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Project 4: Hatchery Trout Evaluations

Subproject #1: Use of Tiger Muskellunge to Remove Brook Trout from High Mountain Lakes

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

Subproject #2: Sterile Trout Investigations: Production of Sterile Trout: Westslope Cutthroat

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

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ABSTRACT

Nonnative brook trout *Salvelinus fontinalis* populations in high mountain lakes threaten the persistence of native fish and often offer limited fishing opportunity because of stunted growth. Elimination of brook trout populations by stocking tiger muskellunge *Esox lucius x masquinongy* 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 2007, nine alpine lakes containing stunted brook trout populations were planted with tiger muskellunge (40 fish/ha) with an average length of 317 mm. Lakes were surveyed in summer 2008, 2009, and 2010 to compare changes in brook trout size and abundance relative to 2005 or 2006 data. Relative abundance of brook trout varied widely among the nine study lakes but declined substantially in most lakes, while average length and weight increased significantly following stocking with tiger muskellunge. Mean catch rates of brook trout declined from 22.8 per net night before planting tiger muskellunge, to 3.9 per net night in 2010. Prior to tiger muskellunge, mean brook trout length and weight was 212 ± 3 mm (n = 519) and 88 ± 5 g. After stocking, mean brook trout length increased to 264 ± 7 mm (n = 138) in 2009, and decreased slightly to 237 ± 7 mm (n = 84) in 2010. Catch rates of brook trout declined slightly in “control” lakes, but size distributions remained largely unchanged. The initial attempt to use habitat characteristics to classify lakes according to the likelihood of eradicating brook trout was generally not an accurate predictor of results, suggesting these characteristics were not the primary factors driving successful tiger muskellunge predation of brook trout. If only using tiger muskellunge, brook trout may overcome eradication efforts by recolonizing lakes from refuge habitats and by density-dependent recruitment success. I recommend combining tiger muskellunge introductions with other suppression methods in lake tributaries or outlets to increase the chances of eliminating brook trout.

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INTRODUCTION

During the early 20th 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 in western North American were fishless prior to human introduction of salmonids. Therefore, 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 downstream fish populations. High elevation streams contain some of the strongest remaining cutthroat *Oncorhynchus clarkii* and bull trout *Salvelinus confluentus* 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 habitats by their ability to disperse downstream through 80% slopes and over 18 m waterfalls. Brook trout have the ability to outcompete cutthroat trout (De Staso and Rahel 1994) and may eventually eliminate some cutthroat trout populations (Kruse et al. 2000). Additionally, brook trout may hybridize with or displace bull trout, thereby reducing or eliminating some 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, fisheries managers may be interested in shifting the size structure of brook trout populations in high mountain lakes to provide higher proportions of quality fish (i.e. those ≥254 mm). 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 have 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 during the ice-free period for a total effort of 108 net days (Knapp and Matthews 1998). This effort effectively removed the entire population (97 fish) 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 and subsequent monitoring. 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. Lake trout established self-sustaining populations in all five lakes, and by the early 1980s, no response in brook trout populations was noted in two lakes, while numbers of brook trout were decreased in two others and eliminated or nearly so from the other. Nelson (1988) also noted that brook trout lakes that contained brown trout had lower densities of brook trout, with 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 at stocking to exert significant predation pressure, and due to high densities of brook trout, were likely not 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 growth rates, ease of hatchery rearing, intermediate angling vulnerability (Weithman and Anderson 1977; Brecka et al. 1995), and because they are functionally sterile (Crossman and Buss 1965). Sterility allows biologists to stock tiger muskellunge with little to no threat of creating self-sustaining populations. Tiger muskellunge are highly effective predators on a variety of fish but prefer soft-rayed fusiform prey (Tomcko et al. 1984). When in high densities, muskellunge have been shown to limit densities of prey species such as white suckers *Catastomus commersonii* and black crappie *Pomoxis nigromaculatus* (Siler and Beyerle 1986), showing promise as a means to manage unwanted brook trout populations.

During 1998 and 1999, IDFG personnel began a management case study to determine if tiger muskellunge could eliminate brook trout from Ice Lake and Rainbow Lake, two high mountain lakes in the Clearwater region. Tiger muskellunge were stocked into Ice Lake at a density of 41 fish/ha (E. Schriever and P. Murphy, IDFG, personal communication). 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 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, brook trout catch decreased to 10 fish per net night. The authors speculated that brook trout would not likely be eliminated from Rainbow Lake with tiger muskellunge predation and backpack electrofishing, due to the size of the inlet and outlet. They anticipated instead that reduced densities would improve the size structure of the remaining brook trout, thereby improving fishery quality (E. Schriever and P. Murphy, IDFG, personal communication).

Tiger muskellunge have also been 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 recently only contained 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 brook trout population had decreased by an estimated 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 future 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, I describe initial efforts to investigate the effectiveness of introducing tiger muskellunge to reduce or eliminate brook trout populations in alpine lakes in Idaho. I compare changes in brook trout populations and relative density following tiger muskellunge introduction.

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 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 and associated inlet or outlet characteristics that influence success/failure of brook trout eradication efforts with 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. Lakes that were known to have brook trout populations and were thought to have limited inlet and outlet spawning habitats were preferentially selected for this study. Steep drainages were preferred, as they most likely possessed barriers that would prevent recolonization by any downstream brook trout populations. Nine lakes throughout central Idaho received tiger muskellunge for this evaluation (Figure 1). Additionally, brook trout densities were monitored at four additional “control” lakes that had established brook trout populations and were in close proximity to the treatment lakes, but did not receive tiger muskellunge.

Lake Sampling

Study lakes were sampled during 2005 or 2006 to determine relative density, age, and size structure of brook trout populations as well as habitat characteristics. All lakes were surveyed with floating gill nets and angling from August 4 to September 29, 2005, except for Grass Mountain #1, Grass Mountain #2, and Corral Lake, which were surveyed in July 2006. The experimental gill nets 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 gill nets were set in the early afternoon and pulled the following morning. While 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. Catch-per-unit-effort (CPUE) of brook trout was calculated by lake as the total brook trout caught per net-night. Angling CPUE was calculated as the total number fish caught per hour angling. Gill net CPUE was used as an estimate of relative abundance before and after stocking tiger muskellunge. See Kozfkay and Koenig (2006) for complete descriptions of age structure, size distributions, and mortality estimates.

Tiger muskellunge were reared at Hagerman State Fish Hatchery. Some authors have indicated that tiger muskellunge reared only on pellet diets are less effective predators on live fish and do not survive well after stocking (Gillen et al. 1981). Tiger muskellunge were therefore converted to live brook trout two weeks prior to stocking to make them more effective predators in the wild and increase their survival after stocking. Tiger muskellunge were stocked on June 12, 2007 into the study lakes. At the time of stocking, the mean length of the tiger muskellunge was 317 mm but ranged from 160 to 400 mm. Tiger muskellunge were planted by helicopter using an adjustable-volume fire bucket set at 946 liters (250 gallons). Tiger muskellunge were counted by hand before each flight, and densities in the fire bucket did not exceed two fish/gallon. Stocking density of tiger muskellunge was held constant across lakes at 40 fish/ha for 2,929 total fish planted (Table 1).

Study lakes were re-sampled first in 2008, approximately 13 months after tiger muskie were planted, and again in 2009 and 2010. Fish were sampled using two floating gill nets (set overnight) and processed according to the methods above. However, only two nets were fished at each lake in an effort to reduce bycatch of tiger muskellunge. Additional samples of brook trout and tiger muskellunge were collected with hook and line techniques using a variety of flies and lures.

Lake habitat and amphibian surveys were conducted at each of the nine lakes at the time fish were sampled in 2008 and 2009. A series of five transects were placed at equal distances perpendicular to the long axis of the lake with the aid of a laser rangefinder. Lake width was measured at each transect using a laser range finder. Depth was measured with a hand-held sonar unit at five equidistant points along each transect. Specific conductivity, pH, and surface temperature were measured at the middle of each transect using Hanna handheld conductivity and temperature/pH meters (Model #HI 98308, DiST 4 and #HI 98127). Lake location and elevation were recorded with the use of a handheld GPS unit. Lake area was calculated with geographic information systems (ArcGIS 9.1). In addition, amphibian surveys were conducted visually by slowly walking the entire perimeter of each lake along the water shore interface and looking near and under woody debris and recording the count, life stage, and species encountered. Basic stream habitat data were collected in inlet and outlets of study reservoirs in an effort to collect information that might help explain eradication success. Measurements were performed over the first 200 m of stream above (inlets) and below (outlets) each lake. Bankfull width was collected every 25 m and the area of suitable trout spawning habitat was estimated using a meter stick. Elevation was measured at the lake level and at the end of each stream reach with a handheld GPS unit. Stream gradient was calculated by dividing the difference in elevation from the start and end of the reach divided by the reach length.

Removal potential (the likelihood that brook trout would be successfully eradicated) at each lake was categorized with a qualitative value 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.

Management Actions

The Region 3 McCall fisheries staff determined conditions in Grass Mountain #1 and #2 in 2010 were satisfactory to justify additional management actions. Brook trout densities were significantly reduced and tiger muskies were in very low numbers in 2009, and none were found in 2010 (Table 4). However, brook trout were still present in the stream connecting the Grass Mountain lakes, and were unlikely to be impacted by tiger muskie. Brook trout were also present below the lakes in the outlet of Grass Mountain #2. Brook trout in these locations were likely to persist without further action and represented a source population that could recolonize each lake and reverse previous efforts to reduce brook trout densities. Nampa Research staff assisted Region 3 McCall with a chemical treatment using rotenone applied to the connecting stream of the Grass Mountain #1 and #2 lakes and the outlet of the lower lake. Details describing the treatment can be found in Appendices A and B.

Stream discharge was estimated on the day of treatment using a fluorescein dye (to estimate mean velocity) and mean width and depth measurements. The outlet of the upper lake (0.16 cfs) was treated with a drip can for 2 hours at 2.0 ppm (70 ml of rotenone). The outlet of the upper lake constituted approximately 300 m of stream before entering the lower lake. No deactivation station was used, as the lake would sufficiently dilute the chemical. A backpack sprayer was used to treat standing pools in the lower segment, just above the lower lake where stream velocities were too low for effective treatment with the drip station. The outlet of the lower lake (0.17 cfs) was treated at 1.5 ppm for 2 hours with a total of 60 ml rotenone. The target treatment reach was approximately 305 m of stream before reaching a natural fish barrier. Below the fish barrier, a potassium permanganate deactivation drip station was set up. The drip station administered a 4.5 ppm potassium permanganate solution for 3 hours.

RESULTS

Lake Sampling

Relative abundance of brook trout varied widely among the nine study lakes, but most lakes saw substantial declines in CPUE and increased average size of brook trout in the years following introduction of tiger muskellunge. In 2008, sampling of the nine high mountain lakes stocked with tiger muskellunge yielded 132 brook trout and 49 tiger muskellunge, which were the only two species collected. The majority of the brook trout sampled were caught at Merriam Lake (n = 70), while catch at all other locations ranged from one to 16 total brook trout. Mean CPUE ranged from one to 17.5 brook trout per net night, with an average of 5.1 per net night overall. Catch-per-unit-effort at several lakes (Black, Corral, and Grass Mountain #2) was equal or less than one trout per net night, indicating very low densities only one year after tiger muskellunge were introduced.

In 2009, sampling the nine treatment lakes yielded 138 brook trout and 30 tiger muskellunge. Merriam Lake again had the largest number of brook trout sampled (*n* = 63), while no brook trout were captured at Black, Corral, and Granite Twin lakes (Table 2). In general, CPUE of brook trout continued to decline in 2009 with some exceptions. Mean catch rates of brook trout were 4.3 per net night, with 301 hours of netting to capture 78 total brook trout. No brook trout were captured in three of the nine lakes, including Black, Corral, and Granite Twin lakes (Table 2). Granite Twin Lake showed a marked decline from 5.5 to zero brook trout from 2008 to 2009, suggesting continued predation by tiger muskellunge, despite no additional stocking. Even though the average gill net catch rate continued to decrease, CPUE actually increased in three other lakes since 2008 (Table 2).

In 2010, sampling yielded 84 brook trout and only 12 tiger muskellunge with a mean catch rate of 3.9 brook trout per net-night. CPUE remained consistent at five lakes, but increased at Granite Twin and Shirts lakes, probably from young brook trout present there in 2009, but too small to recruit to the gill nets until this year. CPUE continued to decline at Grass Mountain #1 and #2 lakes. As in years past, Merriam Lake produced the largest number of brook trout (*n* = 38), while no brook trout were collected at Black and Corral lakes. Overall, these data show much lower trout catch rates compared to pretreatment surveys, which averaged 22.8 brook trout per net-night (Table 2).

After tiger muskellunge were introduced, angling catch rates of brook trout in 2008 were low overall, but heavily reduced in the two lakes where angling data were comparable (Table 2). In 2008, 63.5 total hours of angling were expended which produced 41 brook trout. However, Merriam Lake accounted for 35 of these trout alone, and angling success was poor at most lakes. Without Merriam Lake, the mean angling CPUE for brook trout decreased from 0.55 trout/hour to 0.15 trout/hr. Before introducing tiger muskellunge, mean catch rates of brook trout were 1.5 trout/hr, based on Corral Lake and Shirts Lake. In 2009, mean angling catch rates increased slightly to 0.60 fish per hour, mainly as a result of increased catches from Grass Mountain #1 and Upper Hazard lakes. As with the gill net surveys, no brook trout were caught angling from three lakes, including Black, Corral, and Granite Twin lakes. Angling effort was lower in 2010, but catch rates remained comparable to previous years. As in 2009, no brook trout were caught by angling in Black, Corral, and Granite Twin lakes, and additionally none in Grass Mountain #1 or Upper Hazard lakes (Table 2).

The overall size distribution of brook trout noticeably shifted after tiger muskellunge were stocked (Figure 2). Despite variation in size across lakes, the mean size of brook trout increased slightly compared to 2005/2006 (pre-tiger muskellunge) within one year, based on 95% confidence intervals (Table 3). In 2008, the overall mean brook trout length and weight was 246 ± 6 mm (*n* = 132) and 161 ± 11 g, compared to 212 ± 3 mm (*n* = 519) and 88 ± 5 g before tiger muskellunge were introduced (Table 3). In 2009, mean brook trout size again increased in four out of five lakes where they were caught (no brook trout were caught in three of the study lakes). Brook trout averaged 264 ± 7 mm (*n* = 138) in length, with a mean weight of 181 ± 15 g (*n* = 138).Only Upper Hazard showed a small decline in mean length, although not significant based on 95% confidence intervals (Table 3). Average size decreased slightly in 2010, most likely from small fish caught at Granite Twin Lakes and Shirts Lake. Brook trout averaged 237 ± 7 mm (*n* = 84) in length, with a mean weight of 187 ± 16 g (*n* = 69).

While small sample sizes at some lakes likely precluded meaningful comparisons of mean length (Black, Corral, Grass Mountain #2, and Shirts), the distribution of brook trout sizes indicates an increase in the proportion of larger sized fish, increasing average size and trophy potential at most lakes (Table 2, Figure 3). In the pretreatment surveys, about 40% of the brook trout sampled were ≤200 mm, compared to only 5% in 2008 and 2009. The percent of brook trout ≤200 mm increased to 24% in 2010, mainly from fish found in Shirts, Granite Twin and Upper Hazard lakes. Prior to tiger muskellunge, 18% of brook trout were ≥254 mm, and of those, only 4% were ≥279 mm. Sampling in 2008, 2009, and 2010 showed that on average, 40%, 64%, and 48% of brook trout equaled or exceeded 254 mm, respectively. In 2008, 2009, and 2010, the average percent of brook trout equal or greater than 279 mm was 17%, 38%, and 26%, respectively. These data indicate a marked increase in the proportion of larger brook trout in the sample, corresponding with lower overall abundance.

Tiger muskellunge were more common soon after stocking (as expected) and have become increasingly rare in subsequent samples in all lakes. In 2008, tiger muskellunge were documented in all lakes except Shirts Lake, while in 2010 they were only confirmed in four lakes. Currently, Granite Twin Lakes appears to have the largest number of tiger muskellunge encountered (Table 4), but numbers are too low to compare. The mean length of tiger muskellunge continues to increase from 460 mm in 2008 to 672 mm in 2010 (Table 4).

In contrast to lakes that received plants of tiger muskellunge, the four “control” lakes that were not planted saw little change in brook trout populations. Mean length and weight of brook trout was not markedly different between years (Table 5), and catch rates (both angling and gill nets) of brook trout decreased only slightly from 2009 (Table 6).

Potential study lakes included a wide variety of physical habitat characteristics and inlet and outlet morphologies (Table 1). However, I did not see any consistent patterns of success in relation to perceived potential for elimination at the time the study was initiated. Spruce Gulch and Corral lakes were considered to have “very high” probability for elimination. These lakes had little or no inlet/outlet spawning habitat, limited tributary habitat with barriers nearby, and low complexity lake habitat. At this point in the study, brook trout were heavily reduced in Corral Lake, but are still readily present in Spruce Gulch Lake. The potential for elimination at Granite Twin, Merriam, and Shirts lakes was thought to be “high.” These lakes had only limited spawning habitat in inlet tributaries and possessed migration barriers in outlet tributaries. While brook trout were reduced in Granite Twin and Shirts lakes, CPUE remains consistent in Merriam Lake. The brook trout population in Black Lake, Upper Hazard, Grass Mountain #1, and Grass Mountain #2 lakes were considered to have “moderate” elimination potential because of easily accessible spawning habitat and tributary refuge habitat where brook trout might escape predation from tiger muskellunge. Brook trout were heavily reduced in Black Lake, yet were still present in Upper Hazard, Grass Mountain #1, and Grass Mountain #2 lakes.

Management Actions

No live fish were seen in the outlet of the upper Grass Mountain Lake after the chemical treatment was completed. One live brook trout was found in the lower portion of the treatment reach for the lower lake outlet. There was a small spring seep, which was missed during the primary treatment. This was subsequently treated with an additional 5 ml of rotenone. No dead fish were found below the deactivation station at the end of the treatment. Brook trout were the only species observed during the treatment.

Following treatment, both lakes were stocked by aircraft with trout fry in fall 2010 from the McCall Hatchery. The lower Grass Mountain Lake received 1,500 triploid rainbow trout fry and 700 westslope cutthroat trout fry. The upper Grass Mountain Lake was stocked with 2,200 westslope cutthroat fry.

DISCUSSION

Prior to introducing tiger muskellunge, most lakes in this study generally contained small brook trout. On average, only a small proportion (18%) of the trout sampled were over 254 mm (ten inches). Brook trout populations were characterized by an abundance of younger year classes and slow growth rates, especially after age-4 (Kozfkay and Koenig 2006). Thus, these populations were likely of limited interest to anglers and presented an opportunity for improvement. 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.

Most lakes planted with tiger muskellunge showed substantial declines in CPUE and increased average size of brook trout in the years following stocking with tiger muskellunge. Conversely, "control" lakes that did not receive tiger muskellunge showed little or no change in relative abundance or average size of brook trout. Even though sampling effort was lower in post-treatment surveys compared to pretreatment surveys (in an effort to avoid sacrificing tiger muskellunge), results suggest that brook trout populations were severely reduced following tiger muskellunge introductions in 2007. Tiger muskellunge were highly effective predators on brook trout in most study locations. The effectiveness of the tiger muskellunge was probably improved by their large average size at the time of stocking (>300 mm) and previous experience with live brook trout (in the hatchery). In general, esocids survive better and have higher foraging success when reared on a diet of live fish, and are stocked at larger sizes (>250 mm) in the spring, with high densities of suitable prey (Storck and Newman 1992; Szendrey and Wahl 1996; Larscheid et al. 1999; Wahl 1999). This corresponds well with the design of this study and the conditions found in these lakes.

While brook trout catch rates declined, average length generally increased following tiger muskellunge stocking. Mean brook trout lengths likely increased because of lower density (i.e. reduced competition), or because the largest individuals escaped predation through avoidance or exceeding the gape limitation of the tiger muskellunge. Anderson (1973) suggested that large piscivores could improve the size structure of prey populations by reducing prey densities and triggering compensatory increases in growth. Similarly, Donald and Alger (1989) reported increases in mean weight for all age classes of brook trout in a subalpine lake when subjected to only 20% exploitation. Reductions in brook trout were undoubtedly facilitated by the stocking density of 40 tiger muskellunge per hectare, well beyond the 25 fish/ha considered “high” by Storck and Newman (1992).

Despite the increase in mean brook trout length in most lakes, mean length actually declined in 2010 in Granite Twin, Shirts, and Upper Hazard lakes. Planting tiger muskellunge in Upper Hazard Lake did not seem to have a significant impact, and tiger musky appeared to be in very low densities since 2008, suggesting little impact to brook trout. The decrease in mean size in Granite Twin and Shirts lakes is likely the result of a young year class of brook trout that were present in 2009, but too small to recruit to the gill nets or angling. No brook trout were sampled in Granite Twin Lake in 2009, and only two were caught in Shirts Lake in 2009, despite fry being present in both lakes at that time. These lakes appear to have a new year class of brook trout entering the population. As tiger muskie densities continue to decline, these young brook trout might experience less predation pressure. It is unknown whether the density of tiger muskellunge in these lakes is currently high enough to limit the apparent rebound in the brook trout population. Further sampling in years to come will be needed to monitor whether brook trout can reestablish themselves or if the existing tiger muskie will limit their success.

The initial attempt to classify lakes by the likelihood of eradicating brook trout was generally not an accurate predictor of results. This suggests I have an incomplete understanding of the primary factors driving successful brook trout eradication by tiger muskellunge, at least two years after stocking. For example, Merriam and Shirts lakes were thought to have “high” probability of eradication, when in fact these lakes showed the least success of all. Black Lake was considered to have only “moderate” probability of success, but results suggest much greater impact to brook trout than first anticipated. Only at Corral Lake did results mirror those anticipated by classifying probability of eradication as “very high.”

Eradication appeared to be limited in Merriam and Spruce Gulch lakes. Unlike any other lakes in this study, Merriam Lake actually saw a marked increase in CPUE for both gill nets in 2008, followed by similar catch rates in 2009 and 2010. Merriam Lake sits at the highest elevation of the study lakes and had the lowest average temperature. Conditions here may have been unfavorable for tiger muskellunge, and predation pressure on brook trout may have been light. Regardless of poor tiger muskellunge survival in Merriam Lake, mean brook trout length still increased and smaller size classes were heavily reduced (Figure 2). Removal of brook trout in Merriam Lake is unlikely without future action. Brook trout catch rates in Spruce Gulch declined by about half of pre-tiger muskellunge rates in 2008, but remained similar in 2009 and 2010. Tiger muskie in Spruce Gulch were the smallest captured in 2008, and no tiger muskellunge were caught in Merriam Lake (and only one small specimen was observed during surveys, with none in 2009). Despite the smaller average size of tiger muskellunge in Spruce Gulch, 12 were captured in 2008 and five in 2009, and two in 2010, suggesting moderate survival compared to other lakes. Given the large average size and consistent catch rates of brook trout and low numbers of tiger muskie in the last two years, it is unlikely that brook trout will be eliminated from Spruce Gulch without additional management actions.

Brook trout declined in some lakes more quickly than in others, and declines continued from 2009 to 2010 in some lakes. In 2008, Corral Lake showed the largest reduction in CPUE of BKT of all the lakes planted with tiger muskellunge, and no brook trout were captured in 2009 or 2010. The lake habitat appears ideal for lie-in-wait predators like tiger muskellunge. Corral Lake is shallow, with abundant submerged woody debris and emergent aquatic vegetation. It is a small lake with the lowest elevation of those planted and summer water temperatures that may be suited for tiger muskellunge growth. Faster growing tiger muskellunge would be able to eat progressively larger prey, thereby reducing the fraction of the brook trout population that would otherwise exceed the gape limitation. By 2009, no brook trout were captured in three lakes: Black Lake, Corral Lake, and Granite Twin lakes. Although no brook trout were sampled, young brook trout were seen in Corral and Granite Twin lakes, and were likely still present in the outlet of Black Lake. Brook trout were captured in Granite Twin Lakes in 2010, but none were found Black and Corral Lakes, suggesting very low densities of brook trout (if still present).

Upper Hazard Lake showed only a moderate reduction in brook trout catch rates and a modest increase in mean brook trout length in both survey years. Even with abundant complex shoreline habitat (in terms of boulders and woody debris), tiger muskellunge only had a minor impact to brook trout. This is one of the larger lakes in the study (15.8 ha), with an average depth over 7 m and a maximum recorded depth of 21.4 m, suggesting a large amount of pelagic habitat. Tipping (2001) found tiger muskellunge preferred shallow water macrophytes (3-5 m deep) in summer and fall. He speculated that this habitat preference likely reduced their opportunity to prey on salmonids, which are generally pelagic. This tendency for salmonids to occupy pelagic zones while tiger muskie remain mainly littoral might help explain the lower success of eradication efforts larger lakes like Upper Hazard, despite abundant littoral cover for concealment. This pattern might also apply to Merriam Lake, which despite its smaller surface area, has higher average and maximum depths, corresponding to its steep shorelines and limited littoral habitat.

Despite heavy predation by tiger muskellunge, complete eradication might have only been achieved in Corral Lake at this time, as at least some brook trout were found in all other lakes. Although no brook trout were sampled in 2009 and 2010, brook trout may still be present at very low levels in Corral Lake. No brook trout were sampled at Black Lake in 2009 or 2010, but one was seen in the outlet in 2010, suggesting a very low-density population with tiger muskellunge remaining. At this point in the study, these results are similar to those reported from previous IDFG studies to manage brook trout in Lower Rainbow Lake and Ice Lake in the Clearwater River drainage (Schriever and Murphy, *In Press*). These lakes were stocked with tiger muskellunge in 1999 and affected brook trout with mixed results. Lower Rainbow Lake (4.5 ha) was initially stocked with a low density of tiger muskellunge, then restocked again with a higher density (40.7 fish/ha) a year later. Brook trout densities decreased while mean length increased following treatment, but eradication was never achieved. Failure to remove brook trout was likely a result of lower tiger muskellunge stocking density, abundant complex lake habitat, and extensive inlet and outlet habitat that reduced the effectiveness of tiger muskellunge to consume brook trout. Brook trout were successfully removed from Ice Lake, which is a very small lake (0.54 ha) and was stocked with a high density of tiger muskellunge (40.7 fish/ha surface area). Stocked in 1999, tiger muskie were observed until 2001, but no brook trout were observed in 2002. Small lake size, minimal inlet/outlet habitat, and low complexity lake habitat likely helped to remove brook trout. Within both mountain lakes, tiger muskellunge introductions were coupled with electrofishing removal of brook trout from lake inlets and outlets. Together, these significantly changed the composition of the brook trout population and decreased overall brook trout abundance in these two lakes (Schriever and Murphy, *In Press*).

More recently, Rhodes et al. (2007) reported similar findings in four additional lakes in the Clearwater drainage treated with tiger muskellunge in 2006. Fly, Heather, Platinum and Running lakes range in size from 1.0 ha to 8.4 ha and were stocked with tiger muskellunge at similar densities (40 fish/ha). Their results indicated similar shifts in mean brook trout size, with an overall average increase of 76 mm in length, while catch-per-unit-effort also declined in three of the four lakes simultaneously. Survey results from 2008 showed mean brook trout length again increased, but only in two of the four lakes (Fly and Running lakes), while it decreased in Platinum Lake and remained unchanged in Heather Lake (Rhodes and Dupont 2009). CPUE also decreased in all lakes except Platinum, suggesting that tiger muskellunge continued to reduce brook trout numbers two years after planting. As with previous studies, full eradication was not achieved using tiger muskellunge alone. The current study differs in that no electrofishing removal was conducted to improve eradication efforts at any lakes. However, a chemical treatment was applied to the inlet and outlet complex of Grass Mountain #1 and #2 lakes to aid in reducing brook trout recruitment from nearby refugia. Another important difference with the current study is that tiger muskellunge were only stocked on one occasion across a larger number of lakes. The lakes used in this study were of larger sizes with deeper mean depths, on average, with several lakes over 10 ha in surface area.

At this point in the study, one can only discuss the short-term success of tiger muskellunge to reduce brook trout in mountain lakes. Short-term success is likely dependent on lake morphology and size (shallow, small lakes), while long-term success may likely be a function of brook trout recruitment through reproduction or immigration from inlet/outlet refugia and spawning. The population dynamics and species interactions between tiger muskellunge and brook trout in alpine lakes are poorly understood. Long-term success of eradicating brook trout may hinge on whether tiger muskellunge can live long enough to continue limiting brook trout. If tiger muskellunge exhaust their food resources quickly, they may starve and die off before completely removing brook trout. As with Schriever and Murphy (*In Press*), I also noted that brook trout were present in inlet and outlet streams, away from typical tiger muskellunge habitat. Without further effort to remove brook trout that persist in refuge habitats such as inlet or outlet streams, lakes could be recolonized shortly after tiger muskie have disappeared (Schriever and Murphy *In Press*). Completely eradicating brook trout using tiger muskellunge may require several stockings to maintain adequate predatory pressure to collapse a brook trout population. Additionally, brook trout that escape predation may represent the largest individuals, and would therefore have the highest fecundity to repopulate a mountain lake. In the absence of predatory tiger muskellunge, brook trout may rebound quickly, so multiple suppression methods should be combined for the best chance of success. Evidence currently exists to support this, based on the fact that new year classes of brook trout were captured at Granite Twin and Shirts lakes, from fry that were present in the previous year, despite no adults having been captured.

Introducing tiger muskellunge appears to be an effective means to reduce brook trout densities in alpine lakes, as it requires only a minimal labor investment. After an initial stocking effort, only cursory sampling efforts are needed to document population responses in small, shallow lakes. Tiger muskellunge may live for several years, thereby removing brook trout for extended periods. In larger, more complex lakes, 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.

This study is currently at an important stage, where decisions about using other treatment options should be made. If left untreated, brook trout populations in these lakes are likely to rebound quickly from the few large remaining adults or young brook trout already spawned. In cooperation with Region 3M staff, the inlet/outlets of Grass Mountain #1 and #2 were chemically treated in 2010 and lakes restocked with cutthroat and rainbow trout in an effort to reduce recruitment in the brook trout population and to shift the fishery towards different species less prone to stunting. Current conditions in some lakes (such as Corral Lake) suggest other treatments could be highly successful for eradicating brook trout completely, while conditions in other lakes suggest further efforts are likely futile or too difficult (such as Merriam

Lake). Table 7 lists some of the key attributes of each lake that may help guide future treatment options, and whether further treatments should be considered. Smaller lakes with simple inlet and outlet habitat and few numbers of juvenile fish should be a priority, since they offer great benefit at little effort. Larger lakes with little change in brook trout populations and complex habitats would not be worth the effort necessary for successful eradication.

RECOMMENDATIONS

1. Sample all study lakes in 2011 to evaluate changes in brook trout populations and longevity of tiger muskellunge. Sampling in 2011 will also indicate whether rainbow and cutthroat fry planted in 2010 are likely to recruit to the fishery in the coming years.

2. Consider additional management actions (see Table 7) in fall 2011 at Corral Lake and Shirts Lake to eradicate sources of brook trout in inlets and outlets. Treatments that could be applied to lakes, inlets, and outlets might include rotenone, electrofishing, or high-intensity gill netting to target any remaining fish.

    a. Corral Lake and Shirts Lake should be prioritized for additional treatments, especially inlet and outlets applications.

    b. Merriam, Spruce Gulch, and Upper Hazard lakes should not be given additional treatments because of the remaining adult brook trout population.

3. Corral Lake, or Black Lake, Shirts Lake could be restocked in fall 2011 pending sampling in summer 2011 with rainbow or cutthroat trout.

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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. 1973. Application of theory and research to management of warmwater fish populations. Transactions of the American Fisheries Society 102:164-171.

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

Brecka, B. J., M. L. Hooe, and D. H. Wahl. 1995. Comparison of growth, survival, and body composition of muskellunge and tiger muskellunge fed four commercial diets. The Progressive Fish Culturist 57:37-40.

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.

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.

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.

Gillen, A. L., R. A. Stein, and R. F. Carline. 1981. Predation by pellet-reared tiger muskellunge on minnows and bluegills in experimental systems. Transactions of the American Fisheries Society 110:197-209.

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.

Kozfkay, J., and M. K. Koenig. 2006. Hatchery trout evaluations. Job Performance Report, Project F-73-R-28, Idaho Department of Fish and Game. Boise.

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.

Larscheid, J., J. C. Christianson, T. Gengerke, and W. Jorgensen. 1999. Survival, Growth and abundance of pellet-reared and minnow-reared muskellunge stocked in northwestern Iowa. North American Journal of Fisheries Management. 19:230-237.

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.

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.

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.

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

Rhodes, T., J. Erhardt, and J. Dupont. 2007. Mountain lakes monitoring and management in north central Idaho Mountains. 2007 Annual Report. Idaho Department of Fish and Game, Boise.

Rhodes, T., and J. Dupont. 2009. Mountain lakes monitoring and management in north central Idaho Mountains. 2008 Annual Report. Idaho Department of Fish and Game, Boise.

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.

Siler, D. H., and G. B. Beyerle. 1986. Introduction and management of northern muskellunge in Iron Lake, Michigan. *In* Managing muskies. American Fisheries Society, Special Publication 15, Bethesda, Maryland.

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.

Szendrey, T. A., and D. H. Wahl. 1996. Size-specific survival and growth of stocked muskellunge: effects of predation and prey availability. North American Journal of Fisheries Management. 16:395-402.

Tipping, J. M. 2001. Movement of tiger muskellunge in Mayfield Reservoir, Washington. North American Journal of Fisheries Management 21:683-687.

Tomcko, C. M., R. A. Stein, and R. F. Carline. 1984. Predation by tiger muskellunge on bluegill: Effects of predator experience, vegetation, and prey density. Transactions of the American Fisheries Society 113:588-594.

Wahl, D. H. 1999. An ecological context for evaluating the factors influencing muskellunge stocking success. North American Journal of Fisheries Management. 19:238-248.

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.

Weithman A. S. and R. O. Anderson. 1977. Survival, growth, and prey of esocidae in experimental systems. Transactions of the American Fisheries Society 106:424-430.

Table 1. Physical description of study waters planted with tiger muskellunge in 2007.

Lake name	Number planted	Area (ha)	Elevation (m)	Mean depth (m)	Max depth (m)	Mean spec. cond. (µS/cm)	Mean temp (C)	Mean pH
Black Lake	420	10.5	2199	19.7	37.8	31	16.1	7.72
Corral Lake	104	2.6	2085	4	24	-	15.9	7
Granite Twin	656	16.1	2183	7.4	20.4	6.4	13.2	7.72
Grass Mtn 1	206	5.1	2263	2.9	6.1	4.8	15.1	6.78
Grass Mtn 2	225	5.1	2238	2.6	3.7	5	14	6.52
Merriam Lake	107	2.6	2926	7.5	34	20.8	10	8.52
Shirts Lake	140	3.5	2254	7.7	3	15.6	13.1	8.3
Spruce Gulch	439	10.9	2698	4.9	13	8.8	15.9	6.84
Upper Hazard	632	15.8	2264	7.6	21.4	3	13.8	8.24

Lake name	IDFG catalog	Region	Distance from road (km)	Part of chain?	Inlet spawning habitat?	Outlet spawning habitat?	Outlet barrier w/in 1 km?	Elimination potential	Apparent success
Black Lake	07-00-00-0143	3M	0	No	Yes	Yes	Yes	Moderate	Yes
Corral Lake	07-00-00-0177	3M	0.9	No	No	No	Yes	Very High	Yes
Granite Twin	07-00-00-0193	3M	1.9	No	No	Yes	Yes	High	Yes
Grass Mtn 1	07-00-00-0180	3M	3	Yes	No	No	No	Moderate	No
Grass Mtn 2	07-00-00-0183	3M	3	Yes	Yes	No	No	Moderate	No
Merriam Lake	07-00-00-1308	7	3.1	No	Yes	Yes	Yes	High	No
Shirts Lake	09-00-00-0271	3M	1.9	No	No	Yes	Yes	High	No
Spruce Gulch	07-00-00-1316	7	10.6	No	No	No	Yes	Very High	No
Upper Hazard	07-00-00-0170	3M	3.1	Yes	Yes	Yes	Yes	Moderate	No

Table 2. Mean catch-per-unit-effort by gill nets (fish per net-night) and angling (fish per hour) sampled from nine mountain lakes before and after tiger muskellunge were introduced in 2007. The total trout caught by each method (n) is shown by lake. Dashed lines indicate where angling was not conducted.

	2005-06			2008			2009			2010		
Lake Name	**CPUE**	**Hours**	**n**	**CPUE**	**Hours**	**n**	**CPUE**	**Hours**	**n**	**CPUE**	**Hours**	**n**
Gill Nets												
Black Lake	13.5	55.5	54	1.0	23.5	2	0	58	0	0.0	29.3	0
Corral Lake	60.0	10.0	60	1.0	31.4	2	0	29	0	0.0	38.7	0
Granite Twin	20.0	55.5	80	5.5	31.9	11	0	27	0	2.5	34.4	5
Grass Mtn 1	28.0	16.5	28	6.0	32.8	12	6.5	33	13	3.0	35.1	6
Grass Mtn 2	35.0	17.0	35	0.5	30.9	1	4.5	40	9	1.0	35.0	2
Merriam Lake	9.3	73.3	37	17.5	24.2	35	16.0	31	32	14.5	29.3	29
Shirts Lake	14.3	48.0	57	2.0	25.8	4	0.5	23	1	3.5	25.4	7
Spruce Gulch	15.8	65.5	63	8.0	22.4	16	6.5	26	13	6.0	24.8	12
Upper Hazard	9.0	81.3	36	4.0	28.3	8	5.0	35	10	4.5	37.5	9
Total	22.8	422.5	450	5.1	251.0	91	4.3	301	78	3.9	290.5	70
Angling												
Black Lake	-	0	0	0.05	22	1	0	17.5	0	0.0	1.8	0
Corral Lake	2.0	2	4	0	5	0	0	4.4	0	0.0	2.0	0
Granite Twin	-	0	0	0.80	5	4	0	8.5	0	0.0	11.0	0
Grass Mtn 1	-	0	0	0	2	0	1.1	7	8	-	0.0	-
Grass Mtn 2	-	0	0	-	0	-	0.2	5.5	1	0.0	1.8	0
Merriam Lake	-	0	0	3.33	10.5	35	2.6	12	31	2.1	4.3	9
Shirts Lake	4.3	15	65	0	6	0	0.2	5	1	0.8	1.3	1
Spruce Gulch	-	0	0	0	8	0	0.3	4	1	3.1	1.3	4
Upper Hazard	-	0	0	0.20	5	1	1.1	16	17	0.0	1.8	0
Total	6.3	17	69	0.55	63.5	41	0.6	79.9	59	0.6	25.1	14

Table 3. Mean length (mm) and weight (g) of brook trout (with 95% confidence intervals) sampled from nine mountain lakes by survey year. Tiger muskellunge were introduced into the listed lakes in 2007.

Lake name	Mean length (mm)	n	Mean weight (g)	n	Mean condition	Longest five
			2005-2006			
Black Lake	203 (± 7)	54	80 (± 7)	54	0.93	244
Corral Lake	206 (± 12)	64	94 (± 12)	64	1.09	262
Granite Twin	232 (± 12)	80	124 (± 12)	80	0.90	291
Grass Mtn 1	209 (± 19)	28	104 (± 24)	28	0.99	278
Grass Mtn 2	251 (± 10)	35	161 (± 18)	35	0.98	286
Merriam Lake	205 (± 14)	37	91 (± 18)	37	0.94	265
Shirts Lake	196 (± 5)	122	31 (± 7)	122	1.04	231
Spruce Gulch	207 (± 9)	63	92 (± 11)	63	0.98	260
Upper Hazard	227 (± 14)	36	113 (± 18)	36	0.90	292
Total	212 (± 3)	519	88 (± 5)	519	0.97	268
			2008			
Black Lake	241 (± 87)	3	115 (± 118)	3	0.80	241*
Corral Lake	263 (± 400)	2	170 (± 762)	2	0.89	263*
Granite Twin	265 (± 13)	15	194 (± 25)	15	1.03	290
Grass Mtn 1	283 (± 15)	12	263 (± 34)	12	1.16	303
Grass Mtn 2	287	1	210	1	0.89	287*
Merriam Lake	232 (± 7)	70	124 (± 9)	66	1.04	288
Shirts Lake	225 (± 60)	4	128 (± 100)	4	1.08	225*
Spruce Gulch	264 (± 11)	16	223 (± 29)	16	1.19	283
Upper Hazard	246 (± 29)	9	1567 (± 41)	9	1.01	268
Total	246 (± 6)	132	161 (± 11)	128	1.06	272
			2009			
Black Lake	-	0	-	0	-	-
Corral Lake	-	0	-	0	-	-
Granite Twin	-	0	-	0	-	-
Grass Mtn 1	299 (± 8)	21	243 (± 22)	21	0.90	324
Grass Mtn 2	286 (± 46)	10	244 (± 64)	10	0.95	317
Merriam Lake	251 (± 4)	63	138 (± 8)	63	0.88	280
Shirts Lake	273 (± 121)	2	188 (± 158)	2	0.93	273*
Spruce Gulch	309 (± 12)	14	337 (± 51)	14	1.12	326
Upper Hazard	235 (± 23)	28	130 (± 27)	28	0.88	296
Total	264 (± 7)	138	181 (± 15)	138	0.91	334
			2010			
Black Lake	-	0	-	0	-	-
Corral Lake	-	0	-	0	-	-
Granite Twin	148 (± 32)	5	34 (± 18)	5	0.97	148
Grass Mtn 1	275 (± 93)	6	230 (± 123)	6	0.96	311
Grass Mtn 2	338 (± 32)	2	430	2	1.12	-
Merriam Lake	253(± 5)	38	163 (± 11)	29	1.00	276
Shirts Lake	166 (± 12)	8	41 (± 5)	6	0.92	174
Spruce Gulch	315 (± 20)	16	352 (± 82)	12	1.20	355
Upper Hazard	162(± 33)	9	56 (± 29)	9	1.15	196
Total	237 (± 7)	84	187 (± 16)	69	1.05	243

Table 4. Mean length (mm) of tiger muskellunge (with 95% confidence intervals where possible) sampled from nine mountain lakes by survey year and method. Tiger muskellunge were introduced into the listed lakes in 2007. Dashed lines indicate missing values.

Lake name	Mean length (mm)	n	Gill net	Angling	Visual
		2008			
Black Lake	478 (± 31)	26	1	25	0
Corral Lake	572 (±103)	6	0	6	0
Granite Twin	375	1	1	0	0
Grass Mtn 1	526 (± 114)	2	2	0	0
Grass Mtn 2	605	1	1	0	0
Merriam Lake	-	0	0	0	1
Shirts Lake	-	0	0	0	0
Spruce Gulch	344 (± 51)	12	1	11	0
Upper Hazard	495	1	1	0	0
Mean	460 (± 31)	49	7	42	1
		2009			
Black Lake	540 (± 58)	10	2	7	1
Corral Lake	494 (± 136)	10	0	4	6
Granite Twin	580 (± 108)	6	0	2	4
Grass Mtn 1	431	1	0	0	1
Grass Mtn 2	-	0	0	0	0
Merriam Lake	-	0	0	0	0
Shirts Lake	643	8	1	0	7
Spruce Gulch	370	5	1	0	4
Upper Hazard	-	1	1	0	0
Mean	535 (± 41)	41	5	13	23
		2010			
Black Lake	635	2	0	0	2
Corral Lake	-	0	0	0	0
Granite Twin	713 (±60)	6	0	2	4
Grass Mtn 1	-	0	0	-	0
Grass Mtn 2	-	0	0	0	0
Merriam Lake	-	0	0	0	0
Shirts Lake	710	2	0	0	2
Spruce Gulch	545	2	1	1	0
Upper Hazard	0	0	0	0	0
Mean	672 (±73)	12	1	3	8

Table 5. Mean length (mm) and weight (g) (with 95% confidence intervals), condition and trophy potential (assessed by the mean of the five longest fish) of brook trout sampled from four "control" mountain lakes. These lakes were in the same drainage as most of the treated lakes.

Lake name	Mean length (mm)	n	Mean weight (g)	n	Mean condition	Longest five
			2005			
Hard Creek Lal	226 (± 19)	44	140 (± 38)	44	0.98	340
			2006			
Black Lake #2	192 (± 29)	25	100 (± 33)	25	1.01	270
Hard Creek Lal	217 (± 18)	31	110 (± 24)	31	0.96	287
Lloyds Lake	220 (± 15)	30	120 (± 23)	30	1.04	281
Rainbow Lake	185 (± 18)	30	66 (± 17)	30	0.97	260
Total	210 (± 56)	160	111 (± 88	160	0.99	287
			2009			
Black Lake #2	236 (± 16)	31	131 (± 19)	31	0.95	294
Hard Creek Lal	233 (± 11)	72	130 (± 20)	46	0.93	315
Lloyds Lake	233 (± 13)	45	140 (± 21)	45	1.00	296
Rainbow Lake	213 (± 14)	22	78 (± 14)	22	0.78	253
Total	231 (± 44)	170	125 (± 65)	144	0.93	290
			2010			
Black Lake #2	198 (± 19)	31	94 (± 20)	31	1.05	253
Hard Creek Lal	229 (± 24)	23	117 (± 26)	13	0.54	283
Lloyds Lake	208 (± 20)	17	79 (± 28)	10	0.62	246
Rainbow Lake	194 (± 14)	33	80 (± 13)	33	1.10	234
Total	203 (± 9)	104	91 (± 10)	87	0.88	254

Table 6. Mean catch-per-unit-effort by gill nets (fish per net night) and angling (fish per hour) sampled from four "control" mountain lakes by sample year. These lakes were in the same drainage as most of the treated lakes. Dashed lines indicate where angling was not conducted.

Lake name	**CPUE Gill nets**	**Net-nights**	**n**	**CPUE angling**	**n**	**Angling hours**
			2005			
Hard Creek Lake	22	2	44	-	-	
			2006			
Black Lake #2	11	1	11	7.0	14	2
Hard Creek Lake	31	1	31	-	-	-
Lloyds Lake	30	1	30	-	-	-
Rainbow Lake	30	1	30	-	-	-
Total	22.5	4	90	7.0	14	2
			2009			
Black Lake #2	9.0	2	18	6.5	13	2
Hard Creek Lake	23.0	2	46	5.1	26	5.05
Lloyds Lake	21.5	2	43	1.3	6	4.5
Rainbow Lake	7.5	2	15	1.9	7	3.75
Total	15.25	8	122	3.4	52	15.3
			2010			
Black Lake #2	15.0	2	30	-	-	-
Hard Creek Lake	7.0	2	14	3.2	9	2.8
Lloyds Lake	6.5	2	13	2.0	4	2.0
Rainbow Lake	16.5	2	33	-	0	1.0
Total	11.3	8	90	2.5	13	5.8

Table 7. Summary details to help consider future management options for continued suppression of brook trout (BKT) and the future direction for mountain lakes stocked with tiger muskellunge (TM).

Lake	Area (ha)	Part of Chain?	Refugia	Barrier w/in 1km?	Initial BKT Num	Final BKT Num	TM Remaining	YOY Present 2009	Changed BKT Size	Treatment Potential	Potential Treatment	Treatment Priority
Black Lake	10.5	No	Inlet/Outlet/ Shoreline	Yes	54	0	2	no	-	inlet/outlet good targets very deep, large lake	Yes	3
Corral Lake	2.6	No	Inlet	Yes	60	0	0	fry in inlet	-	easy inlet/outlet treatments maybe whole lake?	Yes	1
Granite Twin Lakes	16.1	No	Inlet	Yes	80	0	6	dozens along shore	- 84 mm*	easy inlet treatment	Maybe	2
Grass Mt Lake #1	5.1	Yes	Outlet	No	28	6	0	1 fry	+ 66 mm	easy outlet treatment but can TM kill all adult BKT?	Treated in 2010	-
Grass Mt Lake #2	5.1	Yes	Inlet/Outlet	No	35	2	0	lots in inlet/outlet	+ 87 mm	easy inlet treatment easy outlet treatment	Treated in 2010	-
Merriam Lake	2.6	No	Inlet/Outlet	Yes	37	29	0	lots in inlet	+ 48 mm	too many adult BKT no TM left	No	-
Shirts Lake	3.5	No	Inlet/Outlet/ Shoreline	Yes	57	7	2	100s on shore	- 30 mm*	easy outlet treatment lots of small inlets	Yes	4
Spruce Gulch Lake	10.9	No	Shoreline	Yes	63	12	2	no	+ 108 mm	too many adult BKT	No	-
Upper Hazard Lake	15.8	Yes	Inlet/Outlet/ Shoreline	Yes	36	9	0	100's on shore	- 64 mm	too many adult BKT	No	-

* Size average has decreased likely because of brook trout present as fry in 2009 recruited to gill nets in 2010 after reaching large enough size.

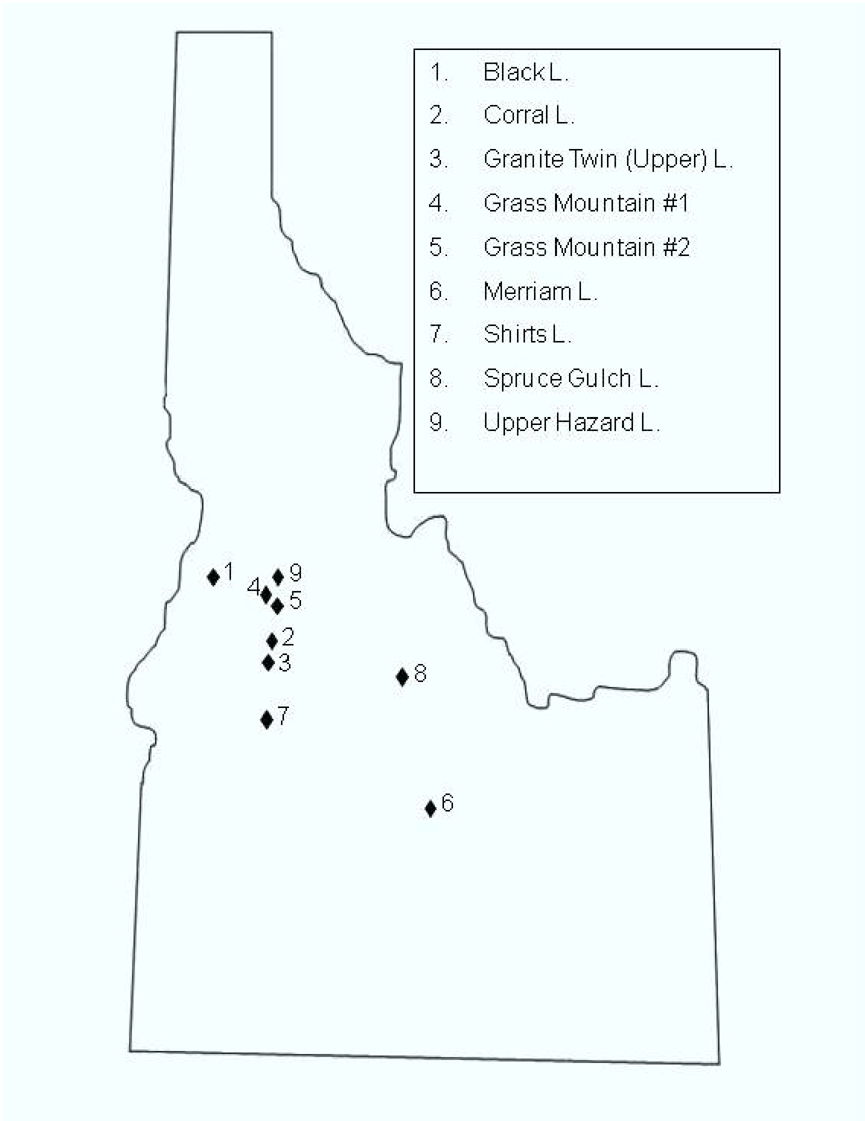


Figure 1. Locations of nine high mountain lakes in Idaho that were chosen for inclusion in a study designed to eliminate brook trout populations by stocking tiger muskellunge. Lakes were initially surveyed in 2005 or 2006 and planted with tiger muskellunge in 2007. Sampling was again conducted in 2008 to investigate subsequent changes to brook trout populations.

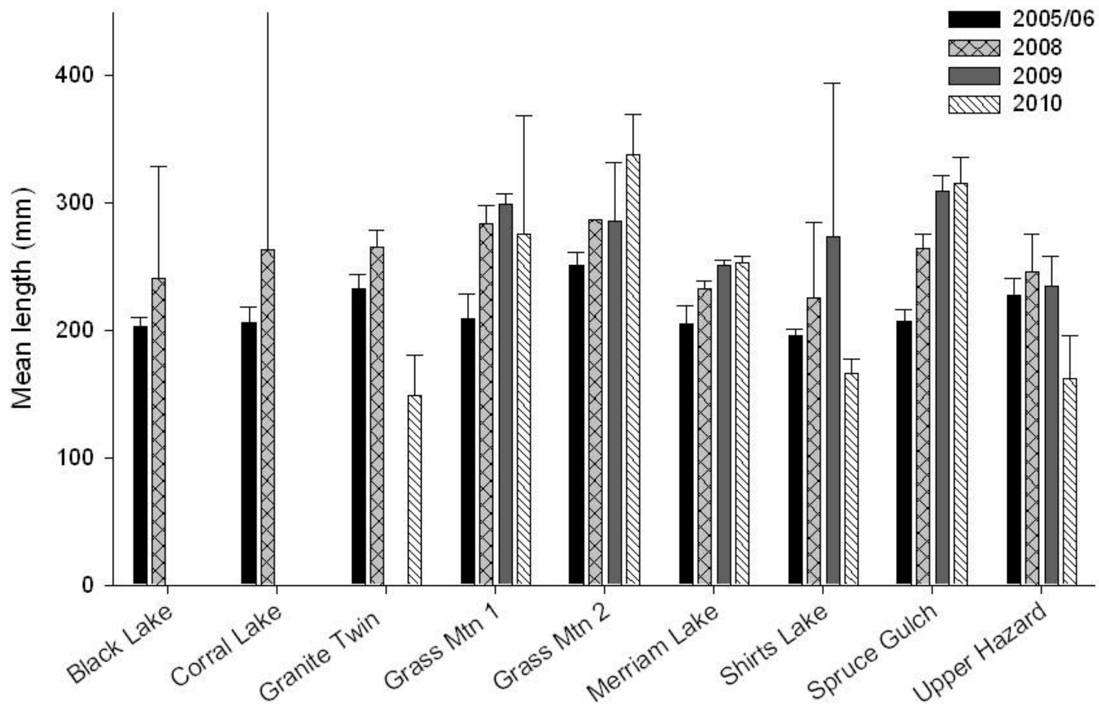
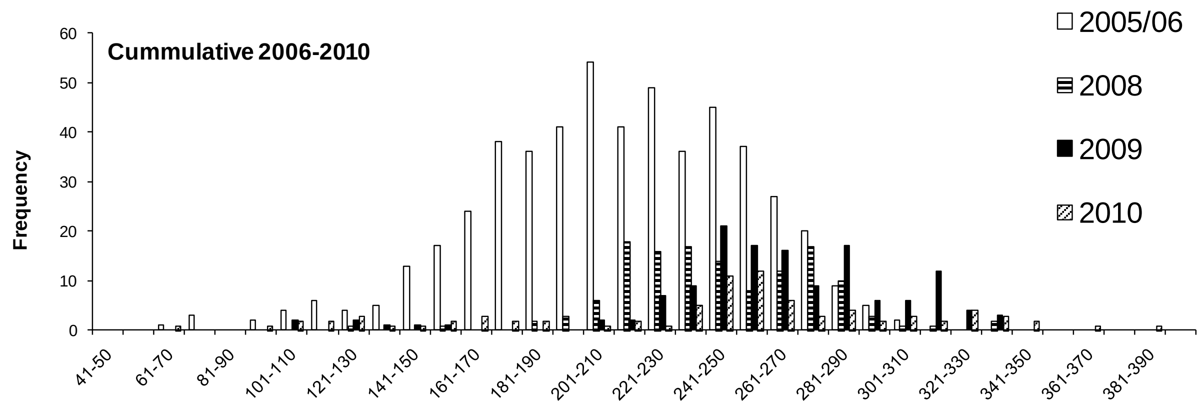


Figure 2. Mean total length of brook trout before (2005-06) and after tiger muskellunge were introduced in 2007. Error bars indicated 95% confidence intervals around the mean, where sample sizes allowed. Wide confidence intervals at Corral Lake are a result of low sample size (*n* = 2). Missing bars indicate no brook trout were captured.



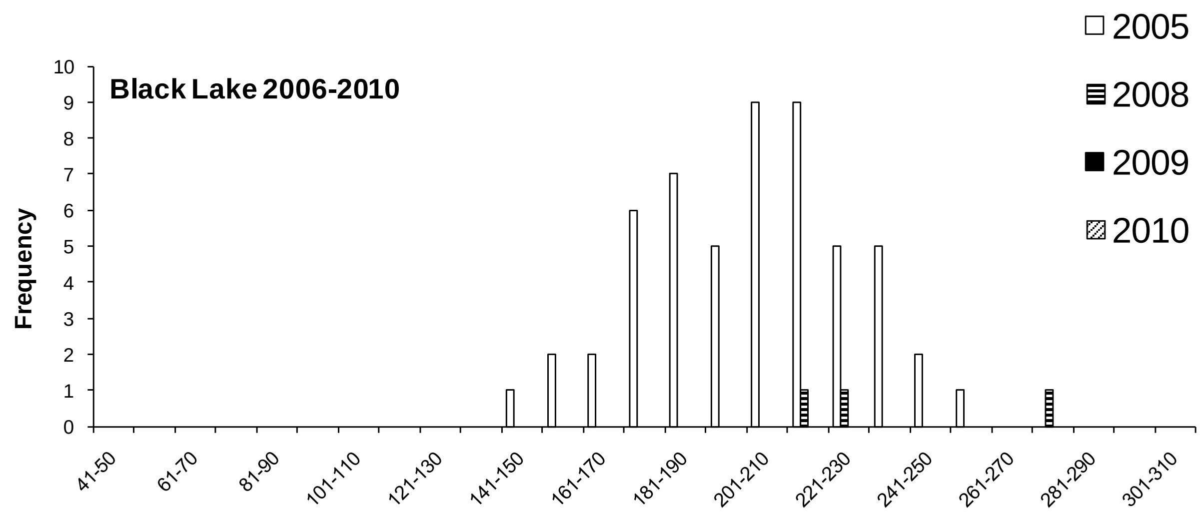
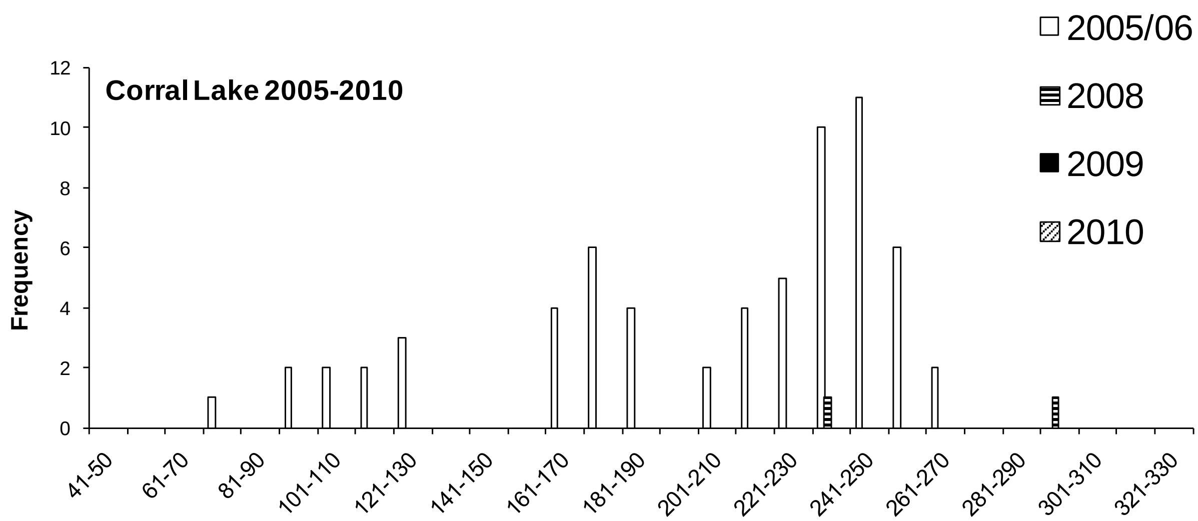
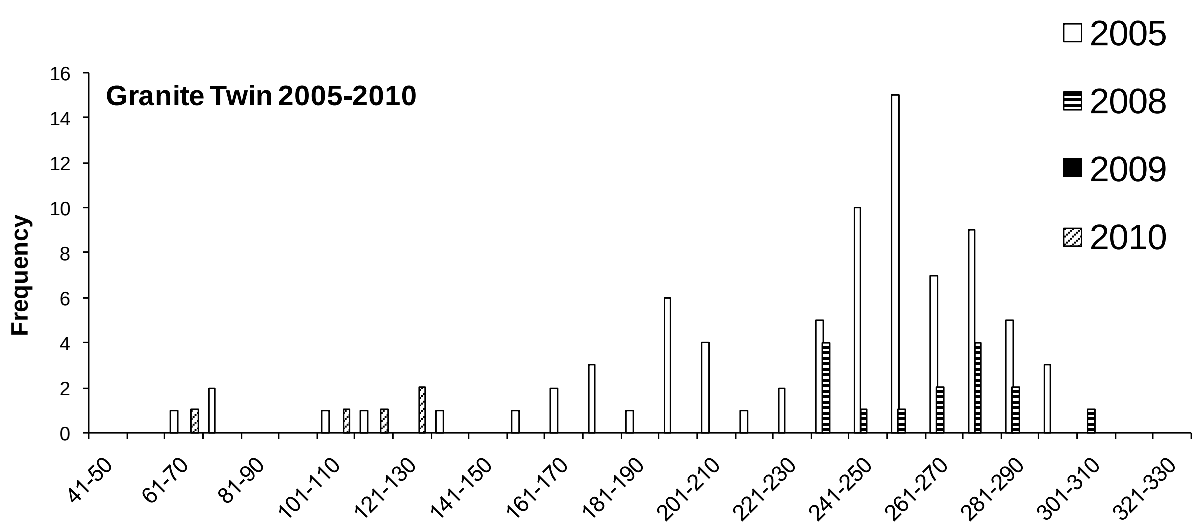


Figure 3. Size distribution (total length, mm) of brook trout captured before (2005 or 2006) and after (2008) introduction of tiger muskellunge. Tiger muskellunge were introduced in 2007.





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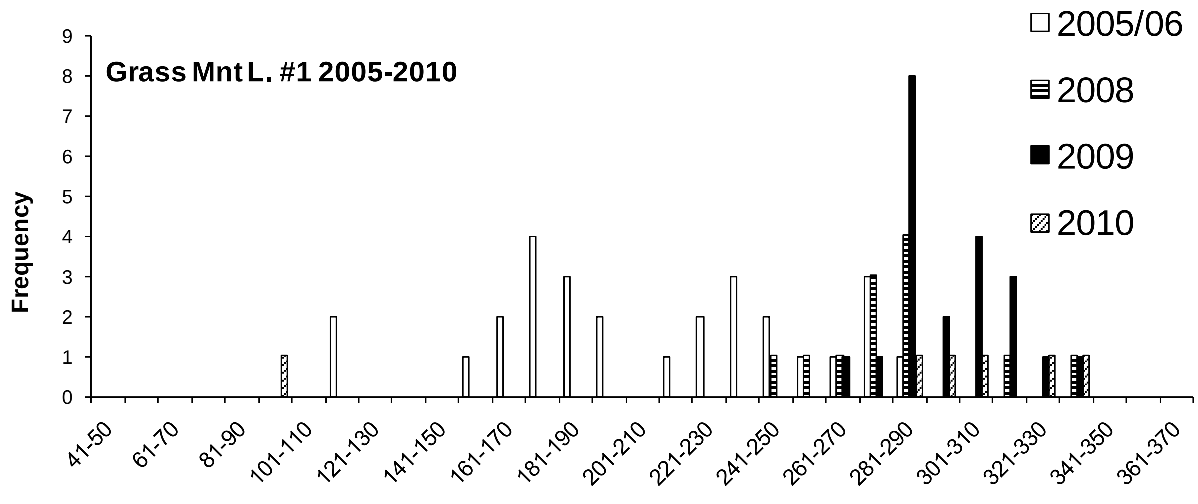

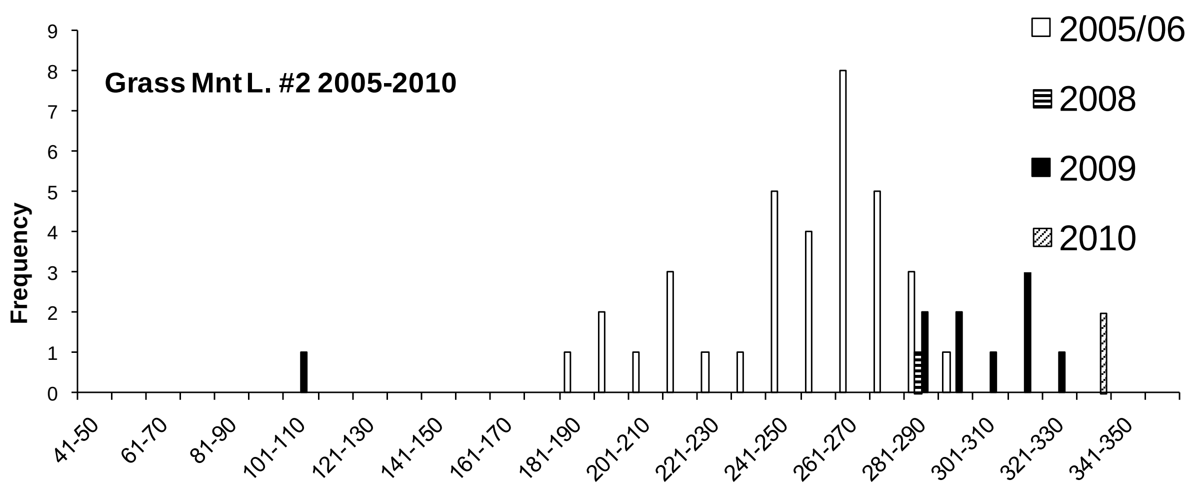

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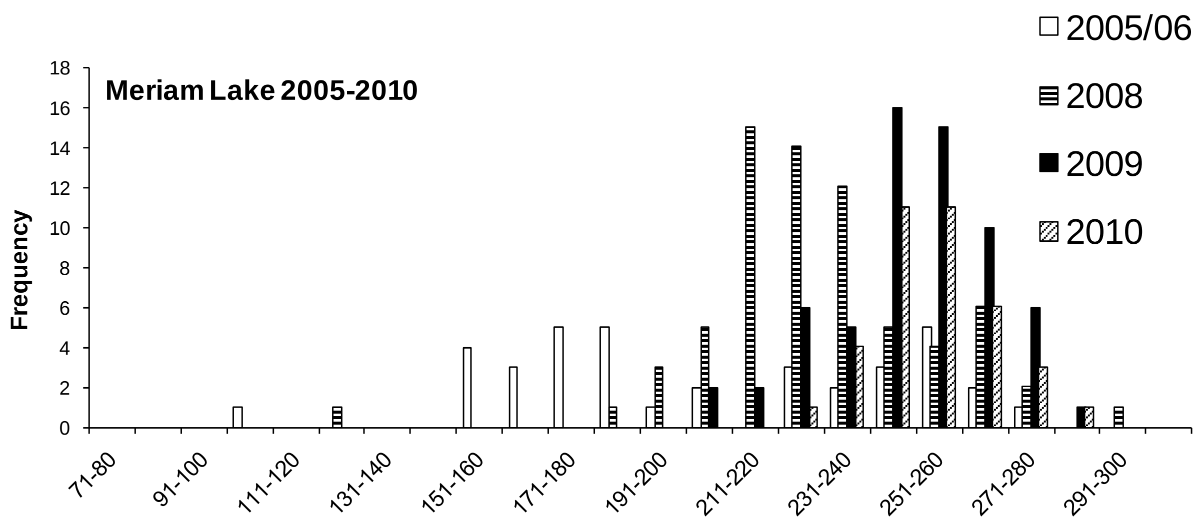

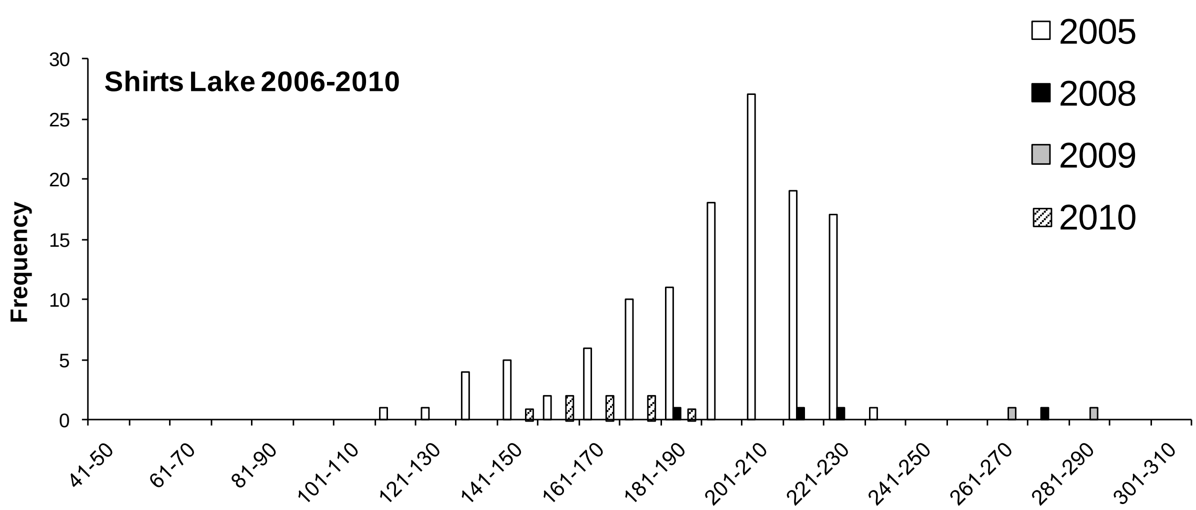

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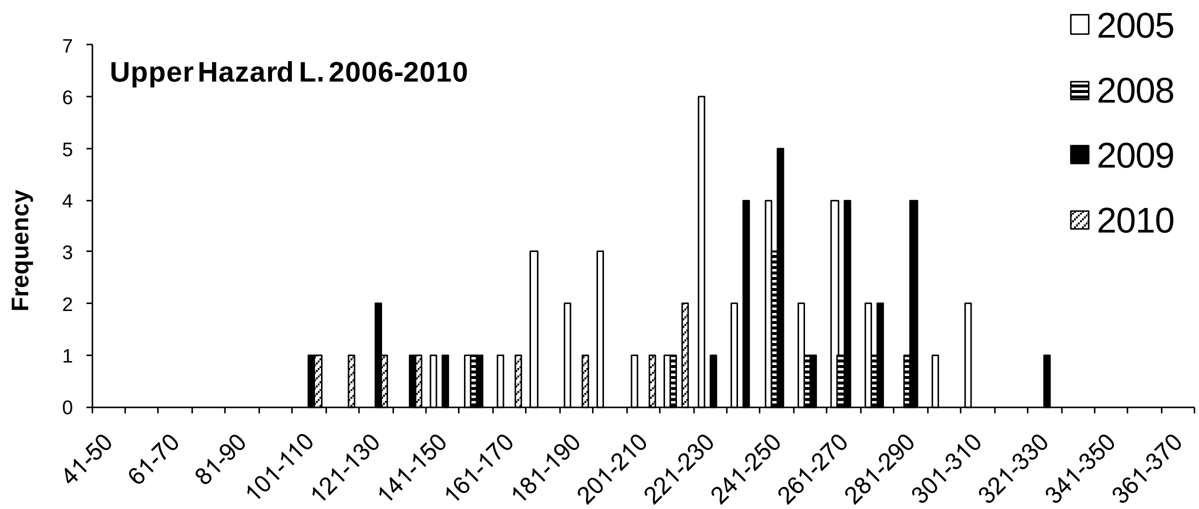

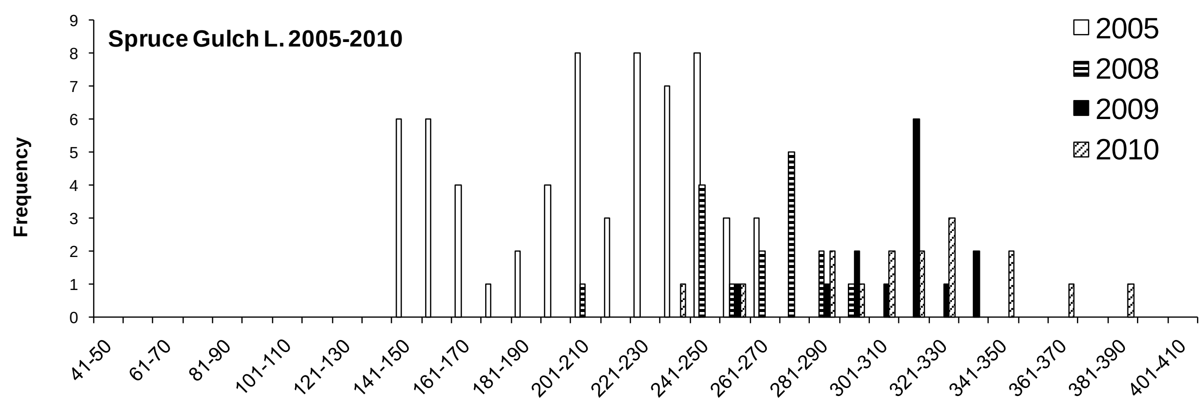

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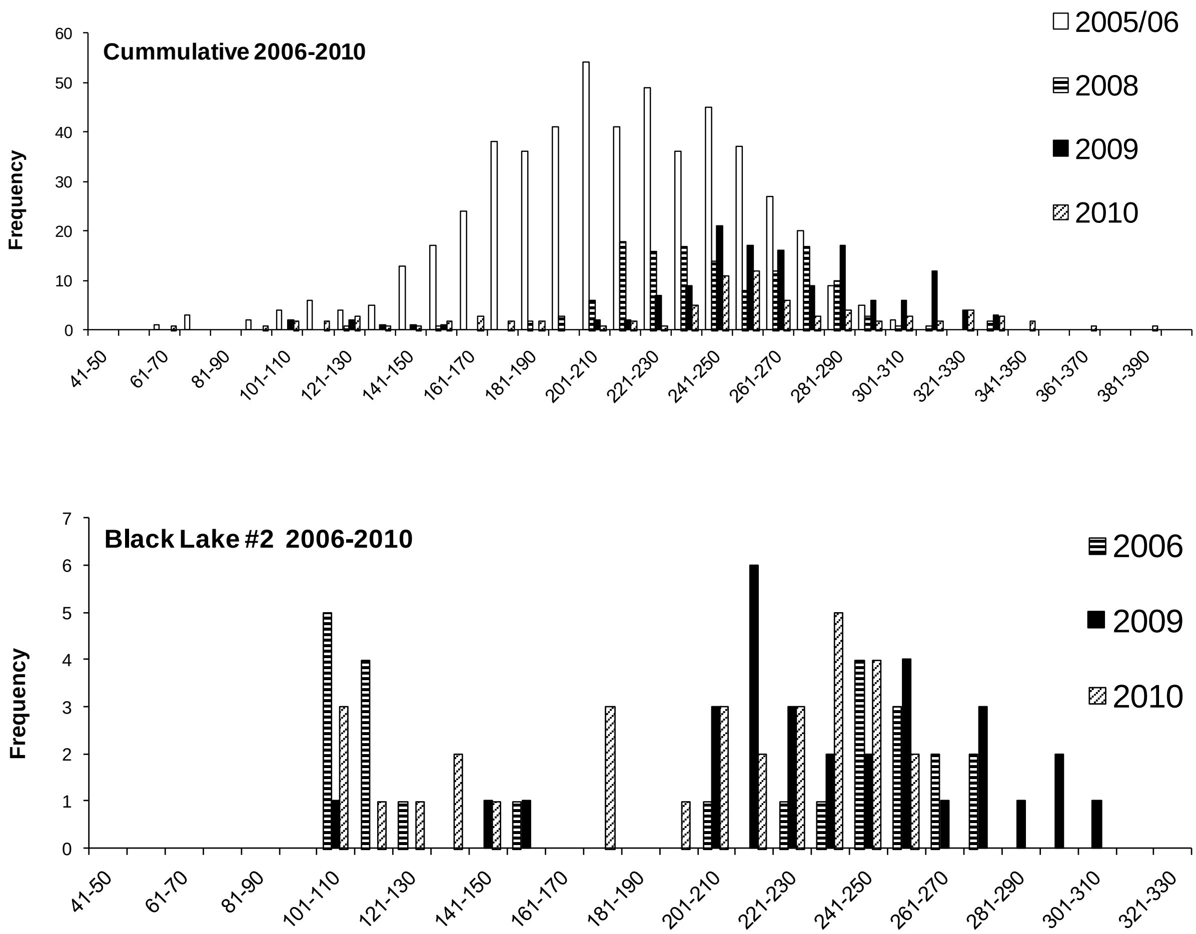
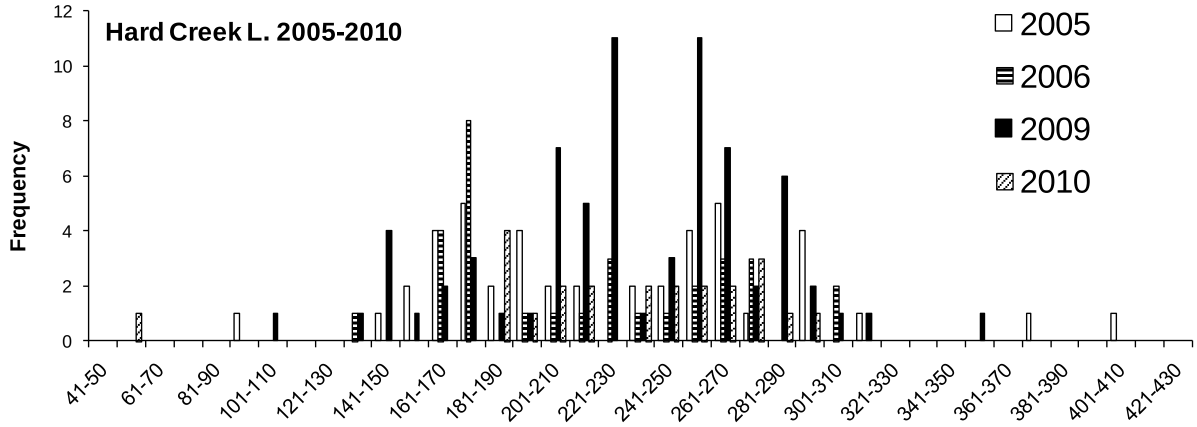
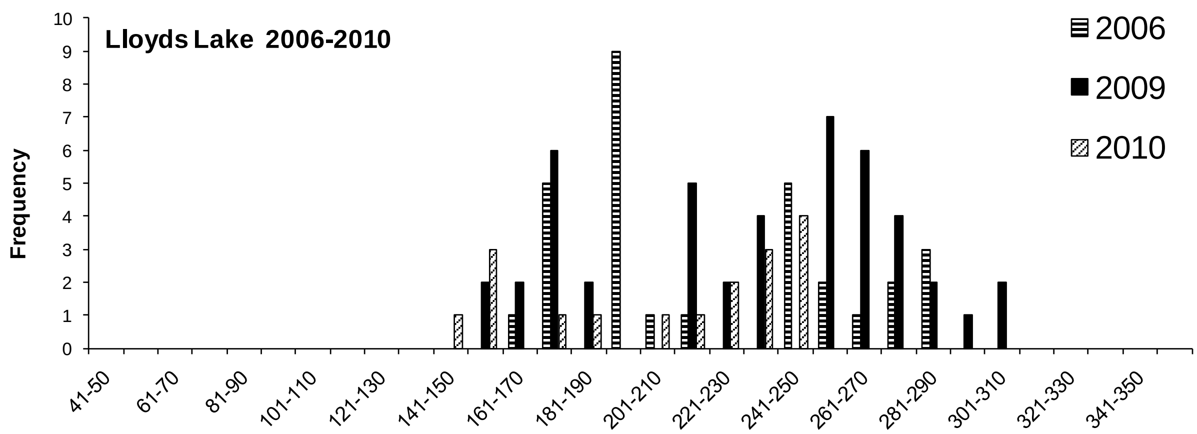


Figure 4. Size distribution (total length, mm) of brook trout from four "control" mountain lakes by sample year. These lakes were in the same drainage as most of the treated lakes, but did not receive any tiger muskellunge.





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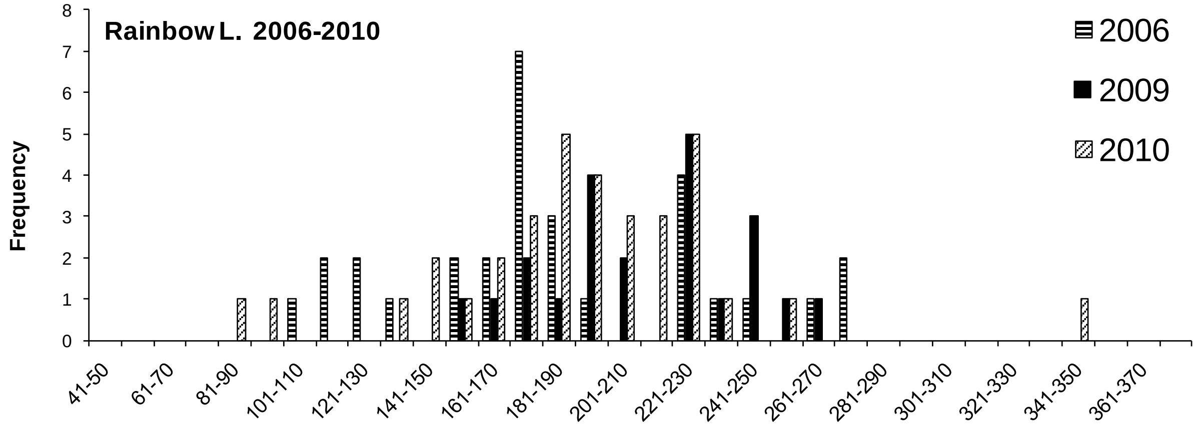

Figure 4. Continued.

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

State of: Idaho Grant No.: F-73-R-33 Fishery Research

Project No.: 4 Title: Hatchery Trout Evaluations

Subproject #2: Sterile Trout Investigations

Contract Period: July 1, 2007 to June 30, 2008

ABSTRACT

Increased growth, improved survival, and genetic protection of wild stocks have been suggested as benefits of stocking triploid (i.e. sterile) salmonids for recreational fisheries. We examined the relative growth and survival of triploid and diploid kokanee salmon *Oncorhynchus nerka* across five lakes and reservoirs stocked in similar numbers during spring 2005. The number of kokanee caught in each study location during 2007 was highly variable, with catch-per-unit-effort ranging from 0.8 to 4.9 fish/hr of netting. In 2007, 1,208 kokanee were captured, with the majority being unmarked nontest fish (95%). Of the 305 fish examined, 56 marked fish were identified (5%) based on fin clips and calcein-marked otoliths. Diploid kokanee accounted for a higher percentage (61%, 34 fish) of the total marked kokanee captured. When catch data were adjusted to reflect the 79% triploid-induction rate, diploids made up 73% (41 fish) of the marked kokanee captured. For the 2007 samples, there was no difference in length (322 ± 20 mm and 316 ± 26 mm) or weight (326 ± 86 g and 304 ± 101 g) between 2N and 3N kokanee, respectively, based on 95% CIs. Eleven fin-clipped kokanee were captured, with six having right ventral clips (triploid) and five with left ventral clips (diploids). Ten of these clipped fish had visible calcein marks present in their otoliths, suggesting a 91% mark retention rate for calcein in otoliths two years after stocking. In 2008, 1,835 kokanee were sampled with gill nets, of which 579 were examined for calcein marks. Only two marked fish were found in 2008; identified as triploid from the calcein mark. In 2009, 1,342 kokanee were sampled with gill nets, of which 272 were examined for calcein marks. No marked kokanee were found in the 2009 samples. Sampling was discontinued after 2009. Capture of marked kokanee were too low to make definitive conclusions about the performance of triploid kokanee. Due to lengthy processing time and uncertainty in interpreting the mark (both while in the field and lab), we do not recommend using calcein as a mass-mark in the future for long-term paired release evaluations.

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INTRODUCTION

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

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

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

OBJECTIVE

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

METHODS

Test Groups

Test groups were spawned using eggs collected at a weir on the Deadwood River from August 23 through September 7, 2004. Ripe kokanee were anesthetized prior to spawning. The eggs of 4-13 females were fertilized with the milt of 4-13 males in each of four spawning bowls.

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

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

Field Sampling

Kokanee were sampled from each of the five study waters using a combination of experimental gill nets and net curtains. Experimental gill nets measured 48 m long by 1.8 m deep and were comprised of six panels of 19, 25, 32, 38, 51 and 64 mm bar mesh, placed in random order when manufactured. Experimental net curtains in two different sizes were used. The “small” mesh net curtains measured 55 m long by 6 m deep and were composed of panels of 19, 25, 32, 38, 51, and 64 mm bar mesh monofilament. “Large” mesh net curtains measured 55 m long by 6 m deep and were composed of panels of 76, 102, 127 mm bar mesh monofilament. Sampling in 2009 was conducted using only “large” mesh net curtains to target only larger, older kokanee to increase the likelihood of capturing marked test fish stocked in 2005. Nets were set overnight for a minimum of 8 hours. Gillnets and net curtains were either set floating on the surface or suspended along the thermocline.

Study sites were sampled in 2007, 2008, and 2009 between May and July. Sampling effort varied across locations. All kokanee captured were measured for total length to the nearest millimeter and weighed to the nearest gram. Sex and maturity level were determined by observing gonads. Sexual maturity was assigned to one of the three levels: immature, developing, or mature. Immature gonads were small, with testes being light-colored, opaque, fine-textured organs, and ovaries being granular and translucent, whereas mature fish were characterized as having testes that were much enlarged and milky white and ovaries with evident well-developed eggs (Strange 1996). Developing gonads were characterized as having characteristics intermediate between immature and mature.

Reading Marks

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

Relative proportions of 2N and 3N kokanee were determined by examining a subsample of the total otoliths collected for calcein marks. The 2007 subsample was chosen based on several criteria intended to narrow the samples to those most likely corresponding to the size range of the marked test fish. We used the size range of fin-clipped kokanee captured from each reservoir and length-frequency histograms. All fish within 50 mm below the minimum size of fin-clipped test fish and all samples larger than fin-clipped test fish were examined for calcein marks. Additionally, any fish that did not fit into these length criteria but were aged as age-2 (based on examination of otoliths) were also included. The 2008 subsample included all kokanee greater than (or equal) in total length to the smallest marked fish identified in the 2007 samples. We examined all samples that met the minimum length of 370 mm (Lucky Peak), 420 mm (Devils Creek Res.), 320 mm (Ririe Res), 265 mm (Twin Lakes), and 260 mm (Mirror Lake). Given the time involved in examining otoliths for calcein, we made an effort to maximize the likelihood of finding marked fish in the 2009 subsample. The 2009 sample was selected by examining all the fish in the longest 25% (total length) of kokanee captured.

Selected samples were initially wet sanded lightly to prevent sanding through the plane of the mark. Initial sanding with 600-grit sandpaper was followed with 1200- and 1500-grit to lightly polish after each sanding. Otoliths were alternately sanded and viewed using a compound microscope at 40X under UV light, using a calcein-specific filter set and dichromatic mirror (Chroma #41012). Iterations of sanding and viewing continued until the mark was clearly visible or until the otolith had been sanded through the plane of the focus.

The total numbers of marked kokanee stocked and later recaptured were adjusted for the 2007 sampling results to reflect the triploid-induction rate (Table 9, Appendix C). The 79% induction rate was first applied to the original number of triploid kokanee stocked to calculate corrected totals for each group. The total number of diploid kokanee stocked and recaptured was used to calculate a relative survival for diploids only. This was then applied to the 21% of triploid kokanee stocked that were likely diploid to determine how many of the marked triploid kokanee recaptured were actually diploid. We assumed that the survival ratio of diploid: triploid kokanee was constant across all the study sites. Last, a ratio of 2N:3N kokanee captured was calculated by dividing the estimated survival rates of each group. See Appendix C for complete breakdown of calculations. No adjustments were made to the 2008 and 2009 data collected, since only two marked fish were identified, precluding any meaningful comparisons.

RESULTS

Test Groups

During spawning, 725 female kokanee were used for creation of the 3N group. Average fecundity was approximately 649 eggs/fish, yielding 470,455 green eggs. From these eggs, 180,946 eggs survived to eye-up for an eye-up rate of 38.5%. Survival to eye-up was highly variable across spawning days and ranged from 35-58%. Although 2N groups were not true controls, they do act as a good reference for comparison of survival between groups. Mean survival to eye-up for 2N and 3N collected on the same day were 57% and 39%, respectively, resulting in 110,946 2N and 102,523 3N kokanee stocked.

Length and weight of kokanee were similar between test groups prior to stocking. Mean length of the 2N ($\bar{X}$ = 86 ± 1 mm; *n* = 100) and 3N ($\bar{X}$ = 88 ± 2 mm; *n* = 100) groups was equal based on 90% CIs. Similarly, mean weight of the 2N ($\bar{X}$ = 3.9 ± 0.2 g; *n* = 100) and 3N ($\bar{X}$ = 4.0 ± 0.2 mm; *n* = 100) groups was equal. Flow cytometry analysis indicated that triploid-induction rate for the 3N group was 79% (n = 99). Approximately equal numbers of kokanee from the 3N and 2N groups were stocked into four of the five study waters (Table 8). Lucky Peak was the only exception, with 49,950 2N kokanee and 41,400 3N kokanee stocked.

Field Sampling

During 2007, nets were fished from 62-131 hours per water body, yielding a total effort of 455 hours, capturing 1,208 kokanee. Catch-per-unit-effort (CPUE) for all kokanee combined ranged from 0.8 fish/hr (Mirror Lake) to 4.9 fish/hr (Lucky Peak), with a mean of 2.5 fish/hr (Table 9). For test fish only, Twin Lake had the highest catch rates for 2N and 3N kokanee at 0.21 fish/hr and 0.15 fish/hr, respectively. Ririe Reservoir had the lowest catch rates of diploids (0.03 fish/hr), while Lucky Peak had the lowest catch of triploids (zero). On average, gill nets and net curtains caught 0.08 marked 2N kokanee/hr and 0.06 marked 3N kokanee/hr across all reservoirs.

During 2008, nets were fished from 170-398 hours per water body, yielding a total effort of 1,341 hours, capturing 1,835 kokanee. CPUE for all kokanee combined ranged from 0.56 to 1.91, with a mean of 1.23 fish/hr (Table 9). One marked kokanee was captured in Ririe Reservoir and one in Devils Creek Reservoir. Both marked kokanee were identified as triploid from calcein marks. During 2009, nets were fished from 151-598 hours per water body, yielding a total effort of 1,393 hours, capturing 1,342 kokanee. CPUE for all kokanee combined ranged from 0.3 to 1.9 fish/hr, with a mean of 0.77 fish/hr (Table 9). No marked kokanee were identified during sampling in 2009.

Mean length (mm) and mean weight (g) of kokanee captured varied between water bodies (Table 10) and across sample years (Figure 5). Figure 5 shows length frequency for all water bodies combined over time. Mean length increased slightly in 2009 as expected, likely resulting from the larger mesh sizes used to select for larger kokanee. Statistical comparisons of mean length and weight between 2N and 3N groups within reservoirs could not be made because of limited samples sizes. For the 2007 samples, there was no difference in length (322 ± 20 mm and 316 ± 26 mm) or weight (326 ± 86 g and 304 ± 101 g) between 2N and 3N kokanee, respectively, based on 95% CIs. Lucky Peak Reservoir and Devils Creek Reservoir had the highest mean lengths (over 400 mm) for marked kokanee, indicating rapid growth in the first two years of age.

Reading Marks

In 2007, the number of kokanee caught in each study location was highly variable, ranging from 78 to 468 (Table 9). Overall, 1,208 kokanee were captured, with the majority being unmarked nontest fish (1152, or 95%). From the total captured, 305 kokanee fit the criteria to be included in the subsample examined for calcein marks. Fifty-six test fish were identified (5%) based on fin clips and/or calcein-marked otoliths. Diploid kokanee made up 61% (34 fish) of the test fish caught and were captured from all five water bodies. However, when corrected for the 79% triploid-induction rate, diploid kokanee comprised 73% (41 fish) of the total marked kokanee captured. Only 22 kokanee marked as 3N were caught, having been caught in all study waters except Lucky Peak Reservoir. Triploid kokanee made up only 27% (15 fish) of the total marked kokanee recaptured when corrected for the triploid-induction rate. The adjusted average survival ratio of 2N:3N kokanee caught in the 2007 sample was 1.61:1 (Appendix C). This survival ratio became 1.43:1 – about a 12% difference – if left uncorrected.

A small percentage (13.2% and 12.5% for 2N and 3N, respectively) of the test fish stocked was double-marked with both calcein and ventral fin clips. Of the marked kokanee caught, six possessed right ventral fin clips (3N) and five had left ventral clips (diploids). Ten of these clipped fish had visible calcein marks present in their otoliths, suggesting a 91% mark retention rate for calcein in otoliths two years after stocking.

In 2008, 1,835 total kokanee were sampled with gill nets, of which 579 were examined for calcein marks. Only two marked fish were found in 2008; identified as triploid from the calcein mark. In 2009, 1,342 total kokanee were sampled with gill nets, of which 272 were examined for calcein marks. No marked kokanee were found in the 2009 samples. The very low numbers of marked kokanee recaptured precluded any meaningful comparisons of the performance of 2N and 3N kokanee in 2008 and 2009. Sampling was discontinued after 2009.

DISCUSSION

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

Although the 79% triploid-induction rate is not ideal, it should not affect the utility of this study. Using the combined total number of fish caught across all reservoirs, we were able to approximate the number of diploid and triploid kokanee captured. Of course, this was calculated assuming that this difference was constant across all the study lakes. We also assume no difference of survival between pressure-shocked diploids (resulting from only 79% 3N-induction rates) and those of control diploids stocked, which remains largely untested (Piferrer et al. 2008). There was not enough data to correct the numbers of marked fish caught at individual study locations. Data from 2007 provide the only opportunity for comparison, as only two marked kokanee were caught in later sampling. The combined totals of marked kokanee captured in 2007 suggest that 2N kokanee survive at higher rates up to age-2 than their 3N counterparts do. Diploid kokanee accounted for a large percentage (61%, N = 34) of the total marked kokanee captured, especially when corrected for the 79% triploid-induction rate (73%, N = 41) (Table 9). When adjusted for the 79% triploid induction rate, the ratio of 2N:3N kokanee in the 2007 sample would be 1.61:1. If the captures are left uncorrected, the ratio of 2N:3N kokanee was 1.43:1. Therefore, this correction accounts for about a 12% difference in the ratio of 2N:3N caught during sampling.

Kozfkay and Koenig (2006) suggested it may be possible to address the lower triploid-induction rate by collecting blood or fin-clip samples that could later be analyzed using flow cytometry to allow development of more precise correction factors (Lamatsch et al. 2000). However, after completing a season of sampling, the feasibility of collecting and later processing fin clip tissue into single cell suspensions for flow cytometry analysis is questionable, given the time, equipment, and scheduling necessary. Without a readily detectible mark to denote test fish in the field, large numbers of kokanee would have to be processed in the hopes of detecting small numbers of 3N fish. All fin clip samples would later be matched to calcein-marked otolith samples to ensure that they belonged to one of the marked test groups. Collecting and processing fin clips would add to the already lengthy processing time for fish sampled in the field. Additionally, processing fin clips would also require the involvement of at least one (and probably two) outside laboratories to prepare the suspensions and then to conduct the flow cytometry analysis. Fin clip tissues would have to be processed within 4-6 weeks of collection, so timing would be critical.

The number of 2N and 3N kokanee captured should be interpreted with caution. Relatively few marked kokanee were recaptured during this evaluation, especially in 2008 and 2009, so spurious conclusions from statistical comparisons are possible. Data from Mirror Lake and Twin Lake suggest that 2N kokanee are captured in higher numbers than triploids at two years of age. However, returns from Lucky Peak, Devils Creek, and Ririe reservoirs are inconclusive. Unfortunately, the number of marked kokanee captured was so low that robust statistical comparisons were not possible. Elrod and Frank (1990) recommended a sample size of 279 fish in order to detect a 20% difference between paired release groups ($\alpha = 0.05$, $1 - \beta = 0.90$). Given that marked fish made up about 5% of the total kokanee captured in 2007, we would have needed to capture approximately 5,200 total kokanee to achieve that sample size. Capturing 5,200 kokanee would have required sampling for 29 nights, at the mean catch rate of 2.7 kokanee/hr (in 2007), and setting six nets for 12 hours per night. Despite a three-fold increase in netting hours from 2007 and adding nets with larger mesh sizes to target older fish, only two marked fish were captured in 2008, and none in 2009. Natural and fishing mortality probably reduced the number of marked kokanee available in sequential years during this study. Many of the 2N group (and males of the 3N group) likely reached sexual maturity and spawned in fall 2007 at age-2. The probability of capturing marked age-3 and age-4 kokanee in 2008 and 2009 could have been prohibitively low and would have required more sampling effort than was feasible. Since the marked fish captured in 2007 were larger than the average size of kokanee caught, we made an effort to target larger fish during subsequent sampling. In 2008 and 2009, we began to incorporate nets with larger mesh sizes. As expected, the mean size of captured kokanee increased (Figure 5, Table 10), although there was little added success in capturing marked fish.

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

1993). Recent efforts to produce quality kokanee fisheries (>400 mm) using triploids in Canada have provided similar results. In recent years, the Canadian Ministry of Natural Resources Operations converted several popular fisheries to 3N kokanee stocks. They found 3N kokanee grew significantly slower than 2N kokanee, and that 3N kokanee did not live significantly longer in highly exploited fisheries (M. Ramsay, personal communication). Multiple year classes of sterile kokanee decreased overall growth as a result density-dependent competition. They also found reducing stocking densities to help increase mean 3N kokanee size decreased angling effort, since catch rates became so low that anglers lost interest (M. Ramsay, personal communication).

Other authors have reported poor results from field experiments using triploid coho salmon and hormone-sterilized Chinook salmon. Rutz and Baer (1996) found that triploid coho salmon grew more slowly and survived poorly compared to diploids, making up only 25% of the catch two years after stocking. In 1986, the Wisconsin Department of Natural Resources experimented with hormone-sterilized Chinook salmon in Lake Michigan (see Kitchell and Hewett 1987 for review), but the results were inconclusive because few fish were ever recaptured (M. J. Hansen, University of Wisconsin, personal communication). At this time, our results are similar to those of Johnston et al. (1993), where sterile kokanee show lower initial survival, but our results are inconclusive when addressing longevity. It is possible that the very low catch rates of kokanee in 2008 and 2009 are related to very low survival to age-3 and age-4. If this is indeed the case, triploid kokanee may not result in any improvement in the fishery in light of this low carryover. Benefits of increased longevity may never be realized if survival rates are very low.

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

The time associated with processing otoliths to read calcein marks limits the effectiveness of calcein for long-term field evaluations. Typically, one technician may be able to mount 80-100 otoliths onto microscope slides during an 8-hour workday, which results in only 40-50 fish if using both otoliths. For reading marks, two trained technicians can read 80-100 slides in a typical 8-hour workday, with one person sanding/polishing while another examines the slides for marks. The time needed to process samples can hinder counting the number of marked fish recaptured. Knowing recapture totals while sampling is useful so that sampling intensity can be adjusted to meet sample size requirements. In this respect, using calcein to distinguish test groups would still require some sort of externally-detectable mark on an adequate proportion of the population. Fin clips and coded-wire tags might be a better option to reduce tag reading error and tag processing time and would easily distinguish marked fish when captured in the field (Elrod and Schneider 1986; Munro et al. 2003; Koenig and Meyer 2011). These types of tags would require higher application costs to tag large numbers of fish but would have lower decoding costs and provide more accurate and timely results.

RECOMMENDATIONS

1. Discontinue using calcein as a mass-mark for long-term paired release experiments for salmonids.

2. Initiate a multiyear study to investigate how annual stocking of only triploid kokanee could affect population age and size structure to improve sportfishing yield and size in a typical kokanee fishery. Although triploid kokanee may have lower mortality rates in early years, this may be offset by increased longevity. Extended longevity might result in a population with multiple overlapping age classes of adult-sized fish. Such a population might result in higher catch rates, with greater numbers of larger, more catchable kokanee increasing over time as age classes overlap.

3. Use pressure treatment instead of heat treatment to induce triploidy in kokanee for any future triploid kokanee experiments.

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LITERATURE CITED

Beattie, W. D., and P. T. Clancey. 1991. Effects of *Mysis relicta* on the zooplankton community and kokanee population of Flathead Lake, Montana. American Fisheries Society Symposium 9: 39-48.

Elrod, J. H., and A. Frank. 1990. Sample size requirements and analysis of tag recoveries for paired releases of lake trout. North American Journal of Fisheries Management 10:196-201.

Elrod, J. H., and C. P. Schneider. 1986. Evaluation of coded wire tags for marking lake trout. North American Journal of Fisheries Management 6:264-271.

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.

IDFG (Idaho Department of Fish and Game). 2007. Resident fish hatcheries 2006 annual report. IDFG, Report 07-04, Boise.

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.

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.

Kitchell, J. F., and S. W. Hewett. 1987. Forecasting forage demand and yield of sterile Chinook salmon (Oncorhynchus tshawytscha) in Lake Michigan. Canadian Journal of Fisheries and Aquatic Sciences 44:384-389.

Koenig, M. K., and K. A. Meyer. 2011. Relative performance of diploid and triploid catchable rainbow trout stocked in Idaho lakes and reservoirs. North American Journal of Fisheries Management, In Press.

Kozfkay, J. 2003. Hatchery trout evaluations. Job Performance Report, Project F-73-R-25, Idaho Department of Fish and Game. Boise.

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

Kozfkay, J., and M. K. Koenig. 2006. Hatchery trout evaluations. Job Performance Report, Project F-73-R-28, Idaho Department of Fish and Game. Boise.

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.

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.

Munro, A. R., T. E. McMahon, S. A. Leathe, and G. Liknes. 2003. Evaluation of batch marking small rainbow trout with coded wire tags. North American Journal of Fisheries Management 23:600-604.

Myers, J. M., and W. K. Hershberger. 1991. Early growth and survival of heat shocked and tetraploid-derived triploid rainbow trout. Aquaculture 96:97-101.

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.

Piferrer, F., A. Beaumont, J. Falguière, M. Flajšhans, P. Haffray, and L. Colombo. 2008. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293:125-156.

Rieman, B. E., and M. A. Maiolie. 1995. Kokanee population density and resulting fisheries. North American Journal of Fisheries Management 15:229-237.

Rieman, B. E., and D. Myers. 1992. Influence of fish density and relative productivity on growth of kokanee in ten oligotrophic lake and reservoirs in Idaho. Transactions of the American Fisheries Society 121:178-191.

Rohrer, R. L., and G. H. Thorgaard. 1986. Evaluation of two hybrid trout strains in Henry's Lake, Idaho, and comments on the potential use of sterile triploid hybrids. North American Journal of Fisheries Management 6:367-371.

Rutz, D. S., and C. C. Baer. 1996. Comparative performance of stocked mixed-sex and all-female rainbow trout and diploid and triploid coho salmon in landlocked lakes in southcentral Alaska, 1994 and 1995. Alaska Department of Fish and Game. Fishery Data Series No. 96-23, Anchorage.

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.

Strange, R. T. 1996. Field examination of fishes. Pages 433-446 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

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.

Wydoski, R. S., and D. H. Bennett. 1981. Forage species in lakes and reservoirs of the western United States. Transaction of the American Fisheries Society 110:764-771.

Table 8. Stocking location, date, and number of kokanee stocked during 2005 in five Idaho lakes and reservoirs to assess relative performance of diploid (2N) and triploid (3N) kokanee. Columns to the right of each stocking group indicate stocking densities in fish per hectare of lake area. “Corrected Total” refers to the total number of 2N and 3N kokanee planted if corrected for the 79% triploid-induction rate of the test groups (see Appendix C).

		Number of kokanee planted					
Water Body	Date Stocked	2N	Fish/ha	3N	Fish/ha	Nontest	Fish/ha
Mirror Lake	5/31/2005	2,516	74	2,520	74	0	0
Twin Lake (Lower)	5/16/2005	20,000	127	20,000	127	20,000	127
Lucky Peak Res.	6/3/2005	49,950	45	41,400	37	108,800	97
Devils Creek Res.	5/19/2005	3,520	101	3,503	100	0	0
Ririe Res.	4/28/2005	34,960	61	35,100	61	140,975	246
Grand Total		110,946		102,523		269,775	
Corrected Total		132,476		80,993			

Table 9. Total kokanee captured by test group using a combination of gill nets and net curtains. “Corrected Total” indicates group totals if adjusted for the 79% triploid induction rate (see Appendix C). Mean gillnet catch-per-unit-effort (total fish per hour of netting) is shown in parentheses. “Adjusted mean CPUE” reflects the total marked kokanee captured (adjusted for the 79% induction rate) divided by the total hours of netting effort. CPUE was not adjusted for induction rate at individual lakes. No adjustments were made to 2008 or 2009 data because of insufficient sample sizes.

Lake name	Total	Nontest	Diploid	Triploid
		2007		
Devils Creek Res	84 (1.4)	78 (1.3)	3 (0.05)	3 (0.05)
Lucky Peak Res	466 (4.9)	462 (4.8)	4 (0.04)	0
Mirror Lake	78 (0.8)	65 (0.7)	8 (0.08)	5 (0.05)
Ririe Res	468 (3.6)	459 (3.5)	5 (0.04)	4 (0.03)
Twin Lake (Lower)	112 (1.7)	88 (1.3)	14 (0.21)	10 (0.2)
Mean CPUE	2.5	2.3	0.08	0.06
Adjusted mean CPUE	-	-	0.09	0.03
Corrected total			41	15
		2008		
Devils Creek Res	95	94 (0.55)	0	1 (0.006)
Lucky Peak Res	523	523 (1.4)	0	0
Mirror Lake	294	294 (1.5)	0	0
Ririe Res	761	760 (1.9)	0	1 (0.003)
Twin Lake (Lower)	163	163 (0.8)	0	0
Mean CPUE		1.2	0	0.0045
		2009		
Devils Creek Res	69	69 (0.3)	0	0
Lucky Peak Res	805	805 (1.4)	0	0
Mirror Lake	2	2 (0.01)	0	0
Ririe Res	409	409 (1.9)	0	0
Twin Lake (Lower)	57	57 (0.3)	0	0
Mean CPUE		0.77	0	0

Table 10. Mean total length (mm) and weight (g) (± 95% confidence interval) for unmarked nontest, diploid, and triploid kokanee by study location.

Survey year	Lake name	Mean length (± CI)						Mean weight (± CI)					
		Nontest	n	2N	n	3N	n	Nontest	n	2N	n	3N	n
2007	Devils Creek Res	326 (± 7)	78	448 (± 48)	3	446 (± 60)	3	366 (± 23)	78	921 (± 266)	3	834 (± 283)	3
	Lucky Peak Res	261 (± 8)	461	400 (± 48)	4	-	0	267 (± 12)	405	649 (± 172)	4	-	0
	Mirror Lake	212 (± 10)	66	274 (± 11)	8	276 (± 18)	5	88 (± 10)	66	170 (± 27)	8	174 (± 49)	5
	Ririe Res	259 (± 4)	460	339 (± 13)	5	336 (± 31)	4	166 (± 7)	459	345 (± 38)	5	341 (± 75)	4
	Twin Lake (Lower)	251 (± 10)	84	293 (± 5)	14	289 (± 8)	10	134 (± 17)	84	189 (± 22)	14	197 (± 18)	10
2008	Devils Creek Res	304 (± 26)	96	-	0	440	1	524 (± 92)	82	-	0	896	1
	Lucky Peak Res	328 (± 5)	523	-	0	-	0	383 (± 15)	503	-	0	-	0
	Mirror Lake	178 (± 5)	294	-	0	-	0	55 (± 5)	271	-	0	-	0
	Ririe Res	283 (± 4)	761	-	0	325	1	232 (± 8)	761	-	0	280	1
	Twin Lake (Lower)	297 (± 5)	163	-	0	-	0	258 (± 14)	163	-	0	-	0
2009	Devils Creek Res	363 (± 13)	69	-	0	-	0	549 (± 66)	69	-	0	-	0
	Lucky Peak Res	379 (± 5)	805	-	0	-	0	592 (± 17)	805	-	0	-	0
	Mirror Lake	288	2	-	0	-	0	161	2	-	0	-	0
	Ririe Res	343 (± 3)	409	-	0	-	0	397 (± 7)	409	-	0	-	0
	Twin Lake (Lower)	393 (± 12)	57	-	0	-	0	621 (± 54)	57	-	0	-	0

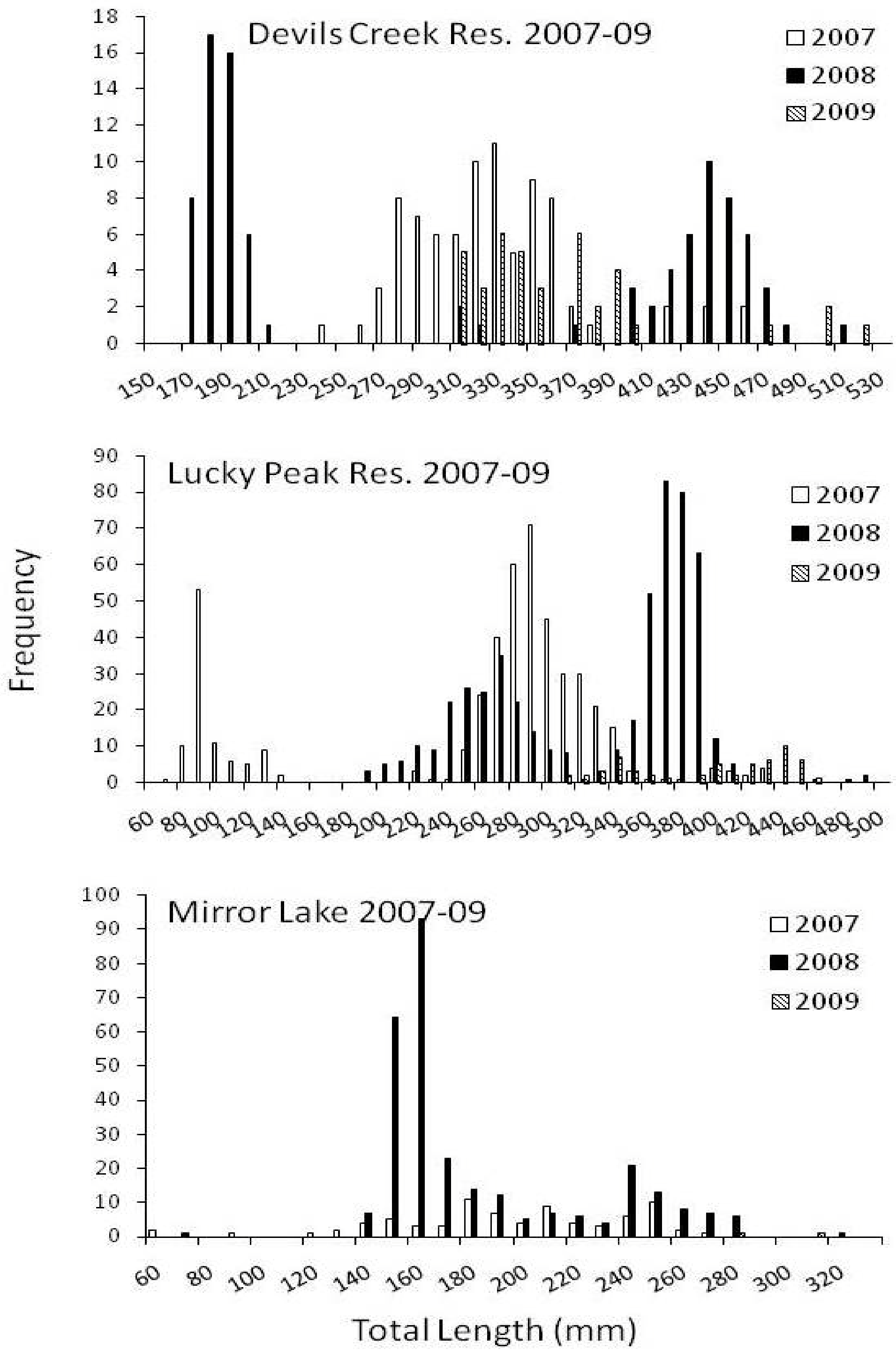

Figure 5. Length-frequency histograms for kokanee sampled from five Idaho lakes and reservoirs during June and July 2007.

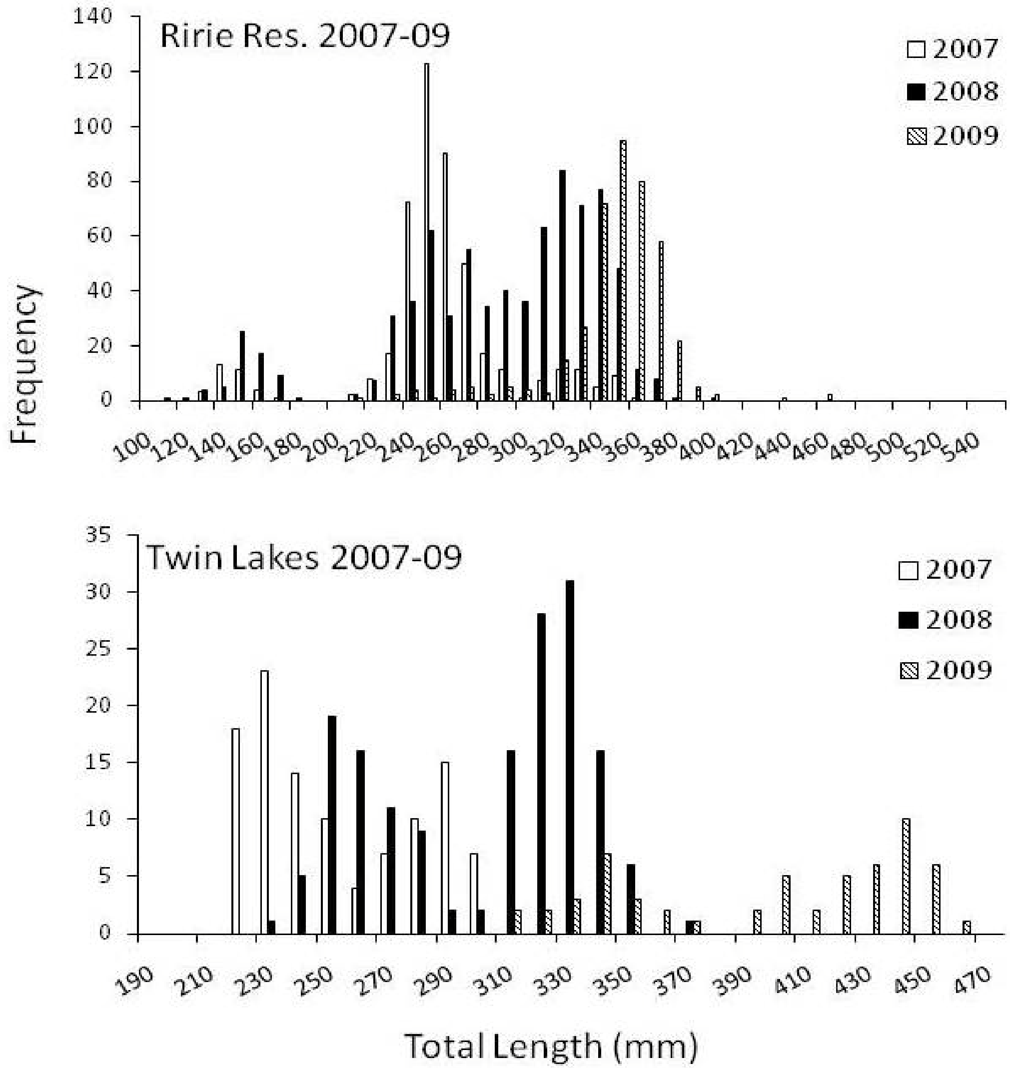

Figure 5. Continued.

ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT INVESTIGATIONS: PRODUCTION OF STERILE TROUT: WESTSLOPE CUTTHROAT

State of: Idaho

Grant No.: F-73-R-33 Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #2: Production of Sterile Trout

Contract Period: July 1, 2010 to June 30, 2011

ABSTRACT

The genetic conservation of wild populations is a management priority for the Idaho Department of Fish and Game (IDFG). However, the majority of trout stocked in Idaho alpine lakes are diploid westslope cutthroat trout *Oncorhynchus clarkii lewisi*, and a sterile cutthroat program at IDFG has yet to be developed. Methods for producing triploid westslope cutthroat were investigated during 2010. Triploid induction rates and survival rates (to eye-up) were tested using 9,500 psi of pressure at three different time periods after fertilization. Control groups had the highest eye-up rates, with an average of 31% overall. Treatments #1 and #2 both showed high mean 3N induction rates, with 100% triploid fish across all replicates. Treatment #3 had only slightly lower 3N induction (98% on average), as one diploid fish was sampled. Survival to eye-up differed slightly across treatments, decreasing as the time after fertilization increased. Survival to eye-up was consistently low for Replicate #1 in all treatments and control groups, suggesting poor egg quality. Eye-up rates were much higher for both Replicate #2 and #3. Treatment #2 (9,500 psi, 5 min, 300 Celsius-minutes after fertilization) appears to be the preferred treatment for westslope cutthroat trout. This combination has shown good results in previous IDFG experiments, and again provided the best overall results during this trial.

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INTRODUCTION

Triploid salmonids are functionally sterile, and the common assertion is that sterility provides a fisheries or aquaculture benefit (Benfey 1999). Several investigators reported enhanced hatchery performance in terms of growth and food conversion for age-1 and older triploids (Lincoln and Scott 1984; Bye and Lincoln 1986; Boulanger 1991; Habicht et al. 1994; Sheehan et al. 1999). However, triploid salmonids produced by temperature or pressure shock may suffer lower fertilization rates, increased mortality, or reduced growth from egg through initiation of feeding (Solar et al. 1984; Happe et al. 1988; Guo et al. 1990; Oliva-Teles and Kaushik 1990; Galbreath et al. 1994; McCarthy et al. 1996). Despite these early rearing disadvantages, triploid performance appears to improve with age. Several authors have studied post-stocking performance of triploid salmonids including rainbow trout *Oncorhynchus mykiss* (Simon et al. 1993, Brock et al. 1994, Koenig 2010), kokanee *O. nerka* (Parkinson and Tsumura 1988), brook trout *Salvelinus fontinalis* (Warrillow et al. 1997) and Atlantic salmon *Salmo salar* (Cotter et al. 2000). However, little data regarding triploid westslope cutthroat trout *O. clarkii lewisi* is currently available.

The Idaho Department of Fish and Game (IDFG) Fisheries Management Plan (IDFG 2007) outlines several guidelines regarding the management of alpine lakes. The genetic conservation of wild populations is a management priority for the Idaho Department of Fish and Game. The IDFG has established a policy to stock only triploid rainbow trout in systems where reproduction between wild or native trout and hatchery fish is possible (IDFG 2007). This policy has resulted in widespread stocking of sterile rainbow trout in most Idaho reservoirs and many alpine lakes. However, the majority of trout stocked in Idaho alpine lakes are westslope cutthroat trout, and a sterile cutthroat program at IDFG has yet to be developed. As a result, diploid cutthroat trout are still routinely stocked across Idaho.

Induced sterility in westslope cutthroat trout could further reduce impacts of stocking on Idaho's native and wild fish populations. The development of sterile westslope cutthroat trout would give managers another alternative for stocking high mountain lakes and would further reduce the potential for intraspecific hybridization throughout central and northern Idaho. Previous IDFG research began to develop techniques for triploid westslope cutthroat trout (Kozfkay 2005), but further refinement in pressure shocking recipes was needed. In this progress report, I compare several pressure shock recipes to induce triploidy in westslope cutthroat trout.

METHODS

I conducted a pressure shock experiment to induce triploidy in westslope cutthroat trout at Cabinet Gorge Fish Hatchery on May 4, 2010. To reduce variation among treatments due to egg quality, eggs from 19-23 females were pooled together for each replicate. Approximately equal numbers of eggs were split four ways (three treatments and one control) from the pooled eggs, and then fertilized using pooled milt from 4-5 males and activated with water. Approximately 89 ml of eggs were used for each replicate. Egg counts indicated approximately 15 eggs/ml at the time of the experiment, with an average fecundity of about 400 eggs per female. Any leftover eggs not used in the treatments were added to normal production lots.

After mixing eggs and milt for several minutes, eggs were then rinsed one or two times before waiting to be pressure treated. All treatments used the same pressure (9,500 psi) and duration (5 minutes), but the time elapsed after fertilization before pressure treatment varied.

Treatments consisted of 300, 350, and 400 Celsius Minutes After Fertilization (CMAF, water temperature x time). Water temperature during the experiments was 7.8°C. The hydraulic pressure chamber, Model HPC™, used during this experiment was built by TRC Hydraulics Inc., Dieppe, New Brunswick, Canada. The 2.7 L chamber was filled with ambient hatchery water before egg treatment. Fertilized eggs were placed in a perforated aluminum cylinder for loading and unloading and transferred to incubation units after the treatment was applied. After receiving pressure treatment, eggs were then water hardened for 60 minutes, followed by disinfection with iodine for 10 minutes. Eggs were then moved to indoor vertical upwelling cylinders for the entire rearing period. Each treatment replicate and controls were reared in separate cylinders at 9.4°C well water.

Triploid induction rates were determined by analyzing blood samples using flow cytometry. Fish were approximately 52 mm (295 fish/pound) at the time the blood samples were collected. Up to 50 blood samples were collected per treatment replicate where possible. Some replicates with poor survival had as few as 20 fish remaining, in which case all fish were sampled. Blood samples were stored in Alsever's solution and shipped to North Carolina State University where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw. The triploid induction rate was determined as the proportion triploid of the total tested in each replicate. Confidence intervals (95%) around the proportion triploid were calculated according to the methods outlined in Fleiss et al. (2003).

RESULTS

Triploid induction rates were very similar across all three treatments. Treatments #1 and #2 both showed high mean 3N induction rates, with 100% triploid fish across all replicates. Treatment #3 had only slightly lower 3N induction (98% on average), as one diploid fish was sampled from egg lot #10 (Table 11). As with previous trials, 9,500 psi for 5 minutes appears to provide consistent induction rates. When comparing the time after fertilization to apply the pressure shock, 300 CMAF appears to provide excellent induction rates, yet seems to return higher average survival to eye-up (Table 11).

Survival to eye-up differed slightly across treatments, decreasing as the time after fertilization increased. Treatment #1 had the highest eye-up rates (27% on average) next to the Control group (31% on average). Mean survival to eye-up was 16% and 18% for Treatment #2 and #3, respectively. Eye-up rates were low for all egg lots from Replicate 1, including controls (Table 11), suggesting low egg quality may have been a problem. When not including eggs from Replicate #1, mean eye-up rates were much higher, ranging from 21% to 38%. Treatment #1 (9,500 psi, 300 CMAF, 5 minutes) returned the best results overall, with excellent 3N induction rates and survival to eye-up comparable to controls.

DISCUSSION

During this experiment, triploid induction rates for pressure treated westslope cutthroat trout eggs were high for all treatment recipes. Previous pressure shock experiments conducted in 2004 using 9,500 psi for 5 minutes produced similar results with a mean triploid induction rate of 99% (Kozfkay 2005). However, Kozfkay (2005) reported much higher average eye-up rates of 34% and 52% for pressure treatments of 200 and 300 CMAF, respectively. My results show very high average triploid induction rates, but mean eye-up survival was lower compared to previous trials. The first group of pooled eggs used in Replicate #1 showed consistently low survival across all treatments and controls, suggesting that poor egg quality may have played a role. I would expect eye-up rates to increase in future years as the Cabinet Gorge broodstock matures and eggs quality improves. This year's egg take consisted mainly of age-2 and some age-3 fish, so younger eggs may have played a role in the lower eye-up rates. Poor eye-up rates could also have been a result of the lack of normal disinfectant treatments, which are usually applied to normal production lots reared in trays. These treatments were not applied to the egg lots reared in the vertical upwelling cylinders.

The time after fertilization appears to be important in determining the correct recipe. While 350 and 400 CMAF provided adequate induction rates, eye-up survival for these treatments were consistently lower than 300 CMAF treatments. Conversely, Kozfkay (2005) showed better results with increased time after fertilization, with 200 CMAF returning lower survival compared to 300 CMAF. Treatment #2 (9,500 psi, 5 min, 300 CMAF) appears to be the preferred treatment for westslope cutthroat trout. This combination has shown good results in previous IDFG experiments, and again provided the best overall results during this trial. Future efforts to produce triploid westslope cutthroat trout could use this recipe and expect good results.

RECOMMENDATIONS

1. Efforts to culture sterile westslope cutthroat trout should use pressure shocking techniques to induce triploidy to ensure consistent high-quality results. I recommend using a combination of 9,500 psi, 5 minutes shock duration at 300 CMAF for best results.

2. Evaluate performance of triploid westslope cutthroat trout in high alpine lakes to determine stocking densities necessary to provide satisfactory catch rates while protecting against genetic impacts to wild stocks.

ACKNOWLEDGEMENTS

John Rankin and Jamie Mitchell of Cabinet Gorge Hatchery did an excellent job helping with spawning and pressure shocking experiments. They also did a fantastic job rearing each test individual test group and were very helpful when it came time to collect blood samples. I would like to thank Joe Kozfkay for his assistance performing these experiments.

Table 11. Percent eye-up and triploid induction rates for three pressure treatments of westslope cutthroat trout eggs at Cabinet Gorge Hatchery.

Treatment	Egg lot	Replicate	% Eye up	Num sampled	Num 3N	%3N	Lower bound	Upper bound
				Control				
	1	1	13	-	-	-	-	-
C	2	2	36	-	-	-	-	-
	3	3	45	-	-	-	-	-
		Mean	*31*					
				9,500psi / 300 CMAF / 5 min duration				
	4	1	7	20	20	100	80	100
1	5	2	37	49	49	100	91	100
	6	3	38	50	50	100	91	100
		Mean	*27*	*119*	*119*	*100*	*96*	*100*
				9,500psi / 350 CMAF / 5 min duration				
	7	1	8	23	23	100	82	100
2	8	2	27	50	50	100	91	100
	9	3	14	48	48	100	91	100
		Mean	*16*	*121*	*121*	*100*	*96*	*100*
				9,500psi / 400 CMAF / 5 min duration				
	10	1	6	48	49	98	88	100
3	11	2	25	50	50	100	91	100
	12	3	24	51	51	100	91	100
		Mean	*18*	*149*	*150*	*99*	*96*	*100*

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.

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'Maoiléidigh, 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.

Fleiss, J. L., B. Levin, and M. C. Paik. 2003. Statistical methods for rates and proportions, 3rd edition. John Wiley & Sons, Inc., Hoboken, New Jersey.

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.

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). 2007. Fisheries Management Plan 2007-2012. Idaho Department of Fish and Game, Boise.

Koenig, M. 2010. Hatchery trout evaluations. Job Performance Report 10-06, Project F-73-R-32, Idaho Department of Fish and Game, Boise.

Kozfkay, J. 2005. Hatchery trout evaluations. Job Performance Report, Project F-73-R-27, Idaho Department of Fish and Game. Boise.

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.

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.

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.

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.

APPENDICES

Appendix A. Application for Short-term Activity Exemption describing chemical treatment of Grass Mountain Lakes #1 and #2 connecting stream and outlet stream in fall 2010.

APPLICATION FOR SHORT-TERM ACTIVITY EXEMPTION

Applicant: Idaho Department of Fish and Game (IDFG)

Contact Person: Dale Allen, Paul Janssen, 634-8137

Body of Water: Grass Mountain Lakes (upper and lower)

Tributary To: Hard Creek (Little Salmon River)

Objective: To chemically eradicate exotic, stunted, brook trout (Salvelinus confluentus) and re-establish rainbow trout.

Date: September 2010

Evidence of protection or promotion of public interest

Two mountain lakes; Grass Mountain Lakes, upper and lower are located in the Hard Creek Drainage of the Little Salmon River drainage (Figure 1). Both lakes have a long history of overpopulation by brook trout (*Salvelinus confluentus*). Surveys of these lakes in 1984 and 1986 indicated the presence of large numbers of small brook trout averaging 215 mm and 200 mm in the lower and upper lakes respectively. Chinook salmon were stocked in both lakes in 1984 in an attempt to reduce the number of brook trout and increase average size. These fish did not survive long enough to consume 200 mm brook trout.

In 2007, tiger muskie were introduced into both upper and lower Grass Mountain lakes to prey on and remove as many brook trout as possible with the hopes of removing this species and allowing the re-establishment of a native trout species such as rainbow trout or cutthroat trout. Fish survey work in August 2010 revealed that tiger muskie had reduced brook trout numbers drastically and most tiger muskie had been removed via nets, angling, and natural mortality. However, many juvenile brook trout were observed in the small creek that connects the two lakes. Juvenile brook trout were also observed in the outlet stream. These two areas are refuges for juvenile brook trout as they are too shallow for tiger muskie to enter and effectively feed on the small brook trout.

To attempt to finish the brook trout removal project, we propose to use rotenone to remove brook trout from the two refuge areas as well as any inlet areas that are found to hold brook trout on the day of the treatment. The outlet stream flowing out of the lower Grass Mountain Lake will be treated downstream for approximately 120 m to a natural fish barrier.

Previous fish surveys in Hard and Hazard Creek drainages, downstream of the Grass Mountain lakes, indicated primarily brook trout and a small rainbow trout presence, therefore a fish kill below the barrier would be acceptable to IDFG. However, with all the tributaries entering Hard

Creek and Hard Creek itself below the lakes, we would anticipate a fish kill down only to the main Hard Creek.

Although a fish kill from Grass Mountain Lakes down to the main Hard Creek is acceptable, (as we would kill primarily brook trout) we would prefer not to kill fish below the barrier. Therefore, a rotenone deactivation drip station (potassium permanganate) will be placed just below this fish barrier to neutralize rotenone and minimize fish kill downstream.

Prevention of long-term injury to beneficial use

The IDFG plans to stock both Grass Mountain Lakes in the fall of 2010 with fingerling rainbow trout to provide a fishery within the next three years.

Flows of the stream between both lakes and the outlet stream of the lower lake have been calculated and will be recalculated on the day of the treatment. Drip stations will be located at the head of the connecting stream and the head of the outlet stream on the lower lake. Drippers will be loaded at label rates and will run for 2 to 4 hours. The deactivation drip station will run until all rotenone has passed the treatment section. Fluorescein dye will be added to the outlet rotenone dripper to enable us to monitor rotenone movement to the deactivation dripper. If we can collect small fish they will also be used as a bio assay to ensure an effective treatment and to monitor effectiveness of the deactivation dripper.

The main body of the two lakes will not be treated.

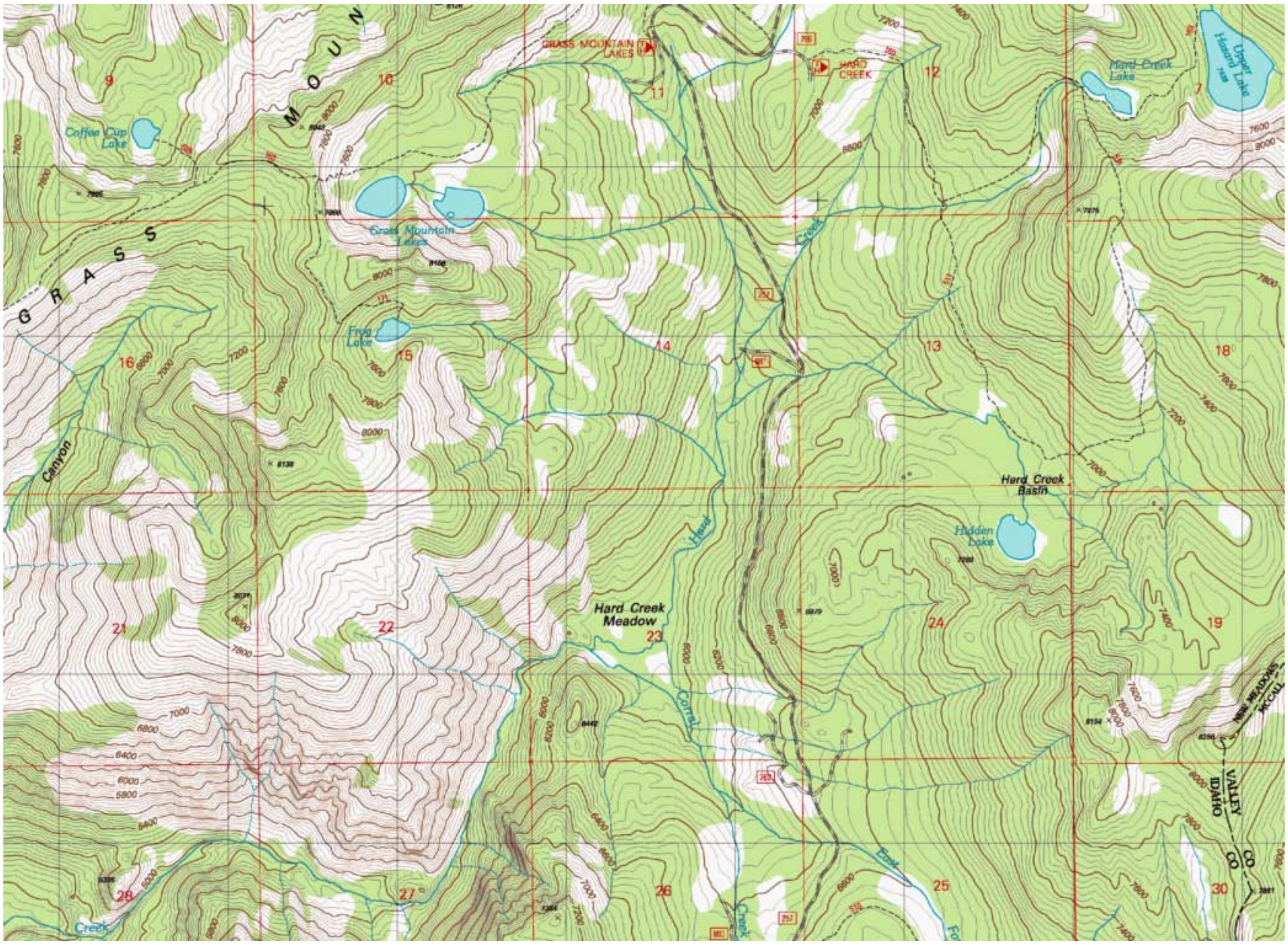

Figure A1. Project area for Grass Mountain Lakes chemical treatment to reduce brook trout.

Appendix B. Rotenone Application Record describing chemical treatment of Grass Mountain Lakes #1 and #2 connecting stream and outlet stream in Fall 2010.

Idaho Department of Fish and Game
555 Deinhard Lane
McCall, Idaho 83638
Rotenone Application Records

Location of Application: Grass Mountain Lakes 1 & 2

Date of Application: 9/27/10

Time: 1100-1900 Fish Species Targeted: brook trout

Brand of Chemical used: Prentiss Synpren-Fish Toxicant EPA Registration #: 655-421
Brand of Chemical used: EPA Registration #:
Brand of Chemical used: EPA Registration #:

Length of Streams Treated: 300 m between upper and lower lakes, and 305 m below lower lake outlet.

Stream Flow Rate (CFS): .016 cfs below upper lake and .017 cfs below lower lake.

Amount of Chem. Applied to Streams: 70 cc's below upper lake, 60 cc's below lower lake and 5 cc's in small spring below lower lake.

Length of Treatment in Hours: One, 2 hour dripper just below the outlet of both the upper and lower lakes.

Area of standing water treated: 00 Amount of Chem. Applied to Standing Water: NA

Amount of Powder Applied to All Areas: 0

Name/License Number of Applicator(s): Martin Koenig/51742, Paul Janssen/43145

Name of Property Owner: USFS

Rate of Application: 1.5 ppm below lower lake outlet, 2 ppm below outlet of upper lake.

Wind Speed and Direction: Calm

Person who Recommended the Product: IDFG

Worker Protection Information Exchange: NA

Comments: A rotenone deactivation drip station was setup approximately 312 m feet below the outlet of the lower Grass Lake to prevent fish kills further down the drainage. The dripper applied a concentration of 4.5 ppm of potassium permanganate ($KmNo^{4)}$ and was operated for 3 hours. The deactivation dripper was started when fluorescein dye (mixed with the rotenone) was observed just above the detoxification dripper. No dead brook trout were observed below the drip station after the treatment concluded.

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

Test Group	Number Stocked	Total Caught
2N	110,946	34
3N	102,523	22

Adjusted 3N stocked = Number stocked x 79% = 80,993
Adjusted 2N stocked = 102,523 – 80,993 = 21,530

Relative survival of 2N kokanee is the number of 2N caught divided by 2N stocked:

2N Relative Survival ($2N_s$) = $\frac{2Ncaught}{2Nstocked} = \frac{34}{110,946} = 3.065 \times 10^{-4}$

Using that survival, we estimate how many of the fish marked as 3N could actually be 2N based on the 79% induction rates (corrected # stocked) and the 2N survival rate:

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

The number of actual 3N in the marked-3N group is then the total captured minus the estimated number of 2N:

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

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

This is the ratio of 2n:3n fish using the survival estimates and the corrected numbers of stocked fish:

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

If we used 22 as the number of 3N kokanee caught and did not correct the stocking numbers, we can look at what the uncorrected survival ratio would have been:

2N:3N Uncorrected survival ratio = $\frac{2Ns}{3Ns} = \frac{3.065 \times 10^{-4}}{2.145 \times 10^{-4}} = 1.13$

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