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Project 4: Hatchery Trout Evaluations

**Subproject 2: Sterile Trout Investigations: Performance of Sterile Kokanee in
Lowland Lakes and Reservoirs**

**Subproject 2: Sterile Trout Investigations: Performance of Sterile Rainbow Trout in
High Mountain Lakes**

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ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT INVESTIGATIONS: PERFORMANCE OF STERILE
KOKANEE IN LOWLAND LAKES AND RESERVOIRS

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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) salmonids for recreational fisheries. We examined the relative growth and survival of triploid and diploid kokanee salmon *Oncorhynchus nerka* across five lakes and reservoirs stocked in similar numbers during spring 2005. The number of kokanee caught in each study location during 2007 was highly variable, with catch-per-unit-effort ranging from 0.8 to 4.9 fish/hr of netting. Overall, 1,208 kokanee were captured, with the majority being unmarked nontest fish (95%). Fifty-six test fish were identified (5%) based on fin clips and calcein-marked otoliths. Diploid kokanee accounted for a higher percentage (61%, 34 fish) of the total marked kokanee captured. When catch data were adjusted to reflect the 79% triploid-induction rate, diploids made up 73% (41 fish) of the marked kokanee captured. Eleven fin-clipped kokanee were captured, with six having right ventral clips (triploid) and five with left ventral clips (diploids). Ten of these clipped fish had visible calcein marks present in their otoliths, suggesting a 91% mark retention rate for calcein in otoliths two years after stocking. Capture totals of marked kokanee were too low to make definitive conclusions about the performance of triploid kokanee at this time. Sampling will continue in 2008 to collect age-3 marked kokanee if available. Due to lengthy processing time and uncertainty in interpreting the mark (both while in the field and lab), we do not recommend using calcein as a mass-mark in the future for long-term paired release evaluations.

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INTRODUCTION

Kokanee salmon *Oncorhynchus nerka* are an important recreational species in reservoirs and lakes across the western United States and Canada (Rieman and Myers 1992). Kokanee may support high yield fisheries or act as a forage base for large piscivores (Wydoski and Bennett 1981). While kokanee are important to the harvest-oriented angling public and for providing trophy fisheries, managing for healthy kokanee populations is often problematic (Beattie and Clancey 1991). Harvest rates of kokanee are heavily influenced by growth rates, population density, and fish size. Since the majority of kokanee populations in Idaho are found in oligotrophic lakes or reservoirs, growth rates are low, especially when population densities exceed 50 fish/ha (Rieman and Maiolie 1995). Additionally, kokanee mature early and typically spawn and die at age-3 or -4 (Johnston et al. 1993). Due to slow growth rates, short life span, and angler's preference for larger fish, kokanee are often only exploited for a short period of time during their last year.

In Idaho, hatchery-reared diploid (2N) kokanee are stocked to supplement depressed wild populations and to provide put-grow-and-take fisheries. Using triploid (3N) salmonids has become increasingly common in hatchery-supported freshwater fisheries. Triploid salmonids are functionally sterile, and the common assertion is that sterility provides a fisheries or aquaculture benefit (Teuscher et al. 2003). Benefits of stocking triploid salmonids may include increased longevity and survival (Ihssen et al. 1990), genetic protection of wild stocks (Rohrer and Thorgaard 1986), as well as increased growth (Habicht et al. 1994; Sheehan et al. 1999). However, drawbacks of stocking triploid salmonids may include higher mortality and reduced growth during early life-history stages (Myers and Hershberger 1991).

While triploid kokanee would be a poor alternative to increase natural production, their increased longevity could be beneficial in extending recreational fisheries opportunities over the long term (Johnston et al. 1993). Enhanced longevity may provide additional sportfishing opportunity in subsequent years after semelparous diploids would have already perished. Additionally, greater longevity could result in increased yield and size, since kokanee are known to be increasingly susceptible to angling as length increases (Rieman and Maiolie 1995). We were therefore interested in whether the benefits of stocking triploid kokanee in put-grow-and-take fisheries would outweigh the detriments of lower egg eye-up rates and poor initial survival (Parkinson and Tsumura 1988). More specifically, the objective of this study was to enhance the longevity of kokanee through sterilization by at least one year and thereby increase harvest rates by 25%.

OBJECTIVE

1. To increase the longevity of kokanee through sterilization by at least one year and thereby increase angler harvest opportunity by 25%.

METHODS

Test Groups

Test groups were spawned using eggs collected at a weir on the Deadwood River from August 23 through September 7, 2004. Ripe kokanee were anesthetized prior to spawning. The

eggs of 4-13 females were fertilized with the milt of 4-13 males in each of four spawning bowls. An equal number of males and females were spawned in 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. Triploid kokanee eggs were produced using a heat bath at 27°C at 20 minutes after fertilization (MAF) for 20 minutes. This treatment worked well in a previous experiment and provided high induction rates (98%) and acceptable survival rates (64% to eye-up, relative to controls; Kozfkay 2003). After heat treatment, eggs were shipped to rear at Mackay Fish Hatchery with 2N production egg lots. Triploid induction rates of the heat-treated group were determined when the fish reached approximately 50 mm, using 100 blood samples stored in Alsever's solution. Samples were shipped to North Carolina State University where ploidy levels were determined using flow cytometry by Dr. Jeff Hinshaw.

A quick and efficient method for applying two distinct batch marks was needed to mark large numbers of 2N and 3N kokanee stocked as fry. Calcein has been shown to be a persistent mark for Atlantic and Chinook salmon as well as steelhead trout (Mohler 1997, 2003a, 2003b). Kokanee fry were marked following the techniques outlined in Mohler (2003a, 2003b), using SE-MARK™ calcein solution diluted to 5g/L and a 1.5% salt bath pretreatment. Based on a pilot study run in 2004 (Kozfkay 2004), we single marked the 3N group and double marked the 2N group. The first mark was applied from February 8-10, 2005. The second mark was applied to the 2N group from April 18-20, 2005, an interval of 70 days. To assess long-term retention of the calcein marks, 13.2% of the 3N group and 12.5% of the 2N group were marked with right and left ventral fin clips, respectively. Approximately equal numbers of kokanee from the 2N and 3N groups were stocked into the five study waters from April 28 through June 3, 2005 (Table 1).

Field Sampling

Kokanee were sampled from each of the five study waters between June 13 and July 26, 2007 using a combination of experimental gill nets and net curtains. Nets were set prior to sunset and fished overnight. During each sample night, one to five gillnets and one or two net curtains were fished at each reservoir. Floating experimental gill nets measured 46 m long by 2 m deep and were composed of panels of 19, 25, and 32 mm stretch mesh monofilament. Experimental net curtains measured 55 m long by 6 m deep and were composed of panels of 19, 25, 32, 38, 51, and 64 mm stretch mesh monofilament. Gillnets and net curtains were either set floating on the surface or suspended along the thermocline.

Sampling effort varied across locations. Devils Creek Reservoir, Mirror Lake, and Lower Twin Lake were sampled two nights, while Lucky Peak Reservoir and Ririe Reservoir were sampled three nights. All kokanee captured were measured for total length to the nearest millimeter and weighed to the nearest gram. Sex and maturity level were determined by observing gonads. Sexual maturity was assigned to one of the three levels: immature, developing, or mature. Immature gonads were small, with testes being light-colored, opaque, fine-textured organs, and ovaries being granular and translucent, whereas mature fish were characterized as having testes that were much enlarged and milky white and ovaries with evident well-developed eggs (Strange 1996). Developing gonads were characterized as having characteristics intermediate between immature and mature.

Reading Marks

To identify marked kokanee, otoliths were collected in the field and stored dry in micro centrifuge tubes and stored indoors away from direct sunlight. Otoliths were mounted whole to a

microscope slide with Crystalbond™ mounting wax. Before looking for calcein marks, otoliths were first photographed in immersion oil using reflective light at 40X power using a Leica (Model DC 500) digital camera and Leica (Model DM 4000B) compound microscope. Typical focus position and annuli patterns were initially determined using known age-2 kokanee from each reservoir based on samples from fin-clipped kokanee that were captured. Ages were estimated using photographs of whole otoliths from both otoliths (when available).

Relative proportions of 2N and 3N kokanee were determined by examining a subsample of the total otoliths collected for calcein marks. The subsample was chosen based on several criteria intended to narrow the samples to those most likely corresponding to the size range of the marked test fish. We used the length frequency histograms and the size range of fin-clipped kokanee captured from each reservoir. All fish within 50 mm below the minimum size of fin-clipped test fish and all samples larger than fin-clipped test fish were examined for calcein marks. Additionally, any fish that did not fit into these length criteria but were aged as age-2 (based on examination of otoliths) were also included. Selected samples were initially wet sanded lightly to prevent sanding through the plane of the mark. Initial sanding with 600-grit sandpaper was followed with 1200-grit to lightly polish after each sanding. Otoliths were alternately sanded and viewed using a compound microscope at 40X under UV light, using a calcein-specific filter set and dichromatic mirror (Chroma #41012). Iterations of sanding and viewing continued until the mark was clearly visible or until the otolith had been sanded through the plane of the focus.

The total numbers of marked kokanee stocked and later recaptured were adjusted to reflect the triploid-induction rate (Table 1, Table 2, Appendix A). The 79% induction rate was first applied to the original number of triploid kokanee stocked to calculate corrected totals for each group. The total number of diploid kokanee stocked and recaptured was used to calculate a relative survival for diploids only. This was then applied to the 21% of triploid kokanee stocked that were likely diploid to determine how many of the marked triploid kokanee recaptured were actually diploid. We assumed that the survival ratio of diploid: triploid kokanee was constant across all the study sites. See Appendix A for complete breakdown of calculations.

Limnology Sampling

Limnological samples were collected in July and August 2007. Samples were collected at three locations equally spaced along the longitudinal axis of each water body and marked using GPS (NAD83 datum). Limnological variables were measured at each location using the Hydrolab MiniSonde 4a and data logger (model Surveyor 4a). Water quality data were collected across the vertical water column at 1 m intervals. At each depth interval temperature (°C), dissolved oxygen (DO, mg/L), pH, and conductivity (µS) were recorded. Mean Secchi transparency was also recorded at each site by two different observers. Zooplankton size and abundance data were collected to describe forage availability for kokanee in each water body. Three samples were collected at each sample location using conical plankton nets of 150, 500, and 750 µm mesh with an opening width of 50.8cm. Samples were collected using a 9.3 m deep vertical tow and stored in 70% alcohol at a concentration 1:1 (sample volume:alcohol) (Teuscher 1999). The zooplankton ratio index (ZPR) (the ratio of preferred to usable zooplankton) and the zooplankton quality index (ZQI) (a total measure of zooplankton abundance corrected with size ratios) was calculated and corrected for tow depth as described by Teuscher 1999. Plankton scores were averaged across locations by lake to determine a mean score for each sample date.

RESULTS

Test Groups

During spawning, 725 female kokanee were used for creation of the 3N 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 2N groups were not true controls, they do act as a good reference for comparison of survival between groups. Over the five days when both 2N and 3N 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, resulting in 110,946 2N and 102,523 3N kokanee stocked.

Length and weight of kokanee were similar between test groups prior to stocking. Mean length of the 2N ($\bar{x} = 86 \pm 1$ mm; $n = 100$) and 3N ($\bar{x} = 88 \pm 2$ mm; $n = 100$) groups was equal based on overlapping 90% CIs. Similarly, mean weight of the 2N ($\bar{x} = 3.9 \pm 0.2$ g; $n = 100$) and 3N ($\bar{x} = 4.0 \pm 0.2$ g; $n = 100$) groups was equal. Flow cytometry analysis indicated that triploidy induction rate for the 3N group was 79% ($n = 99$). Approximately equal numbers of kokanee from the 3N and 2N groups were stocked into four of the five study waters (Table 1). Lucky Peak was the only exception, with 49,950 kokanee diploids and 41,400 triploids being stocked.

Field Sampling

Gillnets and net curtains were fished from 61.7-131.3 hours per water body, yielding a total effort of 455.4 hours. Catch-per-unit-effort (CPUE) for all kokanee combined ranged from 0.8 fish/hr (Mirror Lake) to 4.9 fish/hr (Lucky Peak and Ririe Reservoirs), with a mean of 2.7 ± 0.60 fish/hr (indicating 95% CI) (Table 3). For test fish only, Twin Lake had the highest catch rates for 2N and 3N kokanee at 0.21 fish/hr and 0.15 fish/hr, respectively. Ririe Reservoir had the lowest catch rates of diploids (0.03 fish/hr), while Lucky Peak had the lowest catch of triploids (zero). On average, gill nets and net curtains caught 0.08 marked 2N kokanee per hour and 0.06 marked 3N kokanee per hour across all reservoirs.

Mean length (mm) and mean weight (g) of kokanee captured varied between water bodies (Figure 1), ranging 222-335 mm (Table 4). Likewise, the mean weight (g) of kokanee per site ranged from 101–403 g (Table 3). Figure 2 shows length frequency for all water bodies combined. Statistical comparisons of mean length and weight between 2N and 3N groups within reservoirs could not be made because of limited sample sizes. When combined across all sites, there was no difference in length (322 ± 20 mm and 316 ± 26 mm) or weight (326 ± 86 g and 304 ± 101 g) between 2N and 3N kokanee, respectively, based on 95% CIs. Lucky Peak Reservoir and Devils Creek Reservoir had the highest mean lengths (over 400 mm) for marked kokanee, indicating rapid growth in the first two years of age.

Reading Marks

Numbers of kokanee caught in each study location during 2007 were highly variable, ranging from 78 to 468 (Table 2). Overall, 1,208 kokanee were captured, with the majority being unmarked nontest fish (1152, or 95%). From the total captured, 305 kokanee fit the criteria to be included in the subsample examined for calcein marks. Fifty-six test fish were identified (5%) based on fin clips and/or calcein-marked otoliths. Diploid kokanee made up 61% (34 fish) of the

test fish caught and were captured from all five water bodies. However, when corrected for the 79% triploid-induction rate, diploid kokanee comprised 73% (41 fish) of the total marked kokanee captured. Only 22 kokanee marked as 3N were caught, having been caught in all study waters except Lucky Peak Reservoir. When corrected for the triploid-induction rate, 3N kokanee made up only 27% (15 fish) of the total marked kokanee recaptured. A small percentage (13.2% and 12.5% for 2N and 3N, respectively) of the test fish stocked was double-marked with both calcein and ventral fin clips. Of the marked kokanee caught, six possessed right ventral fin clips (3N) and five had left ventral clips (diploids). Ten of these clipped fish had visible calcein marks present in their otoliths, suggesting a 91% mark retention rate for calcein in otoliths two years after stocking.

Limnology Sampling

Mean zooplankton quality index (ZQI) varied between the five water bodies, ranging from 0.05–0.42 (Table 5). Mirror Lake returned the lowest ZQI score (0.05), while the highest score (0.42) was recorded at Twin Lake (Lower).

DISCUSSION

The variable river water temperatures that seemed to affect survival during production of 3N kokanee groups (Kozfkay 2004) also seemed to affect triploidy induction rates. Higher or lower water temperatures would submit fish to a more mild or intense heat shock than for fish held at a constant temperature as in all of our previous (in-hatchery) sterilization experiments. Flow cytometry indicated that 3N induction rates were relatively low (79%) for the test group created for this study compared to previous experimental kokanee treatments (Kozfkay 2003) and production efforts for other species such as rainbow trout (IDFG 2007). Future efforts to develop 3N kokanee stocking programs should focus on using pressure treatment as a more consistent method.

Although the 79% triploid-induction rate is not ideal, it should not affect the utility of this study. Using the combined total number of fish caught across all reservoirs, we were able to approximate the ratio of diploid to triploid kokanee captured. Of course, this was calculated assuming that this ratio was constant across all the study lakes. There simply was not enough data to correct the numbers of marked fish caught at individual study locations. Kozfkay and Koenig (2006) suggested it may be possible to address the lower triploid-induction rate by collecting blood or fin-clip samples that could later be analyzed using flow cytometry to allow development of more precise correction factors (Lamatsch et al. 2000). However, after completing a season of sampling, the feasibility of collecting and later processing fin clip tissue into single cell suspensions for flow cytometry analysis is questionable, given the time, equipment, and scheduling necessary. Without a readily detectible mark to denote test fish in the field, large numbers of kokanee would have to be processed in the hopes of detecting small numbers of 3N fish. All fin clip samples would later be matched to calcein-marked otolith samples to ensure that they belonged to one of the marked test groups. Collecting and processing fin clips would add to the already lengthy processing time for fish sampled in the field. Additionally, processing fin clips would also require the involvement of at least one (and probably two) outside laboratories to prepare the suspensions and then to conduct the flow cytometry analysis. Fin clip tissues would have to be processed within 4-6 weeks of collection, so timing would be critical.

The combined totals of marked kokanee captured suggest that 2N kokanee survive at higher rates up to age-2 than their 3N counterparts do. Diploid kokanee accounted for a large percentage (61%, N = 34) of the total marked kokanee captured, especially when corrected for the 79% triploid-induction rate (73%, N = 41) (Table 2). In fact, the correction for the triploid induction rate assumes equal survival between the treatment groups, so this is probably a very conservative estimate. Sample size dictates that the number of 2N and 3N kokanee captured should be interpreted with caution. The total number of marked fish captured is so low that spurious conclusions from statistical comparisons are possible. Data from Mirror Lake and Twin Lake suggest that 2N kokanee are captured in higher numbers than triploids at two years of age. However, returns from Lucky Peak, Devils Creek, and Ririe reservoirs are inconclusive. Additional sampling in 2008 should yield some insightful data, since many of the 2N group may have reached sexual maturity and spawned in fall 2007, removing them from the total population. Unfortunately, the number of marked kokanee captured was so low that robust statistical comparisons were not possible.

In order to achieve adequate numbers of marked test fish in 2008, sampling effort will have to be increased dramatically. Elrod and Frank (1990) recommended a sample size of 279 fish was needed in order to detect a 20% difference between paired release groups ($\alpha = 0.05$, $1 - \beta = 0.90$). Given that marked fish made up about 5% of the total kokanee captured in 2007, we would have needed to capture approximately 5,200 total kokanee. Capturing 5,200 kokanee would have required sampling for 29 nights, given the mean catch rate of 2.7 kokanee/hr (in 2007) and setting six nets for 12 hours per night. Additionally, this would assume that samples could be appropriately pooled across all five test waters, a condition that has yet to be examined. Sampling in 2008 is not likely to yield as high a proportion of marked fish, since many of the 2N group likely reached sexual maturity and spawned in fall 2007, and sampling crews have finite time to collect kokanee. Thus, capture probability of marked kokanee in 2008 could be exceedingly low if effort remains unchanged.

Unlike the breadth of work reported for 3N salmonids in aquacultural settings, published literature on the performance of 3N kokanee in natural environments is sparse. Parkinson and Tsumura (1988) sterilized kokanee by applying several levels of 17α -methyltestosterone (MT) to feed. Initial survival of the treated groups was lower than the untreated groups, but proportions of treated fish in the catch increased after age-3, indicating that sterile fish survived longer to older ages. In another study, MT-sterilized kokanee dominated the catch in Salsbury Lake, BC after untreated fish matured at age-3 (Johnston et al. 1993). While the total number of sterile kokanee recaptured was lower than that of untreated kokanee, sterile kokanee had much greater longevity. The authors found sterile kokanee persisted through age-7, whereas only four untreated fish were captured after age-4 and none was captured after age-5. However, despite increased longevity, sterilized kokanee did not outgrow untreated kokanee (Johnston et al. 1993).

Other authors have reported poor results from field experiments using triploid coho and hormone-sterilized Chinook salmon. Rutz and Baer (1996) found that triploid coho salmon grew more slowly and survived poorly compared to diploids, making up only 25% of the catch two years after stocking. In 1986, the Wisconsin Department of Natural Resources experimented with hormone-sterilized Chinook salmon in Lake Michigan (see Kitchell and Hewett 1987 for review), but the results were inconclusive because few fish were ever recaptured (M. J. Hansen, University of Wisconsin, personal communication). At this time, our results are similar to those of Johnston et al. (1993), where sterile kokanee show lower initial survival. Sampling in 2008 will help determine whether triploid kokanee in this evaluation will exhibit longer life spans and make up a larger proportion of marked fish recovered. Future research might include a multiyear study to investigate how annual stocking of only triploid kokanee could affect population age and size

structure to improve sport fishing yield and size in a typical kokanee fishery. Although triploid kokanee may have lower mortality rates in early years, this may be offset by increased longevity. Extended longevity might result in a population with multiple overlapping age classes of adult-sized fish. Such a population might result in higher catch rates, with greater numbers of larger, more catchable kokanee, increasing over time as age classes overlap.

Although calcein does have some advantages as a mass-marking tool, using it for paired release experiments in the future is not recommended. Judging from the double-marked fish recovered, calcein did show high retention rates in otoliths two years after marking (91%, $n = 11$). Calcein can also be applied to large numbers of small fish quickly and economically. However, calcein has several significant disadvantages when compared to other marking techniques that need to be considered. While examining otoliths under UV light, auto-fluorescence of the sample may obscure the calcein mark, causing a false negative. Having recovered only 11 double-marked samples, it is difficult to estimate tag reading error. Ten of these eleven double-marked kokanee had visible calcein marks. In this respect, one could interpret that as either a 91% tag retention rate, or a 9% reading error. Secondly, sanding/polishing otoliths is often required before the mark can be seen. This takes time and allows the possibility of sanding through the plane of the mark, at which point the mark is no longer visible. To avoid this, the sample must be sanded very carefully and then examined. This process must be repeated several times before concluding whether a sample is marked, increasing the processing time for each otolith sample. Both otoliths for a single fish should also be examined to confirm marks. This evaluation could benefit from a mark that requires less interpretation to distinguish groups and less uncertainty in identifying the mark. Additionally, it is difficult to directly estimate mark retention or tag reading error if a secondary (and more reliable) mark is not used in conjunction with calcein.

The time associated with processing otoliths to read calcein marks limits the effectiveness of calcein for long-term field evaluations. Typically, one technician may be able to mount 80-100 otoliths onto microscope slides during an 8-hour workday, which results in only 40-50 fish if using both otoliths. For reading marks, two trained technicians can read 80-100 slides in a typical 8-hour workday, with one person sanding/polishing while another examines the slides for marks. The time needed to process samples can hinder counting the number of marked fish recaptured. Knowing recapture totals while sampling is useful so that sampling intensity can be adjusted to meet sample size requirements. In this respect, using calcein to distinguish test groups would still require some sort of externally-detectable mark on an adequate proportion of the population. Fin clips and coded-wire tags might be a better option to reduce tag reading error and tag processing time and would easily distinguish marked fish when captured in the field (Elrod and Schneider 1986; Munro et al. 2003). These types of tags would require higher application costs to tag large numbers of fish but would have lower decoding costs and provide more accurate and timely results.

RECOMMENDATIONS

1. Discontinue using calcein as a mass-mark for long-term paired release experiments for salmonids.
2. Continue sampling efforts in summer 2008 to capture age-3 marked kokanee. Performance differences between 2N and 3N groups should become more apparent in 2008 after many of the 2N group may have spawned during 2007.

3. Initiate a multiyear study to investigate how annual stocking of only triploid kokanee could affect population age and size structure to improve sport fishing yield and size by developing overlapping age classes of adults.

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Table 1. Stocking location, date, and number of kokanee stocked during 2005 in five Idaho lakes and reservoirs to assess relative performance of diploid (2N) and triploid (3N) kokanee. Columns to the right of each stocking group indicate stocking densities in fish per hectare of lake area. The triploid group is abbreviated as 3N, whereas the diploid group is abbreviated as 2N. "Corrected Total" refers to the total number of 2N and 3N kokanee planted if corrected for the 79% triploid-induction rate of the test groups (see Appendix A).

Water Body	Date Stocked	2N	Number of kokanee planted				
			Fish/ha	3N	Fish/ha	Nontest	Fish/ha
Mirror Lake	May 31, 2005	2,516	74	2,520	74	0	0
Twin Lake (Lower)	May 16, 2005	20,000	127	20,000	127	20,000	127
Lucky Peak Res.	June 3, 2005	49,950	45	41,400	37	108,800	97
Devils Creek Res.	May 19, 2005	3,520	101	3,503	100	0	0
Ririe Res.	April 28, 2005	34,960	61	35,100	61	140,975	246
Grand Total		110,946		102,523		269,775	
Corrected Total		132,476		80,993			

Table 2. Total kokanee captured by test group by lake during summer 2006 using a combination of gill nets and net curtains. "Corrected Total" indicates group totals if adjusted for the 79% triploid induction rate (see Appendix A).

Lake Name	Total	Nontest	Diploid	Triploid	2N/3N Ratio
Mirror Lake	78	65	8	5	-
Twin Lake (Lower)	112	88	14	10	-
Lucky Peak Reservoir	466	462	4	0	-
Devils Creek Reservoir	84	78	3	3	-
Ririe Reservoir	468	460	5	4	-
Grand Total	1208	1152	34	22	1.55
Corrected Total	-	-	41	15	1.61

Table 3. Mean gillnet catch-per-unit-effort (total fish per hour of netting) of kokanee by test group captured during summer 2007 by study location. "Adjusted Mean CPUE" reflects the total marked kokanee captured (adjusted for the 79% induction rate) divided by the total hours of netting effort. CPUE was not adjusted for induction rate at individual lakes.

Lake Name	Total	Nontest	Diploid	Triploid
Mirror Lake	0.8	0.66	0.08	0.04
Twin Lake (Lower)	1.7	1.32	0.21	0.15
Lucky Peak Reservoir	4.9	4.85	0.04	0.00
Devils Creek Reservoir	1.4	1.27	0.05	0.05
Ririe Reservoir	4.9	3.50	0.03	0.04
Mean CPUE	2.74	2.32	0.08	0.06
Adjusted Mean CPUE	-	-	0.09	0.03

Table 4. Mean total length (mm) and weight (g) (\pm 95% confidence interval) for total, diploid, and triploid kokanee by study location.

Lake Name	Mean Length (\pm CI)			Mean Weight (\pm CI)		
	Total	Diploid	Triploid	Total	Diploid	Triploid
Mirror Lake	221 (10)	274 (11)	276 (18)	101 (11)	170 (27)	174 (49)
Twin Lake (Lower)	250 (10)	293 (5)	289 (8)	148 (9)	189 (22)	197 (18)
Lucky Peak Reservoir	262 (8)	400 (48)	-	271 (13)	649 (172)	-
Devils Creek Reservoir	334 (9)	448 (48)	446 (60)	403 (36)	921 (266)	834 (283)
Ririe Reservoir	260 (4)	339 (13)	336 (31)	169 (7)	345 (38)	341 (75)

Table 5. Mean zooplankton quality index (ZQI) scores by study site and sampling dates.

Lake Name	Date Sampled	Mean ZQI
Mirror Lake	7/18/2007	0.05
Twin Lake (Lower)	7/18/2007	0.42
Lucky Peak Reservoir	8/13/2007	0.09
Devils Creek Reservoir	7/23/2007	0.38
Ririe Reservoir	7/24/2007	0.14

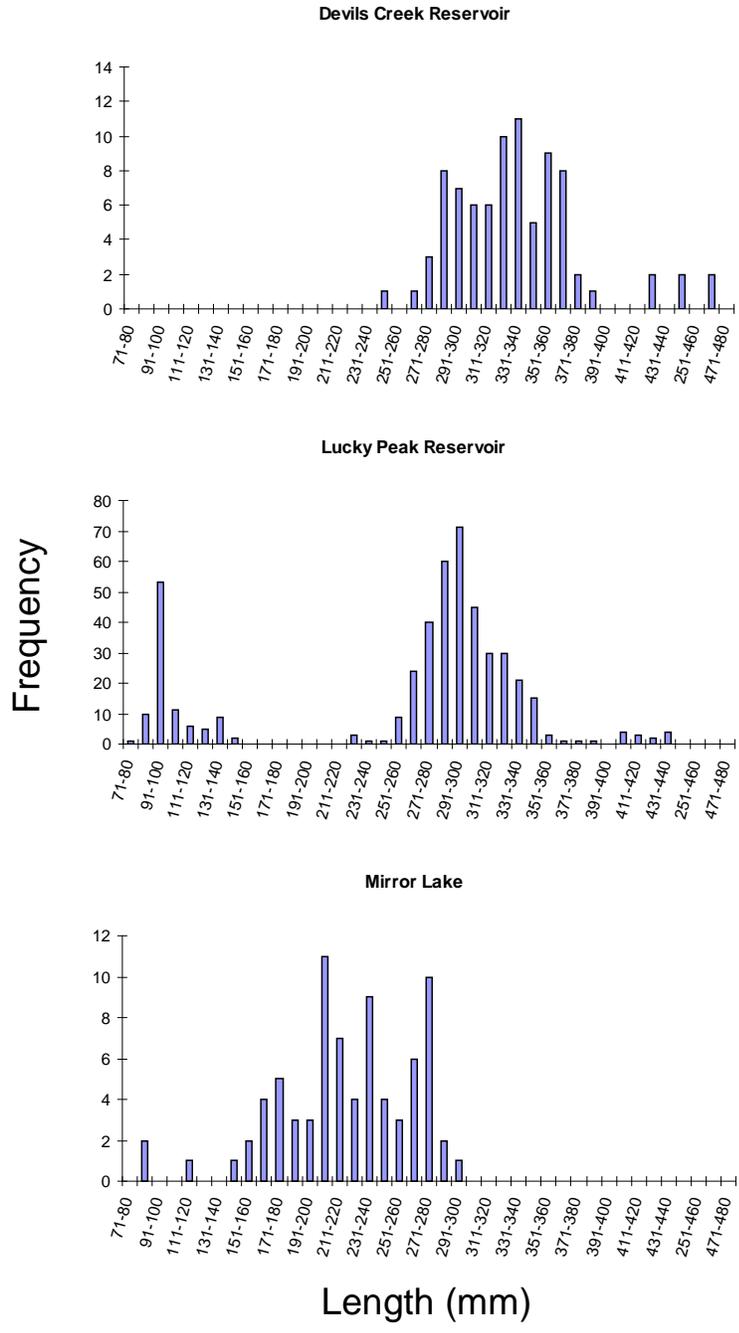


Figure 1. Length-frequency histograms for kokanee sampled from five Idaho lakes and reservoirs during June and July 2007.

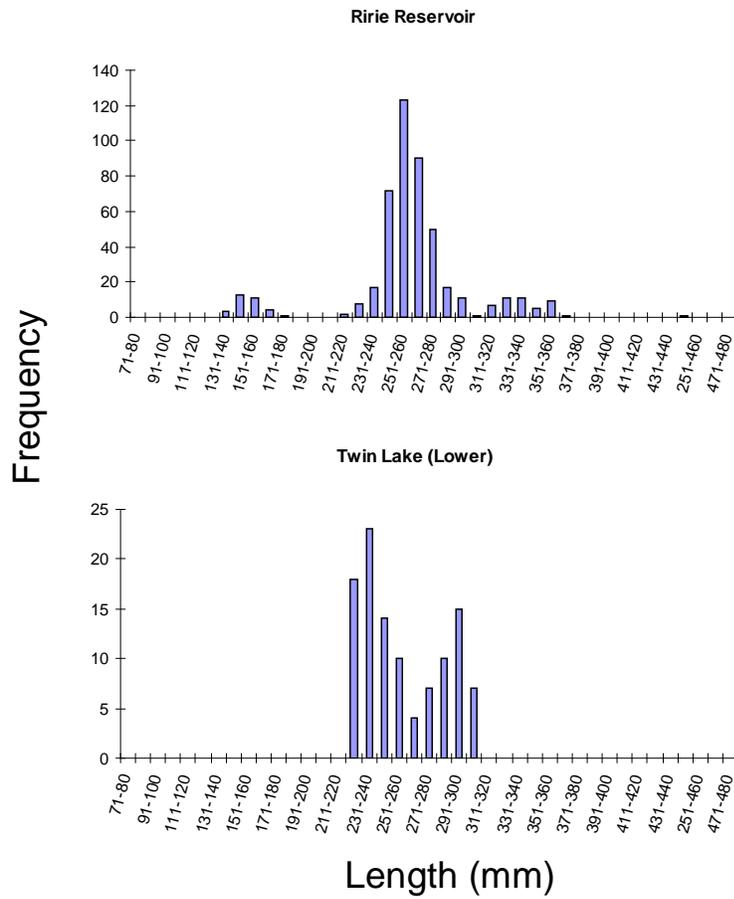


Figure 1 Continued. Length-frequency histograms for kokanee sampled from five Idaho lakes and reservoirs during June and July 2007.

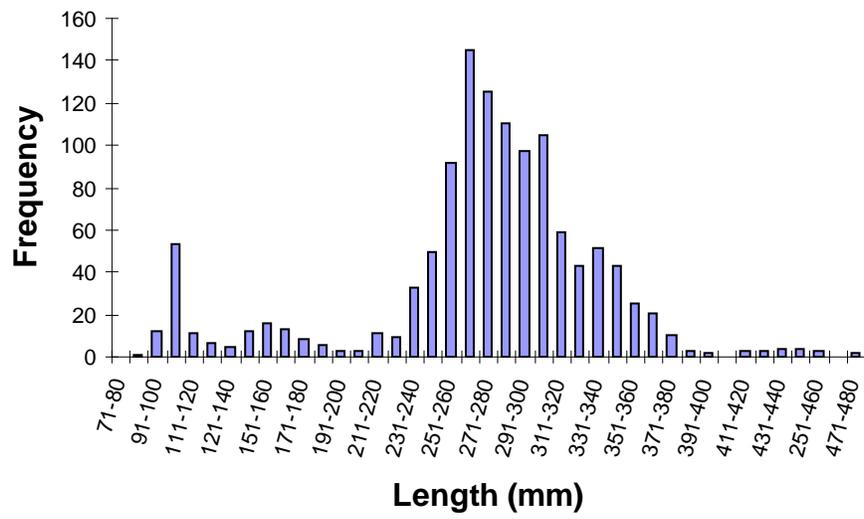


Figure 2. Length frequency histogram for cumulative kokanee sampled from five study waters surveyed during summer 2007.

**ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT INVESTIGATIONS: PERFORMANCE OF STERILE
TROUT IN HIGH MOUNTAIN LAKES**

State of: Idaho Grant No.: F-73-R-30 Fishery Research
Project No.: 4 Title: Hatchery Trout Evaluations
Subproject #2: Sterile Trout Investigations
Contract Period: July 1, 2007 to June 30, 2008

ABSTRACT

Increased growth, improved survival, and genetic protection of wild stocks have been suggested as benefits of stocking triploid (i.e. sterile) salmonids for recreational fisheries. We examined the relative survival and growth of mixed-sex diploid (2N), mixed-sex triploid (3N), and all-female triploid (AF3N) rainbow trout *Oncorhynchus mykiss* across 28 alpine lakes stocked in 2001 and 2003 and sampled 2-4 years later. During 2004, a total of 779 trout were captured, including 59 2N and 29 3N fish with mean lengths of 332 and 322 mm, respectively. During 2005, an additional 295 trout were captured, including 19 2N and seven 3N fish with mean lengths of 348 and 327 mm, respectively. Taken together, the 2N group composed an average of 0.68 of the total marked fish caught, and the combined proportions of marked test fish (including netting and angling) differed significantly between the test groups, and differed consistently across survey years. During 2006 and 2007, 1,195 trout were captured, including 60 2N, 31 3N, and 212 AF3N rainbow trout. Mean length of marked fish was similar between test groups for each year. Overall, the relative survival of triploid rainbow trout in alpine lakes in Idaho was low compared to diploids, while AF3N rainbow appeared to return in higher proportions than both groups. Triploid groups studied in this evaluation did not show any growth advantages over the duration of the study, but ultimate mean size was similar to that of diploids. Fisheries managers concerned with maintaining consistent alpine lake fisheries while minimizing natural reproduction or impact to native stocks should consider all-female triploid salmonids as a viable option to fertile fish.

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INTRODUCTION

Idaho contains approximately 3,000 alpine lakes ranging from small seasonal ponds to lakes over a mile long (IDFG 2007a). Of these lakes, approximately 1,355 are stocked by the Idaho Department of Fish and Game (IDFG) or have self-sustaining fish populations. Fishing opportunities in alpine lakes are highly rewarding, offering solitude, dramatic scenery and a backcountry experience seldom found in other fisheries. Not surprisingly, anglers visiting these lakes typically express the highest level of satisfaction with their fishing experience (WGF 2002; IDFG 2007a). Alpine lake fisheries can provide a high quality angling experience at relatively little investment in stocking costs to management agencies (Wiley 2003; IDFG 2007b). In Idaho, alpine lakes make a significant contribution to Idaho's recreational economy, garnering visits from over 40,000 anglers annually (IDFG 2007a).

Managing alpine lake fisheries presents a challenge, as managers must balance the conflicting mandates of providing recreational fishing opportunities while minimizing impacts to wilderness ecosystems and native fish communities (Knapp et al. 2001; Wiley 2003). The IDFG Fisheries Management Plan (IDFG 2007a) outlines guidelines regarding the management of alpine lakes, with genetic conservation of wild trout populations as a priority. Triploid salmonids, created by heat or pressure shock, are functionally sterile and may be a useful tool for managing alpine lake fisheries. Sterility can help avoid genetic introgression with downstream wild stocks and provide a fishery benefit such as increased growth (Thorgaard 1986; Boulanger 1991; Teuscher et al. 2003) or longevity (Parkinson and Tsumura 1988; Johnston et al. 1993; Warrillow et al. 1997). The IDFG has established a policy to stock only triploid rainbow trout in systems where stocked rainbow trout pose a genetic risk to native trout populations (IDFG 2007a). Implementation of the above-noted policy has resulted in the widespread stocking of sterile rainbow trout in hundreds of Idaho alpine lakes (IDFG 2007b).

Survival, longevity, and growth of triploid salmonids in natural environments are inconsistent relative to diploid fish (Brock et al. 1994; Simon et al. 1993; Parkinson and Tsumura 1988; Warrillow et al. 1997) and may be species- or strain-dependent (Ihssen et al. 1990). Studies describing relative survival and return-to-creel of triploid rainbow trout have been performed in streams, ponds and reservoirs (Simon et al. 1993; Dillon et al. 2000; Teuscher et al. 2003), but literature describing their performance in alpine lakes is sparse. In such lakes, trout may experience low temperatures and severe hypoxia in winter (Gruber and Wieser 1983; Rahel and Kolar 1990), and some authors suggest that triploid salmonids may suffer higher mortality under stressful conditions (see Benfey 1999 for review). Before using sterile fish as a common management tool in alpine lakes, it is important to determine if stocking triploid rainbow trout produces satisfactory fisheries. If not, managers may need to adjust stocking strategies rather than rely on historical stocking levels, as is often practiced (Meyer and Schill 2007). Our objective was to examine growth and relative survival of diploid and triploid rainbow trout stocked in alpine lakes 3 and 4 years after stocking—when trout would normally have reached sexual maturity.

RESEARCH GOAL

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

OBJECTIVE

1. Determine if growth and relative survival of triploid rainbow trout is comparable to that of diploid rainbow trout in stocked alpine lakes.

STUDY SITE

We chose 32 lakes in central Idaho representing the range of sizes and elevations of alpine lakes typically stocked with rainbow trout by IDFG (Table 6). Lakes ranged in elevation from 2138 to 3157 m, in surface area from 0.7 to 23.5 ha, and in maximum depth from 2.8 to 26.6 m. Study lakes were selected from those having a history of rainbow trout stocking and were scheduled to receive plants in the given study year. Additionally, past surveys must have indicated that lakes were capable of supporting trout fisheries. Test fish were not stocked in drainages where conflicts with native or wild populations were likely or in lakes where brook trout *Salvelinus fontinalis* populations were established. All study lakes were managed under the “general” trout regulation of six fish per day with no length restrictions, except for two lakes that were managed under the trophy regulation of two fish per day with none under 508 mm.

METHODS

2001 Stocking

Rainbow trout were obtained from mixed-sex rainbow trout eggs produced from 1:1 pairings at Hayspur Fish Hatchery. Triploid eggs were produced by thermal-shock in a 26°C water bath at 20 minutes after fertilization (MAF) for 20 minutes (Teuscher et al. 1998). The triploid induction rate was estimated at 98% using blood samples and flow cytometry ($n = 40$). Diploid (hereafter 2N) and triploid (hereafter 3N) groups were marked with adipose fin clips and either green or red fluorescent grit dye, respectively. Initial mark retention in the hatchery two weeks post-marking was estimated as 94% for the diploid group (green) and 98% for the triploid group (red). Three hundred fry from each group were then stocked into 16 alpine lakes by fixed-wing aircraft from August 30 to September 15, 2001. At the time of planting, mean size ranged from 65 to 67 mm total length. Previous studies suggest benefits of sterile salmonids are not realized until after the species normally reaches maturity (Simon et al. 1993; Teuscher et al. 2003). Accordingly, lakes were initially surveyed three years after stocking between July 16 and August 24, 2004, with half of the lakes sampled again between July 6 and August 16, 2005.

2003 Stocking

Rainbow trout were obtained from mixed-sex eggs produced from 1:1 pairings at IDFG's Hayspur Fish Hatchery. Half of the eggs were thermal-shocked (using the methods described above) to produce a mixed-sex triploid group (3N), while the other half were untreated and used as a mixed-sex diploid group (2N). In addition, all-female triploid Kamloops strain rainbow trout (hereafter AF3N) eggs were purchased from Troutlodge, Inc. (Sumner, Washington). Fry from the 2N, 3N, and AF3N groups were marked using left ventral, right ventral, and adipose fin clips, respectively. Triploid induction rates were estimated as 100% for the 3N group ($n = 25$) and the AF3N group ($n = 60$) using blood samples analyzed with flow cytometry. Marked trout were stocked in 14 lakes from August 14-22, 2003 using fixed-wing aircraft or all-terrain vehicles.

Each of the lakes received 500 fry from each treatment group except Blue Lake, which received 400 3N fry and 500 of the other two groups. At the time of planting, mean size ranged from 62 to 65 mm total length. As in the 2001 stocking, lakes were initially surveyed three years after stocking between July 7 and October 2, 2006, with half of the lakes sampled again from July 30 to September 26, 2007.

Sample Collection

Lakes were sampled using a combination of angling and gill nets. Floating experimental gillnets consisted of nylon mesh panels of 19, 25, 30, 33, 38, and 48 mm bar mesh and were 46 m long and 1.5 m deep. Typically, three to six gillnets were set perpendicular to the shoreline around the lake and set overnight. All rainbow trout captured were measured to nearest millimeter (total length), weighed to the nearest gram, and examined for fin clips and the presence of grit marking (when applicable) using 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 bag. Gonads of rainbow trout were examined to determine sex and assigned one of three levels of maturity: immature, developing, or mature. Immature gonads were small, with testes being light-colored, opaque, fine-textured organs, and ovaries were granular and translucent; mature fish were characterized as having enlarged testes that were milky white or having ovaries with well-developed eggs (Strange 1996). Developing fish were characterized as having gonads with characteristics intermediate between immature and mature.

Bathymetric and water quality data were collected along three transects 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, conductivity, pH, surface water temperature, and Secchi depth were collected at each of the sampling points. Depth measurements were collected with handheld sonar. Lake temperature was recorded hourly for one year using a thermograph placed in each lake approximately 0.6 m below the water's surface and 2-3 meters from shore. Lake area was determined with ArcGIS 9.2 software.

Data Analysis

Mean length and weight of test groups prior to stocking were compared using 95% confidence intervals (CI) around the mean. Catch-per-unit-effort (CPUE) was calculated for each test group by lake, survey year, and gear type by dividing the total number of fish caught by the total hours of netting or angling effort. Because of the different combinations of fish strains used in the years of stocking, catch data from each stocking year (2001 or 2003) were examined separately. Mean length and weight was compared between groups within each sampling year using mixed-model ANOVA and Tukey's multiple comparisons. To compare relative survival, the total numbers captured from each group were expressed as a proportion of the total combined marked trout caught within each lake. We used the combined total marked trout caught from both angling and gill nets for analysis, with each lake serving as one independent observation. The number of diploid and triploid fish captured was adjusted to reflect the triploid induction rate at the time of stocking. Prior to analysis, catch data were transformed using a log+1 transformation to meet the assumptions of identical, independent, and normally distributed errors. The mean proportion captured was compared between groups using mixed-model ANOVA and Tukey's multiple comparisons with repeated measures to account for multiple survey years at some lakes. The "stocking group" and "survey year" were treated as categorical fixed effects, while "lakes" were treated as random effects. All statistical tests were performed with $\alpha = 0.05$ using Statistical Analysis Software 9.1 (SAS 2003-2004).

RESULTS

2001 Stocking

Size at Stocking

At the time of stocking, the mean length (\pm 95% CI) of the 2N (65 ± 1 mm, $n = 100$) and 3N (67 ± 1 mm, $n = 100$) groups were similar, based on overlapping 95% confidence intervals. Mean weight for the 2N (3.0 ± 0.2 g, $n = 100$) and 3N groups (3.0 ± 0.2 g, $n = 100$) was equal.

CPUE of Test Fish

CPUE of marked test fish caught in 2004 and 2005 was variable across lakes and treatment groups (Table 8). During 2004, a total of 779 trout were captured, including 59 diploid and 29 triploid marked rainbow trout. In 2004, the 3N and 2N groups were caught on average at 0.03 and 0.06 fish/hr of gill netting, respectively. Angling in 2004 produced similar or higher average catch rates (0.11 and 0.03 fish/hr for 2N and 3N, respectively). However, angling was only successful at capturing marked fish in four of the 12 lakes where it was used (Table 7).

During 2005, a total of 295 salmonids were sampled, including 19 diploid and 7 triploid marked rainbow trout. The mean CPUE of in 2005 using gill nets for the 3N and 2N groups was 0.02 and 0.07 fish/hr, respectively (Table 7). Similar to the 2004 sampling, angling provided higher average catch rates for 2N and 3N groups (0.92 and 0.22 fish/hr, respectively), but was only successful in three out of the six lakes sampled with angling.

Size of Test Fish

The 2N and 3N fish captured in 2004 had a mean length of 332 and 322 mm, respectively, but differences in length were not statistically different between test groups (ANOVA: $F_{1, 83} = 1.80$; $P = 0.18$). In 2005, the 2N and 3N groups averaged 348 and 327 mm, respectively. Mean length in 2005 did not significantly differ between test groups (ANOVA: $F_{1, 24} = 0.65$; $P = 0.43$), despite the apparent differences in length (Table 10). This is likely a result of large variation in sizes and limited numbers of recovered test fish for the 3N group. The mean weight of 2N trout captured in 2004 (376 g) was significantly greater than 3N (305 g) (ANOVA: $F_{1, 83} = 7.04$; $P = 0.0095$), but differences were not significant in 2005 (ANOVA: $F_{1, 24} = 1.22$; $P = 0.28$), despite a sizeable disparity in weight (Table 9). Diploid fish captured in 2005 weighed an average of 464 g, while 3N fish weighed an average of 351 g.

Proportions Captured

Overall, the 2N group returned in much higher proportions in both years surveyed. In 2004, the 2N group made up 0.76 of the total marked fish caught on average, while the 3N group made up only 0.24 (Table 7). The results were similar in 2005, with the 2N group composing an average of 0.80 of the total marked fish caught (Table 7). Considered across both survey years, the combined proportions of marked test fish (including netting and angling) differed significantly between the test groups (Table 11) (ANOVA: $F_{1, 24} = 91.93$; $P < 0.0001$), but

“survey year” and the interaction of “survey year” by “stocking group” were not significant effects (ANOVA: $F_{1, 10} = 0.01$; $P = 0.99$ and $F_{1, 10} = 0.09$; $P = 0.77$, respectively).

2003 Stocking

Size at Stocking

At the time of stocking, minor differences in mean length existed between groups. Mean length (\pm 95% CI) of the 2N group (62 ± 0.8 mm, $n = 120$) and 3N groups (62 ± 0.9 mm, $n = 120$) was similar, while the AF3N group was slightly longer (65 ± 1.3 mm, $n = 90$). Mean weight of the AF3N group (2.0 ± 0.2 g, $n = 90$) was similar to the 2N (1.8 ± 0.1 g, $n = 120$) and 3N groups (1.8 ± 0.1 g, $n = 120$).

CPUE of Test Fish

CPUE of marked test fish caught in 2006 and 2007 was variable across lakes and treatment groups (Table 8). During 2006, a total of 993 trout were sampled. The 230 marked trout recovered included 49 2N, 19 3N, and 162 AF3N rainbow trout. In 2006, mean gill net CPUE was highest for the AF3N (0.29 fish/hr), followed by the 2N and 3N groups (0.08 and 0.03 fish/hr, respectively). Angling in 2006 resulted in higher mean catch rates and paralleled the same pattern as netting, with AF3N having the highest catch rates (0.68 fish/hr), followed by the 2N and then 3N groups (0.039 and 0.22 fish/hr, respectively). In 2007, 202 trout were captured, including 50 AF3N, 11 2N, and 12 3N rainbow trout. The 2007 average gill net catch rate was again highest for the AF3N group (0.40 fish/hr), while the 2N and 3N groups were caught at 0.04 and 0.03 fish/hr, respectively. Mean angling catch rates in 2007 were higher, ranging from 0.19 fish/hr for the 2N group to 0.40 fish/hr for the AF3N group (Table 8).

Size of Test Fish

The mean length of marked fish captured in 2006 was similar and ranged from 280 mm to 295 mm. However, mean length of marked trout in 2006 did not significantly differ among test groups (ANOVA: $F_{2, 216} = 1.62$; $P = 0.20$) (Table 9). The mean weight of marked trout in 2006 ranged from 233 g to 275 g, and was also not significantly among between test groups (ANOVA: $F_{2, 214} = 2.00$; $P = 0.14$), despite apparent differences of roughly 40 g. The mean length of marked trout captured in 2007 was similar, ranging from 321 to 340 mm, but was not significantly different among test groups (ANOVA: $F_{2, 70} = 1.33$; $P = 0.27$) (Table 9). The mean weight of marked trout captured in 2007 ranged from 338 g to 391 g and was not significantly different among test groups (ANOVA: $F_{2, 69} = 0.62$; $P = 0.54$).

Proportions Captured

The AF3N group performed best in terms of relative survival, followed by the 2N group. During the 2006 sample, the AF3N female group on average made up 0.67 of the total marked fish captured, followed by the 2N group (0.14) and the 3N group (0.05) (Table 8). In 2007, results were similar, with the AF3N group making up 0.60 of the total marked fish caught on average, followed by the 2N group and the 3N group (0.30 and 0.10, respectively). The ANOVA analysis on the proportion of fish captured indicated that the stocking group was a significant effect (ANOVA: $F_{2, 39} = 19.23$; $P < 0.0001$). However, “survey year” and the interaction term of “survey year” by “stocking group” were not significant effects in the analysis (ANOVA: $F_{1, 21} =$

0.01; $P = 0.98$ and $F_{2, 21} = 0.49$; $P = 0.62$, respectively), suggesting that proportions between stocking groups were not different between survey years and that differences were consistent across years. Results from Tukey's multiple comparisons indicated that the AF3N group was caught in significantly higher proportions than both the 2N and 3N groups, but the proportions of the two Hayspur groups could not be distinguished despite seemingly large disparity in the results (Table 10).

Based on these results, we repeated the ANOVA analysis but removed the AF3N group. With the AF3N group removed from the analysis, differences in the mean proportion caught between the 3N and 2N groups (ANOVA: $F_{1, 26} = 7.46$; $P = 0.01$) became significant. "Survey year" was a significant effect in the model (ANOVA: $F_{1, 14} = 6.66$; $P = 0.02$), suggesting that the proportions caught of the two groups were different between years. However, the interaction term of "survey year" by "stocking group" was not a significant effect in the analysis ($F_{1, 14} = 2.87$; $P = 0.11$), indicating that differences were consistent across years. Tukey's multiple comparisons indicated that the 2N group made up a greater proportion of the catch on average (Table 10).

DISCUSSION

Several authors have reported growth advantages of triploid salmonids over their diploid counterparts. However, we found differences in growth between diploid and triploid stocking groups were less apparent, and when present, favored diploid fish. Although not always statistically significant, results from our study suggest that growth of triploid rainbow trout did not surpass that of other groups at three or four years of age. Individuals of the Hayspur triploid group were consistently shorter and lighter than either the diploid or the all-female triploid group across both stocking years (Table 9). These results contradict much of the literature describing the growth of triploid salmonids. For example, Teuscher et al. (2003) evaluated an all-female stock and found that diploid rainbow trout grew faster through age-3. However, 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). Sheehan et al. (1999) found all-female triploid rainbow trout grew faster than their diploid and mixed-sex counterparts over a 265-day trial in an aquaculture setting. Much of the growth advantage was attributed to low gonad development and the lack of males, which matured earlier at smaller sizes. In this respect, it was unexpected to find that the diploid group attained the largest ultimate sizes in both stocking events. However, Sheehan et al. (1999) suggested caution when comparing triploid growth among 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 may 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 all-female 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 could be expected.

Data from alpine lakes stocked in 2001 suggest that relative survival of diploid rainbow trout far exceeds that of triploid trout from the same stock. On average, diploids made up three and four times the proportion of marked fish captured in 2004 and 2005 surveys (Table 7). These results contradict those of Teuscher et al. (2003) and Dillon et al. (2000), who found similar or better returns from triploid rainbow trout compared to diploids. However, critical differences exist between these studies, including different strains of rainbow (Troutlodge, Inc. and Mt. Lassen, respectively) and the habitats in which they were conducted. Teuscher et al. (2003) conducted a long-term fingerling evaluation in two highly productive reservoirs using all-female rainbow trout, while Dillon et al. (2000) evaluated plantings of mixed-sex catchable-sized rainbow trout in several mountain streams.

Similar to the 2001 stocking, results from alpine lakes stocked in 2003 indicated that relative survival of 2N rainbow trout was higher than 3N. However, the relative survival of the AF3N group far exceeded that of both the 2N and 3N rainbow groups. On average, the AF3N group made up 0.67 and 0.60 of the total marked fish captured in 2006 and 2007, far exceeding the catch of either of the Hayspur stocking groups (Table 8). Not only was the AF3N group captured in higher numbers, these fish were caught in more locations. For example, in 2006, trout from the AF3N group were the only test fish recovered in five of the study waters. This suggests not only better survival relative to Hayspur-strain rainbow, but also more consistent survival across various alpine lakes in general.

The initial ANOVA analysis was unable to distinguish all the treatment groups in the 2003 stocking. While the AF3N group had the highest proportion of marked fish captured, the analysis could not separate the 2N and 3N groups, despite a seemingly large disparity in the proportions captured. Upon removing the AF3N group from the analysis, the 2N and 3N groups were then easily separated. The power of ANOVA to differentiate treatment groups is a function of sample size, the number of levels of each factor, the variability among population means, and the specified α significance level. Removing the AF3N group from the analysis reduced the levels of the “stocking group” factor, and the variability in the proportions captured, while the sample size and α significance level remained constant. This would allow greater power to differentiate the remaining 2N and 3N stocking groups more easily.

Length and weight of test fish at the time of stocking is unlikely to explain the differences seen in relative survival between the stocking groups. In the 2001 stocking, both diploids and triploids were similar length and weight. In 2003, the AF3N group was 3 mm longer on average, but the 2N and 3N groups did not differ in length. Although the AF3N was 0.2 g heavier on average, differences were not statistically significant between any of the groups. A mean length difference of only 3 mm seems unlikely to be responsible for the large differences in relative survival between the Troutlodge, Inc. all-female and Hayspur groups. If a slightly larger size at stocking conferred a survival advantage, we would have expected a smaller disparity in returns in the 2001 stocking, as diploids were stocked slightly smaller, yet returned at much higher numbers.

Alpine lakes are thought to be harsh oligotrophic aquatic habitats, with short growing seasons, cold temperatures, inconsistent food sources, and low dissolved oxygen during winter months (Donald and Anderson 1982; Bailey and Hubert 2003). Some authors have reported poor performance of triploid rainbow triploid under chronically stressful conditions such as high temperatures or low dissolved oxygen (Simon et al. 1993; Ojolic et al. 1995). In contrast, 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 raise 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 the higher relative 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 reduction of red blood cell production (erythropoiesis) may 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 vitellogenin, 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.

It is possible that the 3N rainbow used in this evaluation do not perform well under stressful environmental conditions. However, given the lack of definitive physiological differences of triploid fishes, reduced competitive ability of triploids may be a more likely explanation of poor returns. Poor competitive ability of triploid fishes has been demonstrated for Atlantic salmon (Galbreath et al. 1994) and saugeye (Czesny 2000). If competition was a key factor affecting relative survival of the various stocking groups, we might anticipate higher survival of triploid rainbow trout when stocked separately from diploids.

It is unknown whether spawning-related emigration might account for some of the differences in relative survival between the test 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). 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). 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 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 have been accounted for.

One confounding factor in this study is that the AF3N group was marked with adipose fin clips, while the 2N and 3N 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 81% of the rate as adipose-clipped trout from two separate release groups over several years. 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 contrast those of Mears and Hatch (1976), who 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 left and right ventral-clipped coho salmon *Oncorhynchus kisutch* returned to Bear Lake, Alaska, at 55% and 61% of the rate of adipose-clipped salmon, respectively. Compared to reported values, the differences in relative survival we found between adipose and ventral-clipped trout in this study are considerably higher. Even when considering increased mortality due to ventral fin clips (compared to adipose fin clips), the differences in catch between our test groups remains considerable (3-5 times). Although it is reasonable to expect lower survival in ventral-clipped groups, the differences in performance between our test groups is large enough that it is unlikely to be explained on the basis of fin clips alone.

CONCLUSION

Previous long-term evaluations indicate that field performance of triploid salmonids can be highly variable (Brock et al. 1994; Simon et al. 1993; Parkinson and Tsumura 1988; Warrillow et al. 1997). Our study presents similar findings, with two triploid groups exhibiting large differences in relative survival. Survival of 3N Hayspur-strain rainbow in alpine lakes in Idaho is very low relative to 2N trout, while AF3N rainbow from Troutlodge, Inc. appear to return in higher proportions than either Hayspur groups when stocked concurrently. Unfortunately, the study design was not able to separate the effects of stock and ploidy-level. Triploid groups studied in this evaluation did not show any growth advantages over the duration of the study, and ultimate sizes were similar to that of diploids. In this respect, triploid rainbow (especially all-female stocks) could be expected to return to anglers at sizes similar to diploid trout. Fisheries managers concerned with maintaining consistent alpine lake fisheries while minimizing natural reproduction or impact to native stocks should consider all-female triploid salmonids as a viable option to fertile fish.

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, Inc. all-female triploid rainbow trout. Data collected during this evaluation suggests that Troutlodge, Inc. 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, Inc. all-female rainbow trout with identical marks (like coded-wire tags) and eliminate confounding factors such as fin clips, stocking size, 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.

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Table 6. Stocking sites and selected habitat characteristics grouped by stocking year.

Lake name	Elev. (m)	Stocking density (fish/ha)	Area (ha)	Avg. depth (m)	Max. depth (m)	Mean pH	Mean cond (µS)	Surface temp (°C)	Mean temp (°C)	Max. temp (°C)	Secchi (m)
2001 Stocking Sites											
Blackwell L.	2151	41	14.5	7.3	10.8	7.4	14.3	20.3	-	-	2.8
Blue 1 L.	2222	95	6.3	4.3	5.2	7.5	9	14.2	-	-	4.2
Blue Jay L.	2610	250	2.4	9.4	17.5	7.8	5	18.4	16.0	17.7	6
Brush L.	2179	58	10.4	4.3	8.7	7.3	4	15	15.1	16.0	5.5
Cache Creek L. #1	2370	273	2.2	4.5	8.3	7.7	17	8.3	12.3	14.6	2.1
Cache Creek L. #2	2334	857	0.7	1.6	7.1	7.6	20	12	16.3	18.4	3.1
Josephine L. #2	2262	109	5.5	7.5	17.3	7.6	8	17.2	16.3	17.5	4.5
L. Ingeborg	2723	58	10.4	8.7	14.2	7.6	1.7	16.8	14.2	17.1	6
Long L.	2907	113	5.3	1.6	2.8	8.7	41.3	15.1	15.4	17.7	5.8
NF 20 Mile L. Long	2388	90	6.7	6.1	7.8	7.2	10.3	13.4	15.9	17.5	5
NF 20 Mile L. South	2400	171	3.6	4.5	7.8	7.3	18.3	12.3	15.5	16.8	3.3
Queens River L. #5	2561	176	3.4	6.6	14.4	7.8	3	18.2	12.3	14.1	4.2
Raft L.	2138	214	2.8	5.2	8.6	6.9	8.3	14.8	20.1	22.3	2.7
Shaw Twins L. #1	2213	250	2.4	8.0	10.3	7	6.7	19.7	15.5	17.0	7
Squaw L.	2150	353	1.7	4.5	6.6	7.2	6	22.2	16.4	18.0	4.5
Washington L.	3157	231	2.6	12.5	20.2	7.9	14	10	-	-	3.9
2003 Stocking Sites											
Big L.	2958	224	6.7	6.0	10.5	7.7	-	12.5	16.2	18.0	7.6
Blackwell L.	2151	103	14.5	7.3	10.8	7.2	12	19.9	-	-	2.8
Blue L.-Secesh	2332	203	6.9	12.8	19.8	7.8	11	12.7	17.9	19.2	4.9
Edna L. #2	2563	64	23.5	13.2	21.2	6.2	5	18.8	-	-	-
Heart L.- R.3B	2617	250	6.0	3.6	7.5	7	3	19.3	17.6	18.2	-
Heart L.-R. 4	2542	385	3.9	13.7	26.6	8.9	13.7	17.7	-	-	-
Kane Canyon L.	2813	254	5.9	2.8	4.7	7.7	22	11.9	11.1	13.0	2.2
L. Ingeborg	2723	144	10.4	8.7	14.2	7.2	2	18.1	16.9	17.6	6
Leggit L.	2600	200	7.5	5.3	10.2	8.2	4	15.4	12.8	14.2	-
Lynx Creek L. #1 W.	2569	294	5.1	7.4	10.3	8	12	14.7	-	-	-
Perkons L.	2653	395	3.8	5.8	13.8	7.9	217	17.3	16.2	18.0	-
Rough L.	2925	366	4.1	4.4	5.9	7.5	13.4	13	16.1	17.6	5.7
South Buckhorn L.	2123	221	6.8	9.5	11.9	7.1	3.4	16.3	-	-	-
WF Buckhorn L. #1	2122	123	12.2	12.6	21.9	7.2	3.3	19.6	-	-	-

Table 7. Catch-per-unit-effort (fish per total hours netting or angling) for the 2001 stocking event by survey year for each stocking group. The total proportion of the catch of each stocking group corrected for triploid induction rate. Missing values indicate lakes where angling was not conducted.

Lake	Nets		Angling		Proportion	
	3N	2N	3N	2N	3N	2N
2004						
Blackwell L.	0.17	0.17	0	0.11	0.33	0.67
Blue 1 L.	0	0.09	0	0	0	1.00
Blue Jay L.	0.05	0.09	0	0	0.33	0.67
Brush L.	0	0.03	-	-	0	1.00
Cache Creek L. #1	0.06	0.12	0.31	0.92	0.28	0.72
Cache Creek L. #2	0.02	0.02	-	-	0.49	0.51
Josephine L. #2	0	0.02	0	0	0	1.00
L. Ingeborg	0	0	0	0	-	-
Long L.	0	0.02	-	-	0	1.00
NF 20 Mile L. Long	0.04	0.07	0	0.31	0.25	0.76
NF 20 Mile L. South	0.01	0.01	-	-	0.49	0.51
Queens River L. #5	0.02	0.02	0	0	0.49	0.51
Raft L.	0	0.01	0	0	0	1.00
Shaw Twins L. #1	0.14	0.18	0	0	0.43	0.57
Squaw L.	0	0	0	0	-	-
Washington L.	0.04	0.05	0	0	0.39	0.61
<i>Mean</i>	0.03	0.06	0.03	0.12	0.24	0.76
2005						
Cache Creek L. #1	0.06	0.24	0.50	3.00	0.17	0.83
Josephine L. #2	0.04	0.07	0.80	2.40	0.33	0.67
L. Ingeborg	0.02	0.02	0	0	0.49	0.51
NF 20 Mile L. Long	0.02	0.07	0	0	0.25	0.76
Queens River L. #5	0	0.02	0	0.12	0	1.00
Raft L.	0	0	-	-	-	-
Shaw Twins L. #1	0.01	0.07	0	0	0.16	0.84
Washington L.	0	0.04	-	-	0	1.00
<i>Mean</i>	0.02	0.07	0.22	0.92	0.20	0.80

Table 8. Catch-per-unit-effort (fish per total hours netting or angling) for the 2003 stocking event by survey year and stocking group. Proportions represent those of the total marked fish caught. Missing values indicate lakes where angling was not conducted.

Lake	Nets			Angling			Proportion		
	AF3N	3N	2N	AF3N	3N	2N	AF3N	3N	2N
2006									
Big L.	0.09	0	0	0	0	0	1.00	0	0
Blackwell L.	0.04	0	0	0.33	0	0	1.00	0	0
Blue L.-Secesh	0.04	0	0	-	-	-	1.00	0	0
Edna L. #2	0.10	0	0.07	0.33	0.33	0	1.00	0	0
Heart L.-R.3B	0.11	0.02	0.11	0	0	0	1.00	0	0
Heart L.-R.4	1.47	0.04	0.31	0.73	1.09	2.18	0.57	0.14	0.29
Kane Canyon L.	0.33	0	0.15	0	0	0	0.46	0.08	0.46
L. Ingeborg	0	0	0	0	0	0	0.29	0.14	0.57
Leggit L.	0.36	0.04	0	1.33	0	0	0.69	0.07	0.24
Lynx Creek L. #1 W	0.35	0.12	0	1.00	1.00	2.00	0.62	0.31	0.07
Perkons L.	0.11	0	0.04	-	-	-	0.69	0	0.31
Rough L.	0.83	0.06	0.15	1.67	0	0	0	0	0
South Buckhorn L.	0.49	0.16	0.41	2.75	0.25	0.50	0	0	0
WF Buckhorn L. #1	0	0	0.04	-	-	-	0.92	0.08	0
Blackwell L.	0.04	0	0	0	0	0	0.86	0	0.14
<i>Mean</i>	0.29	0.03	0.08	0.68	0.22	0.39	0.67	0.05	0.14
2007									
Blue L.-Secesh	0.10	0	0	0	0	0	0.63	0.25	0.13
Heart L.-R.3B	0	0	0.11	0.20	0.10	0.30	0.77	0.08	0.15
Heart L.-R.4	0.57	0.16	0	1.74	2.17	0.87	0.75	0	0.25
L. Ingeborg	0	0	0	0	0	0	0.81	0.06	0.13
Leggit L.	0.47	0	0.08	0	0	0	0.67	0.17	0.17
Lynx Creek L. #1 W	0.41	0.04	0.08	-	-	-	0.58	0.13	0.30
Rough L.	0.04	0.04	0.04	0.49	0	0	0	0	1.00
<i>Mean</i>	0.23	0.03	0.04	0.40	0.38	0.19	0.60	0.10	0.30

Table 9. Mean length (mm) and weight (g) of marked diploid (2N), triploid (3N) and all-female triploid (AF3N) test trout by sampling year. Standard deviation (SD) is shown in parentheses. Test groups that share the same letter group are not statistically different. Letter groups correspond to analysis made within each sample year and are not intended for comparison across years.

Stocking	Stock	Length (SD)	n	Group	Weight (SD)	n	Group
2004 Sample							
2001	2N	332 (39)	56	A	376 (133)	56	A
	3N	322 (23)	29	A	305 (73)	29	B
2005 Sample							
	2N	348 (62)	19	A	464 (253)	19	A
	3N	327 (46)	7	A	351 (143)	7	A
2006 Sample							
2003	AF3N	295 (35)	157	A	275 (88)	155	A
	2N	290 (31)	43	A	274 (85)	43	A
	3N	280 (35)	19	A	233 (83)	19	A
	2007 Sample						
	AF3N	336 (31)	50	A	357 (107)	49	A
	2N	340 (39)	11	A	391 (162)	11	A
	3N	321 (28)	12	A	338 (100)	12	A

Table 10. Mean relative proportion captured (combined from angling and netting) of diploid (2N), triploid (3N) and all-female triploid (AF3N) rainbow trout by stocking year. Results show Log+1 transformed value derived from the ANOVA analysis and associated standard error (SE). Comparisons were made within stocking years and are not intended for comparison across stocking years. Treatments with the same letter group are not statistically different when $\alpha = 0.05$.

Stock	Estimate	SE	Group
2001 Stocking			
2N	0.547	0.023	A
3N	0.226	0.023	B
2003 Stocking			
AF3N	0.468	0.047	A
2N	0.183	0.047	B
3N	0.062	0.047	B
2003 Stocking w/o AF3N			
2N	0.385	0.052	A
3N	0.182	0.052	B

APPENDICES

Appendix A. Calculation procedure for adjusting total kokanee catch data to account for the 79% triploid-induction rate. The number of marked diploid kokanee planted and recaptured was used to determine a relative survival rate for diploids. This in turn was used to determine what proportion of the fish marked as triploid was actually likely to be diploid.

<u>Test Group</u>	<u>Number Stocked</u>	<u>Total Caught</u>
2N	110,946	34
3N	102,523	22
Adjusted 3N	80,993	3Nadj
Adjusted 2N	21,530	2Nadj

$$2N \text{ Relative Survival } (2N_s) = \frac{2N_{\text{caught}}}{2N_{\text{stocked}}} = \frac{34}{110,946} = 3.065 \times 10^{-4}$$

$$\text{Adjusted number of 2N caught } (2N_{\text{adj}}) = 2N_s \times \text{Adjusted } 2N = 3.065 \times 10^{-4} \times 21,530 = 6.59$$

$$\text{Adjusted number of 3N caught } (3N_{\text{adj}}) = \text{Total } 3N - 2N_{\text{adj}} = 22 - 6.59 = 15.41$$

$$3N \text{ Relative survival } (3N_s) = \frac{3N_{\text{adj}}}{\text{Adjusted } 3N} = \frac{(22 - 6.59)}{80,993} = 1.903 \times 10^{-4}$$

$$2N:3N \text{ Survival ratio} = \frac{2N_s}{3N_s} = \frac{3.065 \times 10^{-4}}{1.903 \times 10^{-4}} = 1.61$$

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