



WILD TROUT EVALUATIONS: MY BROOK TROUT IN LAKES



Report Period July 1, 2015 to June 30, 2016

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M_{YY} BROOK TROUT IN LAKES

Annual Performance Report July 1, 2015 to June 30, 2016

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ANNUAL STOCKING OF M_{YY} BROOK TROUT TO ERADICATE WILD NONNATIVE BROOK TROUT AT SELECT ALPINE LAKES IN IDAHO

ABSTRACT

Nonnative Brook Trout *Salvelinus fontinalis* were introduced throughout western North America in the early 1900s, resulting in widespread self-sustaining nonnative populations that are difficult to eradicate and often threaten native salmonid populations. A novel approach to eradicating undesirable Brook Trout populations is using YY male (M_{YY}) Brook Trout (created in the hatchery by feminizing XY males and crossing them with normal XY males). When M_{YY} Brook Trout reproduce successfully with wild females, it shifts the sex ratio of the wild population toward males. Through many annual stocking events, model simulations suggest the population would be eradicated after the sex ratio reached 100% males and there are no females left in the population to reproduce. Fingerling-sized M_{YY} Brook Trout were stocked in four lakes and catchable-sized M_{YY} Brook Trout in four lakes in central Idaho in 2015 and 2016. For these two sets of lakes, gillnet removals were conducted at half of the lakes, hereafter suppression lakes, prior to stocking to reduce competition with wild trout, and increase survival of M_{YY} Brook Trout. At all lakes gillnets were fished for a minimum of three net nights to characterize relative abundance. For all wild Brook Trout removed, total lengths, weights, phenotypic sex, were recorded and sagittal otoliths were removed for estimating age composition and mortality. Tissue samples were obtained from each fish to estimate genetic sex ratios of the wild Brook Trout population before stocking. Gill nets were set for an average of 15.7 hours over 57 net-nights. The amount of trout biomass removed from suppression lakes averaged 8.5 kg. In contrast, sampling at other lakes removed an average of 4.0 kg of salmonid biomass. The stocking biomass of fingerling-sized M_{YY} Brook Trout averaged 46.6 kg over two years. In 2016, the biomass of catchable-sized M_{YY} Brook Trout stocked averaged 60.7 kg. Based on uncorrected catch curves, annual mortality rates of wild Brook Trout of all ages averaged 0.59 across all ten lakes, but ranged from a low of 0.34 at Disappointment Lake to a high of 0.78 at Lloyds Lake. Genetic sex ratios of wild Brook Trout averaged 49.4% males over all populations. Sex ratios by age confirmed the assumption that males and females comprise equal proportions of the population at age-0, though after age-1, males comprised a slightly higher proportion of each age group. Concordance between genetic sex and phenotypic sex averaged 93.1% but this lower rate was partially attributed to insufficient training, which will be improved in the future. Though 9-12 net nights were fished at suppression lakes, the gill net removals may not have suppressed the wild population enough to identify a difference in M_{YY} Brook Trout survival between treatment levels (suppression and non-suppression). Future monitoring will identify if stocking rates were sufficient to shift the sex ratio as expected.

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INTRODUCTION

Brook Trout *Salvelinus fontinalis* were originally introduced outside their native range in western United States waters by the U.S. Fish Commission (MacCrimmon and Campbell 1969; MacCrimmon et al. 1971) and continue to colonize new habitats in western North America (Benjamin et al. 2007). Brook Trout have contributed to declines in native fish abundance through hybridization, competition, and predation (Rahel 2000). Thus fisheries managers have worked to suppress or eliminate Brook Trout populations outside of their native range (reviewed in Dunham et al. 2004). There are several methods fisheries managers can use to eradicate nonnative fish. Managers have used piscicides with some success (Gresswell 1991; Lee 2001; Lentsch et al. 2001; Hepworth et al. 2002), though piscicides result in collateral damage to native fish populations (Britton et al. 2011), and other aquatic fauna (e.g., Hamilton et al. 2009; Billman et al. 2012). Multiple-pass electrofishing has been used with mixed results to physically remove Brook Trout from streams (e.g., Thompson and Rahel 1996; Meyer et al. 2006; Shepard et al. 2014), and it has been questioned whether stream electrofishing removal by itself can make meaningful progress in Brook Trout eradication at the landscape scale (Schill et al. 2017). Most recently, sterile predatory fish were introduced in alpine lakes and successfully eradicated Brook Trout in some (but not most) of the lakes (Koenig et al. 2015). The mixed success of these methods identifies the need for an additional method for nonnative fish eradication.

One method suggested decades ago for eradicating undesirable fish populations is to shift the sex ratio of the population toward all males (Hamilton 1967). In this scenario, shifting the population sex ratio over time could be accomplished by annual introductions of hatchery produced male fish with a YY genotype (M_{YY}) to eliminate females, eventually resulting in population eradication (Gutierrez and Teem 2006; Teem and Gutierrez 2010). To create an M_{YY} brood stock, XY males are feminized by exposing them to estrogen. After rearing to maturity, the resulting XY neo-females are crossed with normal XY males and, on average, one-quarter of the subsequent progeny will be M_{YY} (Teem and Gutierrez 2010). Then, by exposing half of the M_{YY} fish to estrogen at an early age, an M_{YY} and F_{YY} broodstock can be created, and all their progeny are M_{YY} . These M_{YY} progeny can then be stocked into wild populations of fish in an effort to drive the sex ratio of the wild population to 100% males (Parshad 2011). Although YY fish culture is occasionally used in commercial hatcheries (e.g. Mair et al. 1997; Liu et al. 2013), a stocking program utilizing YY fish has not yet been tested in the wild to eradicate a non-native fish species (Wedekind 2012; Wedekind in press).

Sex ratios in wild Brook Trout populations would only shift under such a stocking program if the M_{YY} Brook Trout survive and successfully reproduce after stocking. A pilot study estimated an average of 11% of M_{YY} Brook Trout survived for three months and successfully reproduced with wild females after they were stocked in four streams in Idaho (Kennedy et al. 2018). All hatchery trout encounter many challenges upon their liberation into the wild, and due to several factors, often exhibit low survival, especially in streams (e.g., Miller 1952; Bettinger and Bettoli 2002; High and Meyer 2009). Low survival in streams is largely attributed to the stress associated with adjusting to natural stream flows and competition with resident fish (Schuck 1948; Miller 1958; Hochachka and Sinclair 1962). Competition with wild resident fish has been identified as a major factor contributing to the low survival of hatchery trout in streams (Miller 1954). Though rarely evaluated, past studies suggest manual removal (hereafter suppression) of wild fish prior to stocking hatchery fish could markedly improve survival of the stocked hatchery trout (Miller 1958; Horner 1978). In addition, modelling by Schill et al. (2017) suggested that suppression of wild fish may decrease the time-to-eradication in Brook Trout populations, which would result in fewer years of stocking and greater efficiency of an M_{YY} eradication program for fishery managers.

Post-release mortality of hatchery trout is also affected by size-at-release. Adult hatchery trout of catchable-size (>220 mm), hereafter referred to as catchables, generally survive at a much higher rate than juvenile hatchery trout, hereafter referred to as fingerlings (Wiley et al. 1993; Dillon and Jarcik 1994). The greater survival of catchables may result from larger energy reserves and reduced vulnerability to post-release predation and competition with wild fish. On the other hand, catchables are immediately vulnerable to anglers upon release, whereas fingerlings must survive and grow for months or perhaps more than a year before they are large enough to be vulnerable to angling gear. Most work comparing survival between catchables and fingerlings has focused on put-and-take fisheries to evaluate return-to-creel but the difference in short-term survival between fingerlings and catchables is not known. This difference in survival is of much interest in the case of M_{YY} fish. Even four-five month survival of catchables to the first spawning season may result in faster eradication relative to fingerlings.

The Idaho Department of Fish and Game (IDFG) produced a YY Brook Trout brood stock to annually produce M_{YY} Brook Trout for stocking (Schill et al. 2016). Survival and reproductive success of M_{YY} Brook Trout in the wild were evaluated in Kennedy et al. (2018). Recent modelling results suggest that annual stocking of M_{YY} Brook Trout into alpine lakes should result in eradication of the wild population within 10 years if M_{YY} Brook Trout are stocked at a rate of 50% of the initial wild Brook Trout abundance, with faster eradication times likely as suppression of the wild population is increased (Schill et al. 2017). However, these models are theoretical, and need to be tested on wild Brook Trout populations to validate predictions.

OBJECTIVES

1. Monitor the reproductive success of M_{YY} fish and resulting changes in sex ratios in select wild Brook Trout populations to determine if fingerling or catchable M_{YY} Brook Trout are more effective at eradicating wild Brook Trout populations from alpine lakes.
2. Determine if suppressing the abundance of wild Brook Trout populations decreases the mortality of stocked M_{YY} Brook Trout and thereby increases M_{YY} reproductive success and the subsequent rate that sex ratios shift in wild Brook Trout populations in alpine lakes.

METHODS

The IDFG experimentally feminized male Brook Trout fry with estrogen (in the form of 17β -estradiol) to create an adult broodstock of YY Brook Trout. For complete details of YY broodstock production, see Schill et al. (2016). Offspring were produced by crossing F_{YY} and M_{YY} broodstock at the Hayspur Hatchery. Fish were reared to fingerling and catchable-size at Mackay Hatchery in outdoor concrete raceways in 10-12°C single-use spring water until the time of release. For this study, fingerlings and catchables were stocked at approximately 4 and 16 months after hatching, respectively.

Study lakes were selected based on the criteria that Brook Trout comprised a large majority (>80%) of the fish species composition in the lake (Figure 1; Table 1). In 2015, two treatment levels were initiated in lakes for fingerling stocking (suppression and non-suppression), and in 2016 the same treatment levels were initiated in lakes for catchable stocking. Suppression was achieved through the removal of wild trout mainly with gill nets; non-suppression lakes had M_{YY} Brook Trout stocked without suppression of their wild counterparts, except those wild fish that were sampled with gill nets to characterize the wild population. Two control lakes were selected

nearby to monitor the stochastic changes naturally occurring in wild Brook Trout populations at alpine lakes in central Idaho. All treatment lakes will be stocked annually, for a minimum of seven years, unless the population collapses and intensive sampling identifies that no female (F_{xx}) Brook Trout remain. Evaluation of each Brook Trout population's change from wild Brook Trout to M_{yy} Brook Trout at all study lakes will occur approximately every three years until the wild population is considered eradicated.

Sampling

To capture fish at alpine lakes, floating Swedish experimental gill nets (36 m long and 1.8 m deep) consisting of nylon mesh panels of 10, 12.5, 18.5, 25, 33, and 38 mm bar mesh were set overnight. Net locations were selected to maximize catch, based on professional experience. One net per night constituted one unit of effort. Data collected from captured fish included: species, total length (TL; mm), weight (g), any marks, and the mesh size that captured the fish. Gonads were exposed for observation by making a ventral mid-line incision along the entire body cavity. Males were classified as immature if testes were dorsally restricted, opaque, and thread-like, and mature if they were large and milky white. Females observed were classified as immature if their ovaries were small, translucent, granular, and dorsally restricted, and mature if they possessed eggs in advanced stages of development filling much of the abdominal cavity (Downs et al. 1997; Meyer et al. 2003; Schill et al. 2010). Sagittal otoliths were removed from up to ten wild Brook Trout in each 10-mm length class and preserved dry in 1.5-mL microcentrifuge tubes and stored indoors away from direct sunlight for future age determination.

For suppression lakes, at least nine net-nights were conducted to suppress wild Brook Trout abundance in an effort to reduce competition with stocked M_{yy} Brook Trout. Suppression of the wild Brook Trout only occurred prior to the first stocking. The amount of biomass (kg) removed was estimated for each species by adding the weight measurements (g) from all fish removed from each lake. All wild Brook Trout were overdosed with anesthetic, sacrificed, and sunk (by puncturing the air bladder) in the middle of the lake to avoid public concern.

Relative weights (W_r) were calculated (Brouder et al. 2009) for each fish from observed lengths and weights, then averaged to describe growth for each population. Mean W_r for each population was compared to mean W_r of stock-quality size ($W_r = 91.9$) Brook Trout sampled by gill netting in Ecoregion 5 (Brouder et al. 2009) to determine if growth was low, indicating an overabundance of Brook Trout.

We collected tissue samples to estimate genetic sex ratios of the wild Brook Trout populations in treatment lakes prior to stocking. Sex-biased mortality was anticipated in mature Brook Trout due to the stresses associated with spawning and size-selective harvest by anglers (McFadden 1961). Fry were assumed to be exempt from these biases so equal sex ratios for males and females were anticipated (Fisher and Bennett 1999). In lakes, however, fry may not be efficiently recruited to gill nets, so describing the change in sex ratios will be estimated using tissue sampled from all fish recruited to all gear types. Tissue samples from fry were obtained opportunistically with backpack electrofishing when fry were observed at stream inlets or lake outflows. Minnow traps (25.4 x 25.4 x 43.2 cm; 3.2 mm sq. mesh) baited with either dog food, Berkley trout eggs, marshmallow trout bait, Powerbait®, or tuna in oil were also used to target fry along shorelines, and lake inlets and outlets.

Wild Brook Trout were captured by anglers using fly and spinning tackle for future evaluation of angler use at alpine lakes. Angled Brook Trout (>150 mm TL) were tagged using 70 mm (51 mm of tubing) fluorescent orange/green T-bar anchor tags (Dell 1968). Anchor tags were

labeled with “IDFG” and tag reporting phone number (IDFG 1-866-258-0338) on one side, with the tag number on the reverse side. Anglers could report tags using the IDFG “Tag! You’re It!” phone system and website, as well as at regional IDFG offices or by mail (for details see Meyer et al. 2012). Angler exploitation was calculated seven months after the latest stocking (August 2016), following methods from Meyer and Schill (2014). A goal of 30 tags per lake was pursued at each sampling trip, with 10% of tags being \$50 reward tags. Angling and tagging were conducted upon all visits to study lakes. With multiple visits over subsequent years, eventually the stocked fingerling M_{YY} Brook Trout will be tagged (at the same rate as wild fish) when they are large enough to be recruited to angling gear. Reported tags will be pooled across all study lakes to describe angler use at alpine lakes. If public angling effort and tag reporting is high enough, differential harvest between wild and M_{YY} fish will be evaluated.

Abundance and mortality

During the first visit at each lake, three gill nets (all set for one night) were set to describe the fish species composition, and relative abundance estimated by catch-per-unit-effort (CPUE). Catch-per-unit-effort at each lake was calculated as the average catch-rate of Brook Trout (number of Brook Trout/net-night). Relative abundance will be estimated again at three, five, seven, and nine years after the initial stocking, for all study lakes.

Whole otoliths were photographed in immersion oil using reflective light at 40X power for sections and 25X power for whole otoliths 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 the smallest Brook Trout (<50 mm) recruited to electrofishing or minnow traps from each lake. Ages were estimated by two individuals, unaware of fish length, using photographs of the otoliths. When ages were not in agreement between the two readers, fish length was included for consideration to resolve the discrepancy and to determine a consensus age. The age composition and mean length-at-age was estimated for the wild Brook Trout population at each study lake. Instantaneous natural mortality (M) was estimated following methods outlined by Quinn and Deriso (1999) using the equation:

$$M = -\ln(P_s)/t_{max},$$

where t_{max} is the maximum age observed in the population and P_s is the proportion of the population that survives to t_{max} , which was assumed to be 1% (Quinn and Deriso 1999). Conditional natural mortality (n) was estimated from M by:

$$n = 1 - e^{-M}.$$

Following Ricker (1975), n was equal to actual mortality (A) because fishing mortality (m) is assumed to be zero at these lakes until anchor tag return data can provide further information.

$$A = m + n - mn.$$

Stocking

In the western United States, trout in alpine lakes are stocked as fry or fingerlings at densities ranging from 50-250 fish/acre (Meyer and Schill 2007). The Idaho Department of Fish and Game commonly stocks at 200 fish/acre, but at a size of about 35 mm; because the M_{YY} fingerlings available were larger than this, the fingerling stocking density slightly was first reduced from 200/acre to 175/acre. At the time of stocking (August), M_{YY} fingerling Brook Trout were about

1/5 the weight of catchables, so catchable stocking rates were reduced to 35/acre to standardize the amount of biomass stocked in each alpine lake. A 5:1 stocking ratio of fingerlings to catchables in alpine lakes is also supported by the fact that few M_{YY} fingerlings and nearly all M_{YY} catchables are mature in their first year of stocking (P. Kennedy, unpublished data), and wild Brook Trout populations typically exhibit about a 4:1 ratio of immature:mature fish (McFadden 1961; Meyer et al. 2006). Thus, a stocking rate of 4-5 times as many immature fish as mature fish makes sense from both biological standpoints. A review of abundance data for Brook Trout in alpine lakes provided very little guidance, but available data (Hall 1991; Hand et al. 2012) showed high densities of wild adult Brook Trout range from 60-694 fish/acre, averaging 268 fish/acre (Appendix 1) in the western United States. However, lake surface elevations in California, described by Hall (1991; Appendix 1), were much higher than these selected study waters (Table 1), and the Brook Trout densities at those higher elevations were significantly higher than densities in Idaho estimated by Hand et al. (2012). Therefore, the mean densities of adult wild Brook Trout estimated by Hand et al. (2012; 120 fish/acre) was assumed to be typical at alpine lakes in Idaho dominated by Brook Trout. Thus, these stocking rates equate to an assumed stocking density (stocked M_{YY} compared to wild abundance) of 17% for catchables and 146% for fingerlings.

Stocking in August allowed sampling to occur in late July to reduce unnecessary mortality of recently stocked M_{YY} fish. Fingerlings were transported to each lake using a helicopter and bucket (90-100 gal capacity SEI Industries Bambi Bucket®), because they were too large to be stocked with fixed-wing aircraft without significant mortality. Precise preliminary estimates of average fish weight (number of fish/lb) were helpful for the necessary helicopter load calculations. Fish loading densities and water displacement were calculated following Piper et al. (1982; Appendix 2). To maximize fish health during transport, target fish loading densities were less than 1.0 lb fish/gal. Load calculations were estimated for the amount of fish and water needed for each lake (Appendix 2). The number of flights to each lake was determined by the helicopter's (Hughes/MD 500) safe load capacity (700 lbs.) and to keep fish load densities under 1.0 lb of fish/gal, and total flight distances were planned to deliver the required amount of fish and water. The number of fish for each flight (estimated via pound counts) was transferred from a hatchery tanker truck to a 100 gal (379 L) Rubbermaid® stock tank where dissolved oxygen was rigorously maintained at 10 ppm (Piper et al. 1982) using a YSI EcoSense® 200-4 dissolved oxygen probe. At each treatment lake, the pilot submerged the bucket and remotely removed the bottom seals of the bucket to allow the fish to swim free without dropping them. The pilot then filled the stocking bucket with lake water and returned to the helipad. Fish were quickly netted from the stock tanks into the helicopter bucket for transfer to the next lake. For safety purposes, coordinates were programmed into the helicopter GPS, and the pilot navigated to each study lake so that no fisheries personnel were on-board.

Genetic sex ratios and reproductive success

To genetically estimate reproductive success for wild Brook Trout populations, approximately 100 tissue samples were collected from wild Brook Trout from each study population during sampling. Tissue samples were clipped from the caudal fin and were preserved in vials filled with 100% ethyl alcohol.

Sex ratio monitoring

Samples were screened by the IDFG Eagle Genetics lab using two genetic markers that differentiate sex in Brook Trout: SexY_Brook1 (Schill et al. 2016) and the master sex-determining gene sdY (Yano et al. 2013). These two markers were screened in a multiplex PCR reaction along with an autosomal microsatellite marker (Sco102) to act as an internal control. Primer sequences

were as follows: SexY_Brook - Forward: GACAGAGACGTAGCCAG ACAAG, Reverse: CCCACCACACCACTCCTAAG; UsdYMod-Forward (modified from Angles et al. 2014): CCCAGCACTSTTTTCTTRTCTCA, Reverse: CTAAAAACYACTCCACCCTCCAT; and Sco102 (Bettles et al. 2005): Forward: CCATCTCTTCTTACCCTCCTC, Reverse: CCAAAA AGCAGTTGATAGACC. The forward primers of each marker were labeled with the carboxyfluorescein (FAM) fluorophore. Thermal cycling PCR reactions were performed in a 5 μ L volume consisting of 0.50 μ L of primer mix, 2.50 μ L of Qiagen Master Mix (cat. 206143), 1.00 μ L dH₂O, and 1.00 μ L template DNA (unknown concentration). Thermal cycling conditions were 95°C for 15 min followed by 25 cycles of 94°C for 30 s, 60°C for 1 min 30 s, and 72°C for 60 s, and then a final extension of 60°C for 30 min.

Amplification products were electrophoresed on a 3730 genetic fragment analyzer. Genetic sex was determined using the following rules: Individuals that amplified at Sco102 (peak height = ~131-135 base pairs; b.p.) and both SexY_Brook1 (peak height = ~161 b.p.) and UsdYMod (peak height = ~222 b.p.) were scored as “males.” Samples that amplified at Sco102 but not at SexY_Brook1 and UsdYMod were scored as “females.” Individuals that failed to amplify at Sco102 were not scored.

The accuracy of this multiplex marker set for differentiating sex in Brook Trout was previously validated by screening them on samples of known genetic sex from Schill et al. (2016). Tissue from 25 individuals of each sex from each treatment lake, whose phenotypic sex was identified in the field by dissection, was tested to further validate the sex marker described above. Tissue samples from each known-sex wild Brook Trout were processed as above for comparison against the phenotype determined from dissections.

Genetic assignment evaluation

The second method to evaluate reproductive success of M_{YY} Brook Trout involves the use of genetic assignment (GA) tests. Genetic assignment refers to a variety of genetic methods that ascertain population membership of individuals or groups of individuals (Manel et al. 2005). Under a GA approach, a sample is required from putative progeny and parents. This methodology is best used in scenarios where it will be impossible due to cost and time limitations to genetically sample all M_{YY} Brook Trout prior to release. This method is best used when study designs require stocking thousands of M_{YY} Brook Trout into large lakes or rivers.

There are several statistical software programs that can be used to identify progeny from two different populations using GA methodologies. One genetic software program that was used for M_{YY} research to identify offspring of M_{YY} Brook Trout is Structure (Pritchard et al 2000; Kennedy et al. 2018). Structure uses an admixture model that estimates a membership coefficient (Q), which represents the portion of an individual's genotype originating from a defined number of populations or genetic clusters (in this case, two). This is accomplished by genetically screening samples collected from both the M_{YY} population used for stocking and from samples obtained from the receiving wild population, prior to introductions of M_{YY} fish. The expectation is that progeny from M_{YY} adults and wild adults will have approximately equal probability of membership to each population (Q = 0.5).

While not reported on in this report, future monitoring of Brook Trout study populations will use the GA analysis to describe the origin of sampled fish as either progeny of wild or M_{YY} Brook Trout, though changes to sex ratios will be monitored as well. The rate at which the composition of wild Brook Trout changes to M_{YY} Brook Trout between suppression and non-suppression lakes, and for lakes stocked with fingerlings versus catchables, will be compared to describe the

effectiveness of each treatment level. All study lakes will be sampled approximately every three years to monitor the rate of change in wild to M_{YY} Brook Trout.

RESULTS

Sampling

Gill nets were set for an average of 15.7 (Appendix 3) hours per night, over 57 net-nights. Gill nets were fished for 12 net-nights at Black Lake and 9 net-nights at all other suppression lakes (Table 2), and 3 net-nights at each of the other six study lakes. The amount of salmonid biomass removed from suppression lakes averaged 8.5 kg (range 7.0-9.3; Table 2). In contrast, sampling at non-suppression and control lakes removed an average of 4.0 kg (range 1.5-5.7) of salmonid biomass.

The size distribution of wild Brook Trout captured in gill nets was similar at most fingerling study lakes (Figure 2) but was more variable at catchable study lakes (Figure 3). Mean relative weights averaged 88.2 over all populations and ranged from 81.3-96.5 (Figure 4). Compared to wild Brook Trout in Ecoregion 5, wild Brook Trout at study lakes were smaller than stock-quality ($W_r = 91.9$) at 8 lakes; however, the upper quartile (75%) fell below the standard at only 4 of 10 lakes. Mean relative weight was higher than the stock-quality at two study lakes.

Minnow traps were set during each visit to all ten study lakes. A total of 37 Brook Trout were captured with minnow traps over 110 trap-nights (Table 3). Catch was low at an average CPUE of 0.4 fish/trap-night. The average TL of Brook Trout trapped was 84.8 mm. Backpack electrofishing was conducted in the stream inlets at Duck and Upper Hazard lakes where trout fry were observed. At Duck Lake a total of 100 Brook Trout fry were captured by electrofishing the inlet; fish averaged 48 mm TL (range 29-118). At Upper Hazard Lake, a total of 106 juvenile Brook Trout were sampled at the inlet; fish averaged 53 mm TL (range 30-109).

Angling success varied widely among lakes. Catch-per-unit-effort averaged 0.93 fish/hour (range 0.0-8.0) across all lakes (Table 4). All angled wild Brook Trout >150 mm TL were anchor tagged for a total of 74 tags released across all lakes. As of January 2018, no anchor tags have been reported from these study waters to estimate harvest.

Abundance and mortality

Relative abundance of wild Brook Trout at all study lakes averaged 13.9 fish/net-night (range 6-24; Table 5). Phenotypic sex of wild Brook Trout was generally apparent in fish >150 mm TL, and males comprised an average of 51.4% (range 33.9-75.0%; Table 6). At the time of sampling in July, the gonads were not well developed so maturity of wild Brook Trout <150 mm TL could not be reliably determined phenotypically.

Age composition was estimated at each study lake and maximum age varied from age-3 at Lloyds Lake to age-11 at Anderson Lake (Table 7). Sample sizes of otoliths were lower than the goal of 10 per 10 mm length-class at Upper Hazard Lake and were potentially limiting at Lloyds and Hard Creek lakes as well. Confidence intervals around mean length-at-age at individual lakes overlapped for several older age-classes (age-4 to age-6), but length-at-age was often distinct for younger age-classes (age-1 and age-3) (Table 8). Mean length-at-age averaged for fish from all lakes also overlapped for older age-classes with length-at-age distinct for younger age-classes.

Actual mortality of wild Brook Trout of all ages averaged 0.59 across all ten lakes, but ranged from a low 0.34 at Anderson Lake to a high of 0.78 at Lloyds Lake (Table 9).

Stocking

In 2015, the biomass of M_{YY} Brook Trout fingerlings stocked averaged 44.6 kg (range 31.9-65.9; Table 2). In 2016, the biomass of M_{YY} Brook Trout fingerlings stocked averaged 48.5 kg (range 32.8-68.3), and the biomass of M_{YY} Brook Trout catchables stocked averaged 60.7 kg (range 29.4-99.2).

At the time of stocking in 2015, fingerlings averaged 133 mm (range 81-160) and 25 g (range 6-46), or 18.0 fish/lb (Appendix 2). In 2016, fingerlings averaged 126 mm (range 73-161) and 20.5 g (range 4-48), or 22.6 fish/lb at the time of stocking. Catchables averaged 278 mm (range 190-332) and 218 g (range 88-383), or 1.9 fish/lb.

Fish transport densities within the helicopter stocking bucket averaged 0.7 lb fish/gal of water (range 0.5-0.9) and 0.9 lb fish/gal of water (range 0.7-1.0) for fingerlings in 2015 and 2016 respectively, and averaged 0.5 lb fish/gal (range 0.4-0.7) for catchables (Appendix 2). Maintaining fish loading densities below 1.0 lb fish/gal required multiple trips to most lakes. Total flight distance for stocking in 2015 was approximately 59 km, and approximately 723 km in 2016. The substantially greater flight distance in 2016 was due to the greater number of trips to each lake for catchables. The average stocking density for fingerlings in 2015 and 2016 was 169 fish/acre and 172 fish/acre, respectively. A total of 7,256 fingerlings were stocked in 2015, and 7,377 fingerlings stocked in 2016 in four treatment lakes. A total of 1,934 catchables were stocked in four treatment lakes in 2016 and the average stocking density of catchables in 2016 was 36.3 fish/acre.

Genetically determined sex ratios

Our estimation of length-at-age generally characterized fry (age-0) as <90 mm TL (Table 8). At Duck and Upper Hazard lakes an adequate sample of tissue was obtained from fry-sized fish. Males comprised 44% (90% CI, 36-52%) of the fry at Duck Lake and 45% (90% CI, 37-53%) at Upper Hazard. At all other study lakes, juvenile sampling was generally unsuccessful, so genetic sex ratios were estimated from fish of all ages (Table 10). Sex ratios for all wild Brook Trout captured averaged 49% males (range 41-57%) across all populations. Sex ratios by age, combined for all study populations, showed that males comprised a lower percentage of age-0 and age-1 fish, but after age-1, males comprised a larger percentage of the population (Figure 5). Sex ratios by age were also estimated within each population to monitor changes in sex ratios at each study lake, though sample sizes of fish with ages and genetic sex were generally low (Table 11). Concordance between genetic sex and phenotypic sex averaged 93.1% (range 83.1-100.0%; Table 12) over both years of sampling.

DISCUSSION

At suppression lakes, the biomass of trout removed was far less than the biomass of M_{YY} Brook Trout stocked. In general, low capture success was observed with all gear types at alpine lakes. To increase the effectiveness of the M_{YY} program, modelling suggested that 50% or more of the wild Brook Trout population should be removed (Schill et al. 2017). It is not likely that 50% removals were achieved considering the total catch and CPUE at suppression lakes, though the initial abundances of wild Brook Trout, and thus the proportion of the wild Brook Trout removed

(and stocking rates), were also unknown. The time it takes to shift the sex ratio of the wild population is likely to be strongly linked with the stocking rate (Schill et al. 2017). Additionally, survival of hatchery fish is likely to be improved by the suppression of wild fish (Miller 1958). Having a better understanding of the effectiveness of suppression and of the initial abundance of wild fish may be important to evaluating the difference between treatment levels. Nevertheless, it is unlikely that the suppression levels achieved will be high enough to speed the eradication of Brook Trout relative to non-suppression lakes. Plans are now underway to remove some existing lakes from the study design and add new lakes that are accessible by vehicle in order to achieve higher suppression levels at the new lakes.

Relative abundance of wild Brook Trout has been shown to vary widely at alpine lakes in Idaho as shown by Koenig et al. (2015) control lakes. Relative abundance of wild Brook Trout also varied widely among these study lakes, though how relative abundance compared to true abundance was not well understood. Considering the uncertainty with wild abundance, stocking may have occurred at very high or very low rates in comparison to true wild abundances. Assessing the sex ratios for all lake populations in 2018 may help identify if stocking rates were too low, if changes to a population's sex ratio are not detected. If stocking rates were too high, and if stocking exceeds the carrying capacity of the lake, the effects of density dependence might be identified by decreased growth of fish (i.e., stunting). Density dependent growth of Brook Trout in alpine lakes has been well-documented (Hall 1991) and typically results in fisheries that are valued less by anglers (Donald and Alger 1989). Aday and Graeb (2012) define stunting to be a population-level characteristic caused by any combination of slow growth and high mortality, and consistently young maturation. Brook Trout populations commonly exhibit these characteristics at the population level so are thus prone to stunting. Stunting may be empirically characterized by measures of high CPUE, low relative weights of individual fish, and smaller length-at-age (Brouder et al. 2009). Brouder et al. (2009) provide relative weight standards for Brook Trout in lentic waters captured with gill nets in Ecoregion 5, which includes much of the core habitat for Eastern Brook Trout throughout the Upper Midwest and Northern Appalachia and most of Eastern and Central Canada. This study occurred in Ecoregion 6, but this is the closest standard available to compare with these study populations. These estimates characterize all but two populations as stunted before stocking was initiated, so stocking at extremely high rates may simply result in high mortality of stocked fish. If survival of stocked fish was high, the stocked M_{YY} Brook Trout may now comprise a large proportion of each treatment population, which should speed eradication.

Mortality was estimated indirectly using an approximation approach (Quinn and Deriso 1999) because of uncertainties with wild Brook Trout abundance and size composition at study lakes. However, Brook Trout ages were well distributed over the range of fish lengths, thus if selectivity of gill nets for Brook Trout is estimated, adjusting gill-net catch accordingly and using an adjusted catch-curve may provide better estimates of mortality of the population (Hansen et al. 1997). The method used to estimate mortality (Quinn and Deriso 1999) was a quick, less rigorous method, but may provide reliable estimates. Assuming that fishing mortality was zero was not likely a valid assumption, but exploitation by anglers was assumed to be low considering that none of tags at-large have been reported to date. Regardless, the plan is to incorporate exploitation into the mortality estimates as tag reports from anglers are accumulated.

Our goal of 100 tissue samples from juvenile fish was not achieved at 8 of 10 study lakes. At Duck and Upper Hazard lakes, where an adequate sample of juvenile-sized fish to estimate sex ratios and age was acquired, average length-at-age for age-1 Brook Trout suggested fish <90 mm were likely age-0. The CIs around the male proportions estimated from age-0 fish overlapped with the CIs around the male proportions estimated from fish of all ages suggesting there was not a statistical difference between the two estimates. Fry may become more important to monitor as

the population approaches eradication and small proportions become very important, but samples from all ages may be sufficient for monitoring early changes to the sex ratio. Nevertheless, monitoring the effects of M_{YY} stocking by sampling age-0 fish is likely the most efficient approach to avoid sex-biased mortality (McFadden 1961), though monitoring this relationship where both samples are available will be continued. Age-0 otolith samples were important for characterizing the focus and first annulus during age determination.

Necropsies from gillnet surveys conducted in July may not have accurately characterized maturity. At the time of sampling, it could not be determined if age-1 fish were sexually mature because the gonads were not as well developed, as they would have been closer to spawning. This was likely true for all age-classes because a low proportion of mature males was estimated across all ages and populations, and it was likely that age-5 males larger than 250 mm were mature.

Sex ratios have historically been estimated solely from phenotypic observations for wild Brook Trout populations (e.g. McFadden 1961). Here phenotypic and genetic sex ratios were reported and a 15.3% difference for Brook Trout >150 mm (range 2.5-34.3%; data not shown) was observed. These sex ratio estimates compare two different samples from the same wild population, though some fish occurred in both samples. It is important to note the difference between these sex ratio estimates because it highlights the importance of estimating the genetic sex ratio when monitoring an M_{YY} study. The genetic sex determinations were generally very accurate as determined by the concordance between genetic sexes from individual fish with known phenotypic sex. For example, concordance between phenotypic and genetic sex was 100% when phenotype was evaluated in a laboratory with the benefit of microscopes for examining gonadal material and there were no field challenges and data recording distractions ($n = 1,200$; Daniel J. Schill, personal communication). Previously estimated concordance between genetic and phenotypic sex in streams has been slightly lower at 97.5% when the latter was determined in the field ($n = 203$; Kennedy et al. 2018).

Concordance between genotypic and phenotypic sex was considerably lower for samples reported in the present study on alpine lakes (93%; $n = 547$). This specific comparison involves the same individual fish, so concordance should therefore be 100%. Crews were instructed to only tissue sample (for genetic sex) fish of known-sex, determined phenotypically. Plausible sources of error are likely more common in the field, and include errors of transcription and errors of phenotypic sex determination. Errors with phenotypic sex determination are likely to be inversely related to fish size and directly related to time from spawning. For smaller fish, errors with phenotypic sex in the field may be partially attributed to the unavailability of gonadal squashing techniques commonly used in the laboratory to more accurately note sex (Guerrero and Shelton 1974). A closer inspection of the discordant samples reported here point toward a less-experienced crew, which highlights the importance of training, but some low rate of error likely occurred at all levels of experience. Discordance was higher in female fish, and immature egg sacs very high in the body cavity near the head were often observed, which can easily be overlooked. Training should emphasize the importance of looking very high within the body cavity for eggs and also emphasizing the appropriateness of using “unknown” when determining sex. A low rate of error is also possible for the genetic sex determination. Contamination resulting from the contact of two fish of different sex can result in errors during genetic sex determination, and the sex marker needs to be standardized to each study water/population. Quality assurance can be increased through further genetic testing to identify and remove contaminated samples, and standardizing the sex marker by continuing to compare phenotypically determined sex with genetic sex and increasing that sample size. Regardless of training, accurate identification of phenotypic sex in the field is going to occasionally be problematic for even some midsize fish.

Therefore, genetic sex ratio is the preferred method to evaluate sex ratio shifts in Brook Trout populations when conducting M_{YY} evaluations. Additional, painstakingly careful comparisons of phenotype and genotype in alpine lake Brook Trout populations should be undertaken in future years to ensure that actual differences between the two measures are not present. Although unlikely, such a scenario would be problematic for the efficacy of the M_{YY} approach.

Evaluating the success of M_{YY} stocking for Brook Trout eradication at these study lakes will involve genetic assignment testing in the future. By using genetic assignments, the proportion of the population comprised of M_{YY} fish will be estimated along with the population sex ratio. The genetic assignment test is slightly more expensive than tests using the sex marker only, so researchers will have to consider the additional costs with respect to the sample sizes needed to detect changes at the population level.

Currently, IDFG has little data on angler exploitation at alpine lakes. The goal of 30 at-large Floy® tags was not achieved in any lake, but may accumulate with subsequent sampling events over time. Instead of considering angler exploitation by lake, pooling tags from all study lakes and considering overall exploitation of Brook Trout at alpine lakes may be a better option. One concern is that exploitation estimates may be biased because tags might not be reported at the same rate as lowland lake fisheries. Tag return rates have been estimated for tens-of-thousands of lowland lake hatchery trout, and this return rate is used to expand the number of tags returned to estimate angler exploitation (see Meyer et al. 2012). The likelihood of an angler backpacking a pencil and paper (to record a tag number) to alpine lakes is lower, and thus the catch-and-release rates (with tags intact) may be higher. If a fish is harvested, the tag may get reported at a lower rate because of the remoteness and complications associated with transporting a tag from the backcountry. The reward tags (\$50) should help to better understand tag reporting rates at alpine lakes.

The precision was good between observed stocking densities and planned stocking densities in both years. Understanding the accuracy and precision of estimating the average weight of fish (#fish/lb) helped to achieve the goal. In 2015 and 2016, pound counts at Mackay Hatchery and projected weights were accurately estimated, which contributed to the good precision of observed stocking densities.

We intend for hatchery and wild Brook Trout to spawn together which is rarely a goal for resident fish management, particularly in the American West in alpine lakes. There is information for hatchery Brook Trout survival in lakes (Lachance and Magnan 1990a), and for how competition impacts hatchery Brook Trout survival in lakes (Lachance and Magnan 1990b). However, little information is currently available to contrast how fingerling and catchable sized fish reproduce in the wild, and thus which will be more effective in eradicating wild Brook Trout in lakes. Catchables may be more effective if they are ready to spawn a few months after stocking as documented by Kennedy et al. (2018), and they likely have a competitive advantage considering the length-frequencies of wild fish at catchable lakes in this study. The results from this study in future years will help to elucidate many questions regarding non-native Brook Trout management at alpine lakes.

RECOMMENDATIONS

1. Eliminate half of the study lakes since suppression has not been effective.

2. Add two lakes with road access so removals can be conducted with multiple gear types to achieve meaningful suppression of the wild Brook Trout abundance before stocking. Estimate abundance of wild Brook Trout at each of these two lakes and stock marked M_{YY} fish so that selective removal (i.e., only wild fish) can be conducted in future years.
3. Continue to evaluate sex ratios and add genetic assignment analyses in future reports in study lakes approximately every three years to monitor reproductive success of M_{YY} fish. Evaluate sources of discordance between phenotypic and genotypic sex determinations.
4. Estimate size selectivity of floating, experimental gill nets used for sampling Brook Trout at alpine lakes. Use the estimated selectivity to build adjusted catch-curves to be used for more robust estimates of wild Brook Trout mortality at study lakes.
5. Collate demographic information from the wild Brook Trout population at each study lake for better planning of future eradication efforts in alpine lakes.

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TABLES

Table 1. Study lakes in central Idaho selected for M_{YY} Brook Trout evaluations including treatment levels, size, and location.

Lake name	Treatment	Stocked fish size	Surface area (acres)	Surface elevation (m)	Latitude	Longitude
Disappointment	Suppression	Fingerling	15.3	2,093	45.1834503	-116.20735921
Duck	Suppression	Fingerling	12.3	2,177	45.1145991	-116.15726311
Hard Creek	Non-suppression	Fingerling	8.5	2,262	45.1724069	-116.14488943
Lloyds	Non-suppression	Fingerling	7.2	2,092	45.1929080	-116.16370556
Snowslide #4	Control	n/a	12.0	2,188	44.9833739	-115.93431897
Upper Hazard	Control	n/a	39.1	2,265	45.1742372	-116.13500053
Anderson	Suppression	Catchable	9.0	2,227	44.8868600	-115.93111000
Black	Suppression	Catchable	6.4	2,149	45.2453900	-116.19867000
Rainbow	Non-suppression	Catchable	21.7	2,175	45.2540600	-116.19663000
Rapid	Non-suppression	Catchable	16.3	2,206	44.8559200	-115.91288000

Table 2. Catch-per-unit-effort (CPUE; fish/net-night) and estimated biomass of Brook Trout and Rainbow Trout removed using experimental gill nets at ten alpine lakes in central Idaho during 2015 and 2016. Also shown is the biomass of M_{YY} Brook Trout stocked into each lake by year.

Lake name	Trout species	Net nights	Sample dates	Trout caught	CPUE	Biomass removed (kg)	2015 Biomass stocked (kg)	2016 Biomass stocked (kg)
Disappointment	Brook	9	7/21-7/22	79	9	6.7	65.9	68.3
	Rainbow			21	2	2.5		
Duck	Brook	9	7/14-7/15	83	9	9.3	43.7	55.2
Hard Creek	Brook	3	7/28	56	19	2.7	31.9	32.8
Llyods	Brook	3	7/16	38	13	5.7	37.0	37.6
Snowslide #4	Brook	3	8/4	64	21	3.8		
Upper Hazard	Brook	3	7/27	33	11	1.4		
	Rainbow			10	3	0.1		
Anderson	Brook	9	7/19-7/20	105	12	7.0		39.4
Black	Brook	12	7/19-7/20, 7/28	77	6	8.3		29.4
Rainbow	Brook	3	7/19	90	30	5.0		99.2
Rapid	Brook	3	7/21	58	19	5.3		74.6
Total		57		714		57.8	178.5	572.2

Table 3. Catch-per-unit-effort (CPUE) by year for minnow traps and juvenile wild Brook Trout length distribution (TL; mm) at ten alpine lakes in central Idaho during late July.

Lake name	Year	Traps	Brook Trout	CPUE (fish/trap night)	Average TL (mm)
Disappointment	2015	20	4	0.2	52.8
Duck	2015	10	12	1.2	51.8
Hard Creek	2015	10	0	0.0	---
Lloyds	2015	20	7	0.4	42
Snowslide #4	2015	10	0	0.0	---
Upper Hazard	2015	10	6	0.6	107
Anderson	2016	10	0	0.0	---
Black	2016	5	2	0.4	131
Rainbow	2016	5	2	0.4	147
Rapid	2016	10	4	0.4	62
Total		110	37		

Table 4. Catch-per-unit-effort (CPUE; fish/hr.) by year for angling by gear type at ten alpine lakes in central Idaho during late July. The number of fish tagged with non-reward and reward T-bar anchor tags at each lake is included, though only Brook Trout >150 mm were tagged.

Lake name	Year	Gear type	Anglers	Hours	Brook Trout	CPUE	Non-reward	Reward (\$50)	Total
Disappointment	2015	Fly	4	15.0	9	0.15	7	1	8
		Spinning	1	4.0	5	1.25	5		5
Duck	2015	Fly	5	17.0	2	0.02	2		2
		Spinning	1	2.0	0	0.00			0
Hard Creek	2015	Fly	5	10.0	12	0.24	11	1	12
Lloyds	2015	Fly	7	12.5	22	0.25	18	1	19
Snowslide #4	2015	Fly	3	7.5	4	0.18	3		3
Upper Hazard	2015	Fly	3	5.0	4	0.27	2		2
Anderson	2016	Spinner	2	5.0	15	1.50	2	12	14
Black	2016	Spinner	1	1.0	8	8.00		8	8
Rainbow	2016	Spinner	2	2.3	1	0.22	1		1
Rapid	2016	Spinner	1	3.0	0	0.00			0
		Fly	2	4.3	0	0.00			0
Total			37	88.6	82		51	23	74

Table 5. Relative abundance characterized by the catch-per-unit-effort (CPUE; fish/net-night) for the first three net-nights using experimental gill nets at ten alpine lakes in central Idaho during late July in 2015 and 2016.

Lake name	Year	Nets	Brook Trout (>125 mm)	CPUE (fish/night)
Disappointment	2015	3	23	7.7
Duck	2015	3	29	9.7
Hard Creek	2015	3	57	19.0
Lloyds	2015	3	31	10.3
Snowslide #4	2015	3	54	18.0
Upper Hazard	2015	3	19	6.3
Anderson	2016	3	61	20.3
Black	2016	3	29	9.7
Rainbow	2016	3	73	24.3
Rapid	2016	3	41	13.7
Average			42	13.9

Table 6. Phenotypically determined sex and maturity of wild Brook Trout >150 mm TL captured with experimental gill nets during late July at ten alpine lakes in central Idaho in 2015 and 2016.

Lake name	Year	Sample size	Male	Female	Unknown sex	Male (%)	Mature	Immature	Unknown maturity	Mature (%)
Disappointment	2015	61	35	24	2	59.3	17	44	0	27.9
Duck	2015	54	31	19	4	62.0	9	41	4	18.0
Hard Creek	2015	47	23	23	1	50.0	16	31	0	34.0
Lloyds	2015	26	8	11	7	42.1	0	26	0	0.0
Snowslide #4	2015	43	19	23	1	45.2	21	22	0	48.8
Upper Hazard	2015	13	9	3	1	75.0	2	11	0	15.4
Anderson	2016	96	20	39	37	33.9	13	69	1	15.9
Black	2016	77	39	31	7	55.7	63	14	0	81.8
Rainbow	2016	90	35	36	19	49.3	59	31	0	65.6
Rapid	2016	61	17	12	32	58.6	13	47	1	21.7
Total		568	236	221	111		213	336	6	

Table 7. Age composition of wild Brook Trout populations in ten alpine lakes in central Idaho. Ages were determined from a subsample of sagittal otoliths sampled from each lake during late July in 2015 and 2016. Sampling effort was not equal at each lake.

Lake name	Sample size	Observed age proportions								
		0	1	2	3	4	5	6	7	11
Disappointment	63		0.17	0.19	0.32	0.22	0.05	0.03	0.02	
Duck	88	0.01	0.47	0.17	0.25	0.10				
Hard Creek	47		0.06	0.38	0.32	0.17	0.06			
Lloyds	34		0.44	0.32	0.24					
Snowslide	64	0.02	0.14	0.33	0.42	0.09				
Upper Hazard	32		0.47	0.31	0.19	0.03				
Anderson	92		0.10	0.32	0.35	0.20	0.03			0.01
Black	78		0.15	0.41	0.22	0.17	0.04	0.01	0.00	
Rainbow	85		0.19	0.44	0.18	0.11	0.06	0.02	0.01	
Rapid	63	0.05	0.38	0.35	0.08	0.10	0.05			

Table 8. Mean length-at-age of wild Brook Trout captured at ten alpine lakes in central Idaho during late July in 2015 and 2016. Confidence intervals ($\alpha = 0.05$) are provided below each point estimate in parenthesis.

Lake name	Age									
	0	1	2	3	4	5	6	7	11	
Disappointment		95 (90-101)	136 (124-148)	213 (202-223)	258 (247-269)	294 (272-315)	293 (283-303)	319		
Duck	33	98 (91-105)	179 (156-202)	249 (240-258)	286 (273-298)					
Hard Creek		133 (122-145)	162 (154-171)	227 (217-238)	255 (247-263)	264 (255-273)				
Lloyds		137 (124-150)	188 (171-204)	239 (224-254)						
Snowslide #4	91	112 (107-116)	152 (142-162)	209 (204-213)	220 (208-232)					
Upper Hazard		107 (101-113)	166 (150-181)	214 (199-229)	289					
Anderson		118 (100-136)	175 (168-182)	194 (186-201)	210 (201-219)	225 (201-250)				376
Black		132 (114-150)	205 (196-218)	228 (214-244)	256 (250-263)	252 (229-276)	262			
Rainbow		110 (104-116)	173 (165-181)	200 (191-210)	219 (209-228)	219 (208-231)	246 (222-270)	248		
Rapid	52 (47-57)	115 (109-121)	170 (155-185)	250 (185-316)	312 (279-344)	325 (308-343)				
Average	56 (37-75)	112 (108-115)	174 (169-178)	217 (213-222)	247 (240-255)	259 (241-277)	268 (246-290)	284 (214-353)		

Table 9. Mortality estimates for wild Brook Trout at ten alpine lakes in central Idaho. Maximum ages of Brook Trout in the population were estimated from sagittal otoliths sampled during late July in 2015 and 2016.

Lake name	Maximum age	Instantaneous natural mortality	Actual mortality
Disappointment	7	0.66	0.48
Duck	4	1.15	0.68
Hard Creek	5	0.92	0.60
Lloyds	3	1.54	0.78
Snowslide #4	4	1.15	0.68
Upper Hazard	4	1.15	0.68
Anderson	11	0.42	0.34
Black	6	0.77	0.54
Rainbow	7	0.66	0.48
Rapid	5	0.92	0.60
Average	6	0.93	0.59

Table 10. Genetically determined sex ratios used to estimate the sex ratio of the wild Brook Trout population at each study lake prior to stocking M_{YY} Brook Trout. Fish recruited to all gear types over all lengths were included. Confidence intervals (CI; $\alpha = 0.1$) were estimated around the male proportion of each population.

Lake name	Sample size	Female	Male	Male proportion	90% CI
Disappointment	91	39	52	0.57	0.09
Duck	185	97	88	0.48	0.06
Hard Creek	69	41	28	0.41	0.10
Lloyds	67	34	33	0.49	0.11
Snowslide #4	68	33	35	0.52	0.11
Upper Hazard	149	73	76	0.51	0.07
Anderson	95	46	49	0.52	0.09
Black	19	10	9	0.47	0.22
Rainbow	33	19	14	0.42	0.16
Rapid	58	26	32	0.55	0.12
Average				0.49	0.03

Table 11. Genetic sex by age of wild Brook Trout for each population with confidence intervals (CI; $\alpha = 0.1$) estimated around the male proportion.

Lake name	Year	Age	Male	Total	Male proportion	90% CI
Disappointment	2015	0	0	0	0.00	0.00
		1	3	10	0.30	0.29
		2	10	16	0.63	0.23
		3	13	20	0.65	0.20
		4	9	15	0.60	0.24
		5	3	3	1.00	0.17
		6	2	2	1.00	0.25
		7	1	1	1.00	0.50
Duck	2015	0	46	104	0.44	0.08
		1	13	30	0.43	0.17
		2	8	18	0.44	0.22
		3	13	21	0.62	0.20
		4	6	9	0.67	0.31
Hard Creek	2015	0	0	0	0.00	0.00
		1	3	5	0.60	0.46
		2	10	20	0.50	0.21
		3	7	15	0.47	0.25
		4	6	10	0.60	0.31
		5	2	4	0.50	0.54
Lloyds	2015	0	0	0	0.00	0.00
		1	7	16	0.44	0.24
		2	5	11	0.45	0.29
		3	5	10	0.50	0.31
Snowslide #4	2015	0	0	1	0.00	0.00
		1	4	9	0.44	0.33
		2	14	21	0.67	0.19
		3	12	27	0.44	0.18
		4	3	6	0.50	0.42
Upper Hazard	2015	0	47	105	0.45	0.08
		1	8	15	0.53	0.25
		2	7	10	0.70	0.29
		3	4	6	0.67	0.40
		4	1	1	1.00	0.50
Anderson	2016	0	0	0	0.00	0.00
		1	3	8	0.38	0.34
		2	16	30	0.53	0.17
		3	18	32	0.56	0.16
		4	10	18	0.56	0.22
		5	0	3	0.00	0.00

Table 11 continued

Lake name	Year	Age	Male	Total	Male proportion	90% CI
Black	2016	11	0	1	0.00	0.00
		0	0	0	0.00	0.00
		1	4	8	0.50	0.35
		2	3	5	0.60	0.46
		3	1	1	1.00	0.50
		4	1	3	0.33	0.61
Rainbow	2016	5	0	1	0.00	0.00
		0	0	0	0.00	0.00
		1	7	16	0.44	0.24
		2	4	10	0.40	0.31
		3	1	2	0.50	0.83
		4	0	1	0.00	0.00
Rapid	2016	6	1	1	1.00	0.50
		0	0	0	0.00	0.00
		1	12	24	0.50	0.19
		2	12	21	0.57	0.20
		3	3	5	0.60	0.46
		4	3	6	0.50	0.42
		5	1	1	1.00	0.25

Table 12. Concordance between phenotypic sex determined during wild Brook Trout necropsies from gillnet sampled during late July in 2015 and 2016, and genetic sex determined from a tissue sample taken from each necropsied wild Brook Trout.

Lake name	Pedigree	Concordant	Discordant	Total	Concordance (%)
Disappointment	SfoDSPP15C	59	5	64	92.2
Duck	SfoDUCK15C	48	6	54	88.9
Hard Creek	SfoHCRL15C	47	1	48	97.9
Lloyds	SfoLOYD15C	23	1	24	95.8
Snowslide #4	SfoSNSL15C	53	1	54	98.1
Upper Hazard	SfoUHZL15C	18	2	20	90.0
Anderson	ScoANDL16C	49	10	59	83.1
Black	SfoBLAL16C	11	0	11	100.0
Rainbow	SfoRNBL16C	15	0	15	100.0
Rapid	SfoRPDL16C	23	4	27	85.2
Average					93.1

FIGURES

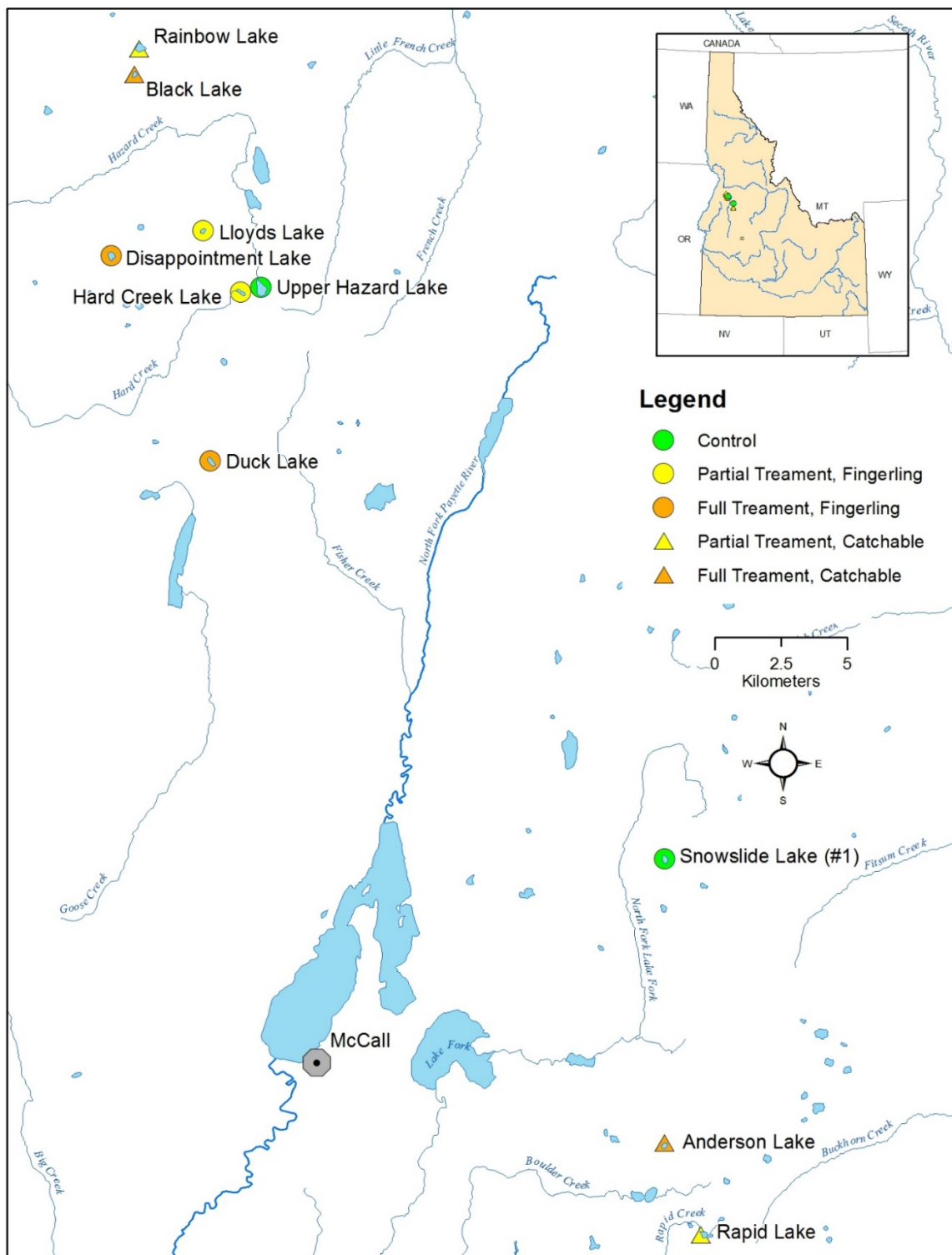


Figure 1. General locations of study lakes selected for evaluating M_{YY} Brook Trout in central Idaho. Treatment levels are identified by color; fish size stocked, fingerling or catchable, are differentiated by circles or triangles, respectively.

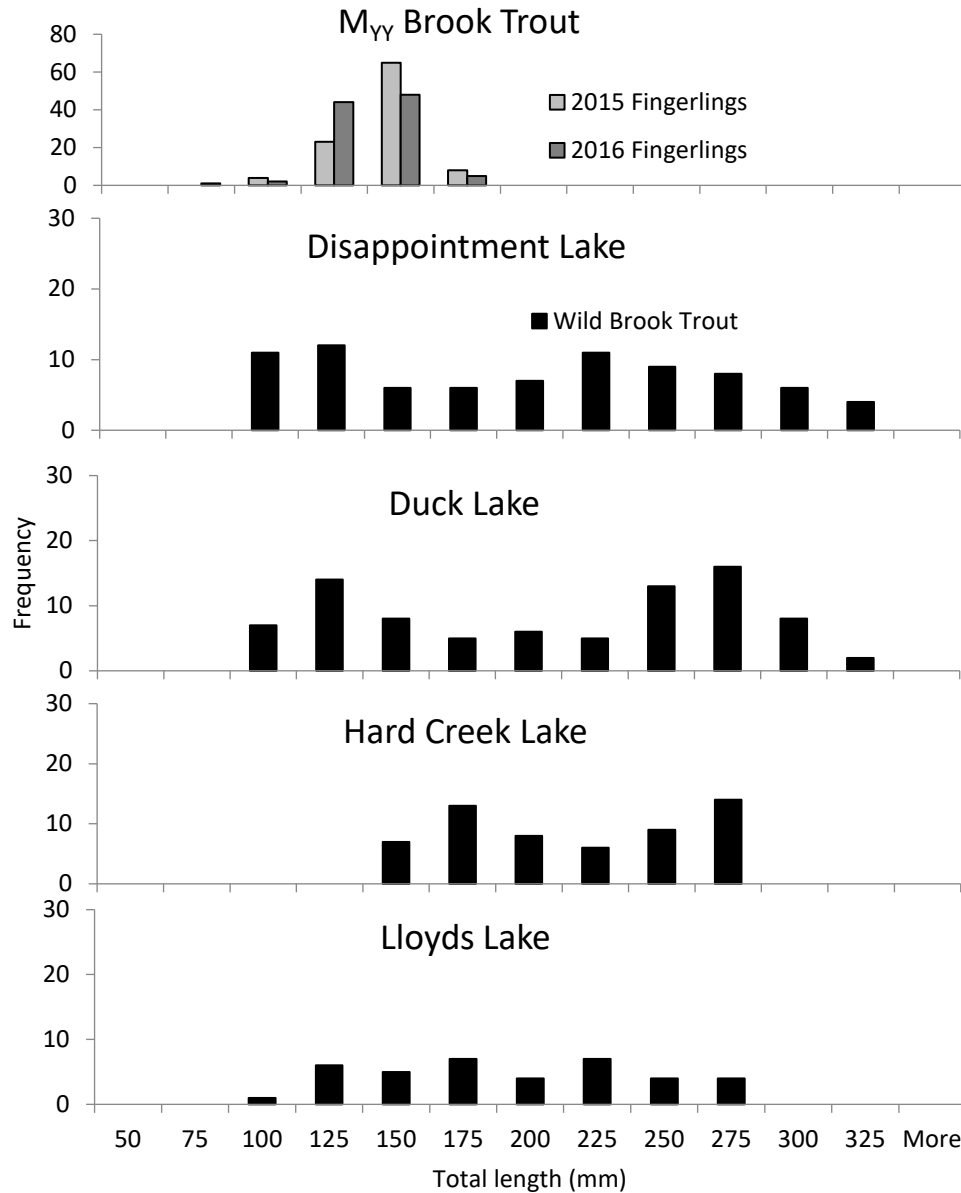


Figure 2. Length-frequencies of a subsample ($n=100$) of fingerling M_{YY} Brook Trout stocked at four lakes in 2015 and 2016 and wild Brook Trout captured with experimental gill nets at each treatment lake in 2015.

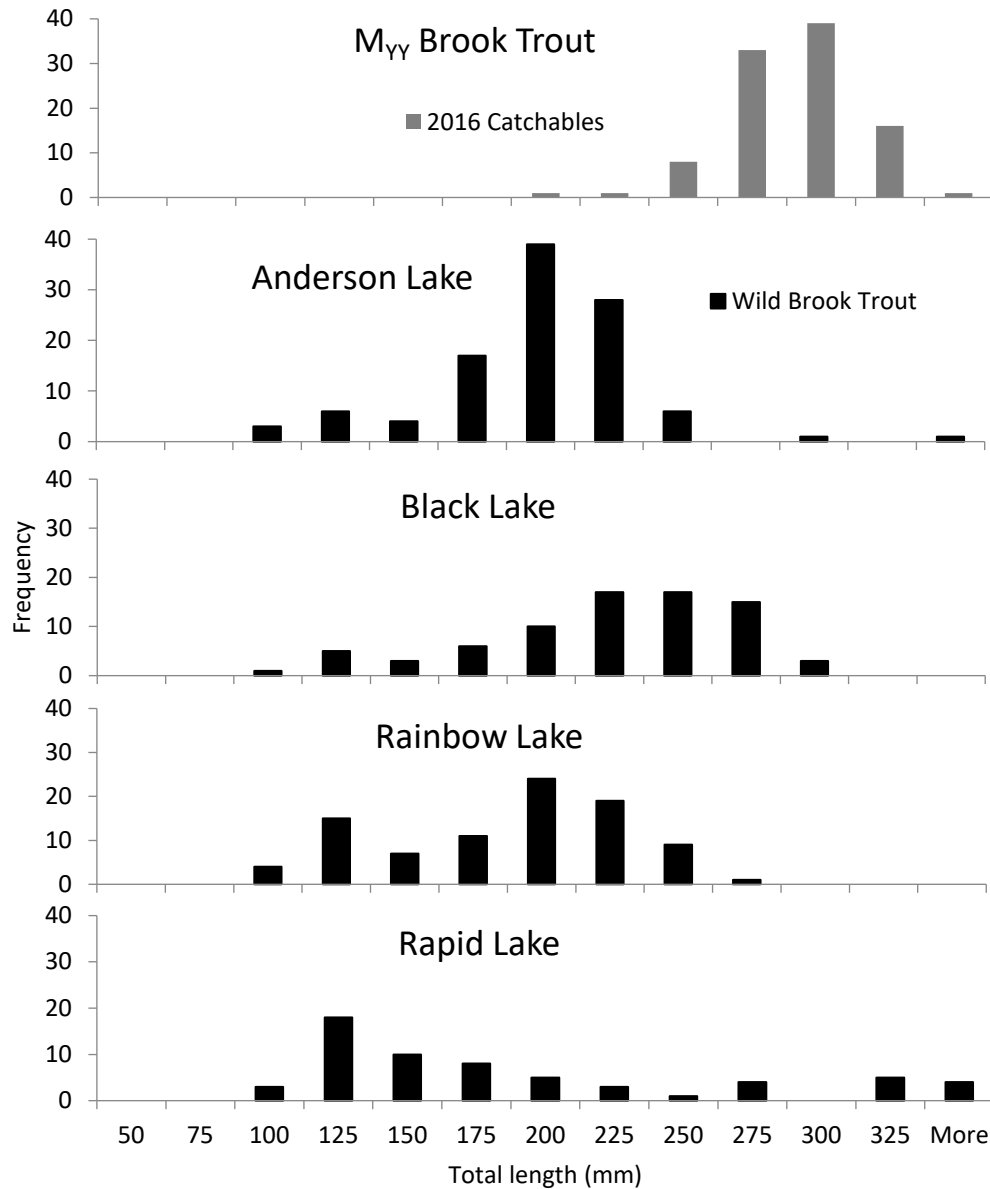


Figure 3. Length-frequencies of a subsample ($n=100$) of catchable M_{YY} Brook Trout stocked at four treatment lakes and wild Brook Trout captured with experimental gill nets at each treatment lake in 2016.

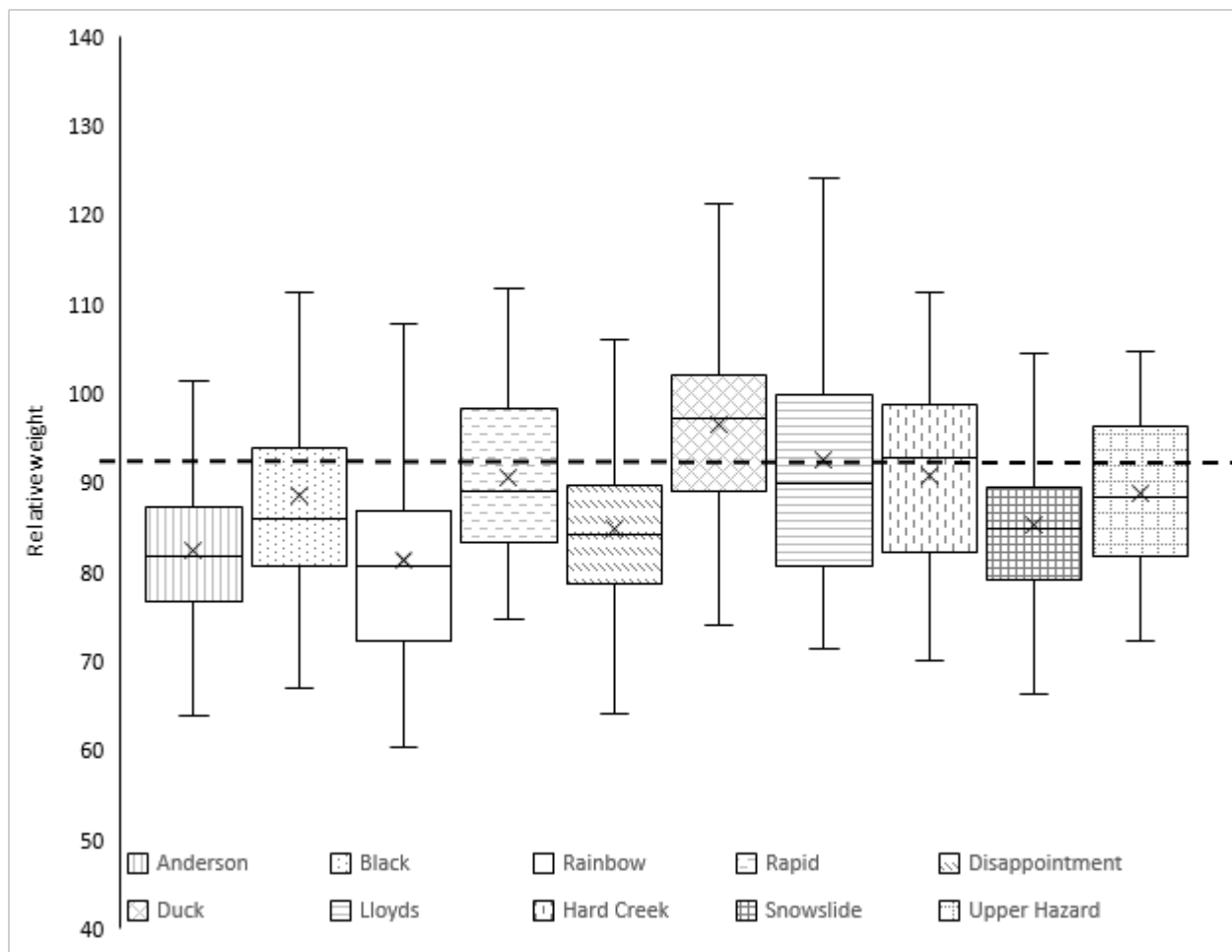


Figure 4. Box-whisker plots of relative weights (W_r) of wild Brook Trout at ten alpine lakes in central Idaho. The top and bottom of the boxes represents the 75th and 25th percentiles, respectively. The line in the interior of the box represents the median and the X identifies the mean. The whiskers identify the maximum and minimum values. A dashed horizontal line at 91.9 shows mean stock-quality W_r (of wild Brook Trout captured with gillnets in lentic waters in Ecoregion 5) and was used to compare wild Brook Trout at study lakes with other wild Brook Trout populations.

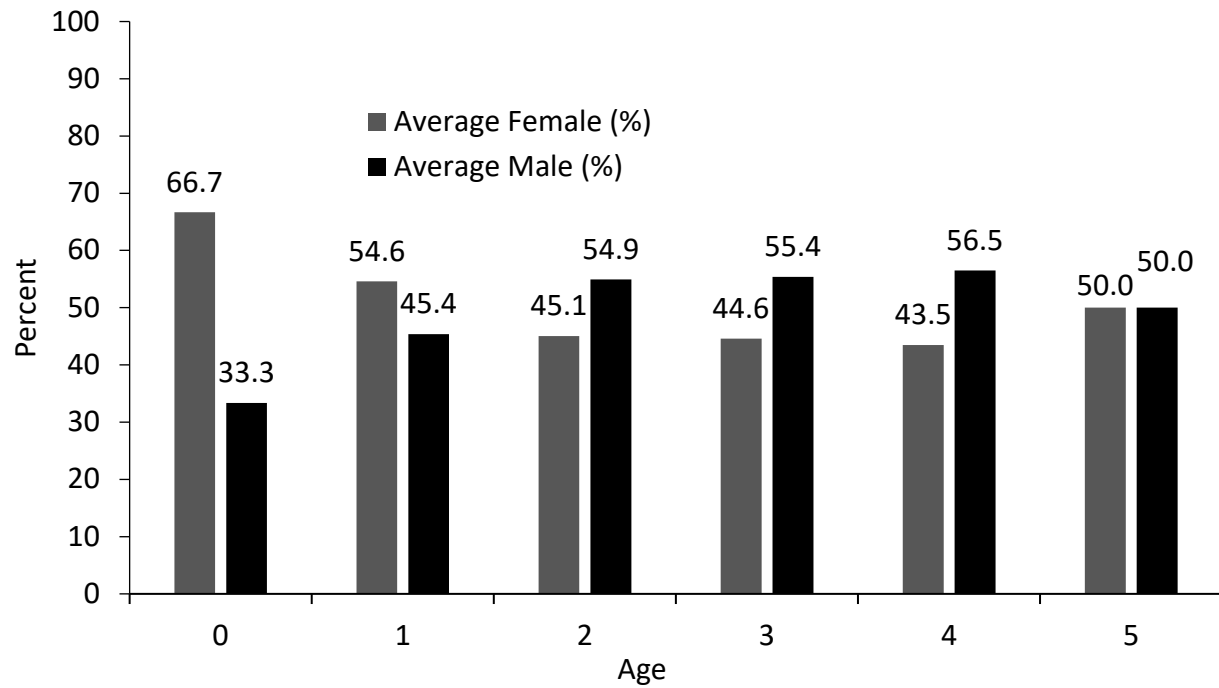


Figure 5. Wild Brook Trout sex (determined genetically) composition by age averaged over ten alpine lakes in Idaho.

APPENDICES

Appendix 1. Abundances of wild adult Brook Trout and physical characteristics for 11 alpine lakes. Data obtained from D. L. Hall's 1991 dissertation and Hand et al. (2012). These estimates were used to characterize a range of adult wild Brook Trout abundances in alpine lakes in the western United States, though the average of mean density at lakes described by Hand et al. (2012) was used to characterize high Brook Trout densities (120 fish/ac) in Idaho lakes.

Lake name	Area (acres)	Brook Trout abundance	Brook Trout density (fish/acre)	Maximum depth (m)	Elevation (m)
Flower	4.6	1,923	416	2.4	3,200
Wonder 3	3.2	1,153	358	7.0	3,375
Fishgut 1	1.6	1,101	694	3.7	3,315
Dingleberry	5.1	2,186	426	6.7	3,195
Hell Diver 3	2.2	168	77	13.1	3,580
Hell Diver 2	1.3	303	234	5.2	3,480
Par Value	5.9	1,460	246	17.7	3,135
Gem 2	1.7	261	150	4.3	3,335
Fly ^a	2.5	299	119	3.3	1,652
Platinum ^a	2.5	148	60	4.1	1,875
Running ^a	20.7	3,389	163	14.0	1,753
Minimum	1.3	148	60	2.4	1,652
Maximum	20.7	3,389	694	17.7	3,580
Average	4.7	1,126	268	7.4	2,900

^a Hand et al. 2012

Appendix 2. Observed number of M_{YY} Brook Trout stocked at each study lake per year, and stocking density (target 35 or 175 fish/ac.). Also shown are the helicopter load calculations (Hughes/MD 500; 700 lbs. maximum) and total flight distance required for stocking each lake in August of 2015 and 2016. The target fish load densities were 1.0 (lbs. fish/gal) to maximize fish health within the stocking bucket (90-100 gal capacity SEI Industries Bambi Bucket®). Water displacement was calculated following Piper et al. (1982; Table 30).

Year	Lake name	Size stocked	Avg. fish weight (#/lb)	Number of fish stocked	Stocking density (fish/ac)	Fish /flight	Fish weight (lbs/flight)	Water weight (lbs/flight)	Total weight (lbs/flight)	# Flights	Total distance (mi)	Loading density (lbs fish/gal)
2015	Disappointment	Fingerling	18.0	2,678	175	1,339	74	625	699	2	7.6	0.9
	Duck	Fingerling	18.0	1,776	145	888	49	414	464	2	18	0.6
	Lloyds	Fingerling	18.0	1,297	180	1,297	72	605	677	1	2.2	0.9
	Hard Creek	Fingerling	18.0	1,505	176	753	42	351	393	2	8.8	0.5
2016	Disappointment	Fingerling	22.6	3,334	218	1,667	74	620	693	2	7.6	0.7
	Duck	Fingerling	22.6	2,695	220	1,348	60	501	560	2	18	0.6
	Lloyds	Fingerling	22.6	1,598	222	1,598	71	594	665	1	2.2	0.7
	Hard Creek	Fingerling	22.6	1,832	215	1,832	81	681	762	1	2.4	0.8
	Anderson	Catchable	1.9	181	20	60	32	267	298	3	138.6	0.6
	Black	Catchable	1.9	135	21	68	36	298	334	2	20.4	0.7
	Rainbow	Catchable	1.9	456	28	114	60	504	564	4	57	1.0
	Rapid	Catchable	1.9	343	16	69	36	303	339	5	203.2	0.9
Average												0.7

Appendix 3. Gill net set and retrieve times (24 hr), and total time in water at ten study lakes in central Idaho in 2015 and 2016.

Lake name	Treatment	Net number	Time in	Time out	Total time (hr)
Disappointment	Suppression	1	1945	915	13.50
		2	2015	1000	13.75
		3	2045	1150	15.08
		4	2115	1237	15.37
		5	2145	1430	16.75
		6	1530	730	16.00
		7	1545	800	16.25
		8	1600	830	16.50
		9	1615	918	17.05
Duck	Suppression	1	1700	1005	17.08
		2	1730	1045	17.25
		3	1800	1350	19.83
		4	1830	1230	18.00
		5	1600	815	16.25
		6	1630	835	16.08
		7	1700	915	16.25
		8	1730	940	16.17
		9	1800	1000	16.00
Hard Creek	Non-suppression	1	1800	745	13.75
		2	1830	900	14.50
		3	1900	945	14.75
Lloyds	Non-suppression	1	1715	910	15.92
		2	1730	930	16.00
		3	1745	1015	16.50
Snowslide #4	Control	1	1530	700	15.50
		2	1600	900	17.00
		3	1630	1015	17.75
Upper Hazard	Control	1	1630	900	16.50
		2	1700	1000	17.00
		3	1730	1115	17.75
Anderson	Suppression	1	2000	800	12.00
		2	2015	1015	14.00
		3	2031	1155	15.24
		4	2045	1240	15.55
		5	2130	1320	15.50
		6	1700	710	14.10
		7	1720	730	14.10
		8	1750	750	14.00
		9	1810	810	14.00

Appendix 3. continued.

Appendix 5: continued.

Lake name	Treatment	Net number	Time in	Time out	Total time (hr)
Black	Suppression	1	1820	1122	17.02
		2	1842	1135	16.53
		3	1854	1140	16.46
		4	1907	1143	16.36
		5	1717	917	16.00
		6	1731	921	15.50
		4	1739	1001	16.22
		8	1753	1031	16.38
		9	1801	1042	16.41
		10	1650	1013	17.23
		11	1712	1045	17.33
		12	1731	1110	17.39
Rainbow	Non-suppression	1	2205	1010	12.05
		2	2220	1030	12.10
		3	2115	821	11.06
Rapid	Non-suppression	1	1945	825	12.40
		2	2037	931	12.54
		3	2100	1015	13.15
Average					15.72

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