FISHERY RESEARCH

Project 4: Hatchery Trout Evaluations

Grant # F-73-R-28

Report Period July 1, 2005 to June 30, 2006

Joseph R. Kozfkay
Senior Fisheries Research Biologist

Martin Koenig
Fisheries Research Biologist

IDFG Report Number 06-06
February 2006

Annual Performance Report

July 1, 2005 to June 30, 2006

Grant # F-73-R-28

Project 4: Hatchery Trout Evaluations

Subproject 1: Use of Tiger Muskellunge to Remove Brook Trout from High Mountain Lakes
Subproject 2: Sterile Trout Investigations
Subproject 3: Predator Training

By

Joseph R. Kozfkay

and

Martin Koenig

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

IDFG Report Number 06-06
February 2006

TABLE OF CONTENTS

Page

Table of Contents, continued.

Page

LIST OF TABLES

List of Tables, Continued.

Page

LIST OF FIGURES

List of Figures, Continued.

Page

ANNUAL PERFORMANCE REPORT
SUBPROJECT #1: USE OF LAKE TROUT OR TIGER MUSKELLUNGE TO REMOVE BROOK TROUT FROM HIGH MOUNTAIN LAKES

State of: Idaho

Grant No.: F-73-R-28 Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #1: Use of tiger muskellunge to remove brook trout from high mountain lakes

Contract Period: July 1, 2005 to June 30, 2006

ABSTRACT

Nonnative brook trout populations in high mountain lakes threaten the persistence of native fish and often offer little fishing opportunity. Elimination of brook trout populations with the use of stocked lake trout or tiger muskellunge may be an efficient means for eliminating some populations, especially in low complexity habitats. Elimination of brook trout populations could contribute to conservation efforts by allowing lakes to be restocked with western salmonids. In this progress report, we document efforts to select study waters, describe characteristics of brook trout populations prior to proposed lake trout or tiger muskellunge introductions, and describe morphological characteristics of lakes and associated inlets and outlets. Out of the 12 lakes sampled, 11 lakes possessed stunted brook trout populations with few individuals over 254 mm. Of the 742 brook trout sampled, only 89 fish (12%) equaled or exceeded 254 mm (10”). The only exception was Hard Creek Lake, where a larger portion (90%) of trophy-sized brook trout (exceeding 254mm) was caught. Analysis of length-at-age data indicated that brook trout grew relatively rapidly to age-4, but then essentially ceased growing. Mean length at ages 0, 1, 2, 3, and 4 equaled 69, 143, 182, 205, and 236 mm, respectively. At age-5 and afterwards, no substantial change in mean length was noted. For the seven lakes with sufficient sample sizes, annual mortality rates estimates averaged 47 ± 9%. The highest mortality rate of 72% was for Emerald Lake, whereas the lowest mortality rate of 32% was observed at Moose Lake (Wildhorse). All other mortality rate estimates ranged between 41% and 51%. Based on lake characteristics, we recommend using nine of the 12 lakes sampled in future studies on the effectiveness of using tiger muskee to eliminate brook trout from mountain lakes. Based on a stocking density of 40 fish per hectare, 4,329 lake trout or tiger muskellunge would be required to initiate this study on a broad geographical scale.

Authors:

Joseph R. Kozfkay
Senior Fisheries Research Biologist

Martin Koenig
Fisheries Research Biologist

INTRODUCTION

During the early twentieth century, brook trout *Salvelinus fontinalis* were introduced to high mountain lakes throughout the western United States and Canada, including Idaho. Most introduction efforts ceased by the 1950s, but by this time, brook trout had established self-sustaining populations in many lakes. Although some of these populations have sustained recreationally important fisheries (Donald et al. 1980), the vast majority do not offer quality fishing opportunities. More importantly, some of these populations threaten the persistence of native fish and amphibian populations.

According to Bahls (1992), over 95% of the deep, high mountain lakes were fishless prior to introduction of salmonids by man. So, establishment of salmonids, including brook trout, in high mountain lakes did not likely reduce numbers of native fish substantially within these habitats; however, these introductions have been linked to declines in other native biota such as amphibians (Pilliod 2001; Murphy 2002) and to declines of downstream fish populations. High elevation streams contain some of the strongest remaining cutthroat and bull trout populations. Headwater lakes within these drainages often contain nonnative trout and may act as source populations for colonization of nonnative fish into downstream habitats (Adams et al. 2001). These authors found that brook trout were capable of invading most downstream habitats by being able to disperse through 80% slopes and over 18 m waterfalls. Brook trout have the ability to outcompete cutthroat trout *Oncorhynchus clarkii* (DeStaso and Rahel 1994) and may eventually eliminate some cutthroat trout populations (Kruse et al. 2000). Additionally, brook trout may hybridize or displace bull trout *Salvelinus confluentus*, thereby reducing or eliminating bull trout populations (Kitano et al. 1994; Kanda et al. 2002).

Within high mountain lakes, brook trout are capable of spawning in inlet and outlet tributaries, as well as lake margins (Fraser 1989). Due to a combination of abundant spawning habitats, early age at maturity, and few predators, brook trout populations often reach very high densities (Donald and Alger 1989). Since most high mountain lakes are low in productivity, high-density brook trout populations are often prone to stunting (Donald and Alger 1989; Hall 1991; Parker et al. 2001), at which point they become of marginal interest to anglers (Rabe 1970; Donald et al. 1980; Donald and Alger 1989). In this case, there is some interest in shifting the size structure of brook trout populations in high mountain lakes to provide higher proportions of quality fish greater than 254mm. In high mountain lakes where complete removal of brook trout is unlikely, investigating techniques to improve the size structure of brook trout populations may be a practical secondary objective.

Biologists have employed several techniques to reduce or eliminate brook trout and other nonnative trout populations from high mountain lakes. Such techniques included high-intensity gill netting, rotenone application, electrofishing, and introducing piscivorous salmonids. During a brook trout removal effort from a 1.6 ha high mountain lake in the Sierra Nevada mountains in California, three to six gill nets were set per night for a total effort of 108 net days (Knapp and Matthews 1998). This effort effectively removed the entire 97 fish population at an estimated cost of $5,600. However, the authors speculated that this technique would not be effective in lakes exceeding 3 ha. With a similar effort, Parker et al. (2001) were able to remove an entire brook trout population (261 fish) from a 2.1 ha lake in Banff National Park, Alberta, Canada. The majority of fish were removed within the first week of netting (54%). Furthermore, within the first year of netting, they suspected that the entire adult population was removed by the time nets were retrieved after ice-off, and only a few juvenile fish were caught thereafter. Walters and Vincent (1973) used rotenone to eliminate brook trout from 1.1 ha Emmaline Lake, Colorado. However, biologists rarely use this method in high lakes due to cost and difficulty of application and subsequent detoxification of outflow, and the negative perception associated with applying chemicals in remote, relatively pristine areas or designated wilderness.

Using piscivorous fish is an attractive alternative for managing brook trout populations in that little effort is needed besides an initial stocking effort. However, results for this technique have been inconsistent. The state of Colorado occasionally stocked lake trout *Salvelinus namaycush* and brown trout *Salmo trutta* in high mountain lakes to control brook trout populations (Nelson 1988). From 1960-1964, experimental plants of lake trout were made in five lakes: Blue, Lower Twin, Katherine, Sugar Bowl, and Upper Camp. Lake trout established self-sustaining populations in all five lakes. By the early 1980s, no response in brook trout populations was noted in two lakes (Sugar Bowl and Upper Camp) while numbers of brook trout were decreased in Blue and Lower Twin lakes and eliminated or nearly so from Katherine Lake (0 fish sampled in 1983; Nelson 1988). He also noted that brook trout lakes that contained brown trout had lower densities of brook trout and more brook trout over 250 mm.

Similar attempts have been made in Idaho using Kamloops rainbow trout *O. mykiss*, bull trout, and brown trout. In 1993, Idaho Department of Fish and Game personnel stocked 702 Kamloops rainbow trout in Carlson Lake in an effort to improve the size structure of stunted brook trout (Brimmer et al. 2002). Unfortunately, this attempt was unsuccessful, as the brook trout size structure in the lake was unchanged. Kamloops rainbow trout were stocked at an average weight of 133 g (3.7 per lb) and an approximate length of 200 mm (8”). These fish were likely too small to exert significant predation pressure and due to high densities of brook trout would not be able to grow large enough to do so. Similar efforts were made in several lakes within Region 4 and the McCall subregion, but to date, none of these efforts has been successful in eliminating or even reducing brook trout densities from their respective lakes (P. Janssen and F. Partridge, IDFG, personal communication).

Tiger muskellunge *Esox lucius X masquinongy* are a cross between a male northern pike *E. lucius* and a female muskellunge *E. masquinongy*. Tiger muskellunge have been stocked in lakes and reservoirs throughout the northern United States to provide trophy angling opportunities (Storck and Newman 1992) and to control prey, rough, and pan-fish populations (Wahl and Stein 1993). Tiger muskellunge are preferred over their parental species due to their superior performance under hatchery conditions (Graff 1978; Pecor 1978) and because they are functionally sterile (Crossman and Buss 1965). Sterility allows biologist to stock tiger muskellunge with little to no threat of creating self-sustaining populations.

During 1998 and 1999, IDFG personnel in the Clearwater Region began a management case study to determine if tiger muskellunge could eliminate brook trout from two high mountain lakes Tiger muskellunge were stocked into Ice Lake at a density of 41 fish/ha (Schriever and Murphy, *In Press*). To suppress brook trout further, IDFG personnel removed fish from inlet and outlet habitats with backpack electrofishing gear. From 1998 to 2001, catch in a single gill net declined from 17 fish to zero fish per net night. Although some fry were seen in the inlet and outlet, the brook trout population in Ice Lake had been substantially reduced and possibly eliminated with one tiger muskellunge stocking. In Rainbow Lake, tiger muskellunge were stocked during 1999 and 2000 and at densities of 6.1 and 33.6 fish/ha, respectively. An initial survey during 1998 indicated that brook trout densities were high (85 fish per net night). By 2001, two years after the initial introduction of tiger muskellunge, catch decreased to 10 fish per net night. Due to the size of the inlet and outlet, we speculated that brook trout populations would not be eliminated from Rainbow Lake with tiger muskellunge predation and backpack electrofishing, but that reduced densities would improve the size structure of the remaining brook trout, thereby improving fishery quality (Schriever and Murphy, *In Press*).

Tiger muskellunge are also being used by IDFG personnel in region 7 to improve the size structure of brook trout in Carlson Lake. Carlson Lake once produced trophy size brook trout but now only contains small stunted fish (Brimmer et al. 2002). Prior to introduction of tiger muskellunge, a population estimate indicated that the lake contained 9,900 brook trout. During 2002, forty-one tiger muskellunge were introduced. By 2003, the population had decreased by 8.5% (Esselman et al. 2004). No additional population assessments have been attempted due to high mortality of tiger muskellunge in gill nets, but will be attempted in several years.

Although encouraging, the results of the two IDFG management efforts above do not provide the scope necessary to reach firm conclusions regarding the utility of tiger muskellunge for eliminating undesirable brook trout populations. In this progress report, we describe initial efforts to select study lakes for a study examining the effects of lake trout or tiger muskellunge introduction on brook trout populations across a broad geographical area of Idaho. Introduction of lake trout or tiger muskellunge may occur during 2006 or 2007. In order to compare changes in brook trout populations post-introduction, we document pre-introduction age and size structure, as well as relative density. Additionally, we describe morphological characteristics of lakes that allow us to prioritize lakes for stocking and eventually determine characteristics that allow successful removal of brook trout.

RESEARCH GOAL

1. To eliminate or improve the size structure of brook trout populations from high mountain lakes, thereby reducing threats to native species and allowing restocking of lakes with sterile western salmonids to improve recreational angling opportunities.

OBJECTIVES

1. To determine if lake trout or tiger muskellunge stocked at densities of 40 fish per hectare into high mountain lakes with stunted brook trout populations can cause recruitment failure and eventual elimination of populations within five years.

2. To determine lake as well as associated inlet or outlet characteristics that influence success/failure of brook trout eradication efforts with lake trout or tiger muskellunge.

METHODS

During 2005, IDFG regional fisheries personnel and U.S. Forest Service personnel provided high mountain lake information that facilitated study site selection. We preferentially selected lakes that were known to have brook trout populations and were thought to have limited inlet and outlet spawning habitats. To avoid biasing results, steep drainages were preferred, as they most likely possessed barriers that will prevent recolonization by any downstream brook trout populations. Twelve lakes were selected throughout northern and central Idaho (Figure 1) as potential study waters for this evaluation.

Potential study lakes were sampled during 2005 to determine relative density, age, and size structure of brook trout populations as well as habitat characteristics. Lakes were surveyed with floating gillnets and angling from August 4 to September 29, 2005. The experimental gillnets used had 19, 25, 30, 33, 38, and 48 mm bar mesh panels and were 46 m long by 1.5 m deep. Typically, three to five 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 (total length), and weighed to the nearest gram. Condition factors were calculated as C = (Weight / Length^3) X 100,000 (Anderson and Neumann 1996). Mean values and 90% confidence intervals (CIs) were calculated to compare size and condition factor across lakes. Additionally, mean length values for the five largest brook trout caught per lake were calculated to index trophy potential. Otoliths samples were collected from approximately 50 brook trout per lake if available and stored dry. In the laboratory, otoliths were viewed whole (Boxrucker 1986) with a dissecting microscope on 2-4 power by two viewers, independently. If a consensus was not reached, the structures were re-examined by a pair of experienced readers to determine a final age estimate. Annual mortality rates were estimated from catch curve analysis. Mortality rates were only estimated for lakes that had at least two fish per age class for three consecutive years. Annual mortality rates were estimated using the following equations: $N_t = N_0e^{-Zt}$, where N_t is the number sampled at age t, N_0 is the number alive initially, and Z is the instantaneous mortality rate. Annual mortality rates were calculated as A = 1 – S where S = e^{-Z}, where A = annual mortality rate and S = survival (Van Den Avyle 1993).

Similar to IDFG standard high lake monitoring protocols, abiotic measurements were collected at each of the 12 lakes. A series of three transects were placed at equal distances perpendicular to the long axis of the lake with the aid of a laser rangefinder. Three sampling points were equally spaced along each transect. Depth measurements 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 conductivity and surface water temperature measurements were collected. Elevation and location were determined with the use of a handheld GPS unit. Lake area was determined with global information systems (GIS). In addition, amphibian surveys were conducted by slowly walking the entire perimeter of each lake along the water shore interface and looking near and under woody debris. Removal potential at each lake was categorized according the qualitative value of “very high,” “high,” “moderate,” and “low” based on the following criteria:

Very High: lakes with no inlet and/or outlet spawning habitat, low habitat complexity within the lake.

High: lakes contain only limited inlet and/or outlet spawning habitat, lake outlets possess migration barriers.

Moderate: lakes contain some accessible inlet and/or outlet spawning habitat.

Low: lakes contain abundant inlet and/or outlet spawning habitat; low gradient outlets with spawning habitat present, connections to lentic habitats with established brook trout present.

RESULTS

During sampling of 12 high mountain lakes known to possess brook trout populations, 760 fish were caught and processed, including 742 brook trout (98%; Table 1), 5 westslope cutthroat trout *O. c. lewisi*, 12 rainbow trout, and 1 bull trout (Table 1). Rainbow trout were only caught in Black and Moose lakes (Region 6), whereas westslope cutthroat trout were only caught from Moose Lake (Region 1). The bull trout, a 737 mm individual, was caught in Upper Hazard Lake and was likely stocked during 1992 or 1993 during an unsuccessful earlier attempt to reduce brook trout populations with piscivores (P. Jansen, IDFG, personal communication). Catch in all other lakes consisted entirely of brook trout. The average catch of brook trout was 62 fish per lake (95% Confidence Interval [CI] ±14). The highest catch was 122 brook trout from Shirts Lake, whereas the lowest catch was 34 from Gem Lake.

Gill nets were fished for 718 h over 49 net nights and caught 657 trout. Relative abundance as indexed by catch per unit effort (CPUE) varied widely among the 12 study lakes. Mean CPUE ranged from 5.8 to 25.3 fish caught per gill net night and averaged 13.7 (Figure 2). The lowest CPUE was recorded from Gem Lake, whereas the highest was recorded from Emerald Lake. Angling surveys were also conducted in five of the 12 lakes surveyed. Total angling effort was 42 hours during 13 angler days. Catch per unit effort for angling equaled 0.8, 8.2, 2.5, 4.3, and 12.7 fish per hour in Gem, Moose (Wildhorse Ck.), Moose (Lightning Ck.), Shirts, and Upper Box Canyon lakes, respectively.

Brook trout appeared to become fully recruited to experimental gill nets at approximately 190 mm (Figures 3 and 4). For all lakes combined, average length and weight was 209 ± 2.4 mm (n = 742) and 81 ± 4 g. Average lengths for individual lakes ranged from a low of 185 ± 7 mm in Shirts Lake to a high of 232 ± 10 mm in Granite Twin Lakes (Table 2). Average weight for individual lakes ranged from a low of 67 ± 6 mm in Shirts Lake to a high of 124 ± 10 mm in Granite Twin Lakes. About 40% of the brook trout we sampled were 200 mm (8”) or less. Very few large fish were caught. Only 89 fish (12%) equaled or exceeded 254 mm (10”), and of those, only 26 fish (4%) equaled or exceeded 279 mm (11”). Only six lakes produced fish longer than 279 mm, including Granite Twin (n = 11), Hard Creek (n = 7), Upper Hazard (n = 4), Moose-Lightning Ck. (n = 2), Gem (n = 1), and Emerald (n = 1) lakes. The three largest fish were all caught from Hard Creek Lake and measured 317, 380, and 417 mm, respectively.

Consensus age estimates were determined for 535 brook trout. Summed across all lakes, 4, 42, 109, 159, and 121 brook trout were estimated to be ages 0, 1, 2, 3, and 4, respectively. An additional 99 fish were estimated to be between five and nine years old. One brook trout, a 248 mm individual, from Merriam Lake was estimated to be 14 years old. Using pooled age estimates from all lakes, mean length at age increased steadily through age-4, after which growth increments decreased to near zero (Figure 5). Mean length at ages 0, 1, 2, 3, and 4 equaled 69, 143, 182, 205, and 236 mm, respectively. At age-5 and afterwards, no substantial change in mean length was noted.

Seven lakes had sufficient numbers of fish within at least three consecutive age classes to estimate mortality rates. In these seven lakes, mortality rate estimates averaged 47 ± 9%. The highest mortality rate of 72% was observed for Emerald Lake (Figure 6), whereas the lowest mortality rate of 32% was observed at Moose Lake (Wildhorse). All other mortality rate estimates ranged between 41% and 51%.

Potential study lakes included a wide variety of physical habitat characteristics and inlet and outlet morphologies (Table 3). Based on these characteristics, lakes have varying potentials for successful elimination of brook trout populations. Spruce Gulch, Upper Box Canyon, and Merriam lakes had “very high” probabilities for elimination of brook trout populations due to the total lack of inlet and outlet spawning habitats and low complexity in-lake habitats. All reproduction occurred within the lakes themselves. The potential for elimination in Gem, Granite Twin, Hard Creek, and Shirts lakes were considered “high.” These lakes possess only very limited spawning habitat in inlet tributaries and possessed migration barriers in outlet tributaries. The potential for elimination in Moose (Lightning), Black, and Upper Hazard lakes was “moderate.” These lakes possess less limited but easily accessible amounts of spawning habitat in inlet and outlet tributaries. In contrast, there was probably almost no possibility for brook trout elimination in Moose (Wildhorse) and Emerald lakes due to abundant inlet spawning habitat, low gradient outlets with abundant spawning habitat, and had connected lentic habitats with brook trout present.

DISCUSSION

With the exception of Hard Creek Lake, all lakes sampled during 2005 generally contained small brook trout. Only a small proportion (12%) of these populations contained fish over 254 mm (10”) and were characterized by an abundance of younger year classes and slow growth rates, especially after age-4. Thus, these populations may be of limited interest to anglers. Removal of these stunted brook trout populations could help conserve native species and may improve recreational fishing opportunities if the lakes are restocked with native salmonids after brook trout removal. Alternatively, introduction of tiger muskellunge may improve the size structure of brook trout in these lakes—and subsequently angler interest—in the event that a complete removal is unsuccessful. If such future removal efforts prove successful, restocking decisions will be based on stakeholder input, potential interactions with amphibians, and other issues identified in both statewide and regional high mountain lake management plans.

Although several methods may be used to eliminate brook trout from high mountain lakes, introducing predatory fish seems to be the most efficient alternative as it requires only a minimal labor investment. After an initial stocking effort, only cursory sampling efforts are needed to document population responses, especially in systems with low habitat complexity such as Spruce Gulch, Upper Box Canyon, and Merriam lakes. In systems with higher complexity, additional effort may be needed to eliminate brook trout not accessible to tiger muskellunge. Such fish might include those inhabiting outlet and inlet tributaries or near seep springs, unless they are forced to move to the main lake during winter. Both electrofishing and chemical treatment could prove useful in such scenarios.

A primary concern for using tiger muskellunge in Idaho is the availability of tiger muskellunge for stocking. Tiger muskellunge are not reared by IDFG hatcheries nor are they commercially available. They may only be acquired through agreements with other agencies. Currently, IDFG acquires tiger muskellunge through the Washington Department of Fish and Wildlife (WDFW) if available after they meet their own stocking requests. From 2000 through 2005, IDFG has received fish in three of the six years. Average allocation during the three years that we received fish was 1,004 fish. At this point, tiger muskellunge stocking densities necessary to eliminate brook trout population in high mountain lakes have not been optimized. Stocking densities used by Region 2 personnel for Ice Lake and for Rainbow Lake during 1999 and 2000 were 41, 6.1, and 33.6 fish per hectare, respectively (P. Murphy, personal communication). Assuming a stocking density of 40 fish per hectare, 640 lake trout or tiger muskellunge would be necessary to stock the lakes with very high elimination potential, another 1,401 fish to stock the lakes with high potential, and 2,628 fish to stock the three lakes with moderate elimination potential. In total, to stock the nine lakes with moderate to very high elimination potential (excluding Hard Creek Lake) at 40 fish per hectare, 4,329 tiger muskellunge would be required at the time of stocking. In order to stock all 12 lakes (excluding Hard Creek Lake) at 40 fish per hectare, 5,553 tiger muskellunge would be required at the time of stocking. Based on these numbers, additional sources of tiger musky would need to be identified to fully stock these study waters. Alternatively, lake trout may be available in most years through the Bear Lake lake trout sterilization program. Sterile lake trout might provide a suitable substitute if tiger muskellunge are not available in sufficient numbers. A combination of lake trout and tiger musky could be used in separate lakes as part of the study to assess the effectiveness of each species in removing brook trout.

RECOMMENDATIONS

1. Do not stock lake trout or tiger muskellunge in Moose (Wildhorse) or Emerald Lake due to their lack of barriers, abundant spawning habitats in inlet and outlet streams, and connection to adjacent lentic habitats, which make it highly unlikely that brook trout populations can be eliminated or even reduced.

2. Do not stock lake trout or tiger muskellunge in Hard Creek Lake. This unique fishery provides an opportunity to catch trophy-sized brook trout from a high mountain lake.

3. Make arrangements so that 5,553 lake trout and/or tiger muskellunge are available for stocking in 2007. Stocking numbers are based on a stocking density of 40 fish per hectare. It is doubtful that this many tiger muskellunge alone could be secured solely from WDFW. Therefore, other sources of tiger muskellunge should be considered, as well as the possibility of using a combination of lake trout and tiger muskellunge. If sufficient numbers of predator fish cannot be attained, consider removing some study lakes with low elimination potential first, then larger lakes with moderate elimination potential. Last, reducing stocking densities to 20 fish per hectare in a portion of the study waters may be necessary to execute the study.

4. Coordinate stocking efforts with local U.S. Forest Service personnel.

ACKNOWLEDGEMENTS

We would like to acknowledge the hard work and dedication of Chris Sullivan, John Cassinelli, and Lars Alsager during the surveying of these 12 lakes. We would also like to thank Joe DuPont, Ed Schriever, Paul Janssen, Brian Flatter, Tom Curet, Bob Esselman, and Bart Gamett for providing information that facilitated study site selection. Tim Cochnauer and Patrick Murphy provided guidance in developing this study, which is essentially a broader-scale extension of work they began in the Clearwater Region during 1998.

LITERATURE CITED

Adams, S. B., C. A. Frissell, and B. E. Riemann. 2001. Geography of invasion in mountain streams: consequences of headwater lake fish introductions. Ecosystems 296-307.

Anderson, R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Bahls, P. 1992. The status of fish populations and management of high mountain lakes in the western United States. Northwest Science 66:183-193.

Boxrucker, J. 1986. A comparison of the otolith and scale methods for aging white crappies in Oklahoma. North American Journal of Fisheries Management 6:122-125.

Brimmer, B., K. Andrews, T. Curet, and A. Brimmer. 2002. Regional fisheries management investigations. Project F-73-R-26, Idaho Department of Fish and Game, Boise, Idaho.

Crossman, E. J., and K. Buss. 1965. Hybridization in the family Esocidae. Journal of the Fisheries Research Board of Canada 22:1261-1292.

De Staso III, J., and F. J. Rahel. 1994. Influence of water temperature on interactions between juvenile Colorado River cutthroat trout and brook trout in a laboratory stream. Transactions of the American Fisheries Society 123:289-297.

Donald, D. B., R. S. Anderson, and D. W. Mayhood. 1980. Correlations between brook trout growth and environmental variables for mountain lakes in Alberta. Transactions of the American Fisheries Society 109:603-610.

Donald, D. B., and D. J. Alger. 1989. Evaluation of exploitation as a mean of improving growth in a stunted population of brook trout. North American Journal of Fisheries Management 9:177-183.

Esselman, B., K. Andrews, T. Curet, and A. Brimmer. 2004. Regional fisheries management investigations. Project F-73-R-28, Idaho Department of Fish and Game, Boise, Idaho.

Fraser, J. M. 1989. Establishment of reproducing populations of brook trout after stocking of interstrain hybrids in Precambrian Shield lakes. North American Journal of Fisheries Management 9:352-363.

Graff, D. R. 1978. Intensive culture of esocids-the current state of the art. Transactions of the American Fisheries Society Special Publication 11:195-201.

Hall, D. L. 1991. Age validation and aging methods for stunted brook trout. Transactions of the American Fisheries Society 120:644-649.

Kanda, N., R. F. Leary, and F. W. Allendorf. 2002. Evidence of introgressive hybridization between bull trout and brook trout. Transactions of the American Fisheries Society 131:772-782.

Kitano, S., K. Maekawa, S. Nakano, and K. D. Fausch. 1994. Spawning behavior of bull trout in the upper Flathead drainage, Montana, with special reference to hybridization with brook trout. Transactions of the American Fisheries Society 123:988-992.

Knapp, R. A., and K. R. Matthews. 1998. Eradication of nonnative fish by gill netting from a small mountain lake in California. Restoration Ecology 6:207-213.

Kruse, C. G., W. A. Hubert, and F. J. Rahel. 2000. Status of Yellowstone cutthroat trout in Wyoming waters. North American Journal of Fisheries Management 20:693-705.

Murphy, P. D. 2002. The effects of different species of introduced species of introduced salmonids on amphibians in headwater lakes of North-central Idaho. Master's thesis. Idaho State University, Pocatello, Idaho.

Nelson, W. C. 1988. High lake research and management in Colorado, special report. High Lake Research and Management in Colorado. Fort Collins, Colorado.

Parker, B. R., D. W. Schindler, D. B. Donald, and R. S. Anderson. 2001. The effects of stocking and removal of a nonnative salmonid on the plankton of an alpine lake. Ecosystems: 334-345.

Pecor, C. H. 1978. Intensive culture of tiger muskellunge in Michigan during 1976 and 1977. Transactions of the American Fisheries Society Special Publication 11:202-209.

Pilliod, D. S. 2001. Ecology and conservation of high-elevation amphibian populations in historically fishless watersheds with introduced trout. PhD dissertation. Idaho State University, Pocatello, Idaho.

Rabe, F. W. 1970. Brook trout populations in Colorado beaver ponds. Hydrobiologia 35:431-448.

Schriever, E. B., and P. D. Murphy. *In Press.* Utilization of tiger muskellunge for controlling self-sustaining populations of introduced brook trout in mountain lakes. Regional Fisheries Management Investigations. Idaho Department of Fish and Game. Boise, Idaho.

Storck, T W., and D. L. Newman. 1992. Contribution of tiger muskellunge to the sport fishery of a small, centrarchid-dominated impoundment. North American Journal of Fisheries Management 12:213-221.

Van Den Avyle, M. J. 1993. Dynamics of exploited fish populations. Pages 105-136 *in* C. C. Kohler and W. A. Hubert, editors. Inland fisheries management in North America. American Fisheries Society, Bethesda, Maryland.

Wahl, D. H., and R. A. Stein. 1993. Comparative population characteristics of muskellunge (*Esox masquinongy*), northern pike (*E. lucius*), and their hybrid (*E. masquinongy* x *E. lucius*). Canadian Journal of Fisheries and Aquatic Sciences 50:1961-1968.

Walters, C. J., and R. E. Vincent. 1973. Potential productivity of an alpine lake as indicated by removal and reintroduction of fish. Transactions of the American Fisheries Society 102:675-697.

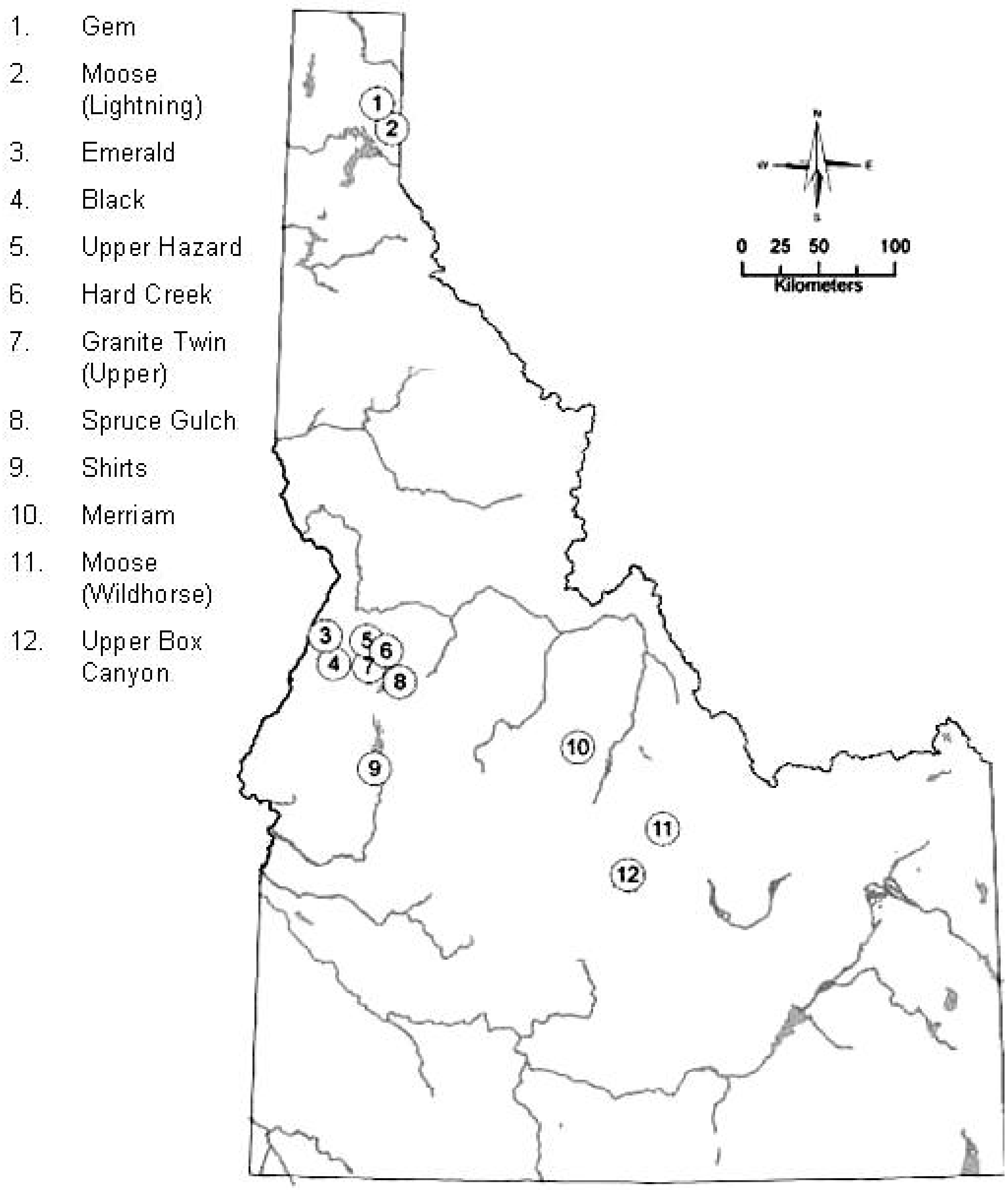


Figure 1. Locations of 12 high mountain lakes in Idaho that were sampled to assess the status of brook trout populations and inventory lake characteristics. Information was gathered to determine whether lakes would be good candidates for inclusion in a study designed to eliminate brook trout populations by stocking tiger muskellunge. The eventual number of lakes used will depend on tiger muskellunge availability.

Table 1. Sample composition by species of 12 Idaho high mountain lakes sampled during 2005 to assess fish population status and lake characteristics.

		Sample composition by species			
Lake name	**Sample size**	**Bull trout**	**Brook trout**	**Cutthroat trout**	**Rainbow trout**
Black	59		54		5
Emerald	101		101		
Gem	34		34		
Granite Twin (upper)	80		80		
Hard Creek	44		44		
Merriam	37		37		
Moose (Lightning)	77		72	5	
Moose (Wildhorse)	68		61		7
Shirts	122		122		
Spruce Gulch	63		63		
Upper Box Canyon	38		38		
Upper Hazard	37	1	36		
Totals	760	1	742	5	12

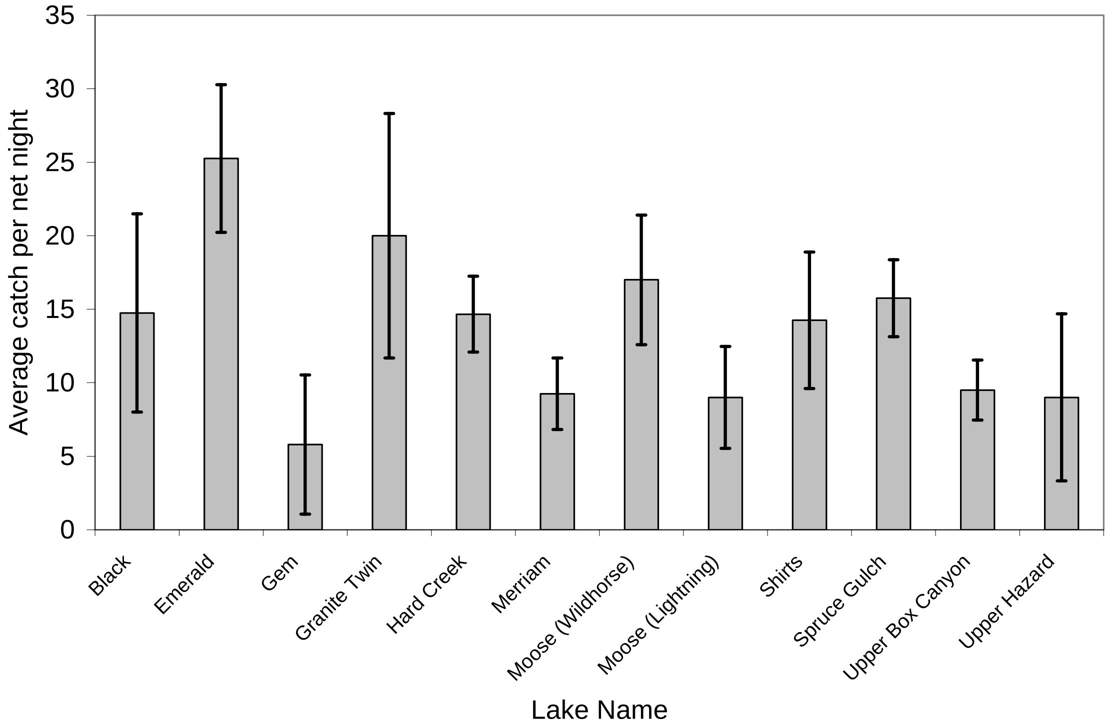


Figure 2. Relative abundance of brook trout in 12 Idaho high mountain lakes measured as average catch per gill net night. Error bars represent 90% confidence intervals.

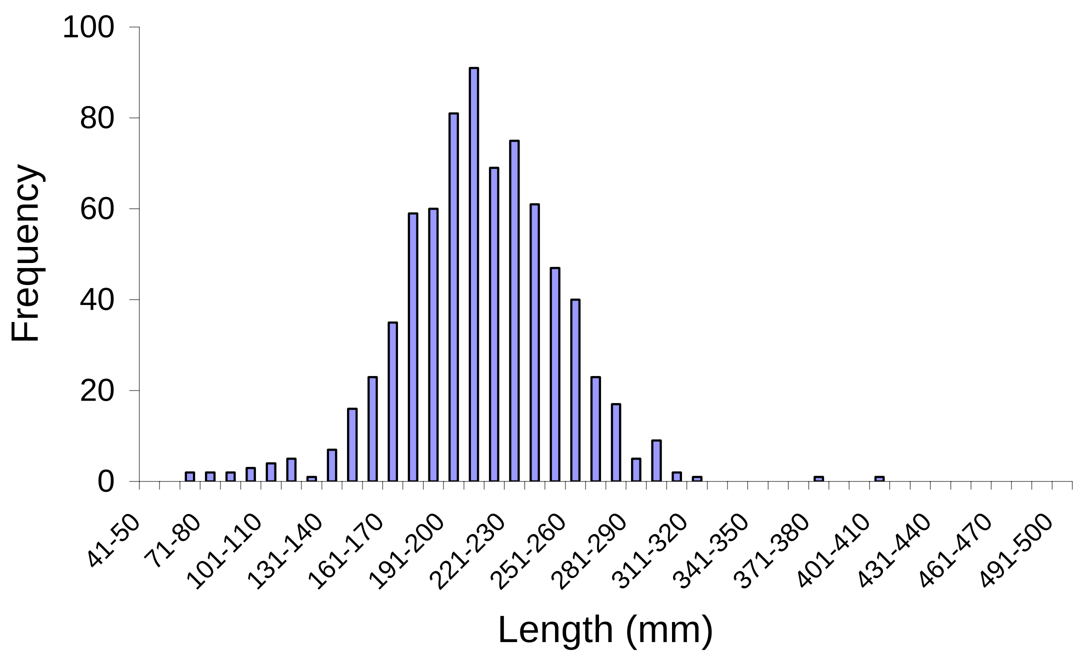


Figure 3. Length frequency histogram for 740 brook trout sampled from the 12 study waters surveyed during 2005.

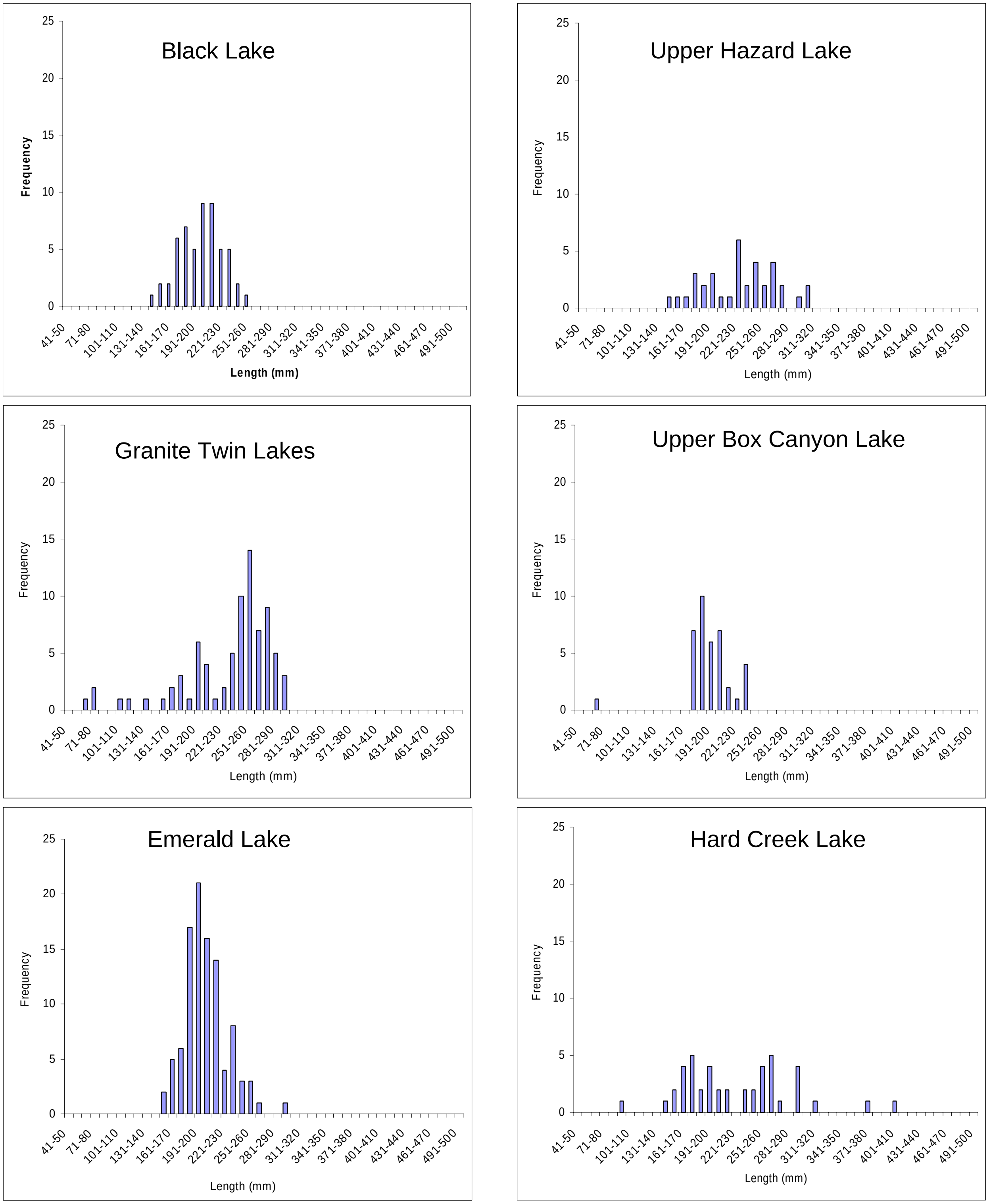
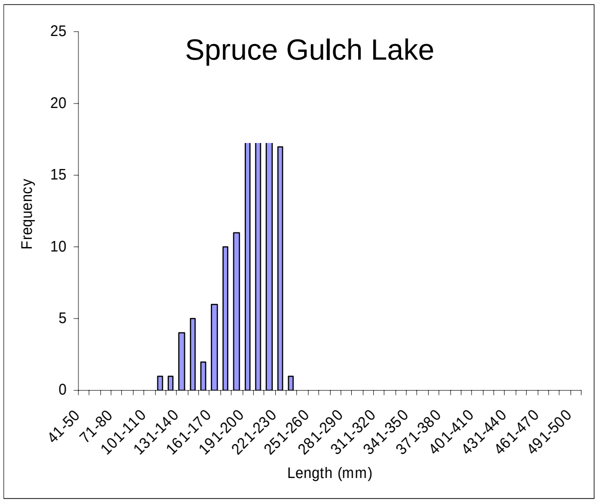
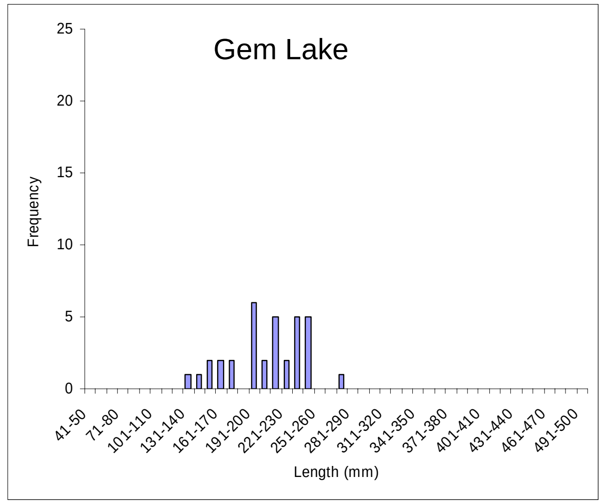
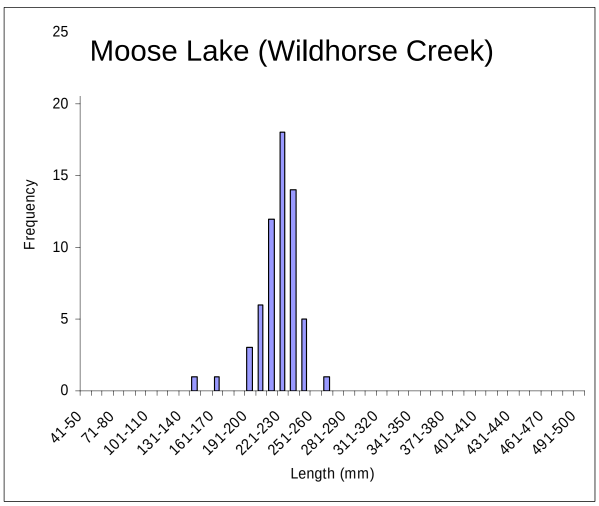


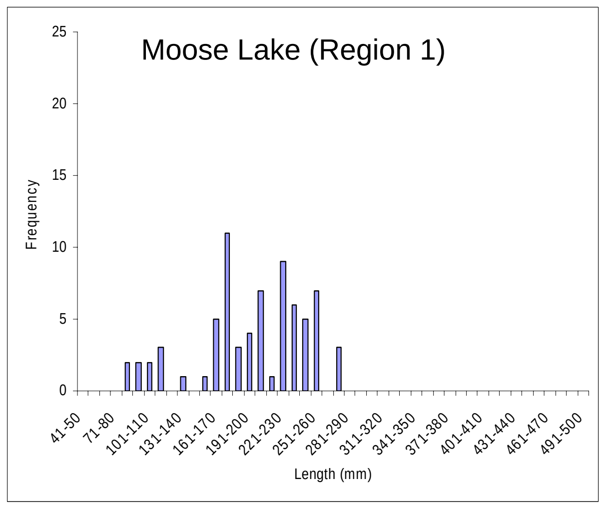
Figure 4. Length frequency histograms for brook trout sampled from 12 Idaho high mountain lakes during 2005. The remaining lakes are continued on the next page.

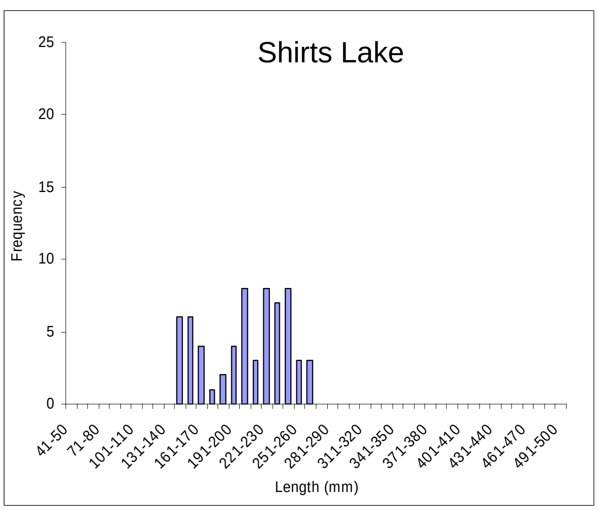
Figure 4. Continued.











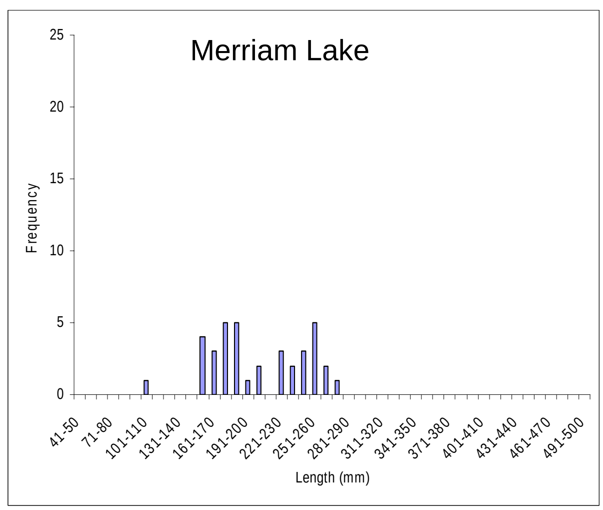


Table 2. Length, trophy potential assessed by the mean of five longest fish, weight, and condition factor of brook trout sampled from 12 Idaho high mountain lakes during 2005.

Lake Name	Length (mm)			Mean of 5 Longest (mm)	Weight (g)			Mean Condition Factor
	Mean	LCL	UCL		Mean	LCL	UCL	
Black	203	198	209	244	80	75	86	0.93
Emerald	204	200	208	266	78	73	83	0.9
Gem	205	195	215	252	95	82	108	1.03
Granite Twin	232	222	242	291	124	114	134	0.9
Hard Creek	226	210	242	340	140	109	172	0.98
Merriam	205	194	217	265	91	77	106	0.95
Moose (Lightning)	194	182	206	269	85	71	99	1.01
Moose (Wildhorse)	222	218	226	247	100	96	104	0.92
Shirts	185	178	192	231	67	61	72	1.06
Spruce Gulch	207	199	215	260	92	83	101	0.98
Upper Box Canyon	193	185	201	233	68	61	75	0.88
Upper Hazard	227	215	239	292	113	98	128	0.91

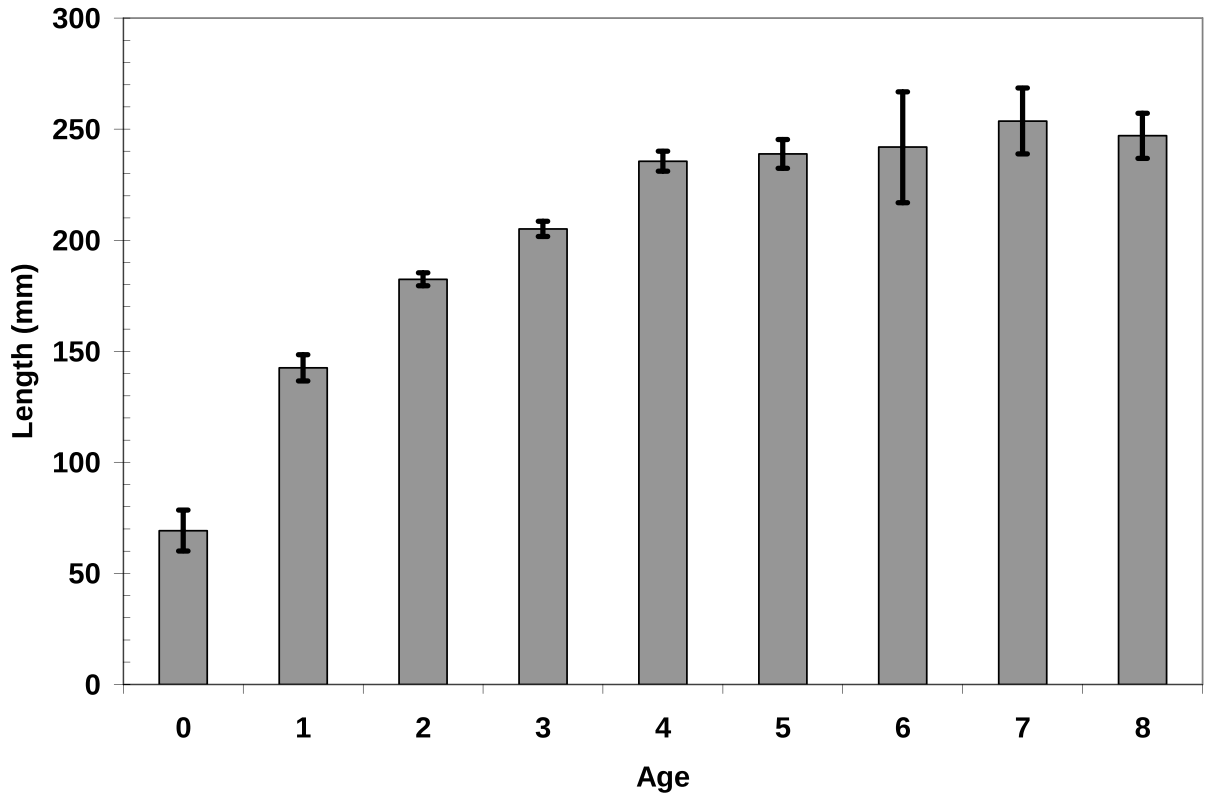


Figure 5. Average length of brook trout by age class. Age estimates were determined by viewing otolith samples whole with a dissecting microscope. Samples were pooled from all 12 lakes sampled during 2005.

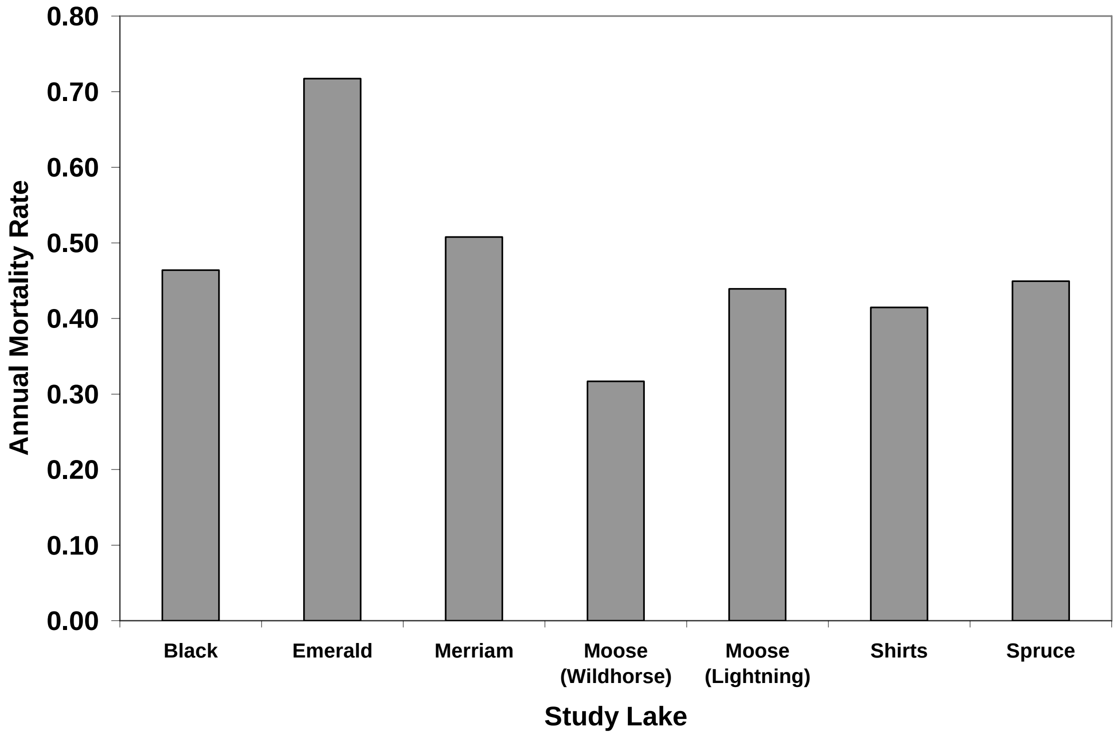


Figure 6. Annual mortality rates for brook trout in seven of the 12 Idaho high mountain lakes sampled during 2005 and known to have introduced brook trout populations. Estimates were determined through analysis of catch curves. Estimates were only made for lakes that had greater than two fish per age class for at least three consecutive years.

Table 3. Physical description of study waters in Idaho surveyed to assess their potential for use in determining whether tiger muskellunge can be used to eliminate brook trout populations from high mountain lakes. Lakes were surveyed during 2005 and will be stocked depending on availability of tiger muskellunge during 2006.

Lake Name	Region	Size (ha)	Elevation (meters)	Average Depth (meters)	Max Depth (meters)	Avg Conductivity (μmho/cm)	Distance from Road (km)	Part of a Chain?	Inlet spawning habitat?	Outlet spawning habitat?	Outlet barrier within 1 km?	Elimination Potential
Black	3M	10.5	2199	27	37	38.7	0.0	No	No	Yes	Yes	Moderate
Emerald	3M	26.7	2072	11	17	31	4.0	Yes	Yes	Yes	No	None
Gem	1	6.9	1760	4	5	—	1.8	No	Yes	Yes	Yes	High
Granite Twin (Upper)	3M	16.1	2183	9	18	9.5	1.9	No	No	Yes	Yes	High
Hard Creek	3M	8.5	2256	3	7	6	4.0	No	No	Yes	Yes	High
Merriam	7	2.6	2926	9	10	165	3.1	No	No	No	Yes	Very high
Moose (Wildhorse)	6	3.9	2848	3	6	59.3	6.8	Yes	Yes	Yes	No	None
Moose (Lightning)	1	16.1	1657	2	3	—	2.4	No	Yes	Yes	Yes	Moderate
Shirts	3M	3.5	2254	3	4	—	1.9	No	No	Yes	Yes	High
Spruce Gulch	7	10.9	2698	7	13	7.3	0.0	No	No	No	Yes	Very high
Upper Box Canyon	4	2.5	2947	5	8	41.3	3.9	No	No	No	Yes	Very high
Upper Hazard	3M	39.1	2264	12	21	3	3.1	No	Yes	Yes	Yes	Moderate

ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT EVALUATIONS

State of: Idaho Grant No.: F-73-R-28 Fishery Research

Project No.: 4 Title: Hatchery Trout Evaluations

Subproject #2: Sterile Trout Investigations

Contract Period: July 1, 2005 to June 30, 2006

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 16 high mountain lakes during 2001. During 2004, 779 fish were sampled, including 99 test fish. Of these 99 test fish, 56 diploids and 29 triploids were identified. During 2005, an additional 329 salmonids were sampled from eight lakes. A total of 295 non-test fish (90%) and 34 test fish (10%) were sampled. From the 34 test fish, 19 diploids, 7 triploids, and 8 unmarked fish were identified.

Over the two-year study, 1,108 salmonids were sampled. This included 133 test fish from the 2001 stocking event (12%), 7 fish from the 2003 stocking event (1%), and 968 non-test fish (87%). Of the 133 test fish sampled, 36 triploids and 75 diploids were positively identified, yielding a catch ratio of 2.1 diploids sampled for every one triploid sampled.

Methods for producing triploid walleye *Sander vitreus* and kokanee *O. nerka* were investigated during 2005. For walleye, treatment at 9,500 psi: 10 min duration resulted in egg survival of 54%, whereas that of untreated was 46%. Triploid induction rates for this treatment were 95% (38 triploids out of 40 fish tested).

To evaluate the relative performance of diploid and triploid kokanee, approximately equal numbers of fish from the triploid and diploid groups were stocked into five study waters. Due to a relatively low triploidy induction rate (79%), stocking numbers and the return of these test groups in several years will have to be adjusted to account for the presence of diploid fish in the triploid group. Secondary marks (pelvic fin clips) on approximately 12-13% of the two test groups will allow assessment of long-term retention rates of single and double calcein marked fish in the field.

Author:

Joseph R. Kozfkay
Senior Fisheries Research Biologist

Martin Koenig
Fisheries Research Biologist

INTRODUCTION

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

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

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

Although walleye *Sander vitreus* 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 kokanee fisheries, as triploid kokanee 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 largescale study in 16 central Idaho high mountain lakes during 2001. We also summarize efforts to refine techniques for inducing triploidy in walleye and kokanee. Finally, we document initial efforts to examine the performance of diploid and triploid kokanee in lowland lakes and reservoirs.

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 high mountain lakes by 25% by stocking triploid fish while maintaining growth rates equal to that of diploid rainbow trout. Assessments will include four high mountain lakes during 2001-2003, an additional 16 lakes during 2004 and 2005, and 15 other lakes in 2006 and 2007.

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

3. To increase the longevity of kokanee through sterilization by at least one year, and thereby increase harvest rates by 25%.

METHODS

Performance of Sterile Trout in High Mountain Lakes

During 2001, IDFG regional fishery managers and U.S. Forest Service personnel provided high mountain lake information that facilitated study site selection. Only lakes scheduled for stocking in 2001 were considered for stocking with test fish. Test fish were not stocked in drainages where conflicts with native or wild populations were possible or in lakes where brook trout populations were established. We preferentially selected lakes that were from five to 10 acres in surface area and had reasonable access from roads, yet were remote enough to keep harvest to a minimum level. Additionally, past surveys must have indicated that lakes were capable of supporting trout fisheries. Sixteen lakes were selected throughout central Idaho (Figure 7). All test lakes are managed under the general trout regulation of six fish per day with no length limit, except for Blackwell and Brush lakes, which are managed under the trophy regulation of two fish per day with none under 508 mm.

Mixed-sex rainbow trout eggs were produced from 1:1 pairings at Hayspur Fish Hatchery. After fertilization, egg lots were split. Half the eggs were reared normally and were used as the control in this study. The other half were placed in a 26°C water bath at 20 minutes after fertilization (MAF) and thermal-shocked for 20 minutes to induce triploidy (Teuscher et al. 1998). Eggs were incubated, reared in 1 m tanks, and then transferred to raceways. Prior to grit marking, both groups were adipose clipped to indicate inclusion in this study when sampled in the field, and the diploid and triploid groups were grit marked with green and red fluorescent dye, respectively.

Lakes were surveyed with floating gillnets and angling from July 16 to August 24, 2004 and from July 6 to August 16, 2005. The experimental gillnets used had 19, 25, 30, 33, 38, and 48 mm bar mesh panels and were 46 m long by 1.5 m deep. Typically, 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. During 2005, only eight of the 16 lakes stocked during 2001 were sampled. The subsample of lakes was selected to include four lakes that yielded test fish during 2004 sampling efforts and four lakes that did not. No sampling was conducted in the other eight lakes to prevent excessive mortality rates from additional netting efforts.

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 within an industrial-strength black plastic garbage bag. Gonads of rainbow trout were examined to determine sex and assigned one of three levels of maturity: immature, developing, or mature. Scale and otoliths samples were collected from most test fish. Scales were stored in coin envelopes, whereas otoliths were stored dry in microcentrifuge tubes. In the laboratory, scales were embedded in acetate, whereas otoliths were viewed whole (Boxrucker 1986).

Similar to IDFG standard high lake monitoring protocols, abiotic measurements were collected at each of the 16 lakes. A series of three transects were placed at equal distances perpendicular to the long axis of the lake with the aid of a laser rangefinder. Three sampling points were equally spaced along each transect. Depth measurements 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 GIS. In addition, amphibian surveys were conducted by slowly walking the entire perimeter of each lake along the water shore interface and looking near and under woody debris.

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

Production of Sterile Fish

Walleye

Experiments designed to induce triploidy in walleye were performed in cooperation with personnel from Montana Department of Fish Wildlife and Parks' (MT FWP) Fort Peck Office and Miles City Fish Hatchery. Brood fish were captured with trap nets by MT FWP personnel from the Dry Arm of Fort Peck Reservoir, Montana and held in net pens until ready to spawn. On April 12, 2005, fertilized eggs were produced by combining the gametes of four females with four males. After one minute to allow fertilization, a slurry of Fuller's earth (clay) and lake water was added to the spawning bowl to make the eggs nonadhesive. After three minutes in the slurry, eggs were split in two and half were pressure treated while the other half was not treated to allow comparisons of survival between treated and untreated eggs. Fertilized eggs were placed in a perforated aluminum cylinder for loading and unloading. The hydraulic pressure chamber, Model HPC™, used during this experiment was built by TRC Hydraulics Inc., Dieppe, New Brunswick, Canada. The 2.7 liter chamber was filled with ambient reservoir water before egg treatment. The process was completed three times with additional groups of males and females to allow testing of three pressure treatments. The treatments consisted of 8,000 psi for 20 minute duration, 8,000 psi for 30 min duration, and 9,500 psi for 12 min duration. The eggs for all treatments were introduced to the pressure chamber at 4 MAF (minutes after fertilization). 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. When fish reached approximately 50 mm, 40 blood samples were collected from each treatment by replicate group, stored in Alsever's solution, and shipped to North Carolina State University where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw.

Kokanee

We conducted a pressure shock experiment to induce triploidy in kokanee collected from the Deadwood River on August 25, 2005. A total of 16 males and 16 females were collected from normal spawning operations. These fish were then loaded on a stocking truck and transported to the wet lab of Eagle Fish Hatchery. During the afternoon of August 25, three females were fertilized with the milt from three males for each of the three replicates. Approximately equal numbers of fertilized eggs were split five ways (four treatments and one control). Pressure treatments were 9,000 psi at 300 CMAF (Celsius minutes after fertilization), 9,500 psi at 250 CMAF, 9,500 psi at 300 CMAF, and 9,500 psi at 350 CMAF. All treatment durations were 5 minutes. After treatment, all eggs were incubated in the wet lab at Eagle Fish Hatchery. Ambient water temperature was 13°C. Eggs were enumerated at the eyed, hatch, and swim-up stages. When fish reached approximately 40-45 mm, 25 blood samples were collected from each treatment by replicate group, stored in Alsever's solution, and shipped to North Carolina State University where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

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

To facilitate evaluation of the performance of large numbers of diploid and triploid kokanee stocked as fry, a quick and efficient method for applying two distinct batch marks was needed. Calcein has been shown to be a persistent mark for Atlantic and Chinook salmon as well as steelhead trout (Mohler 1997; Mohler 2003a; Mohler 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 a pilot study ran in 2004 (Kozfkay 2004), we single marked the triploid group and double marked the diploid group. The first mark was applied from February 8-10, 2005. The second mark was applied to the diploid group from April 18-20, 2005, an interval of 70 days. To assess long-term retention of calcein in the double marked group, 12.5% of the diploid group was given a secondary mark by removing the left ventral fin. To assess long-term retention of the single mark, 13.2% of the triploid group was given a secondary mark by removing the right ventral fin. To assess the triploidy induction rate of the triploid group, when the fish reached approximately 50 mm, 100 blood samples were collected, stored in Alsever's solution, and shipped to North Carolina State University where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw. Approximately equal numbers of kokanee from the diploid and triploid groups were stocked into the five study waters from April 28 through June 3, 2005.

RESULTS

<u>Performance of Sterile Trout in High Mountain Lakes</u>

During 2004 surveys, 779 salmonids were sampled from 16 lakes for a mean of 48.7 ± 10.4 (indicating 90% confidence limit) fish per lake (Table 4). The majority of fish collected (673 or 86%) were non-test fish that originated from previous stocking events or natural recruitment. Non-test fish sampled included rainbow trout, westslope cutthroat trout *O. clarkii lewisi,* Yellowstone cutthroat trout *O. clarkii bouvieri,* and hybrids, as well as lesser numbers of brook by bull trout hybrids *Salvelinus fontinalis* x *confluentus*, Arctic grayling *Thymallus arcticus*, kokanee, and golden trout *O. mykiss aguabonita*.

Of the 779 fish caught during 2004, 99 test fish were caught from the 2001 stocking event. Nearly twice as many Hayspur-strain mixed-sex diploid rainbow trout (56) were sampled as Hayspur-strain mixed-sex triploid rainbow trout (29; Table 4). This yielded a mean catch rate (with 90% confidence interval) of 1.8 triploid (±2.1) and 3.5 diploid fish per lake (±2.6). The highest catch of test fish (43) was collected from Raft Lake and was comprised of 19 triploids and 24 diploids. Catch for diploids and triploids was <9 and <5, respectively, in all other lakes. No test fish from either group were caught in Brush, Cache Cr. #2, Josephine #2, and Squaw lakes. Moreover, no triploid fish were caught from six other lakes. Fourteen adipose clipped fish were caught for which no grit mark could be found; therefore, these fish could not be certainly identified to test group. This yielded a mark retention rate of 86% for the three-year period since test groups were marked. Additionally, seven test fish were caught as age-1 fish from the 2003 stocking event. These fish consisted of six Troutlodge all-female triploid rainbow trout and one diploid Hayspur-strain rainbow trout.

During 2005 surveys, 329 salmonids were sampled from eight lakes for a mean of 41.1 fish per lake. A total of 295 non-test fish (90%) and 34 test fish (10%) were sampled. From the 34 test fish, 19 diploids, 7 triploids, and 8 unmarked fish were identified (Table 5). Test fish were captured from six of the eight lakes sampled including Josephine #2 (*n* = 4), Ingeborg (*n* = 10), 20 Mile Long (*n* = 4), Raft (*n* = 7), Shaw Twins #1 (*n* = 1), and Washington (*n* = 8) lakes. No test fish were caught from Queens River #5 and Cache Ck. #1 lakes. Catch decreased from 2004 to 2005 as expected in five of the eight lakes (Table 5). The only exceptions were Ingeborg, Washington, and Josephine #2 lakes. In fact, no test fish were caught in Josephine during 2004, whereas four were caught during 2005.

Over the two year study, 1,108 salmonids were sampled. This included 133 test fish from the 2001 stocking event (12%), 7 fish from the 2003 stocking event (1%), and 968 non-test fish (87%). Of the 133 test fish sampled, 36 triploids and 75 diploids were positively identified (Table 5), yielding a catch ratio of 2.1 diploids sampled for every 1 triploid sampled. No grit dye could be found on 22 of the 133 test fish. Based on these numbers, mark retention was 83% overall.

During 2004, gill nets were fished for 41-162 h per lake for a combined total effort of 1091 h (Table 6). Catch per unit effort (CPUE) for all fish combined with gill nets ranged from a low of 0.17 fish per hour in Blue Jay Lake to a high of 1.06 fish per hour in Long Lake. Angling surveys were conducted in 15 of the 16 lakes surveyed. Effort ranged from one to 12 hours per lake for a combined total effort of 72.3 h. CPUE for angling equaled zero in Brush, Josephine Lake #3, Raft, and Washington lakes. The highest CPUE recorded for angling was 7.14 fish per hour in Queens River Lake # 5. When only test fish were considered, there was a disparity in the ratio of triploid:diploid fish caught between the two methods. The ratio for gill nets was 1.79 diploids for every 1 triploid, whereas the ratio for angled samples was 6 diploids for every 1 triploid.

During 2005, three to five gill nets were fished from 24 to 52 h per lake. Total gill netting effort equaled 266 h over 36 gill net nights. CPUE for all fish combined with gill nets ranged from a low of 0.25 fish per hour in Raft Lake to a high of 1.8 fish per hour in Lake Ingeborg (Table 7). Angling surveys were conducted in four of the eight lakes sampled. Hook and line effort ranged from two to ten hours. The highest CPUE of 2.5 fish per hour was recorded from Cache Creek Lake #1. CPUE for angling was low in the other three waters sampled and equaled zero in Shaw Twins #1 and Raft lakes and 0.5 fish per hour in Josephine Lake.

For fish sampled during 2004, length and weight for diploid and triploid fish could only be compared across four lakes (due to small sample sizes): Blue Jay, Ingeborg, Long, and Raft. Due to wide confidence intervals at least partially caused by the low number of samples, there were no statistical differences between any of the paired comparisons, except that the weight of triploid fish was significantly less than the weight of diploids in Raft Lake (Figure 8 and 9). For Raft Lake, the only lakes with adequate numbers from both groups, mean length of diploids was 19 mm and 87 g more than that of triploids.

For fish sampled during 2005, length for all test fish (known age-4 fish) averaged 337 mm (± 16) and included a minimum length of 257 mm (Washington Lake) and a maximum length of 494 mm (Raft Lake). For this same group of fish, weight averaged 413 g (± 62) and included a minimum of 170 mm and a maximum of 1220 g. Potential differences in growth between diploid and triploid fish could only be compared across two lakes. Based on mean values alone, length of diploid ($\bar{X}$ = 320 ± 19 mm; *n* = 6) and triploid ($\bar{X}$ = 321 ± 30 mm; *n* = 3) rainbow trout was essentially equal in Lake Ingeborg. For these same fish, mean weight of diploids ($\bar{X}$ = 349 ± 92 g; *n* = 6) was not different from triploids ($\bar{X}$ = 310 ± 77 g; *n* = 3). In Washington Lake, based on mean values, diploids ($\bar{X}$ = 305 ± 15 mm; *n* = 3) were not longer than triploids ($\bar{X}$ = 288 ± 79 mm; *n* = 2). Similarly, diploids ($\bar{X}$ = 315 ± 31 g; *n* = 3) were not heavier than triploids ($\bar{X}$ = 260 ± 126 g; *n* = 2) in Washington Lake. Sample sizes in these lakes were very small and, therefore, did not allow meaningful statistical interpretation of differences in growth between diploid and triploid rainbow trout.

Habitat variables for the 16 lakes sampled in 2004 are listed in Tables 8 and 9. Statistically significant, positive correlations were found between mean depth and average depth ($r = 0.89$; $p < 0.0001$), elevation and pH ($r = 0.76$; $p = 0.0006$), conductivity and pH ($r = 0.63$; $p = 0.0086$), and surface water temperature and Secchi depth ($r = 0.46$; $p = 0.07$). Statistically significant, negative correlations were found between stocking density and size ($r = -0.85$; $p < 0.0001$) as well as average depth and conductivity ($r = -0.46$; $p = 0.07$).

For the length of test fish at age-3, stepwise regression analysis indicated that the CPUE for all fish (test and non-test) and stocking density had the most effect on length achieved; combined they explained 64% of the variation in length among the 16 lakes sampled. For the total number of fish caught per lake, including test and non-test fish, no abiotic or biotic variables were associated, and thus, no model fit the data. For the logistic regression analysis to determine what factors were associated with lakes where triploid fish were sampled, stocking density and elevation were initially selected for the model but were removed, and no model was determined to fit the data. Plots of these data indicated no obvious pattern for elevation; however, a pattern for stocking density was evident. Although there was obvious overlap, triploid fish were never present in lakes that were stocked at densities greater than 261 fish per hectare (FPH) (Figure 10). For the total number of test fish caught (diploid and triploid combined), no logistic model fit the data. Plots of abiotic and biotic habitat variables and whether test fish were present or absent indicated that there was strong overlap.

For known age-3 fish, consensus scale and otolith estimates were available from 63 fish. Mean ages for scales ($\bar{x}$ = 3.0 ± 0.1) and otoliths ($\bar{x}$ = 2.9 ± 0.1) were not statistically different across the 63 fish. From scales, 50 out of 63 fish were correctly aged (79%; Figure 11). Of the remainder, seven were estimated as age-2 (11%), five as age-4 (8%), and one as age-5 (2%). From otoliths, 56 out of 63 fish were correctly aged (89%). Of the remainder, six were estimated as age-2 (10%) and one as age-4 (1%).

For known age-1 fish, consensus scale and otolith estimates were available from six fish. Mean ages for scales ($\bar{x}$ = 1.2 ± 0.3) and otoliths ($\bar{x}$ = 1.7 ± 0.4) were not statistically different. From scales, five out of six fish were correctly aged (83%). Of the remainder, one was aged as age-2 (17%). From otoliths, two out of six fish were correctly aged (33%). Of the remainder, four fish were estimated as age-2 (67%).

Consensus ages for both scales and otoliths were reached for 313 fish sampled during 2004, including known and unknown age fish. Mean ages for scales ($\bar{x}$ = 2.8 ± 0.1) and otoliths ($\bar{x}$ = 2.9 ± 0.1) were not statistically different across the 313 fish. However, this alone is misleading. Scales tended to give higher estimates than otoliths for fish aged as one and two, estimates were equal for age three, and estimates from scales were much lower than from otoliths for ages four through eight (Figure 12). For fish aged as one from otoliths (n = 25), average age from scales equaled 1.6 ± 0.2. For fish aged as two from otoliths (n = 85), average

age from scales equaled 2.2 ± 0.1. For fish aged as three from otoliths (n = 134), average age from scales equaled 3.0 ± 0.1. For fish aged as four from otoliths (n = 43), average age from scales equaled 3.3 ± 0.3. For fish aged as five through eight from otoliths (n = 24, $\bar{x}$ = 5.4 ± 0.3), average age from scales equaled 3.9 ± 0.4.

Production of Sterile Fish

Walleye

Survival of individual groups of treated and untreated walleye eggs were highly variable. For the 8,000 psi for 20 min duration treatment, survival of controls was 64%, whereas survival of treated groups was 18%. For the 8,000 psi for 30 min duration treatment, survival of controls was 51%, whereas survival of treated groups was 6%. For the 9,500 psi : 10 min duration treatment, survival of treated eggs was 54%, whereas that of untreated was 46%, by far the best survival to eye-up of any treatment tested during 2004 or 2005. However, mortality at hatch was higher than expected; whether this was caused by the treatment or other factors is unknown.

Induction rates were high for all three treatments. For the 8,000 psi for 20 min duration treatment, triploidy induction rate was 90% (36 triploids out of 40). For the 8,000 psi for 30 min duration treatment, triploidy induction rate was 97.5% (39 triploids out of 40 fish tested). For the 9,500 psi for 10 min duration treatment triploidy induction rate was 95% (38 triploids out of 40 fish tested). All ten controls analyzed were diploids.

Kokanee

Relative to successful treatments identified for other species, the gap in survival between the control and the best identified treatment was larger. Survival of control or untreated eggs to eye-up and feeding fry stage was 79%, whereas the best treatment for sterilization (9,500 psi at 250 CMAF for 5 minutes duration) provided survival rates of 54% (Table 11). This gap remained through the feeding fry stage where control survival was 70% and the best treatment survival was 42%. Triploidy induction rates were high across all treatments tested. Of the 169 samples analyzed from pressure treated groups, only one sample was determined diploid and found in the least intense treatment of 9,000 psi.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

Final preparations before stocking of kokanee test groups required that diploid and triploid groups be measured to determine relative size, tested to determine triploidy induction rates, and stocked in relatively equal numbers. Relative size within test groups was equal prior to stocking. Mean length of the diploid ($\bar{X}$ = 86 ± 1 mm; n = 100) and triploid ($\bar{X}$ = 88 ± 2 mm; n = 100) groups was equal based on overlapping 90% CIs. Similarly, mean weight of the diploid ($\bar{X}$ = 3.9 ± 0.2 g; n = 100) and triploid ($\bar{X}$ = 4.0 ± 0.2 mm; n = 100) groups was equal. Flow cytometry analysis indicated that triploidy induction rate for the triploid group was 79% (n = 99). Approximately equal numbers of kokanee from the triploid and diploid groups were stocked into four of the five study waters (Table 12). Lucky Peak was the only exception, with 49,950 kokanee diploids and 41,400 triploids being stocked.

DISCUSSION

Performance of Sterile Trout in High Mountain Lakes

In terms of natural reproduction and whether stocked fish were a substantial part of existing fish populations, lakes fell into one of three categories: 1) high percentage of fish from previous stocking events and limited or no natural reproduction, 2) intermediate percentage of fish from previous stocking events and intermediate levels of natural reproduction, and 3) nearly all fish from natural production and limited or no contribution from previous stocking events. Raft and Blue Jay lakes would fall into category 1 (Table 10). Only two age classes of fish were present, age estimates coincided with past stocking events, and marked fish represented a high proportion of the catch, 98 and 65%, respectively. Blackwell, Blue, Long, and Shaw Twins most likely also fell into category 1. However, marked fish represented a smaller portion of the catch, 6-15%. Since these lakes are stocked at frequent intervals, 1-2 years, it was impossible to separate wild and hatchery fish based on age estimates (due to aging error). Cache Cr. # 2, Josephine # 2, Ingeborg, NF 20 mile # 3 and # 4, and Washington lakes fell into category two. For these lakes, a combination of factors was present such as a considerable number of hybrids (indicative of at least some successful spawning), intermediate number of marked fish present in the catch, and fish populations that did not closely mimic past stocking records. Brush, Cache Cr. # 1, Queens River # 5, and Squaw lakes fell into category three. Fish populations were characterized by one or a combination of factors, such as considerable number of hybrids, lack of marked fish present, or near total lack of fish present from previous stocking events. Cache Creek Lake # 2 was not placed into any of the categories because it lacks a stocking history, and the lake is too shallow to hold substantial fish populations.

Gill net and angling surveys for high mountain lakes stocked in 2001 indicated that the relative survival of Hayspur-strain diploid fish far exceed that of Hayspur-strain triploid fish. The overall ratio was 2.1 diploids caught for every 1 triploid caught. This result very closely mimics the results from the pilot study, where over three years of sampling (2001-2003), the ratio of diploid: triploid fish caught was 2.3 : 1 (Kozfkay 2003). These results contradict previous work designed to determine the relative survival of triploid and diploid rainbow trout in Idaho streams (Dillon et al. 2000) and reservoirs (Teuscher et al. 2003). There are some key differences between these studies. For instance, both previous studies used different strains of rainbow trout: Mt. Lassen Mixed Sex and Trout Lodge All-Female rainbow trout, respectively. Secondly, these studies were conducted in different habitats. The reservoir study was conducted in two of the most productive systems in Idaho, whereas the stream study encompassed streams with varying levels of productivity during the summer months using catchable size fish. High mountain lakes are thought to be more harsh environments than streams in summer and reservoirs and the poor survival of triploid fish was likely due to these differences.

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

Triploid fish have lower hemoglobin-oxygen ratios than diploid fish (Graham et al. 1985), which does not allow them to store enough oxygen during times of sustained high demand. This factor is thought to contribute to higher mortality and poorer growth of triploid fish during period of high water temperatures (Ojolick et al. 1995). Currently, we do not know if high water temperatures during late summer were a factor in the poor survival of triploid fish in some lakes. Other chronic environmental stressors may include low temperatures or low dissolved oxygen levels; however, we are aware of no studies that looked at the effect of these characteristics on triploid survival and were unable to measure oxygen levels throughout the study period.

Stocking density was associated with the survival of triploid fish in our study lakes, albeit weakly. Lakes that were stocked at lower stocking densities tended to have better triploid fish survival. This suggests to some degree that competition was affecting survival of triploids. So presumably at higher stocking densities (>261 FPH) competition for limited resources was intense enough to negatively influence the survival of triploids. Secondly, visual interpretation of plots showed that high densities of non-test fish seemed to negatively affect the survival of triploid fish and to a lesser extent diploid fish also, though this factor was not deemed statistically significant during our analysis. For instance, the two lakes with the highest number of test fish caught, Raft and Blue Jay lakes, were also the lakes with the lowest number of non-test fish caught. Lack of statistical significance was likely caused by the large number of lakes for which no test fish were caught, forcing us to analyze the information as presence/absence data. This observation seems to support the notion that triploid fish were being out-competed by diploid fish, either the diploid test group or non-test fish. However, if the majority of the mortality of the triploid group occurred immediately after stocking, then predation cannot be ruled out either.

Small sample sizes prevented meaningful statistical comparisons of diploid and triploid growth rates among lakes. Based on mean values alone, there was a tendency for the diploid group to be about 20 mm longer and 75 grams heavier. The few additional samples collected during 2005 seemed to support the notion that diploid fish are usually heavier and slightly longer, through the disparity seemed to decrease from 2004. The larger numbers of fish stocked during 2003 should allow greater returns and a better assessment of this question when lakes are sampled in 2006. Relative growth of diploid and triploid fish may be age specific. Teuscher et al. (2003) noted that diploid fish grew faster through age-3, but afterwards as diploid fish started to put more energy into gonadal development, growth of triploid fish exceeded that of diploids age-4. For rainbow trout in Alaska lakes, Brock et al. (1994) found that all triploid females were smaller than mixed-sex diploids produced from the same parents in each of six lakes. This trend continued though age-2. Unfortunately, no samples were collected from older fish to see if relative growth changed after sexual maturation.

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

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

Production of Sterile Fish

Survival and induction rates for pressure treatments tested on walleye during 2005 showed a marked improvement over those treatments tested during 2004. This may in part be due to when the experiment was performed. The 2005 experiment was performed over a week earlier than during 2004, April 12 as opposed to April 21. This likely meant that during 2005 eggs were taken closer to the peak of the spawning period when eggs are typically of higher quality. Furthermore, the most efficient treatment identified during 2005 had higher survival rates relative to its respective control. During 2004, the most efficient treatment, 9,500 psi for 5 min duration, in terms of survival, provided survival rates of 73% relative to controls, whereas the most efficient treatment identified during 2005, 9,500 psi for 10 min duration provided survival of 85% relative to its control. The longer duration used during 2005 also provided higher triploidy induction rates. Triploidy induction rates for the 9,500 pressure levels compared across years increased from 47% to 95% when treatment duration was increased from 5 minutes to 10 minutes. An additional increase in treatment duration of two minutes may increase experimental triploidy induction rates to near 100%.

The use of pressure to induce triploidy in kokanee provided very high induction rates. Across the four treatments tested, all but one of the 169 samples tested were determined to be triploid. The one exception came from the least intense pressure treatment of 9,000 psi. In terms of survival rates for high induction rate treatments, pressure provided an improvement over heat treatment tested previously (Kozfkay 2002). However, survival rates for the best treatment identified were still below survival rates for other species that have been tested over the last several years. There are several possible explanations for this disparity. Simply, this effect may be species specific. Kokanee eggs may not respond well to additional handling or treatment. Alternatively, the potential for high survival rates may be lower for eggs collected for feral brood fish in a remote setting. Lastly, we may have yet to identify the best treatment. Over the range of treatments tested, survival rate was inversely related to the time the treatment was applied (CMAF). Based on this observation, it may be worthwhile to examine an even lower CMAF of 200. Since 100% sterility rates are not an important management requirement, then the best treatment was 9,000 psi applied at 250 CMAF for five-minute duration. This treatment maximized survival and provided sufficiently high induction rates of 98%. However, if 100% induction rates are required, the 9,500 psi applied at 250 CMAF for five-minute duration should be used.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

The variable river water temperatures that seemed to affect survival during production of triploid 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 cytometric indicated that triploidy induction rates were relatively low for the test group created for this study, 79%, compared to more controlled production efforts for other species. Although this induction rate is not ideal, it should not affect the utility of this study if adjustments are made or extra steps are taken when kokanee are collected in the field. Most simply, proportional correction factors could be applied to the number of fish stocked and the number of fish sampled. However, while this adjustment would work well for adjusting the number of diploid and triploid fish stocked, it is less preferable for adjusting the number of test fish sampled, especially if differential mortality occurs between test groups. To address this concern, blood or tissue samples should be collected from sampled fish. Blood or tissue samples (Lamatsch et al. 2000) could then be analyzed using flow cytometry to allow development of more precise correction factors.

RECOMMENDATIONS

1. Re-evaluate current stocking strategies for sterile Hayspur rainbow trout in high mountain lakes. From the pilot study and the present evaluation, it is evident that triploid Hayspur rainbow trout survive at about half the rate of diploids when stocked together. A simple solution to this problem would seem to be to double our current stocking rates. However, there did seem to be an effect of stocking density, so increasing stocking density might not increase the number of stocked fish available to anglers. Alternatively, other strains of rainbow trout may perform better. Results from an additional stocking in 2003 will become available during 2006-2007 and will provide information as to whether Troutlodge all-female rainbow trout provide better performance in high lakes.

2. Standard high mountain lake surveys should include an assessment of natural recruitment and contribution of stocked fish to existing populations using batch marked fish. During this study, the use of visual estimation of spawning habitat was inefficient in predicting whether there was sufficient reproduction to maintain populations without further stocking. For instance, for the subset of lakes used in this study, at least four and as many as 10 of the 16 study lakes studied would sustain fishable populations without further stocking.

3. Stock Hayspur triploids at densities of 250 fish per hectare or less in high mountain lakes, and evaluate the benefit of stocking any Hayspur rainbow (diploid or triploid) in mountain lakes less than 2,334 m (7,660 ft) in elevation, given the possibility of poor returns in these lakes.

4. Redesign and retest pressure treatment to induce triploidy in walleye and assess whether using Fuller’s Earth or tannic acid is more efficient for reducing egg adhesion.

5. Since 100% sterility rates are not normally as important in the management of kokanee populations in Idaho, the best treatment should maximize survival while providing reasonably high induction rates. Under this scenario, 9,000 psi applied at 250 CMAF for

five-minute duration was the best treatment identified as it maximized survival and provided a sufficiently high induction rate. Should additional testing be completed, a further decrease to 200 CMAF may further improve survival rates. If 100% induction rates are deemed necessary, the 9,500 psi applied at 250 CMAF for five minute duration should be used.

ACKNOWLEDGMENTS

We would like to thank John Cassinelli, Lars Alsager, Mike Biggs, Matt Wilson, Brett High, Kevin Meyer, Art Butts, Sarah Nelson, Matt Kreizenbeck, Travis Parrill, and Dan Garren for their field assistance during high mountain lake surveys. Gene McPherson, Steve Kammeyer, and Joel Patterson reared and stocked the high mountain lake test groups in 2001. Keith Johnson and Doug Burton provided rearing space in the Eagle Wet Lab for rearing rainbow trout fry for the calcein marking pilot study. Brad Dredge, Jarrett Page, and Jamie Mitchell spawned and reared experimental groups of westslope cutthroat and rainbow trout at Hayspur Fish Hatchery and painstakingly enumerated survival. Mike Ruggles, Jeff Remus, and Paul Santavy from MT FWP and their crews trapped and spawned walleye for our sterilization experiment. Mike Rhoades from MT FWP's Miles City Fish Hatchery and his crew reared experimental walleye groups and enumerated eggs for survival estimates. Rick Alsager, Brian Malaise, Bob Turik, Ken Felty, and Chuck Keister maintained a weir on the Deadwood River for collection of kokanee eggs. Phil Coonts, Mick Hoover, and Brian Grant picked the kokanee eggs, enumerated survival, reared, and stocked the test groups at Mackay Fish Hatchery.

LITERATURE CITED

Benfey, T. J. 1999. The physiology and behavior of triploid fishes. Reviews in Fisheries Science 7:39-67.

Boulanger, Y. 1991. Performance comparison of all-female, diploid and triploid brook trout. Canadian Technical Report in Fisheries and Aquatic Sciences 1789:111-121.

Boxrucker, J. 1986. A comparison of the otolith and scale methods for aging white crappies in Oklahoma. North American Journal of Fisheries Management 6:122-125.

Brock, I. V., P. A. Hansen, D. N. McBride, and A. C. Havens. 1994. Culture and performance of triploid rainbow trout in Alaska. Transactions of the North American Wildlife and Natural Resource Conference 59:263-273.

Bye, V. J., and R. F. Lincoln. 1986. Commercial methods for the control of sexual maturation in rainbow trout *Salmo gairdneri* R. Aquaculture 57:299-309.

Cotter, D., V. O'Donovan, A. O'Maoileidigh, G. Rogan, N. Roche, and N. O. Wilkins. 2000. An evaluation of the use of triploid Atlantic salmon *Salmo salar* L. in minimizing the impact of escaped farmed salmon on wild populations. Aquaculture 186:61-75.

Czesny, S. J. 2000. Biochemical, physiological, behavioral evaluation of the quality of purebred and hybrid walleye (*Stizostedion vitreum*) eggs, larvae, and juveniles. Ohio State University, Columbus, Ohio, PhD Dissertation.

Dillon, J. C., D. J. Schill, and D. M. Teuscher. 2000. Relative return to creel of triploid and diploid rainbow trout stocked in eighteen Idaho streams. North American Journal of Fisheries Management 20:1-9.

Donald, D. B., and R. S. Anderson. 1982. Importance of environment and stocking density for growth of rainbow trout in mountain lakes. Transactions of the American Fisheries Society 111:675-680.

Donald, D. B., R. S. Anderson, and D. W. Mayhood. 1980. Correlations between brook trout growth and environmental variables for mountain lakes in Alberta. Transactions of the American Fisheries Society 109:603-610.

Galbreath, P. F., W. St. Jean, V. Anderson, and G. H. Thorgaard. 1994. Freshwater performance of all-female diploid and triploid Atlantic salmon. Aquaculture 128:41-49.

Graham, M. S., G. L. Fletcher, and T. J. Benfey. 1985. Effect of triploidy on blood oxygen content of Atlantic salmon. Aquaculture 50:133-139.

Guo, X., W. K. Hershberger, and J. M. Meyers. 1990. Growth and survival of intrastrain and interstrain rainbow trout *Oncorhynchus mykiss* triploids. Journal of World Aquaculture Society 21:250-256.

Habicht, C., J. E. Seeb, R. B. Gates, I. R. Brock, and C. A. Olito. 1994. Triploid coho salmon outperform diploid and triploid hybrids between coho salmon and Chinook salmon during their first year. Canadian Journal of Fisheries and Aquatic Sciences 51:31-37.

Happe, A., E. Quillet, and B. Chevassus. 1988. Early life history of triploid rainbow trout *Salmo gairdneri* Richardson. Aquaculture 71:107-118.

IDFG (Idaho Department of Fish and Game). 2001. Fisheries management plan 2001-2006. Idaho Department of Fish and Game. Boise, Idaho.

Ihssen, P. E., L. R. McKay, I. McMillan, and R. B. Phillips. 1990. Ploidy manipulation and gynogenesis in fishes: cytogenetic and fisheries applications. Transactions of the American Fisheries Society 119:698-717.

Isermann, D. A., J. R. Meerbeek, G. D. Scholten, and D. W. Willis. 2003. Evaluation of three different structures used for walleye age estimation with emphasis on removal and processing times. North American Journal of Fisheries Management 23:625-631.

Johnston, N. T., E. A. Parkinson, and K. Tsumura. 1993. Longevity and growth of hormone-sterilized kokanee. North American Journal of Fisheries Management 13:284-290.

Kozfkay, J. 2002. Hatchery trout evaluations. Job Performance Report, Project F-73-R-25, Idaho Department of Fish and Game. Boise, Idaho.

Kozfkay, J. 2003. Hatchery trout evaluations. Job Performance Report, Project F-73-R-26, Idaho Department of Fish and Game. Boise, Idaho.

Kozfkay, J. 2004. Hatchery trout evaluations. Job Performance Report, Project F-73-R-27, Idaho Department of Fish and Game. Boise, Idaho.

Lamatsch, D. K., C. Steinlein, M. Schmid, and M. Schartl. 2000. Noninvasive determination of genome size and ploidy level in fishes by flow cytometry: detection of triploid *Poecilia formosa*. Cytometry 39:91-95.

Lincoln, R. F., and A. P. Scott. 1984. Sexual maturation in triploid rainbow trout *Salmo gairdneri Richardson*. Journal of Fish Biology 25:385-392.

McCarthy, I. D., C. G. Carter, D. F. Houlihan, R. Johnstone, and A. I. Mitchell. 1996. The performance of all-female diploid and triploid Atlantic salmon smolts on transfer together to seawater. Journal of Fish Biology 48:545-548.

Mohler, J. W. 1997. Immersion of larval Atlantic salmon in calcein solutions to induce a non-lethally detectable mark. North American Journal of Fisheries Management 17:751-756.

Mohler, J. W. 2003a. Producing fluorescent marks on Atlantic salmon fin rays and scales with calcein via osmotic induction. North American Journal of Fisheries Management 23:1108-1113.

Mohler, J. W. 2003b. Guidelines for use of calcein to produce non-lethally detectable marks in early-life-stage salmonids. U.S. Fish and Wildlife Service, Northeast Fishery Center, Lamar, Pennsylvania.

Ojolick, E. J., R. Cusack, T. J. Benfey, and S. R. Kerr. 1995. Survival and growth of all-female diploid and triploid rainbow trout reared at chronic high temperature. Aquaculture 131:177-187.

Oliva-Teles, A., and S. J. Kaushik. 1990. Growth and nutrient utilization by 0+ and 1+ triploid rainbow trout *Oncorhynchus mykiss*. Journal of Fish Biology 37:125-133.

Parkinson, E. A., and K. Tsumura. 1988. Growth and survival of hormone-sterilized coho *Oncorhynchus kisutch* and kokanee salmon *O. nerka* in a lacustrine environment. Canadian Journal of Fisheries and Aquatic Sciences 45:1490-1494.

Rahel, F, J. 2004. Unauthorized introductions: fisheries management of the people, for the people, or by the people? Propagated fish in resource management. American Fisheries Society, Symposium 44, American Fisheries Society, Bethesda, Maryland.

Rieman, B.E. and D. Myers. 1990. Kokanee population dynamics. Influence of density on potential yield: prediction of stocking rates. Idaho Department of Fish and Game, Job Performance Report, Project F-73-R-12, Boise.

Schindler, D. E., R. A. Knapp, and P. R. Leavitt. 2001. Alteration of nutrient cycles and algal production resulting from fish introductions into mountain lakes. Ecosystems 308-321.

Sheehan, R. J., S. P. Shasteen, A. V. Suresh, A. R. Kapuscinski, and J. E. Seeb. 1999. Better growth in all-female diploid and triploid rainbow trout. Transactions of the American Fisheries Society 128:491-498.

Simon, D. C., C. G. Scalet, and J. C. Dillon. 1993. Field performance of triploid and diploid rainbow trout in South Dakota ponds. North American Journal of Fisheries Management 13:134-140.

Solar, I. I., E. M. Donaldson, and G. A. Hunter. 1984. Induction of triploid rainbow trout by heat shock, and investigation of early growth. Aquaculture 57:57-64.

Teuscher, D., C. B. Alexander, J. C. Dillon, and D. J. Schill. 1998. Job performance report. Project F-73-R-21, Idaho Department of Fish and Game. Boise, Idaho.

Teuscher, D. M., D. J. Schill, D. J. Megargle, and J. C. Dillon. 2003. Relative survival and growth of triploid and diploid rainbow trout in two Idaho reservoirs. North American Journal of Fisheries Management 23:983-988.

Warrillow, J. A., D. C. Josephson, W. D. Youngs, and C. C. Krueger. 1997. Differences in sexual maturity and fall emigration between diploid and triploid brook trout *Salvelinus fontinalis* in an Adirondack lake. Canadian Journal of Fisheries and Aquatic Sciences 54:1808-1812.

Welch, T. J., M. J. Van den Avyle, R. K. Betsill, and E. M. Driebe. 1993. Precision and relative accuracy of striped bass age estimates from otoliths, scales, and anal fin rays and spines. North American Journal of Fisheries Management 13:616-620.

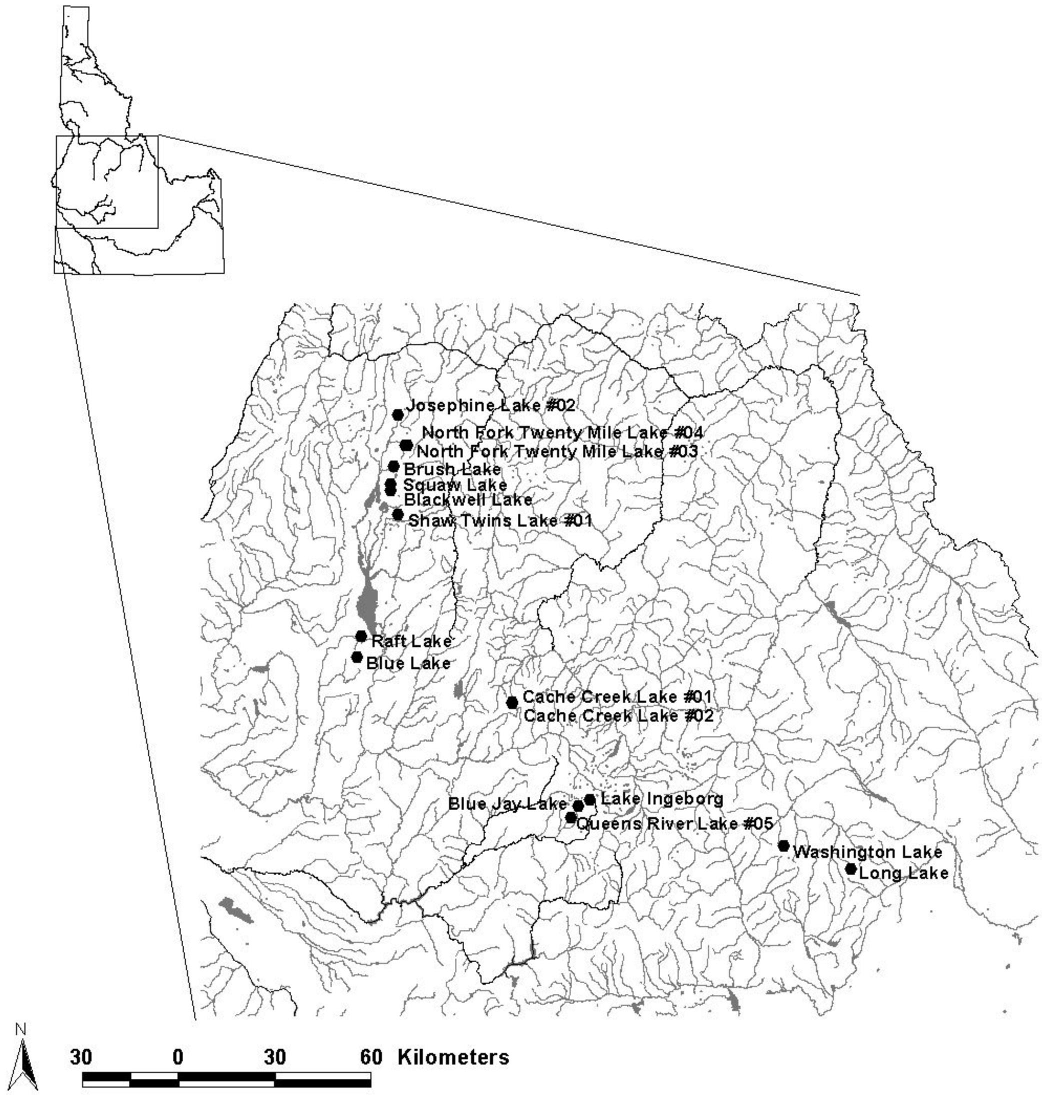


Figure 7. Locations of 16 high mountain lakes in central Idaho used to compare the relative performance of mixed-sex diploid and mixed-sex triploid rainbow trout. Lakes were stocked with 300 fish of each groups during 2001 and sampled during 2004 and 2005.

Table 4. Total catch of non-test fish, diploid, and triploid rainbow trout caught during surveys conducted on 16 high mountain lakes during 2004. Test fish were stocked as fry during summer 2001. The triploid group is abbreviated as 3N, whereas the diploid group is abbreviated as 2N.

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

Table 5. Catch of test fish from eight high mountain lakes that were stocked in 2001 and sampled during 2004 and 2005. The triploid group is abbreviated as 3N, whereas the diploid group is abbreviated as 2N.

	2004			**2005**		
Lake Name	**3N**	**2N**	**No mark w/ ad clip**	**3N**	**2N**	**No mark w/ ad clip**
Cache Creek #1	0	1	0	0	0	0
Josephine #2	0	0	0	0	3	1
Ingeborg	2	6	0	3	6	1
NF Twenty Mile #4	0	3	3	1	1	2
Queens River #5	0	1	0	0	0	0
Raft	19	24	6	1	5	1
Shaw Twins #1	0	2	1	0	1	0
Washington	0	2	0	2	3	3
Totals	**21**	**39**	**10**	**7**	**19**	**8**

Table 6. Catch per unit effort (CPUE) for non-test fish, as well as diploid and triploid rainbow trout surveys conducted during 2004 on 16 high mountain lakes. Test fish were stocked as fry during summer 2001.

	Angling CPUE				Gill Net CPUE			
Lake Name	**Total**	**Non-test fish**	**Hayspur Triploid**	**Hayspur Diploids**	**Total**	**Non-test fish**	**Hayspur Triploid**	**Hayspur Diploids**
Blackwell	0.67	0.67	0.00	0.00	0.86	0.72	0.00	0.02
Blue Jay	5.60	2.40	0.80	2.40	0.17	0.07	0.04	0.07
Blue	1.45	1.45	0.00	0.00	1.32	1.29	0.02	0.02
Brush	0.00	0.00	0.00	0.00	0.59	0.59	0.00	0.00
Cache Creek #1	0.00	0.00	0.00	0.00	1.37	1.35	0.00	0.02
Cache Creek #2	0.33	0.33	0.00	0.00	0.33	0.33	0.00	0.00
Josephine #2	0.00	0.00	0.00	0.00	0.88	0.88	0.00	0.00
Ingeborg	1.09	1.09	0.00	0.00	0.34	0.25	0.02	0.07
Long	0.77	0.46	0.00	0.31	1.06	0.95	0.04	0.07
NF 20 Mile #3	2.67	2.67	0.00	0.00	0.76	0.72	0.02	0.02
NF 20 Mile #4	0.59	0.47	0.00	0.12	0.93	0.91	0.00	0.02
Queens R. #5	7.14	7.14	0.00	0.00	0.80	0.79	0.00	0.01
Raft	0.00	0.00	0.00	0.00	0.31	0.04	0.12	0.15
Shaw Twins #1	0.67	0.67	0.00	0.00	0.38	0.34	0.00	0.03
Squaw	0.67	0.67	0.00	0.00	0.41	0.41	0.00	0.00
Washington	0.00	0.00	0.00	0.00	0.67	0.64	0.00	0.04

Table 7. Catch per unit effort (CPUE) for non-test fish, as well as diploid and triploid rainbow trout surveys conducted during 2005 on eight of the 16 high mountain lakes. Test fish were stocked as fry during summer 2001.

	Angling CPUE					Gill Net CPUE				
	Total	**Non-test fish**	**3N**	**2N**	**Unmarked test fish**	**Total**	**Non-test fish**	**3N**	**2N**	**Unmarked test fish**
Cache Creek #1	2.40	2.40	0.00	0.00	0.00	0.79	0.79	0.00	0.00	0.00
Josephine #2	0.50	0.50	0.00	0.00	0.00	1.43	1.30	0.00	0.10	0.03
Ingeborg	—	—	—	—	—	1.79	1.40	0.12	0.23	0.04
NF 20 Mile Long	—	—	—	—	—	1.38	1.29	0.02	0.02	0.04
Queens River #5	—	—	—	—	—	1.13	1.13	0.00	0.00	0.00
Raft	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.04	0.18	0.04
Shaw Twins #1	0.00	0.00	0.00	0.00	0.00	1.00	0.97	0.00	0.03	0.00
Washington	—	—	—	—	—	1.64	1.35	0.07	0.11	0.11

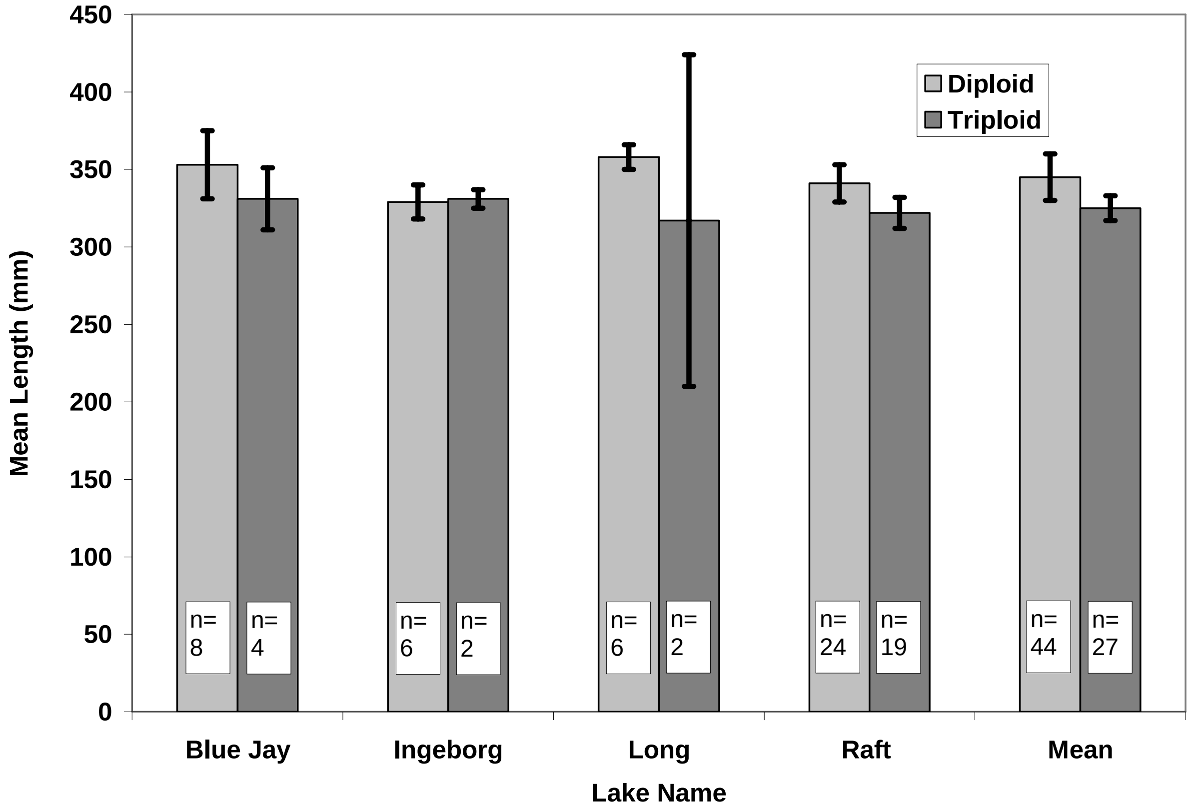


Figure 8. Mean length of triploid and diploid rainbow trout for lakes where two or more test fish were caught from each group. Lakes were stocked during 2001 and sampled during July 2004. Sample sizes are listed in Table 1. Errors bars indicate 90% confidence intervals.

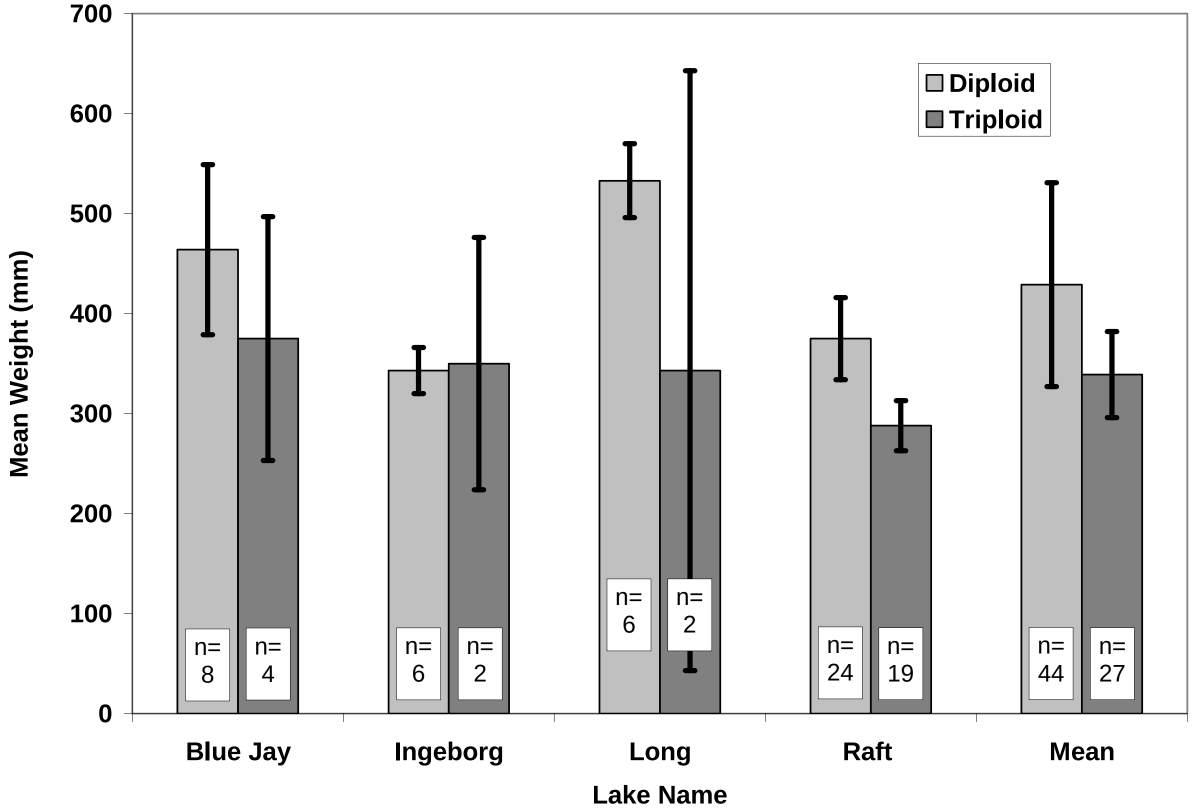


Figure 9. Mean weight of triploid and diploid rainbow trout for lakes where two or more test fish were caught from each group. Lakes were stocked during 2001 and sampled during July 2004. Sample sizes are listed in Table 1. Errors bars indicate 90% confidence intervals.

Table 8. Description of study waters in Idaho stocked with diploid and triploid rainbow trout fry in 2001 and sampled during 2004 and 2005.

Lake Name	Stocking Density	Avg Depth (m)	Avg Depth (m)	Max Depth (m)	Max Depth (m)	Size (ha)	Elevation (m)	Avg pH	Avg Conduct-ivity	Avg Temp	Secchi
Blackwell	113	23.9	7.3	35.4	10.8	5.3	2151	7.4	14.3	20.3	2.8
Blue Jay	261	14.1	4.3	17.1	5.2	2.3	2614	7.8	5	18.4	6
Blue	120	30.7	9.4	57.3	17.5	5	2250	7.5	9	14.2	4.2
Brush	80	14.1	4.3	28.4	8.7	7.5	2179	7.3	4	15	5.5
Cache Creek #1	429	14.9	4.5	27.2	8.3	1.4	2370	7.7	17	8.3	2.1
Cache Creek #2	333	5.1	1.6	23.4	7.1	1.8	2334	7.6	20	12	3.1
Josephine #2	120	24.7	7.5	56.7	17.3	5	2263	7.6	8	17.2	4.5
Ingeborg	63	28.7	8.7	46.7	14.2	9.5	2723	7.6	1.7	16.8	6
Long	107	5.1	1.6	9.2	2.8	5.6	2907	8.7	41.3	15.1	5.8
NF Twenty Mile #3	167	14.7	4.5	25.7	7.8	3.6	2403	7.3	18.3	12.3	3.3
NF Twenty Mile #4	92	20	6.1	25.6	7.8	6.5	2388	7.2	10.3	13.4	5
Queens River #5	194	21.6	6.6	47.4	14.4	3.1	2561	7.8	3	18.2	4.2
Raft	240	17.1	5.2	28.3	8.6	2.5	2043	6.9	8.3	14.8	2.7
Shaw Twins #1	214	26.2	8.0	33.9	10.3	2.8	2213	7	6.7	19.7	7
Squaw	286	14.8	4.5	21.7	6.6	2.1	2150	7.2	6	22.2	4.5
Washington	500	40.9	12.5	66.4	20.2	1.2	3157	7.9	14	10	3.9

Table 9. Parameter estimates, confidence intervals, and correlation coefficients for habitat measurements collected during 2004 high mountain lake sampling. All units are metric. Statistically significant correlations are bolded. Correlations were deemed significant at p = 0.10.

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

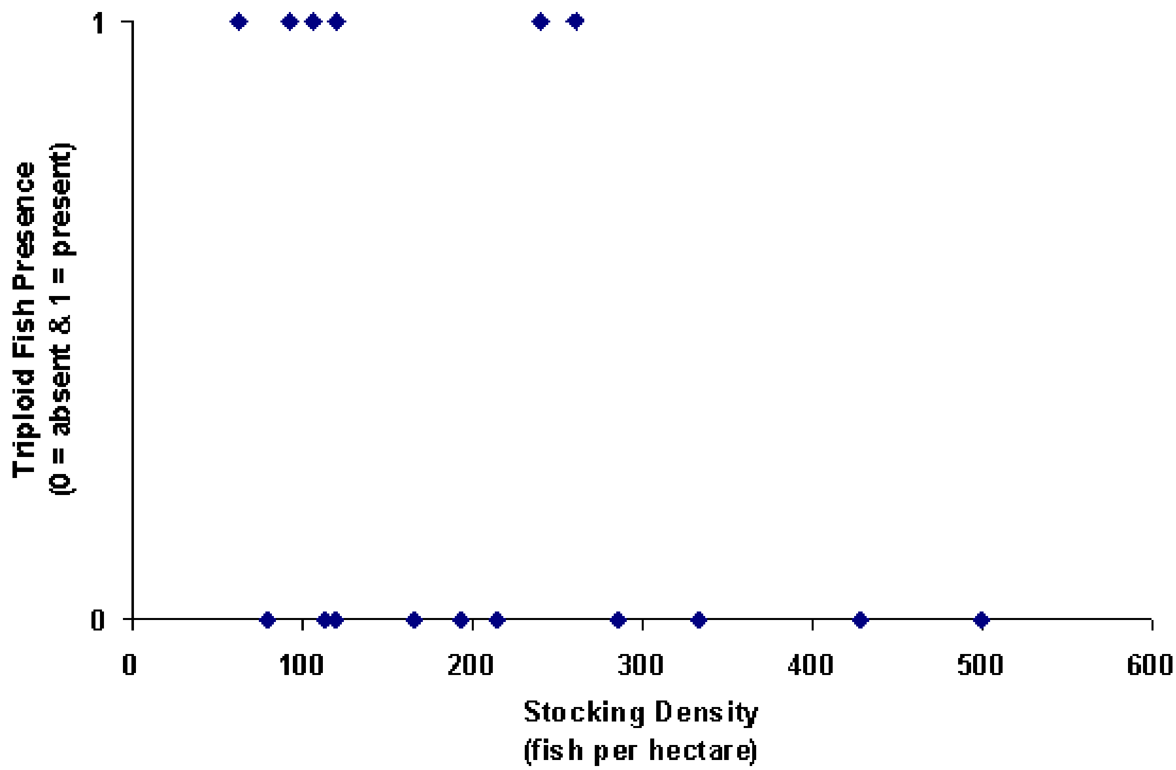


Figure 10. Relationship between stocking density and presence/absence of triploid Hayspur-strain rainbow trout in 16 high mountain lakes stocked during 2001 and sampled during 2004.

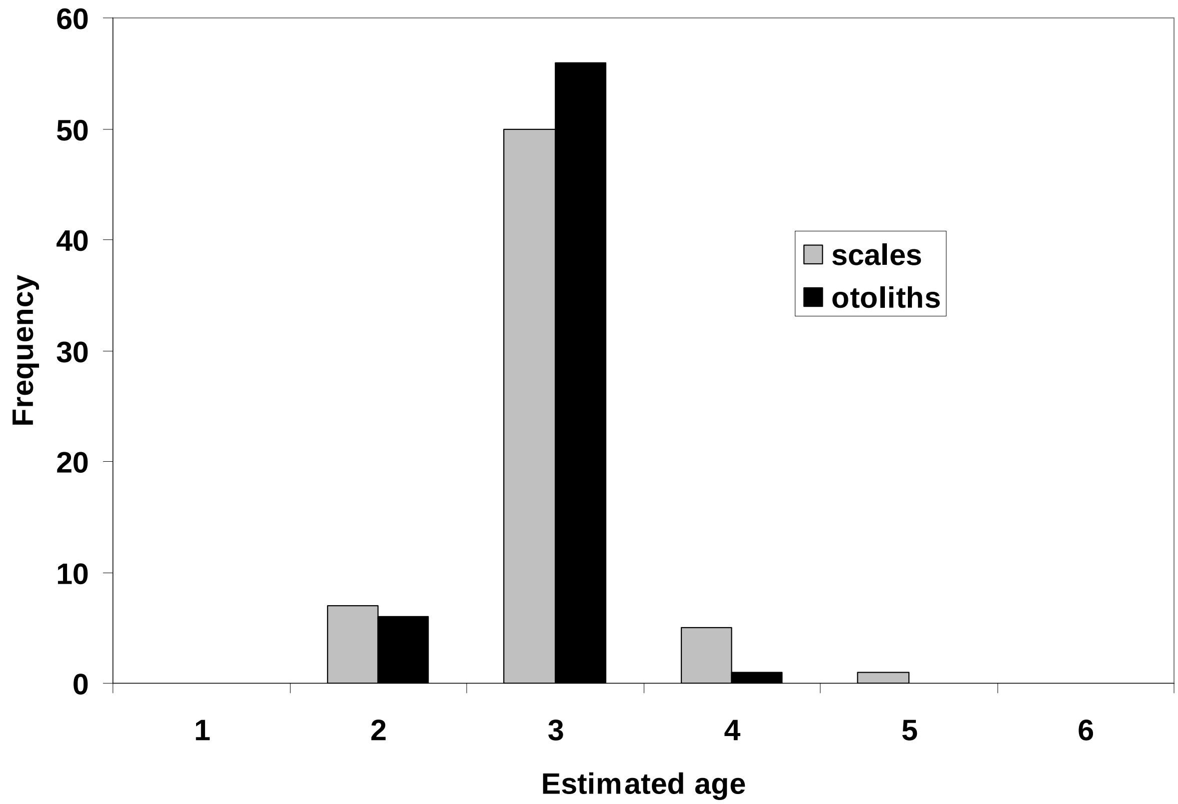


Figure 11. Frequency of age estimates from scales and otoliths for known age-3 fish sampled from high mountain lakes during 2004.

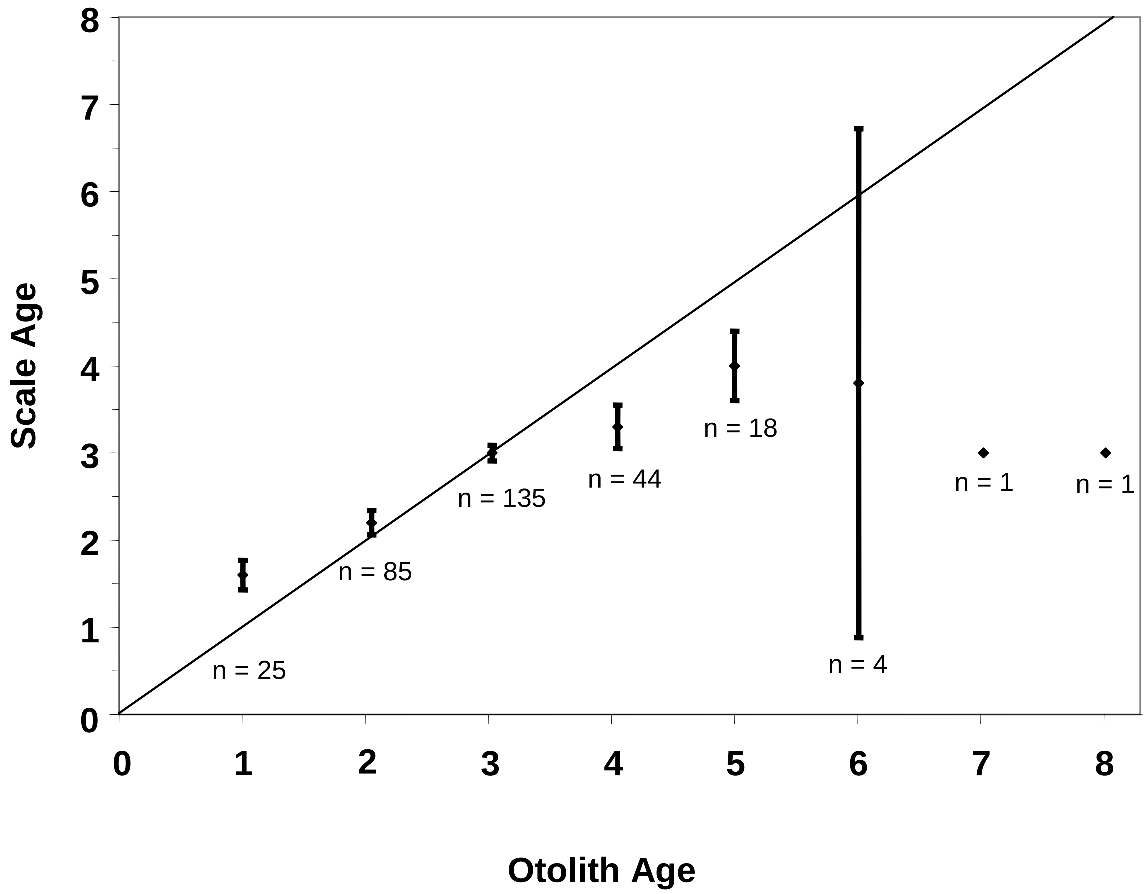


Figure 12. Comparison of consensus scale and otolith ages for known and unknown age trout sampled from high mountain lakes during 2004. Otoliths (x-axis) were used as a reference to compare corresponding consensus scale estimates across the range of ages sampled.

Table 10. Sample and species composition, stocking history, and importance of stocking to fish populations in 16 Idaho high mountain lakes stocked in 2001 and sampled in 2004. 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.

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

Table 11. Survival and triploid induction rates from an experiment designed to test the effectiveness of pressure treatments for inducing triploidy in kokanee salmon. Experiments were conducted on eggs and milt collected at the Deadwood weir and later fertilized at Eagle Fish Health Lab for the experiment.

Pressure	CMAF	MAF	Duration	Survival to eye-up (%)	Mean survival to eye-up (%)	Survival to FF (%)	Mean survival to FF (%)	Induction rates (%)	Mean induction rates (%)	Sample size for flow cytometry
				62		55		10		20
	Control			96	79	86	70	0	3	20
				80		69		0		20
				55		45		100		20
9,000	300	23:42	5	86	58	79	46	95	98	20
				33		13		100		14
				50		33		100		20
9,500	250	17:12	5	64	54	59	42	100	100	20
				48		34		100		20
				18		4		100		6
9,500	300	23:42	5	47	26	33	14	100	100	20
				13		4		100		4
				46		20		100		20
9,500	350	27:00	5	9	19	3	8	100	100	5
				3		0		—		—

Table 12. Stocking location, date, and the number of kokanee stocked during 2005 in five Idaho lakes and reservoirs to assess the relative performance of diploid and triploid kokanee. The triploid group is abbreviated as 3N, whereas the diploid group is abbreviated as 2N.

			# of kokanee stocked		
Water Body	**Date Stocked**	**Region**	**2N**	**3N**	**Non-test fish**
Devils Creek Reservoir	May 19, 2005	5	3,520	3,503	0
Lucky Peak Reservoir	June 3, 2005	3	49,950	41,400	108,800
Mirror Lake	May 31, 2005	1	2,516	2,520	0
Ririe Reservoir	April 28, 2005	6	34,960	35,100	140,975
Twin Lake (Lower)	May 16, 2005	1	20,000	20,000	20,000

ANNUAL PERFORMANCE REPORT
SUBPROJECT #3: PREDATOR TRAINING

State of: Idaho

Grant No.: F-73-R-28 Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #3: Predator Training

Contract Period: July 1, 2005 to June 30, 2006

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 yielded no recaptures of marked test fish. Therefore, we were unable to address whether more predator-trained fingerlings survived than control fingerlings. In order to fully answer this question, additional sampling in spring and fall 2006, and possibly 2007, is needed.

Authors:

Joseph R. Kozfkay
Senior Fisheries Research Biologist

Martin Koenig
Fisheries Research Biologist

INTRODUCTION

By eliminating piscine and avian predation along with other causes of natural mortality, production fish hatcheries are able to supply large numbers of salmonids to habitats that would support few or no fisheries. However, by removing early life-history survival constraints, the behavior of stocked trout is altered from that of their wild counterparts (Dickson and MacCrimmon 1982; Berejikian et al. 1996). Hatchery trout are often more aggressive (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). He observed that conditioned fish moved 0.5 m laterally when the model approached and speculated that this behavior had no survival benefit. Berejikian et al. (1999) were able to train Chinook 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. For instance, creel surveys conducted in Cascade Reservoir from November 1990 through 1992 indicated that less than 1% of fingerlings stocked returned to the creel (Dillon and Alexander 1995). Similarly, the return to creel rate of fingerlings in Magic Reservoir is often very low. From 1992-1995, return to creel rates ranged from 0.1% to 5.8% (Teuscher et al. 1998). Given such low return rates and the results of previous studies, increased post-release survival associated with predator training could dramatically increase the efficiency of the resident hatchery trout program in systems where predation limits survival.

RESEARCH GOAL

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

OBJECTIVES

1. To undertake a 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.

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 13). 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 13).

During spring 2005, approximately 12 months after stocking, experimental gill nets, trap nets, and electrofishing were used to assess the relative survival of the two test groups. Floating gill nets measured 46 m long by 2 m deep and were comprised of six panels of 19, 25, and 32 mm stretch mesh monofilament. Trap nets had a 15.2 m lead that was 0.9 m deep with two 0.9 x 1.8 m frames and four 0.9 m hops with a 10 cm diameter throat; all mesh was 25 mm bar knotless nylon. 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. Otoliths were stored in microcentrifuge tubes. Otoliths were viewed whole (Boxrucker 1986). Each structure was examined with a dissecting microscope on 2-4 power by two readers.

RESULTS

In Lucky Peak Reservoir during 2005, sampling consisted of 468 minutes of boat electrofishing effort. A total of 238 rainbow trout were sampled. Rainbow trout averaged 307 ± 6 mm and 309 ± 21 g. No test fish were identified by presence of grit dye. The vast majority of rainbow trout sampled, over 90%, appeared to originate from recent catchable plants. From 21 otolith samples (taken from noncatchables), 10 rainbow trout were estimated to be age-2, seven as age-3, three as age-4, and one as age-5.

In CJ Strike Reservoir, sampling consisted of 324 minutes of boat electrofishing effort. A total of 198 rainbow trout were sampled. Approximately half of these fish appeared to originate from catchable plants, whereas the other half appeared to be from fingerling plants. Rainbow trout averaged 355 ± 5 mm and 544 ± 23 g. No test fish were identified by the presence of grit dye. From 26 otolith samples (taken from noncatchables), one rainbow trout was estimated to be age-1, eight as age-2, 11 as age-3, six as age-4, and one as age-5.

In Lake Walcott, sampling consisted of 360 minutes of boat electrofishing effort, and three gill nets were fished for three nights for a total of 132 h of gill netting effort. Seventy rainbow trout were sampled. The majority of these fish appeared to originate from fingerling plants. Rainbow trout averaged 416 ± 14 mm and 1,031 ± 114 g. No test fish were identified by presence of grit dye. From 27 otolith samples (taken from noncatchables), seven rainbow trout were estimated to be age-2, 15 as age-3, four as age-4, and one as age-5.

In Hayden Lake, sampling consisted of boat electrofishing effort, and seven or eight gill nets were fished over four nights for a total of 511 h gill netting effort. Nine rainbow trout were sampled. The majority of these fish appeared to originate from fingerling plants. Rainbow trout averaged 324 ± 118 mm and 784 ± 826 g. No test fish were identified by presence of grit dye; however, four grit dyed fish were identified from a separate evaluation. These fish ranged in length from 190 to 260 mm. No otolith samples were collected from Hayden Lake rainbow trout.

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. Based on age estimates, test fish had not yet fully recruited to the sampling gears used in this study. It seemed that during spring 2005 surveys, spring-planted fingerlings had not yet fully recruited to the gears or behaved differently than larger, older rainbow trout that were caught readily. An additional 12-24 months of growth should allow better catches of test fish. Nearly all fish sampled were 1-3 yrs older than the age-1 test fish. In order to address this question, additional sampling in spring and fall 2006 and 2007 is needed, when test fish should be more catchable. Unfortunately, during fall 2004 one of the four study waters, Lucky Peak Reservoir, was drained to less than 1% of total storage volume. The effect that this drawdown had on test groups is unknown at this time. However, it is likely that test groups suffered higher-than-average mortality rates.

RECOMMENDATIONS

1. Complete additional surveys on Lake Walcott, Hayden Lake, and CJ Strike Reservoir during 2006 and 2007 to increase sampling effort in order to recapture test fish.

ACKNOWLEDGEMENTS

Rick Alsager, Brian Malaise, Bob Turik, and Ken Felty raised rainbow trout fingerlings and the predators for use in CJ Strike and Lucky Peak Reservoirs. Joe Chapman, Brian Thompson, and Tim Klucken reared the test fish and predators for Lake Walcott. Dwight Aplanalp, Jeff Seggerman, and Tom Kent reared the test fish for Hayden Lake. Without their cooperation, this project would not have been possible. We would also like to thank John Cassinelli, Lars Alsager, Craig Walker, Rick Bogar, Lance Hebdon, Andy Pristavok, and Rob Ryan for providing assistance during netting and electrofishing surveys. Paul Janssen provided assistance and equipment for grit marking.

LITERATURE CITED

Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry *Oncorhynchus mykiss* to avoid a benthic predator. Canadian Journal of Fisheries and Aquatic Sciences 52:2476-2482.

Berejikian, B. A., S. B. Mathews, and T. P. Quinn. 1996. Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout *Oncorhynchus mykiss* fry. Canadian Journal of Fisheries and Aquatic Sciences 53:2004-2014.

Berejikian, B. A., R. J. F. Smith, E. P. Tezak, S. L. Schroder, and C. M. Knudsen. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of Chinook salmon *Oncorhynchus tshawytscha* juveniles. Canadian Journal of Fisheries and Aquatic Sciences 56:830-838.

Boxrucker, J. 1986. A comparison of the otolith and scale methods for aging white crappies in Oklahoma. North American Journal of Fisheries Management 6:122-125.

Brown, G. E., D. P. Chivers, and R. J. F. Smith. 1997. Differential learning rates of chemical versus visual cues of a northern pike by fathead minnows in a natural habitat. Environmental Biology of Fishes 49:89-96.

Brown, G. E., and R. J. F. Smith. 1998. Acquired predator recognition in juvenile rainbow trout *Oncorhynchus mykiss*: conditioning hatchery-reared fish to recognize chemical cues of a predator. Canadian Journal of Fisheries and Aquatic Sciences 55:611-617.

Dickson, T. A., and H. R. MacCrimmon. 1982. Influence of hatchery experience on growth and behavior of juvenile Atlantic salmon *Salmo salar* within allopatric and sympatric stream populations. Canadian Journal of Fisheries and Aquatic Sciences 39:1453-1458.

Dillon, J. C., and C. B. Alexander. 1995. Put and grow hatchery trout evaluations. Annual Progress Report, Project F-73-R-17. Idaho Department of Fish and Game. Boise, Idaho.

Fenderson, O. C., W. H. Everhart, and K. M. Muth. 1968. Comparative agonistic and feeding behavior of hatchery-reared and wild salmon in aquaria. Journal of the Fisheries Research Board of Canada 25:1-14.

Fraser, J. M. 1974. An attempt to train hatchery reared-brook trout to avoid predation by the common loon. Transactions of the American Fisheries Society 4:815-818.

Fraser, J. M. 1981. Comparative survival and growth of planted wild, hybrid, and domestic strains of brook trout *Salvelinus fontinalis* in Ontario lakes. Canadian Journal of Fisheries and Aquatic Sciences 38:1672-1684.

Healey, M. C., and U. Reinhardt. 1995. Predator avoidance in naive and experienced juvenile Chinook and coho salmon. Canadian Journal of Fisheries and Aquatic Sciences 48:614-622.

Huntingford, F. A. 1993. Development of behaviour in fish. Pages 57-80 *in* T. J. Pitcher, editor. Behavior of teleost fish. Chapman & Hall, 2-6 Boundary Row, London, England.

Johnson, J. E., M. G. Pardew, and M. M. Lyttle. 1993. Predator recognition and avoidance by larval razorback sucker *Xyrauchen texanus* and northern hog sucker *Hypentelium nigricans*. Transactions of the American Fisheries Society 6:1139-1145.

Kozfkay, J. 2002. Hatchery trout evaluations. Job Performance Report, Project F-73-R-25, Idaho Department of Fish and Game. Boise, Idaho.

Mesa, M. G. 1991. Variation in feeding, aggression, and position choice between hatchery and wild cutthroat trout in an artificial stream. Transactions of the American Fisheries Society 6:723-727.

Miller, R. B. 1951. Survival of hatchery-reared cutthroat trout in an Alberta stream. Transactions of the American Fisheries Society 81:35-42.

Miller, R. B. 1953. Comparative survival of wild and hatchery-reared cutthroat in an Alberta stream. Transactions of the American Fisheries Society 83:120-130.

Olla, B. L., and M. W. Davis. 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon *Oncorhynchus kisutch* juveniles. Aquaculture 76:209-214.

Patten, B. J. 1977. Body size and learned avoidance as factors affecting predation on coho salmon *Oncorhynchus kisutch* fry by torrent sculpin *Cottus rhotheus*. Fishery Bulletin, U.S. 75:451-459.

Suboski, M. D., S. Bain, A. E. Carty, L. M. McQuoid, M. I. Seelen, and M. Seifert. 1990. Alarm reaction in acquisition and social transmission of simulated-predator recognition by zebra danio fish *Brachydanio rerio*. Journal of Comparative Psychology 104:101-112.

Teuscher, D., C. B. Alexander, J. C. Dillon, and D. J. Schill. 1998. Fingerling and catchable hatchery trout evaluations. Annual Progress Report, Project F-73-R-20. Idaho Department of Fish and Game. Boise, Idaho.

Thompson, R. B. 1966. Effects of predator avoidance conditioning on the post-release survival rate of artificially propagated salmon. PhD Thesis, University of Washington. Seattle, Washington.

Table 13. 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:

Joseph R. Kozfkay
Senior Fisheries Research Biologist

Martin Koenig
Fisheries Research Biologist

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

Steve Yundt, Chief
Bureau of Fisheries

Daniel J. Schill
Fisheries Research Manager