

Methods for producing triploid walleye *Sander vitreus* were investigated during 2006. Triploid induction rates and survival rates (to eye-up) for three treatments were tested using three time periods with 9,500 psi of pressure. Survival was similar across nontreated groups, while the 15-minute treatment had the best survival of treated groups (21%) with 100% triploid induction. Based on these results and those from previous years, experiments should be repeated using 9,500 psi over a range of 10-15 minutes with several replicates to address variability between egg batches and to better identify an optimum treatment combination.

Author:

Martin Koenig
Fisheries Research Biologist

INTRODUCTION

<u>Sterile Trout</u>

Triploid salmonids are functionally sterile, and the common assertion is that sterility provides a fisheries or aquaculture benefit (Benfey 1999). 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). However, 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.

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 IDFG Fisheries Management Plan (IDFG 2007) outlines several guidelines regarding the management of alpine lakes. The genetic conservation of wild populations is a management priority for the Idaho Department of Fish and Game (IDFG). The IDFG has established a policy to stock only triploid rainbow trout in systems where reproduction between wild or native trout and hatchery fish is possible (IDFG 2007). Implementation of the above-noted policy has resulted in the widespread stocking of sterile rainbow trout in hundreds of Idaho alpine 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 alpine lakes. If not, fisheries managers may need to adjust stocking strategies rather than rely on historical stocking levels, as is currently being done. In addition to identifying sterile fish as a management tool in mountain lakes, the IDFG Fisheries Management Plan guidelines indicate that self-sustaining trout populations in mountain lakes be maintained (IDFG 2007). Therefore, natural reproductive capacity should be evaluated when lakes are surveyed so that appropriate adjustments to stocking strategies can be made.

Idaho Department of Fish and Game began a series of research projects to investigate sterile rainbow trout performance in mountain lakes in 1999. In fall 1999, a pilot study was initiated using diploid and triploid all-female rainbow trout in four mountain lakes using Kamloops all-female diploid and triploid rainbow trout from Troutlodge, Inc. (Kozfkay 2004). A second study was initiated in 2001 using mixed-sex rainbow trout (diploid and triploid) from IDFG's Hayspur Hatchery and included 16 mountain lakes. The 2001 stocking of test fish was also marked with adipose fin clips and grit dye. Results from these studies indicated that the relative survival of diploid trout was about twice that of the triploid group (Kozfkay 2004; Kozfkay and Koenig 2006). In 2003, a third stocking event introduced test fish into 15 mountain lakes, 13 of which had not been previously stocked in 2001. This experiment contained mixed-sex Hayspur rainbow trout (diploids and triploids) and a third group of all-female triploid Kamloops rainbow trout from Troutlodge, Inc. These fish were marked with a combination of fin clips to signify inclusion in the study and to determine ploidy level. Sample collection from the 2003 stocking event began in 2006 and the initial results are presented below.

<u>Sterile Walleye</u>

The Hatchery Trout Project focuses mainly on salmonids raised in IDFG hatcheries, but also incorporates other hatchery fishes including walleye *Sander vitreus*. 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 Wyoming (Rahel 2004) and elsewhere in the western United States. Stocking sterile walleye could greatly reduce or nearly eliminate 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 walleye fisheries, as triploid fish tend to live longer (Rieman and Meyers 1990; Johnston et al. 1993). Increased longevity would provide additional harvest opportunity in subsequent years when diploids would have already perished.

In this progress report, we compare survival and growth of triploid and diploid rainbow trout that were stocked as part of a large-scale study in 15 central Idaho alpine lakes during 2003 and sampled during 2006. We also summarize efforts to refine techniques for inducing triploidy in walleye during 2006.

RESEARCH GOAL

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

OBJECTIVES

1. To increase survival of rainbow trout in alpine lakes by 25% by stocking triploid fish while maintaining growth rates equal to that of diploid rainbow trout.

2. To develop and refine techniques for inducing triploidy in walleye that provide high induction rates (95-100%) while maintaining adequate survival (not less than 75% of untreated fish).

METHODS

Performance of Sterile Trout in High Mountain Lakes

Fifteen lakes were selected that were from five to 10 acres in surface area and had reasonable access from roads, yet were far enough from roads to keep angler harvest to a minimum level (Figure 1). Study lake selection criteria included that they were on the normal stocking rotation and had been stocked historically with rainbow trout. Additionally, past surveys must have indicated that lakes were capable of supporting trout fisheries. All test lakes are managed under the general trout regulation of six fish per day with no length limit except for Blackwell Lake, which is managed under the trophy regulation of two fish per day with none under 508 mm. Two of the lakes had been previously been used in the 2001 stocking event and included Lake Ingeborg and Blackwell Lake.

Eggs for the three test groups used in this study were obtained from Troutlodge and IDFG's Hayspur Fish Hatchery. The Troutlodge group was a Kamloops strain that was genetically modified to produce all-female eggs and then pressure treated by Troutlodge to induce triploidy. 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 minutes after fertilization (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 ventral fin, whereas the right ventral fin was removed from the mixed-sex triploid group, and the adipose fin from the Troutlodge all-female group. Unlike previous evaluations, no grit dye was used for the 2003 stocking 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.

Out of the 15 lakes stocked in 2003, 14 were sampled between July 7 and October 2, 2006. Oreamnos Lake was omitted due to prohibitively difficult access. Fish were captured using floating experimental gill nets. Nets were 46 m long by 1.5 m deep with nylon thread mesh and consisted of various panels with 19, 25, 30, 33, 38, and 48 mm bar mesh. 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.

Captured fish were identified to species, measured to the nearest millimeter, and weighed to the nearest gram. Since two lakes from the 2001 stocking event were also included in the 2003 stocking (Ingeborg and Blackwell), all rainbow trout captured from those lakes were examined for grit mark presence under a portable fluorescent lantern (Model #UVL-4, UVP, Inc.). Gonads of rainbow trout were examined to determine sex and assigned one of three levels of maturity: immature, developing, or mature. Immature gonads are small, with testes being light-colored, opaque, fine-textured organs, while ovaries are granular and translucent (Strange 1996). Mature fish were characterized as having testes that are much enlarged and milky white, and ovaries with evident well-developed eggs (Strange 1996). Developing fish were characterized as having gonads with characteristics intermediate between immature and mature.

Scale and otoliths samples were collected from most test fish to investigate the typical accuracy of using scales and otoliths to correctly estimate rainbow trout age in alpine lakes. Otoliths from nontest fish were also collected to estimate nontest fish ages. Otoliths were stored dry in microcentrifuge tubes and viewed whole (Boxrucker 1986). Scales were stored in coin envelopes then placed between two microscope slides and viewed whole using a dissecting microscope. Sample labels contained an index number but no information regarding fin clips or species, thus readers could not distinguish test fish (fin clipped, known age 3) from nontest fish. Otoliths and scales were examined independently by at least two trained readers. If there was a disagreement in the estimated fish age, the sample was reexamined concurrently by both readers until a consensus age was reached. In the case when a consensus could not be reached, the structure was determined unreadable.

Similar to IDFG standard high lake monitoring protocols, bathymetric and water quality parameters were collected at each of the 14 lakes. A series of three transects was 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 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, surface water temperature, 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 and location were determined with the use of a handheld GPS unit. Lake area was determined with ArcGIS software. 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.

Catch-per-unit-effort (CPUE) was calculated for each test group by lake and survey method by dividing the total number of fish caught by the total hours of netting or angling effort. The overall mean CPUE for each test group was calculated by taking the average CPUE across all lakes. Mean lengths and weights of test-fish were calculated, and differences between groups were addressed by comparing 95% confidence intervals (CIs).

Marked experimental trout (test fish) were stocked from August 14-22, 2003 (Table 1). 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. Fifteen alpine 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. 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, n = 120) and mixed-sex Hayspur triploid (62 ± 1 mm, n = 120) groups, but the Troutlodge all-female group was slightly longer (65 ± 1 mm, n = 120). 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). Statistical comparisons were made by examining overlap of the 95% CIs around the mean.

Production of Sterile Walleye

Experiments designed to evaluate methods to induce triploidy in walleye were conducted in cooperation with personnel from Montana Department of Fish Wildlife and Parks' (MFWP) Fort Peck Office and Miles City Fish Hatchery. Brood fish were captured with trap nets by MFWP personnel from the Dry Arm of Fort Peck Reservoir, Montana and held in net pens until ready to spawn. On April 14, 2006, fertilized eggs were produced by combining the gametes of four females with four males for a total egg volume of 2.8L (3 qts). After 30 seconds to allow fertilization, a mixture of Fuller's earth (clay) and lake water was added to the spawning bowl to make the eggs nonadhesive. After one minute in the slurry, eggs were rinsed in lake water for one minute. Each batch of eggs was split equally, with one-half receiving pressure treatment, while the other half was not treated, to allow comparisons of survival between treated and untreated eggs. Fertilized eggs to be pressure treated were placed in a perforated aluminum cylinder for loading and unloading into the pressure chamber. Treatment egg batches were loaded into the pressure cylinder and brought up to pressure within four minutes of fertilization. I used a hydraulic pressure chamber, Model HPC™, built by TRC Hydraulics Inc., Dieppe, New Brunswick, Canada. The 2.7 liter chamber was filled with ambient reservoir water before egg treatment.

The process was completed three times with different batches of eggs to test three pressure treatment regimes. The three treatments consisted of 9,500 psi for 12 minutes duration, 9,500 psi/15 minutes duration, and 9,500 psi/18 minutes duration. Treatments were evaluated based on egg survival rates to eye-up and the proportion of triploid fry after treatment. Due to the difficulty in rearing small groups of walleye at Miles City Fish Hatchery, we were unable to run replicates for any of the treatment-control combinations. Eggs were hatched in upwelling jars and, as sac fry, were transferred to outside rearing ponds. Due to constraints on hatchery rearing space, after survival to eye-up rates were determined, nontreated egg batches (controls) were combined into a single pond. Fish were reared until large enough to obtain blood samples—about 40 mm total length. On June 26, 2006, 40 blood samples were collected from each treatment group and 10 samples from the combined nontreatment group, stored in Alsever's solution, and shipped to North Carolina State University where triploid-induction rates were determined with flow cytometry by Dr. Jeff Hinshaw. Mean eye-up rate and triploid induction rate was calculated for each treatment group.

RESULTS

Performance of Sterile Trout in High Mountain Lakes

The number of salmonids captured in 2003 in alpine lakes was highly variable (Table 2). Overall, 993 salmonids were captured, yielding an average catch of 71.0 ± 30.4 per lake. Most trout captured were nontest fish (763, or 76.8%) that originated either from previous plantings or from natural production. Of the nontest fish collected, cutthroat trout (westslope and Yellowstone combined; *O. clarkii lewisi, O. clarkii bouvieri*, respectively) made up 65% of the catch, while rainbow trout made up 28%. The remaining catch of nontest salmonids was comprised of grayling *Thymallus arcticus* (2.5%), kokanee salmon *O. nerka* (0.3%), and rainbow/cutthroat hybrids (3.7%).

Of the 993 total salmonids captured in 2006, 230 test fish were collected (Table 2). Troutlodge all-female triploid rainbow trout made up the majority of test fish captured (162 or 70.4%) and were present in 12 of the 14 lakes sampled. Hayspur diploids made up the next largest number captured (49 or 21.3%), and were caught in nine of the 14 lakes. Nineteen (8.3%) Hayspur triploid rainbow trout were captured from seven of the 14 lakes sampled. The combined sex ratio of mixed-sex Hayspur 2N fish was approximately equal, with 22 females, 20 males, and 7 "unknown." The overall sex ratio of mixed-sex Hayspur 3N trout was five females, eight males, and six "unknown."

Gillnets were fished from 24.1–47.2 hours per lake, yielding a total effort of 442.1 hours (Table 3). Catch-per-unit-effort (CPUE) for all fish combined ranged from 0.4 fish/hr (Lake Ingeborg) to 6.0 fish/hr (Kane Canyon Lake), with a mean of 1.9 ± 0.86 fish per hour. Catch rates from gill nets of test fish were highly variable between lakes and test groups, with the Troutlodge all-female group having the highest average CPUE of 0.3 fish/hour (Tables 3 and 4). Hayspur 2N and 3N test fish were captured at an average of 0.09 and 0.03 fish/hour, respectively. On average, gill nets caught 3.4 Troutlodge all-female triploid rainbow trout per hour for every one Hayspur 2N rainbow trout, and 9.9 trout/hr for every one Hayspur 3N caught (Table 4). Gill nets caught 2.9 Hayspur 2N rainbow trout for every one Hayspur 3N caught.

Angling surveys were conducted at 12 of the 14 lakes sampled. The overall angling CPUE for all fish combined was 4.2 ± 2.4 fish per hour. Angling CPUE for all fish combined ranged from zero (4 lakes) to 10.0 (Lynx Creek Lake #1) fish per hour. Catch rates from angling of test fish were variable between lakes and test groups, with the Troutlodge all-female group having the highest average CPUE of 0.68 fish/hour (Table 3). Hayspur 2N and 3N test fish were captured at an average of 0.39 and 0.22 fish/hour, respectively. On average, angling caught 1.7 Troutlodge all-female triploid rainbow trout per hour for every one Hayspur 2N rainbow trout, and three for every Hayspur 3N caught (Table 4). However, these ratios should be interpreted with caution, as they are based on few comparisons, since not all lakes produced fish of each test group (Table 3).

Mean total length (mm) of test groups varied slightly across lakes (Figure 3) and between groups (Figure 4). The overall mean length of test fish was similar between Troutlodge all-female rainbow trout and Hayspur 2N rainbow trout, while Hayspur 3N rainbow were slightly shorter than both other groups. Troutlodge all-female averaged 295 ± 6 mm in length, Hayspur 2N averaged 291 ± 10 mm, and Hayspur 3N averaged 281 ± 17 mm. Troutlodge all-female test fish were significantly longer than Hayspur 3N test fish, but not significantly longer than Hayspur 2N test fish. Hayspur 2N and 3N test fish were not significantly different in mean total length. Troutlodge all-female rainbow trout weighed on average 275 ± 14 g, while Hayspur 2N and Hayspur 3N rainbow trout weighed on average 274 ± 26 g and 233 ± 40 g, respectively. Differences in lengths and weights of all three groups within lakes could only be compared for six lakes, as fish from each group were not caught at all lakes. Statistical comparisons between groups within lakes were not possible due to low sample sizes.

From known age-3 test fish, consensus age estimates were available from 162 scale samples and from 216 otolith samples. From these samples, paired scale and otolith samples (from the same fish) were available from 157 fish. The mean ages for known age-3 trout determined from scales ($\bar{x}$ = 3.04 ± 0.05, n = 162) and otoliths ($\bar{x}$ = 3.07 ± 0.06, n = 216) were similar. Paired otolith/scale samples from the same fish produced correct consensus age estimates 87% of the time (Figure 7). Both otoliths and scales accurately estimated the age of test fish, yet scales were more often correct. From the 162 scale samples, 97% were aged correctly, while 1% were estimated at age-4, and 2% were estimated at age-5 (Figure 8). Of the

216 otolith samples, 87% were aged correctly, while 7% were estimated at age-4, 2% were estimated at age-5, and 4% were estimated at age-2 (Figure 8).

Production of Sterile Walleye

Triploid induction rates of the three treatments were variable, ranging from 90% to 100%. Survival to eye-up of individual groups of treated and untreated walleye eggs were similar (Table 7). For the 9,500 psi/12 min treatment, the eye-up rate of controls was 51%, whereas survival of treated groups was 17%. The 9,500 psi/15 min treatment yielded the highest treatment survival, with an eye-up rate of nontreated eggs and treated eggs and 67% and 21%, respectively. For the 9,500 psi/18 min treatment, eye-up rates of nontreated and treated groups were 68% and 15%, respectively. Induction rates were high for all three treatments (Table 7). For the 9,500 psi/12 min treatment, the triploid induction rate was 98%, compared to 100% for the 9,500 psi/15 min treatment and 90% for the 9,500 psi/18 min treatment.

DISCUSSION

Performance of Sterile Trout in High Mountain Lakes

Gill net and angling surveys for alpine lakes stocked in 2003 indicated that the relative survival of Troutlodge all-female triploid rainbow trout far exceeds that of both Hayspur-strains (diploid and triploid). For Hayspur-strain fish, diploid survival exceeded triploid survival almost 3:1. 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). In addition, Kozfkay and Koenig (2006) found the diploid: triploid ratio across 16 mountain lakes (stocked in 2001) over two years of sampling (2004, 2005) was 2.1:1. These results strongly suggest that diploids survive at higher rates than triploids of the Hayspur-strain stock in alpine lakes, but Troutlodge all-female triploid rainbow trout appeared to have the highest survival. Unfortunately, the study design does not allow separation of the effects of stock and ploidy.

In contrast to differences in survival, growth between strains, in terms of both length and weight, appeared to be similar, although the biggest differences were between Troutlodge all-female triploids and Hayspur triploids. In an aquaculture setting, all-female triploid rainbow trout grew faster than their diploid and mixed-sex counter parts over a 265-day trial (Sheehan et al. 1999). Much of the growth advantage in the all-female triploids was attributed to low gonad development and the lack of males, which matured earlier at smaller sizes. Several researchers have concluded that growth advantages of triploid trout do not become apparent until after sexual maturity would normally have occurred. Teuscher et al. (2003) noted that diploid rainbow trout grew faster through age-3, but afterwards as diploid fish reached sexual maturity, growth of triploid fish exceeded that of diploids at age-4. Similar results were noted for Eastern brook trout and rainbow trout, where triploids showed growth advantages over diploids after reaching 600-700 g (Thorgaard 1986; Boulanger 1991). This pattern of growth may become clearer by collecting more samples in 2007. In addition, Sheehan et al. (1999) suggested caution when comparing triploid growth with different strains of rainbow trout, as some strains may reach sexual maturity at small sizes, which may increase the disparity in growth rates between diploids and triploids.

Although triploids may outperform diploids when grown separately (Sheehan et al. 1999), such growth advantages might disappear when triploids and diploids are reared together. For example, triploid Atlantic salmon *Salmo salar* outperformed diploids when reared separately, but showed no growth advantage when reared together with diploids (Galbreath et al. 1994), apparently a result of competition with diploids. In one respect, the results of our study would contradict those of Galbreath et al. (1994) in that Troutlodge triploids achieved similar size and greater numbers than did their diploid competitors. However, when comparing only Hayspur-strain fish, diploids did outperform triploids as Galbreath et al. (1994) concluded. The poor returns and smaller sizes of Hayspur triploid test fish may be indicative of poor competitive ability against their diploid counterparts. If so, poor returns of Hayspur triploid fingerlings stocked in lakes with established populations of diploid trout may be expected.

Other factors that may explain the large disparity in returns may include size at stocking, spawning behavior, and differences in fin clips. Length and weight of test fish at the time of stocking is an unlikely explanation of the differences seen in relative survival between the groups. Although the Troutlodge group was 3 mm longer on average, the Hayspur 2N and 3N fish did not differ in length, and there was no difference in weight between any of the groups. Additionally, a size difference of only 3 mm seems unlikely to be responsible for the large differences of catch between the Troutlodge all-female and Hayspur groups. Spawning-related emigration by adult brook trout has been well documented in Adirondack lakes and can result in significant losses to stocked fisheries (Josephson and Youngs 1996; Warrillow et al. 1997; Josephson et al 2001), although sterile, male triploid rainbow trout may still exhibit spawning behaviors and undergo spawning-related migration. Consequently, sex ratios in lakes with mixed-sex triploid stocks may shift towards higher proportions of females as males emigrate or die as a result to spawning behavior (Warrillow et al. 1997; Josephson et al 2001). This behavior could present an advantage to planting all-female triploid stocks. Such stocks could provide greater angling opportunity, as few triploid females would be lost to spawning-related emigration and mortality. Additionally, triploid females do not develop mature gonads as do triploid males, and do not divert energy resources into secondary sexual characteristics and reproductive behavior. As a result, triploid females are thought to have higher dress-out weights and improved flesh quality than their triploid male counterparts (Sheehan et al. 1999).

During 2006, the combined sex ratio of mixed-sex Hayspur 2N fish was approximately equal, with 22 females, 20 males, and 7 "unknown." It is unknown whether spawning-related emigration might account for some of the differences in catch between the Hayspur 2N and Troutlodge all-female groups. The overall sex ratio of mixed-sex Hayspur 3N trout was five females, eight males, and six "unknown." The small number of mixed-sex triploid trout captured makes it impossible to draw meaningful conclusions about how spawning behavior might affect long-term persistence of mixed-sex triploid rainbow trout in these mountain lake fisheries. However, the current data indicate that relative survival of triploid Hayspur rainbow trout is so low that emigration of males is likely an insignificant factor contributing to low catches of Hayspur triploids, assuming that the initial sex ratio was 1:1 at stocking. In this study, we have waited to sample test fish until three years after planting. In this respect, any differences in performance between the diploid and triploid groups attributable to spawning-related physiological changes and migration should be accounted for.

One confounding factor in this study is that the Troutlodge all-female group was marked with adipose fin clips, while both Hayspur strain groups were given ventral fin clips. Mortality in salmonids associated with ventral fin clips can be highly variable and is generally higher compared to adipose-clipped and unmarked salmonids of the same group (Nicola and Cordone 1973; Mears and Hatch 1976; Jacobs 1990; PSC 1995; PSC 1997). In one alpine lake in

California, Nicola and Cordone (1973) found that rainbow trout with ventral clips were recovered at lower rates than adipose-clipped rainbow trout from two separate release groups over several years. They found that on average, ventral-clipped trout (both right and left) were recovered at 81% of the rate as adipose-clipped trout. Although fewer ventral-clipped rainbow trout were recovered, differences in returns between adipose-clipped trout and ventral-clipped trout were only significantly different in one of the two release groups. These results were quite different from those of Mears and Hatch (1976). Their study found that overwinter survival of ventral-clipped Eastern brook trout in a shallow, reclaimed pond survived at only 43% of adipose-clipped brook trout. Similarly, Vincent-Lang (1993) found that returns of coho salmon *Oncorhynchus kisutch* to Bear Lake, Alaska, were much lower for ventral-clipped salmon than for adipose-clipped salmon over four different brood years. Coho salmon with left and right ventral fin clips returned at 55% and 61% of the rate of adipose-clipped salmon, respectively.

Compared to values reported in the literature, the differences in relative survival between adipose-clipped and ventral-clipped trout in this study are much higher than expected. Even if one conservatively estimates a 50% reduction in returns from ventral fin clips compared to adipose fin clips, the differences in catch between these test groups remains considerable. Hayspur 2N and 3N (ventral clips) rainbow trout were caught at 30% and 12% of the Troutlodge all-female groups, respectively. Although it is reasonable to expect lower survival in ventral-clipped groups, the differences in performance between these groups is large enough that it is unlikely to be explained by fin clips alone.

Some studies have indicated that triploid fish may not perform well under chronically stressful conditions such as high temperatures or low dissolved oxygen (Ojolick et al. 1995). Other research has shown that triploid brook trout, rainbow trout, and Atlantic salmon do not differ from diploids in their stress response in terms of increased blood hematocrit, plasma cortisol, and glucose levels (Benfey and Biron 2000; Sadler et al. 2000). Similarly, Benfey et al. (1997) found no differences in critical thermal maxima of diploid and triploid brook trout. Triploid fish have lower hemoglobin-oxygen ratios than diploid fish, yet maintain equal hematocrit volume. As a result, triploid fish have a lower maximum blood oxygen capacity, which is hypothesized to reduce their overall aerobic capacity and susceptibility to chronic stress (Graham et al. 1985; Ojolick et al. 1995). However, Stillwell and Benfey (1997) found no difference in critical swimming velocity of diploid and triploid brook trout, suggesting that triploidy does not necessarily result in lower aerobic capacity. In fact, some have concluded that triploid trout should not be limited by blood-oxygen carrying capacity (Stillwell and Benfey 1998) and should be equivalent to diploids in acute hypoxia (Benfey and Sutterlin 1984).

Stillwell and Benfey (1998) proposed a physiological mechanism that may help explain higher survival of all-female triploid trout we observed. Diploid female trout may experience lowered hemoglobin production because of increased estrogens occurring at or following spawning. Estrogen-related erythropoiesis inhibition may lead to reduced red blood cell production and subsequently result in lowered blood hemoglobin concentrations. Such a decrease would not occur in triploid females, as they show no such increases in estrogens. In addition, estrogens also stimulate the production of vitellogin, a yolk-protein that binds to iron. The high energetic demands of vitellogenesis and the subsequent binding of iron in the process could suppress hemoglobin production in diploid females. If increases in estrogens associated with spawning coincide with periods of low dissolved oxygen (such as during the winter), triploid female trout may be less susceptible to hypoxia than diploid females.

On average, scales and otoliths from known age-3 test fish were accurate and returned similar ages. However, when comparing structures from the same fish, scales better estimated the true age. Estimated ages from otoliths had wider variation than scales, and tended to overestimate ages when incorrect. These results are similar to those of Kozfkay and Koenig (2006), who found otoliths correctly estimated the ages of known-age trout from mountain lakes 89% of the time. However, they found scales to be less accurate, being correct only 79% of the time. The difference in the results could be a function of the experience of different readers processing the samples from different years. Additionally, Kozfkay and Koenig (2006) had a much smaller sample size of paired scale/otolith samples (n = 63) from only two test-fish types. Some of the discrepancy between scales and otoliths may also be a result of unusual annulus patterns that may be present in fish raised in hatcheries and later stocked in the wild (Mackay et al. 1990). Our larger number of paired samples across three types of test fish may have included greater variation in scale and otolith appearance. Scales take very little time to collect, but takes more time to prepare before they can be read. Otoliths require more time to collect in the field but can be viewed whole, reducing the amount of preparation time required in the lab. Otoliths may provide some advantage to scales when estimating the ages of fish greater than age-5, as scales tended to underestimate ages for older trout (DeCicco and Brown 2006).

Based on the results, it appears that these lakes could be qualitatively assigned to one of three categories to describe the level of natural reproduction and the contribution of stocked fish to the existing fishery. Heart (R4), Leggit, and South Buckhorn lakes fell into category 1 (Table 6). The catch from these lakes contained a high percentage of test fish, very few age classes, and species compositions and age classes that closely mimic stocking records. At Blackwell Lake, the high frequency of stocking made it difficult to distinguish fingerling plantings from natural production. However, since some test fish were recovered, it was determined that stocking was contributing to the fishery but natural reproduction could not be ruled out.

Big, Edna, Ingeborg, Lynx Creek #1, Perkons, and Rough lakes fell into category 2. For these lakes, a combination of factors suggested that natural production and stocking were contributing to the fishery. These factors included intermediate number of marked fish present in the catch, the presence of hybrids, or several year classes that did not closely mimic past stocking records. In Edna Lake, multiple year classes of cutthroat were captured, even though the stocking record indicates cutthroat have not been planted for ten years. Additionally, the presence of hybrids also suggests natural production. However, 16% of the total catch was composed of marked test fish, suggesting that stocking was still contributing to the fishery. In Big Lake, the fish population seems to match stocking records well, yet there is still evidence of natural production from the presence of hybrids.

Blue, Kane, and WF Buckhorn #1 lakes fell into category 3. Fish populations were characterized by one or a combination of factors such as considerable number of hybrids, low numbers of marked fish present or near total lack of fish present from previous stocking events. Blue Lake showed no signs of a 2002 golden trout planting–-suggesting that few fish of this planting survived—yet there are year classes of cutthroat trout and rainbow trout that are not explained by stocking records. Stocking records indicate that WF Buckhorn Lake #1 has not been stocked with cutthroat trout for 10 years, yet contains several year classes of cutthroat trout, as well as rainbow/cutthroat hybrids, and returned very few marked test fish. These observations suggest a significant proportion of alpine lakes in Idaho may support self-sustaining trout populations. In California, Armstrong and Knapp (2004) found that 68%-70% of alpine lakes in two wilderness areas contained reproducing trout populations long after stocking had discontinued. Poor returns of stocked fish from lakes where self-sustaining trout populations exist suggest that stocking densities and schedules may need to be adjusted to maximize the cost and benefits of mountain lake fisheries.

Production of Sterile Walleye

Triploid induction rates for pressure treatments tested on walleye during 2006 were similar to those treatments tested during 2005 (Kozfkay and Koenig 2006), and survival to eye-up rates were less variable. In 2005, the 8,000 psi treatments (20 and 30 minutes) had induction rates of 90% and 97.5%, with survival rates of 18% and 6%, respectively. Survival for the 9,500 psi (10 minutes) was much higher (54%) with 95% triploid induction. Treatments applied in 2006 had more consistent eye-up rates for treated eggs, with triploid induction rates ranging from 90% to 100% (Table 7). These results suggest that treatments of higher pressure and shorter duration may provide high triploid induction rates and improved eye-up survival rates. However, these results should be interpreted with caution due to the single sample of each treatment group.

Eye-up rates of nontreated control eggs were slightly better in 2006. Perhaps average egg quality or rearing conditions were better than in previous years. Eggs were collected within two days of the same date during 2005, but year-to-year variation in spawn timing could affect egg quality at the time of the experiment. However, without any replication of treatments and controls, there is no way to address how variability between egg batches could have affected the results. Replication may also be able to control for other sources of variation, including rearing conditions, transportation and handling conditions, and minor differences in pressure chamber operation.

Eggs exposed to pressure for longer periods did not always result in lower survival and higher induction rates. For example, in 2006 9,500 psi resulted in high induction rates for 12 min and 15 min duration (98% and 100%), but only 90% induction when extended to 18 minutes (Table 7). This was unexpected, since an 8,000 psi/30 min treatment in 2005 resulted in 97.5% induction (even though survival was poor). In addition, in 2005, the 9,500/10 min treatment had the highest survival, but in 2006, 9,500/15 min had higher survival than 12 min treatment.

Treatments using 9,500psi for a duration ranging from 10-15 minutes should be tested in the future using several replicates to make more robust conclusions regarding the best recipe. So far, 9,500 psi/10 min treatment from 2005 shows best application for hatchery production, since it had the highest eye-up rate of 54% with fairly high triploid induction rates of 95%. However, if higher triploid induction rates are required, increasing the time under pressure to 15 minutes should bring induction rates close to 100%. However, these longer-duration treatments may not return eye-up rates sufficient to meet production goals.

RECOMMENDATIONS

1. Re-evaluate current stocking strategies using sterile Hayspur rainbow trout in alpine lakes. This study suggests that triploid Hayspur rainbow trout survive at less than half the rate of diploids when stocked together, and even less when stocked with both diploids and Troutlodge all-female triploid rainbow trout. Data collected in 2006 suggests that Troutlodge all-female triploid rainbow trout provide better performance in high lakes and should be considered a viable alternative to Hayspur-strain rainbow trout.

2. Conduct a diploid/triploid alpine lake evaluation using only Troutlodge all-female rainbow trout with identical marks to eliminate confounding factors such as fin clips and stock origin.

3. Conduct an evaluation where the performance of triploid rainbow trout in alpine lakes can be evaluated without a possible competitive interaction with diploid trout.

4. Standard alpine lake surveys should include a more comprehensive assessment of natural recruitment and the contribution of stocked fish to existing populations using marked hatchery fish. During this study, it was difficult to accurately determine whether there was sufficient reproduction to maintain populations without further stocking. For instance, for the subset of lakes sampled in 2006, at least three and as many as 11 of the 14 study lakes studied would sustain fishable populations without further stocking.

5. Retest pressure treatments using 9,500 psi over a range of 10-15 minutes duration to induce triploidy in walleye. Treatments must be replicated to address variability and to better identify an optimum treatment combination. Such treatments will be undertaken directly by MTFWP in the future without the aid of IDFG personnel.

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Table 1. Description of 15 study waters in Idaho stocked with Hayspur mixed-sex diploid, Hayspur mixed-sex triploid, and Troutlodge all-female triploid 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)
Big	15-0000-0183	8/20/03	500	500	500	269934	4845567	12	5.8	2958
Blackwell	09-0000-0366	8/14/03	500	500	500	578944	4979995	11	5.3	2101
Blue	07-0000-0370	8/22/03	400	500	500	601076	4998164	11	5.0	2222
Buckhorn #3	07-0000-0494	8/22/03	500	500	500	590225	4972271	11	12.9	2123
Edna #2	09-0000-0241	8/21/03	500	500	500	661147	4870395	11	4.3	2563
Heart -Reg 3B	10-0000-0292	8/19/03	500	500	500	660767	4866896	11	5.2	2617
Heart-Reg 4	10-0000-0164	8/18/03	500	500	500	658388	4822996	11	4.2	2542
Ingeborg	10-0000-0306	8/21/03	500	500	500	657127	4868027	11	9.5	2723
Kane Canyon	15-0000-0208	8/18/03	500	500	500	728605	4851742	11	4.8	2813
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
Oreamnos	09-0000-0169	8/21/03	440	500	500	654770	4879486	11	4.1	2486
Perkons	10-0000-0170	8/18/03	500	500	500	663171	4846357	11	4.1	2653
Rough	15-0000-0186	8/20/03	500	500	500	270957	4844656	12	3.3	2925
WF Buckhorn #1	07-0000-0484	8/22/03	500	500	500	590225	4972271	11	12.9	2123

Table 2. Total trout captured and by test group from mountain lakes surveyed during 2006 using a combination of gill nets and angling. See comments in tables 5 and 6.

Lake Name	Total Fish	Nontest Fish	Hayspur 2N	Hayspur 3N	Troutlodge AF 3N
Big Lake	158	154	0	0	4
Blackwell Lake	59	57	0	0	2
Blue Lake	22	21	0	0	1
Edna Lake	45	38	2	1	4
Heart Lake R4	72	14	14	4	40
Heart Lake R3B	97	84	6	1	6
Kane Canyon Lake	165	152	4	0	9
Lake Ingeborg	15	11	4[a]	0	0
Leggit Lake	32	19	0	1	12
Lynx Creek Lake #1	39	23	2	4	10
Perkons Lake	37	33	1	0	3
Rough Lake	158	104	7	3	44
South Buckhorn Lake	56	16	12	5	23
WF Buckhorn Lake #1	39	38	1	0	0
Grand Total	993	763	49	19	162

[a] These were test fish from the 2001 planting. No fish from the 2003 planting were captured.

Table 3. Catch-per-unit-effort (fish/hour) of alpine lake test fish and nontest fish captured by angling and gill nets during 2006. Dashes indicate where angling was not attempted.

	Angling CPUE					Gill Net CPUE				
Lake Name	Total	Nontest fish	Hayspur 2N	Hayspur 3N	Troutlodge AF 3N	Total	Nontest fish	Hayspur 2N	Hayspur 3N	Troutlodge AF 3N
Big Lake	0	0	0	0	0	3.7	3.6	0	0	0.1
Blackwell Lake	2.7	2.3	0	0	0.3	1.9	1.8	0	0	0.04
Blue Lake-Secesh	-	-	-	-	-	0.8	0.8	0	0	0.04
Edna Lake #2	5.3	4.7	0	0.3	0.3	1.0	0.8	0.07	0	0.1
Heart Lake - Reg 3	6.7	6.7	0	0	0	1.3	1.1	0.1	0.02	0.1
Heart Lake - Reg 4	8.0	4.0	2.2	1.1	0.7	1.9	0.1	0.3	0.04	1.5
Kane Canyon Lake	0	0	0	0	0	6.0	5.5	0.1	0	0.3
Lake Ingeborg	0	0	0	0	0	0.4	0.4	0	0	0
Leggit Lake	9.3	8.0	0	0	1.3	0.7	0.3	0	0.04	0.4
Lynx Creek Lake #1	10.0	6.0	2.0	1.0	1.0	1.1	0.7	0	0.1	0.3
Perkons Lake	-	-	-	-	-	1.3	1.2	0.04	0	0.1
Rough Lake	4.0	2.3	0	0	1.7	3.1	2.0	0.1	0.06	0.8
South Buckhorn Lake	4.8	1.3	0.5	0.3	2.8	1.5	0.4	0.4	0.2	0.5
WF Buckhorn Lake #1	0	0	0	0	0	1.5	1.5	0.04	0	0
Mean CPUE	4.2	2.9	0.4	0.2	0.7	1.9	1.4	0.1	0.03	0.3

Table 4. Ratios of catch-per-unit-effort (CPUE) of alpine lake test fish groups captured by angling and gill nets during 2006. Groups include Troutlodge all-female triploid rainbow trout (TLAF), Hayspur diploid rainbow trout (H2N) and Hayspur triploid rainbow trout (H3N). Comparisons could only be made when at least one fish of each group was captured with each method.

	Angling			Gill Net		
Lake Name	TLAF:H2N	TLAF:H3N	H2N:H3N	TLAF:H2N	TLAF:H3N	H2N:H3N
Big Lake	-	-	-	-	-	-
Blackwell Lake	-	-	-	-	-	-
Blue Lake-Secesh	-	-	-	-	-	-
Edna Lake #2	-	1	-	1.5	-	-
Heart Lake - Reg 3	-	-	-	1	6	6
Heart Lake - Reg 4	0.3	0.7	2	4.8	38	8
Kane Canyon Lake	-	-	-	2.3	-	-
Lake Ingeborg	-	-	-	-	-	-
Leggit Lake	-	-	-	-	10	0
Lynx Creek Lake #1	0.5	1	2	-	3	0
Perkons Lake	-	-	-	3	-	-
Rough Lake	-	-	-	5.6	13	2.3
South Buckhorn Lake	5.5	11	2	1.2	3	2.5
WF Buckhorn Lake #1	-	-	-	-	-	-
Mean Ratio	1.7	3.0	1.8	3.4	9.9	2.9

Table 5. Description of study waters stocked in 2003 with diploid, triploid, and all-female rainbow trout and sampled in 2006.

Lake Name	Stocking Density	Survey Date	Size (ha)	Elevation (m)	Avg Depth (ft)	Avg Depth (m)	Max Depth (m)	Avg Temp (°C)	Avg pH	Avg Conductivity
Big	259	7/12/2006	5.8	2958	19.7	6	11	12.5	7.7	0.0
Blackwell	283	7/5/2006	5.3	2151	23.9	7	11	19.9	7.2	12.0
Blue (Secesh)	203	10/2/2006	6.9	2332	42	13	20	12.7	7.8	11.0
Edna #2	349	7/28/2006	4.3	2563	43.3	13	21	18.8	6.2	5.0
Heart (Reg. 4)	288	8/14/2006	5.2	2617	11.8	14	27	19.3	7.0	3.0
Heart (Reg 3)	357	7/30/2006	4.2	2542	45.1	4	8	17.7	8.9	13.7
Kane Canyon	312	7/26/2006	4.8	2813	9.2	3	5	11.9	7.7	22.0
Ingeborg	158	7/29/2006	9.5	2723	28.7	9	14	18.1	7.2	2.0
Leggit	195	8/23/2006	7.7	2600	17.3	5	10	15.4	8.2	40.0
Lynx Creek #1	283	9/13/2006	5.3	2569	24.4	7	10	14.7	8.0	12.0
Perkons	366	8/21/2006	4.1	2653	19.1	6	14	17.3	7.9	217.0
Rough	455	7/11/2006	3.3	2925	14.5	4	6	13.0	7.5	13.4
South Buckhorn (#3)	221	7/18/2006	6.8	2123	31.1	9	12	16.3	7.1	3.4
WF Buckhorn #1	116	7/17/2006	12.9	2122	41.3	13	22	19.6	7.2	3.3

Table 6. Sample and species composition, stocking history, and importance of stocking to fish populations in 14 Idaho lakes stocked in 2003 and sampled in 2006. Lakes were qualitatively placed into 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.

Lake Name	% Non Test fish	% 2003 Test fish	% 2001 Test Fish	% CUT	% RBT	% RXC	% GRA	% KOK	Rotation (yrs)	Recently stocked species	Preceding stocking to 2003 (spp, yr)	Evidence of Natural Reproduction	Stocking Contributes to Fishery?	Category	Comments
Big Lake	97	3	-	92	3	6	-	-	3	CUT, 2004	CUT, 2001	Yes	Yes	2	2004 Henry's Lake CUT, hybrids present
Blackwell Lake	97	3	-	-	98	-	-	2	1	RBT, 2005	RBT, 2002	Unkown	Yes	2	2005 Hayspur 3N RBT
Blue Lake	95	5	-	55	45	-	-	-	1-2	Test RBT	GNT, 2002	Yes	Some*	3	*CUT from 1999 planting, but no GNT captured; Unexplained CUT year classes; Unexplained RBT year classes
Edna Lake #2	84	16	-	56	24	20	-	-	3	Test RBT	RBT, 2000	Yes	Yes	2	Self-sustaining CUT; No CUT stocking in last 10yrs
Heart Lake - Reg 3	87	13	-	61	36	3	-	-	3	CUT, 2005	CUT, 2001	Yes	Some	2	Some reporduction of RBT and CUT; No RBT stocked in at least 10 yrs, high RBT%, Unexplained year classes of CUT
Heart Lake - Reg 4	19	81	-	-	100	-	-	-	2	Test RBT	CUT, 2000	No	Yes*	1	*CUT stocking not contributing? Possible winter kill? High RBT returns
Kane Canyon Lake	92	8	-	88	11	1	-	-	3	Test RBT	CUT, 2000	Yes	Yes	3	Self-sustaining CUT; Multiple unexplained CUT year classes
Lake Ingeborg	73	0	27	60	40	-	-	-	2-3	CUT, 2005	RBT, 2001	Yes	Some*	2	*No 2003 Test-RBT captured; CUT year classes match stocking and some unexplained CUT year classes
Leggit Lake	59	41	-	-	100	-	-	-	2	RBT, 2005	RBT, 2001	No	Yes	1	2005 stocked Hayspur 3N
Lynx Creek Lake #1	59	41	-	26	74	-	-	-	2	Test RBT	RBT, 2001	Yes	Yes	2	2001 Hayspur 3N RBT; Unexplained CUT and RBT year classes
Perkons Lake	89	11	-	3	97	-	-	-	1-4	Test RBT	RBT, 2000	Yes	Some*	2	Unexplained RBT year classes; Some 2003 test-RBT returned
Rough Lake	65	35	-	38	50	-	12	-	3	CUT, 2004	CUT, 2001	Unkown	Yes	2	2004 Henry's Lake CUT; Unexplained CUT year class
South Buckhorn Lake	29	71	-	-	100	-	-	-	3	Test RBT	RBT, 2000	No	Yes	1	2000, Hayspur 3N RBT; Very few age classes
WF Buckhorn #1	97	3	-	82	3	15	-	-	3	Test RBT	RBT, 2000	Yes	No	3	Self-sustaining CUT; No CUT stocking in last 10yrs, RXC hybrids

Table 7. Eye-up rates and triploid induction rates from an experiment designed to test the effectiveness of pressure treatments for inducing triploidy in walleye. Experiments were conducted on eggs and milt collected and fertilized at Fort Peck Reservoir, Montana in April 2006. Results from previous years' experiments are included for comparison.

Treatment Group	**Eye-up Rate**	**% Triploid**	**Cytometry Sample**
	2006		
9,500 psi - 12min	17%	98%	n = 40
Nontreated	51%		
9,500 psi - 15min	21%	100%	n = 40
Non-treated	67%		
9,500 psi - 18min	15%	90%	n = 40
Nontreated	68%		
Combined non-treatment		0%	n = 10
	2005		
8,000 psi - 20min	18%	90%	n = 40
Nontreated	64%		
8,000 psi - 30min	6%	97.5%	n = 40
Nontreated	51%		
9,500 psi - 10min	54%	95%	n = 40
Nontreated	46%		
	2004		
8,000 psi - 30min	<1%	-	-
Nontreated	<1%		
9,000 psi - 12min	1%	94%	n = 60
Nontreated	17%		
9,500 psi - 5min	32%	47%	n = 60
Nontreated	43%		

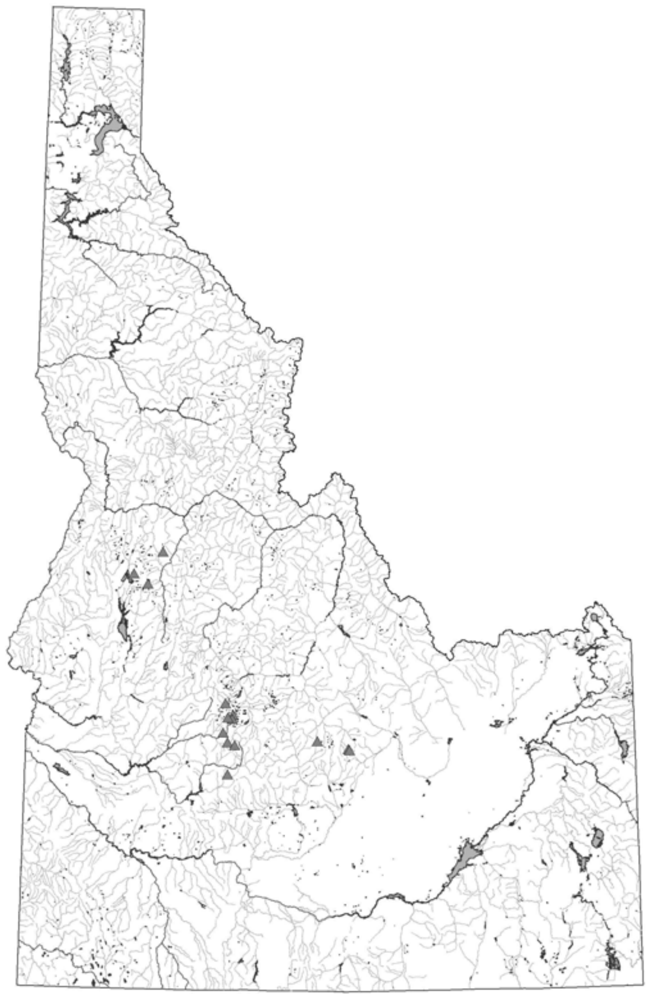

Figure 1. Locations of 15 mountain lakes in Idaho stocked during 2003 and used to compare the relative performance of Hayspur mixed-sex diploid, Hayspur mixed-sex triploid, and Troutlodge all-female rainbow trout.

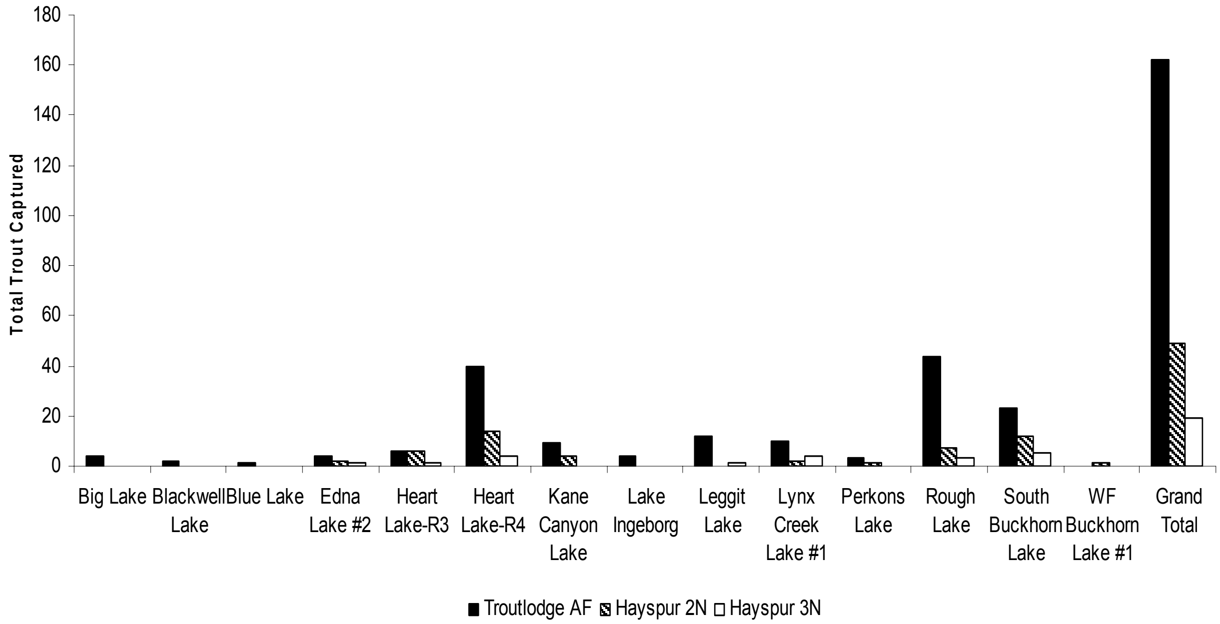

Figure 2. Total numbers of test fish captured by gill nets and angling by lake during 2006.

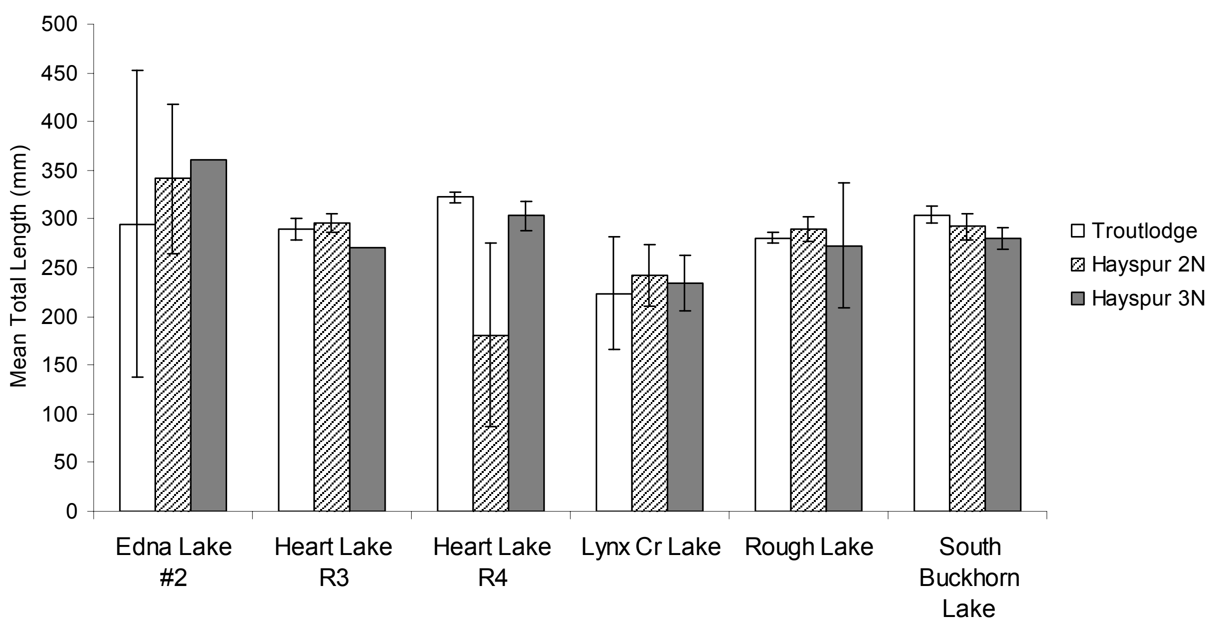

Figure 3. Mean total length (mm) of test fish captured during 2006 with gill nets and angling for lakes where all three groups were captured. Error bars indicate 95% confidence bounds except Edna Lake #2, where only one Hayspur 3N fish was captured.

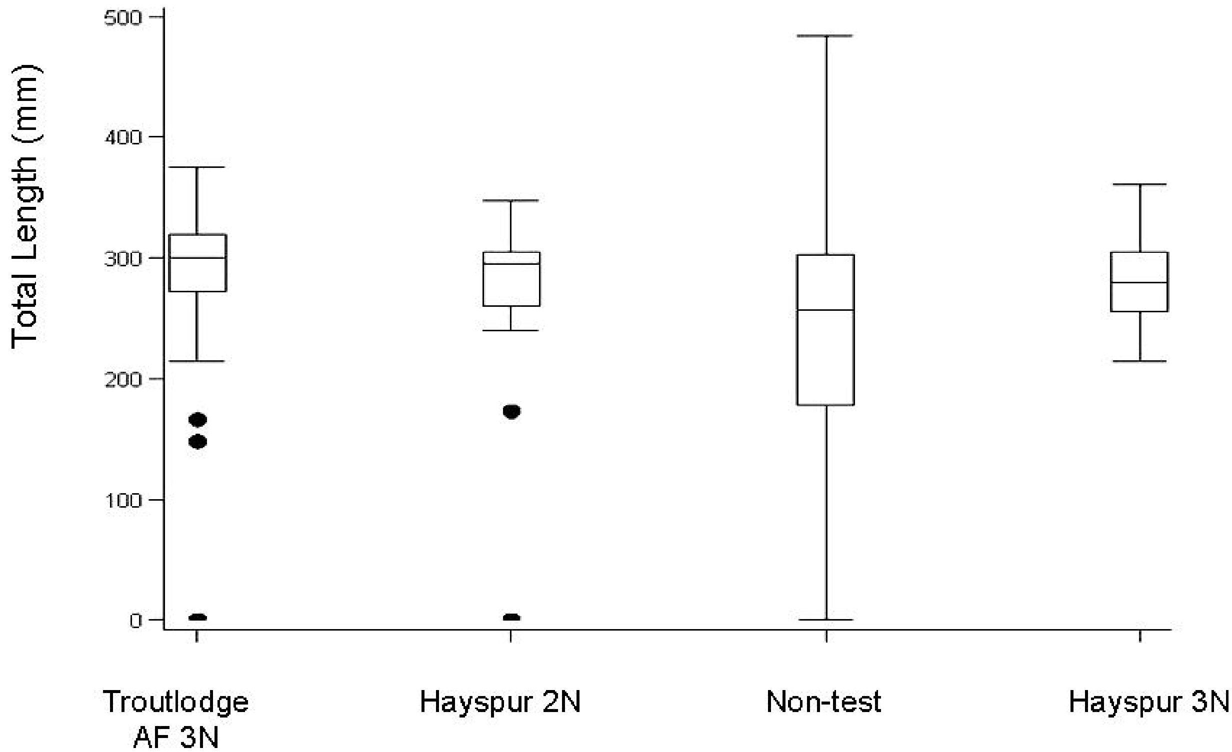

Figure 4. Box plot of total length (mm) of fish captured during 2006 across all lakes. Whiskers indicate 1.5 times the interquartile range.

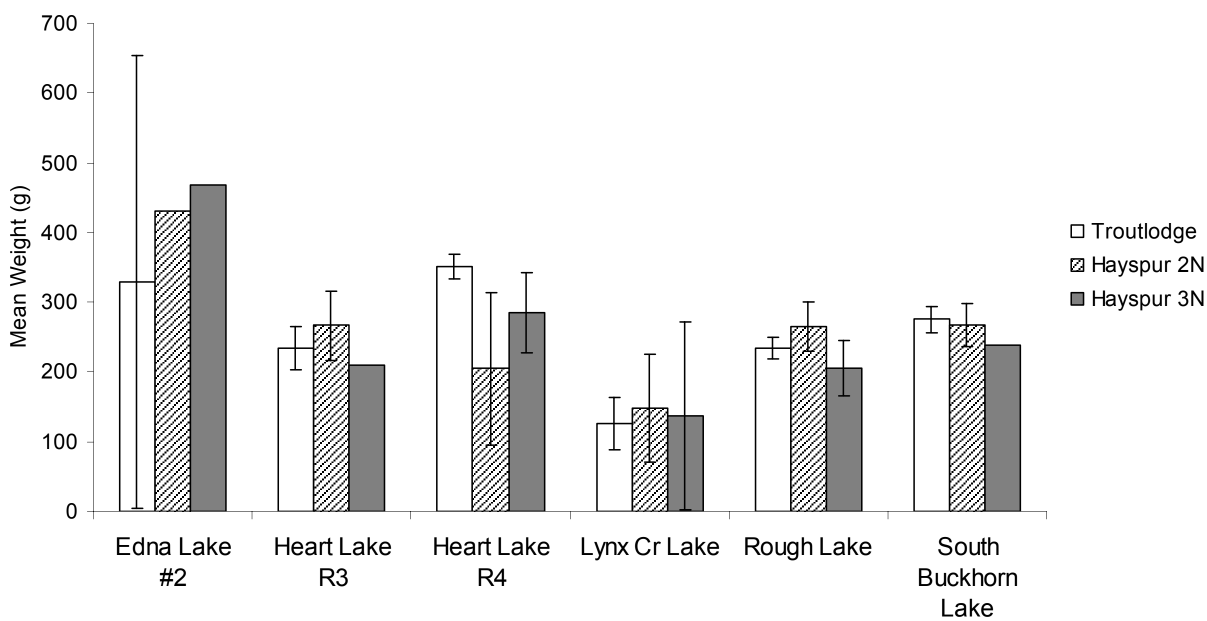

Figure 5. Mean weight (g) of test fish captured during 2006 for lakes where all three groups were captured. Error bars indicate 95% confidence bounds.

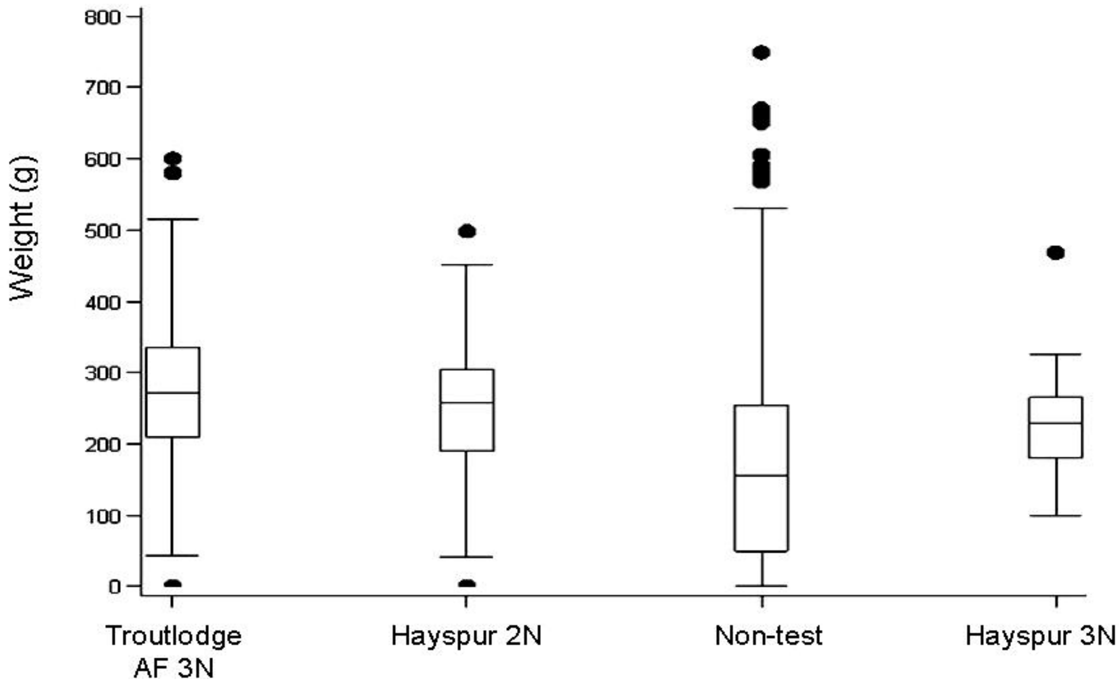

Figure 6. Box plot of weight (g) of fish captured during 2006 across all lakes. Whiskers indicate 1.5 times the interquartile range while outliers are indicated by solid points.

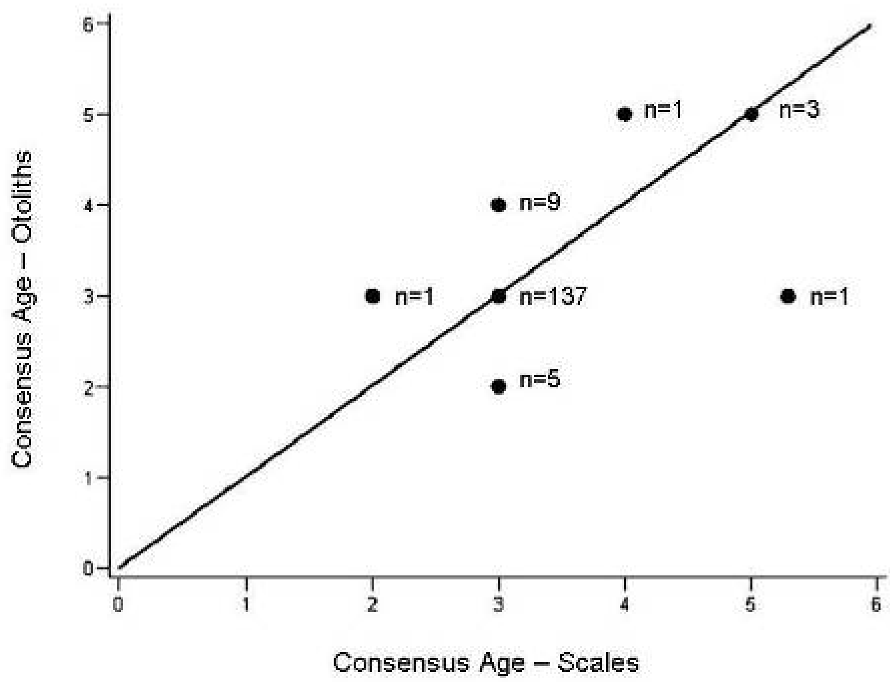

Figure 7. Comparison of consensus ages from both scales and otoliths for known age 3 rainbow trout sampled from alpine lakes during 2006. The solid line represents one-to-one agreement of scales and otolith ages.

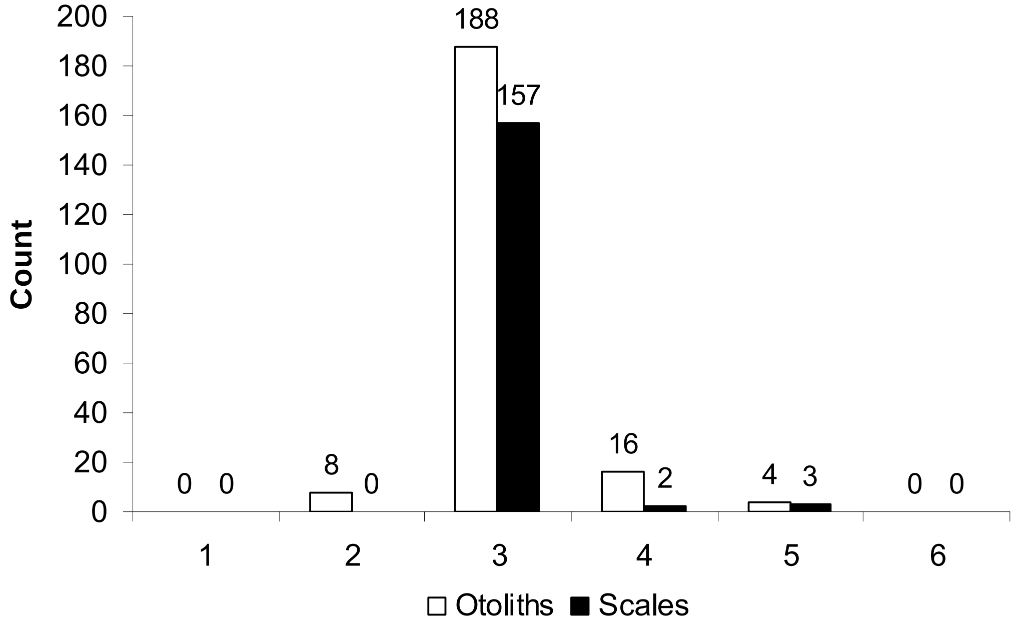

Figure 8. Comparison of consensus ages from both scales and otoliths for known age-3 rainbow trout sampled from alpine lakes during 2006.

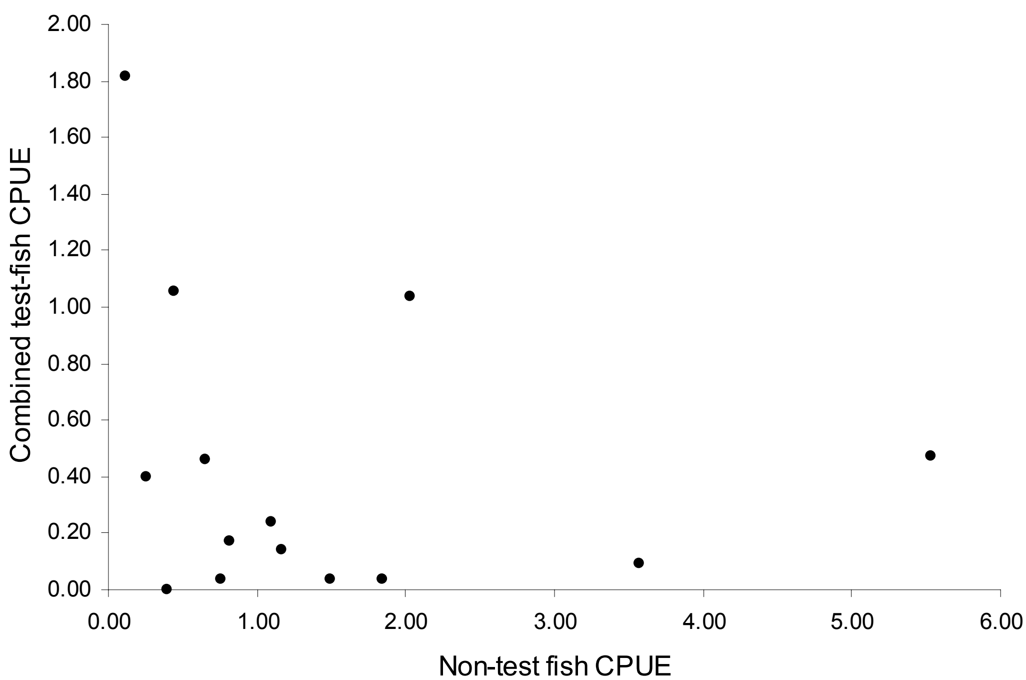

Figure 9. Scatter plot of combined catch-per-unit-effort (CPUE) of all test-fish against CPUE of all nontest fish captured from alpine lakes during 2006.

ANNUAL PERFORMANCE REPORT
SUBPROJECT #3: PREDATOR TRAINING

State of: Idaho   Grant No.: F-73-R-29 Fishery Research

Project No.: 4   Title: Hatchery Trout Evaluations

Subproject #3: Predator Training

Contract Period: July 1, 2006 to June 30, 2007

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, research was 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 for 15-day periods prior to release of fingerlings during spring 2004. Approximately equal numbers of predator trained and control fingerlings were stocked into Lucky Peak, Lake Walcott, and CJ Strike reservoirs, as well as Hayden Lake, during May 2004. Sampling of these four water bodies during spring 2005, spring 2006, and fall 2006 yielded no recaptures of marked test fish. Therefore, we were unable to address whether more predator-trained fingerlings survived than control fingerlings. Additional sampling in 2007 is probably not justified.

Author:

Martin Koenig
Fisheries Research Biologist

INTRODUCTION

By removing early life-history survival constraints, production fish hatcheries are able to supply large numbers of salmonids to habitats that would support few or no fisheries. However, 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 (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 (Miller 1951; Miller 1953; Fraser 1981).

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). Learning to recognize predators while rearing in hatcheries may increase the survival and eventual return to creel rate of fingerling rainbow trout *Oncorhynchus mykiss* after release.

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. Several researchers have trained naive prey of various species 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 *Oncorhynchus 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 *Oncorhynchus 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). Conditioned fish moved 0.5 m laterally when the model approached and Fraser speculated that this behavior had no survival benefit. Berejikian et al. (1999) were able to train Chinook salmon 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, which may contribute to low return-to-creel rates for anglers. 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.

OBJECTIVE

1. To undertake a study that by 2007 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, 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 (Evenson and Ewing 1985, Nielson 1990). Grit dye was applied by placing approximately 200 fingerlings in a wooden frame marking box with a wire mesh bottom. Dye was applied with a modified sandblasting gun using compressed air (460-490 kg/m^2) as a propellant from a distance of about 30 cm. The process was repeated until the entire raceway was marked.

Rainbow trout that would be used as predators were selected from catchable-size groups (200-250 mm) and held an additional three to four 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 Fish Hatchery, seven predators were introduced into raceway 2 ($\bar{X}$ = 308 ± 6 mm, 293 ± 15 g, shown with 90% CI) and 11 predators were introduced into raceway 3 ($\bar{X}$ = 304 ± 7 mm, 286 ± 18 g, shown with 90% CI). The fingerlings from these raceways were stocked into CJ Strike Reservoir. On April 27, 2004 at Hagerman Fish Hatchery, 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 ± 6 mm, 320 ± 19 g) and raceway 12 ($\bar{X}$ = 304 ± 11 mm, 354 ± 35 g) at Nampa Fish Hatchery. Fingerlings from these raceways were stocked into Lucky Peak Reservoir. On May 4, 2004 at Grace Fish Hatchery, 13 predators were introduced into raceway S4 ($\bar{X}$ = 313 ± 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, we 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. Marking success and short-term mark retention were also evaluated at this time by examining these subsamples of fish with a black light in the absence of direct light. 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 8). 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 8).

In spring 2005, and again in spring and fall 2006, experimental gill 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. We 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.

Captured 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 either at night or within an industrial-strength black plastic garbage bag. Otolith samples were collected from a portion of rainbow trout collected to estimate the age of rainbow trout captured. Otoliths were stored in microcentrifuge tubes. Otoliths were viewed whole (Boxrucker 1986) using a dissecting microscope on 2-4 power. Otoliths were examined independently by at least two trained readers. If there was a disagreement in the age of the sample, the sample was then reexamined concurrently by both readers until a consensus age was reached. In the case when a consensus could not be reached, the otolith was determined to be unreadable.

RESULTS

Overall, few rainbow trout of the appropriate age to be test fish were captured across the study reservoirs. Lucky Peak Reservoir was not sampled in 2006 due to the massive drawn-down event that occurred in 2004 (drawing the reservoir to <1% of total), and because no test-fish were recovered in 2005.

In CJ Strike Reservoir, sampling consisted of 421 minutes of boat electrofishing effort and 464.5 hours of gill net effort. Sampling was conducted over three nights in spring and three nights in the fall. A total of 210 rainbow trout were sampled. Rainbow trout averaged 293 $\pm$ 7 mm and 333 $\pm$ 27 g (shown with 95% CIs). No test fish were identified by the presence of grit dye. From 105 otolith samples collected, three rainbow trout were estimated to be age-1, 21 as age-2, 59 as age-3, 17 as age-4, four as age-5, and one as age-6.

In Lake Walcott, sampling consisted of 291 minutes of boat electrofishing effort and 239 hours of gill net effort. Sampling was conducted over two nights in spring and one night in fall. Sixteen rainbow trout were collected. The majority of these fish appeared to originate from fingerling plants. Rainbow trout averaged 392 $\pm$ 78 mm long and averaged 948 $\pm$ 509 g in weight. No test fish were identified by presence of grit dye. From 10 otolith samples, five rainbow trout were estimated to be age-3, three as age-4, and two as age-5.

In Hayden Lake, sampling was conducted using gill nets over six nights (three in spring, three in fall) for a total of 517 hours of gill netting effort. Five rainbow trout were sampled. The majority of these fish appeared to originate from fingerling plants. Rainbow trout averaged 412 $\pm$ 114 mm and 889 $\pm$ 707 g. No test fish were identified by presence of grit dye. Otoliths were used to estimate the age of four of the rainbow trout collected, of which two were estimated as age-2 and two as age-3.

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. We attempted 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 were 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. Since no test fish were recovered, we were unable to address whether more predator-trained fingerlings survived than control fingerlings.

No predator-trained rainbow trout were recovered in either 2005 or 2006. It seemed that during spring 2005 surveys, the age-1 test fish had not yet fully recruited to the gears or behaved differently than larger, older rainbow trout that were caught readily. In 2006, test fish would have been two years old. An additional 12-24 months of growth should have resulted in higher catches of test fish. Based on age estimates from the rainbow trout collected in 2006, our sampling gear was effective at capturing age-2 fish, although age-3 fish were caught more frequently. However, most of the age-2 rainbow trout were captured from CJ Strike, while only two age-2 rainbow trout were collected form Hayden Lake and none from Lake Walcott. These data show that even with considerable electrofishing and gill netting effort, catch rates of rainbow trout were low, especially in Hayden Lake and Lake Walcott. It is unlikely that meaningful numbers of predator-trained test fish could be recovered without significantly increasing sampling effort and efficiency or increasing the number of experimental fingerling stocked in the future.

Low rates of mark retention could decrease apparent survival rates. The grit marking process used in this study has been successful in the past and typically exhibits high retention rates. Rainbow trout marked with grit dye and released into mountain lakes as fingerlings showed retention rates of 90% after two years (Kozfkay 2003) and 86% after three years in the wild (Kozfkay and Koenig 2006). Other studies have reported similar results. Pauley and Trout (1988) reported juvenile steelhead retained grit dye at 100% for a 90-day period in circular tanks. Nielson (1990) reported that grit dye was retained in 90% of cutthroat trout recaptured from Bear Lake, Idaho and Utah, and some fish retained the marks for up to 12 years. Based on these retention rates, it is unlikely that poor mark retention affected the results, and that rainbow trout that were captured were indeed not experimental fish.

In order to recover any predator-trained test fish at all, additional sampling in spring and fall 2007 would be required. It may be necessary to schedule sampling earlier in the spring to increase sampling effort to capture even a small number of test fish. Assuming that predator-trained and control groups were equally catchable, a minimum of 172 grit-marked fish from each reservoir would have to be collected in order to obtain sufficient power for a chi-squared test ($1 - \beta = 0.80$) to detect a change of 20% from a stocking ratio of 50:50 (Elrod and Frank 1990). Considering the lack of success in capturing any grit-marked rainbow trout, the amount of effort needed to capture enough test fish needed to make meaningful comparisons between survival rates of the two groups is likely to be cost and time prohibitive.

RECOMMENDATIONS

1. Additional surveys on Lake Walcott, Hayden Lake, and CJ Strike Reservoir during 2007 to increase sampling effort in order to recapture test fish is probably not warranted given the results to date.

2. Discontinue predator-training experiments designed to increase fingerling hatchery rainbow trout survival rates.

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Table 8. Name, date stocked, rearing facility, and the number of fingerlings stocked to compare the performance of predator trained and control fingerlings during 2004.

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

Prepared by:

Martin Koenig
Sr. Fisheries Research Biologist

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

Steve Yundt, Chief
Bureau of Fisheries

Daniel J. Schill
Fisheries Research Manager