



**WILD TROUT EVALUATIONS: POST STOCKING
SURVIVAL AND REPRODUCTIVE SUCCESS OF YY
MALE BROOK TROUT IN STREAMS**



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SURVIVAL AND REPRODUCTIVE SUCCESS OF HATCHERY YY MALE BROOK TROUT STOCKED IN STREAMS

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). If M_{YY} Brook Trout survive after stocking, and reproduce successfully with wild females, in theory this would eventually drive the sex ratio of the wild population to 100% males, at which point the population would become functionally eliminated. We stocked M_{YY} Brook Trout in four streams in central Idaho in June 2014; in two of these streams, the wild Brook Trout population was suppressed *via* electrofishing prior to stocking to determine if diminished competition with wild fish would increase survival of M_{YY} fish. We conducted electrofishing surveys in October 2014 to estimate (1) abundance of wild and M_{YY} Brook Trout, (2) apparent mortality for M_{YY} fish, and (3) apparent emigration of M_{YY} fish from the stocking reaches. We tissue sampled wild Brook Trout fry in 2014 to genotype the wild population and estimate wild sex ratios in all study streams. We tissue sampled Brook Trout fry again in 2015 and conducted genetic assignment testing to identify successful reproduction of the stocked M_{YY} fish. Apparent survival of M_{YY} Brook Trout averaged 11% (range 7-21%), and may have been improved in one stream by removing wild fish. We estimated that M_{YY} fish comprised 2.7% (range 2.1-3.2%) of all adult spawning Brook Trout in the fall of 2014. Genetic assignment tests in 2015 identified successful reproduction of M_{YY} fish in all streams in which they were stocked, with an average of 3.7% (range 2.1-5.0%) of fry being the progeny of M_{YY} fish. These results confirm that M_{YY} Brook Trout stocked in streams can survive and spawn successfully with wild fish, suggesting that M_{YY} Brook Trout may indeed be a useful tool to eradicate undesirable nonnative Brook Trout populations.

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INTRODUCTION

Nonnative Brook Trout *Salvelinus fontinalis* have been artificially introduced in many lakes and streams (Bahls 1992) 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 (Dunham et al. 2004). There are several methods fisheries managers can use to eradicate nonnative fish. Managers have used piscicides with some success, 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). 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 alternative method for eradicating nonnative fish.

Gutierrez and Teem (2006) modelled a method to eradicate non-native fish populations using a species-specific genetic approach that would reduce impacts to non-target fish species. They modelled shifting the sex ratio of a population toward males (Fisher and Bennett 1999) to the extent that one sex is functionally eliminated from the population, eventually resulting in population eradication (Hamilton 1967). The sex ratio of the population is shifted by introducing hatchery produced male fish with a YY genotype (M_{YY}). To create M_{YY} brood stock, XY males are feminized by exposing them to estrogen. The resulting XY females are crossed with normal XY males and $\frac{1}{4}$ of the subsequent progeny are M_{YY} (Teem and Gutierrez 2010). By exposing $\frac{1}{2}$ of the M_{YY} fish to estrogen, an M_{YY} and F_{YY} brood stock can be created, and all offspring are M_{YY} . These M_{YY} offspring 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). Such a stocking program has not yet been tested in the wild to eradicate a non-native fish species (Wedekind 2012), though monosex culture is commonly used in commercial hatcheries for artificial fish production (Thresher et al. 2013).

The Idaho Department of Fish and Game (IDFG) experimentally produced a YY Brook Trout broodstock which subsequently produced a large number of YY male Brook Trout for eventual stocking into the wild (Schill et al. 2016). Recent modelling suggests that annual stocking of YY male Brook Trout into streams can result in eradication of the wild population within 10 years if 1) YY male Brook Trout are stocked at a rate of 50% of the initial wild Brook Trout abundance, and 2) the population is suppressed by 50% using electrofishing (Schill et al. in review). Only slightly less optimistic results are anticipated in alpine lakes. However, these models are theoretical, and need to be tested on wild Brook Trout populations to validate predictions.

Sex ratios in wild Brook Trout populations would only change under such a stocking program if the M_{YY} Brook Trout survive and successfully reproduce after stocking. Hatchery trout encounter many challenges upon their liberation into the wild, and due to several factors, often a low proportion survive more than a few weeks in wild conditions, especially in streams (e.g., Miller 1952; High and Meyer 2009). High mortality in streams is largely attributed to the accumulation of lactic acid from adjusting to stream flows, coupled with starvation and competition with resident fish (Schuck 1948; Miller 1958; Hochachka and Sinclair 1962). Competition with wild trout is a factor that can be minimized by reducing the abundance of the wild population. Throughout the 1950s, R. B. Miller conducted several experiments to help

understand the high mortality observed by hatchery trout released in streams in Alberta (Miller 1952, 1954, 1958). Though there have been many studies on the high mortality of hatchery trout in streams, Miller (1954) tested how mortality changed when the wild population was removed. These studies identified that competition with resident fish was a factor contributing to the high mortality of hatchery trout in streams. For this study, the following objectives were outlined to guide our work.

OBJECTIVES

1. Estimate post-stocking mortality rates for catchable-sized M_{YY} Brook Trout released in central Idaho streams.
2. Investigate whether reducing the abundance of wild Brook Trout in streams would decrease post-stocking mortality of M_{YY} fish.
3. Determine if M_{YY} Brook Trout spawned successfully with wild Brook Trout.

METHODS

At the Ashton Fish Hatchery, 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 in November 2013. Fish were reared to catchable-size (mean = 229 mm total length at the time of stocking) at Mackay Hatchery in outdoor concrete raceways on 10-12°C single-use spring water until the time of release.

Study streams were selected for known Brook Trout populations and relatively narrow stream width so we could efficiently conduct backpack electrofishing surveys. All treatment and control streams were located within the Big Lost River basin in south-central Idaho (Figure 1). The Big Lost River is the largest (with a watershed covering 5,159 km²) of several hydrologically isolated streams located in south-central Idaho, collectively termed the Sinks Drainages (IDFG 2007). The Big Lost River originates in the Pioneer, Boulder, Lost River, and White Knob mountain ranges and flows onto the Snake River Plain where it terminates at the Big Lost River Sinks (IDFG 2007). Twelve species of fish have been documented in the basin; of these, it is thought that only three species – Mountain Whitefish *Prosopium williamsoni*, Shorthead Sculpin *Cottus confusus*, and Paiute Sculpin *C. beldingii* - are native to the Big Lost River basin (Gamett 2003).

Two experimental treatment levels were implemented for this study: 1) full treatment, where wild Brook Trout were manually suppressed (*via* electrofishing capture and removal) prior to M_{YY} stocking to presumably create open habitat for stocked fish; and 2) partial treatment, where wild trout were not suppressed. Full treatment streams were Wildhorse and Bear creeks, and partial treatment streams were Iron Bog and Cherry creeks (Figure 1). Control streams were the East Fork Big Lost River and Alder Creek. With the exception of Alder and Cherry creeks, which were third-order streams (1:24,000), all other streams were second-order streams originating at over 2,500 m above sea level.

Removals

For full treatment streams, all captured wild Brook Trout were removed using single-pass electrofishing over an average of 2.3 km of stream (range 1.9-2.6; Table 1), prior to stocking in 2014. Electrofishing crews consisted of 2-3 people (depending on stream flow) with backpack electrofishers, and 2-3 people with nets and buckets. We used a pulsed-DC waveform operated at 40-60 Hz, 350-900 V, and a 1-6 ms pulse width. During removals, persons with backpack electrofishers covered all available habitats within the removal reach, moving methodically upstream. All salmonids were measured to the nearest mm for total length (TL). All wild Brook Trout captured in the full treatment streams were euthanized with a lethal dose of anesthetic. Salmonids other than Brook Trout comprised less than 1% of the total catch among all study streams and were not included in any of our analyses.

Stocking

On June 17, 2014, lengths and weights were measured from 100 M_{YY} Brook Trout at Mackay Hatchery prior to stocking. M_{YY} Brook Trout averaged 229 mm TL (range 148-300; Figure 3) and 130 g (range 35-295) or 3.5 fish/lb. All M_{YY} fish were adipose fin clipped prior to stocking so they could be differentiated from wild Brook Trout in the stream. On June 26-27, 2014, approximately 500 (range 492-512) M_{YY} Brook Trout were carried in 19 L pails from a hatchery truck to each treatment stream (Table 2). Release locations were systematically selected so stocked fish were evenly dispersed throughout the treatment reach (Figure 1).

To evaluate angler exploitation, approximately 10% ($n=50$) of the M_{YY} Brook Trout were tagged immediately before stocking using 70 mm (51 mm of tubing) fluorescent orange/green T-bar anchor tags (Table 2). 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. We calculated angler exploitation seven months after stocking following Meyer and Schill (2014).

Abundance

From October 6-15, 2014, mark-recapture backpack electrofishing surveys were conducted within each treatment reach to estimate the abundance of adult wild and M_{YY} Brook Trout. Two separate 300 m survey transects were randomly selected within each treatment reach (Figure 1). One to seven days prior to the recapture effort, wild and M_{YY} Brook Trout (≥ 100 mm) were captured using electrofishing within the experimental reaches of stream, anesthetized as described above, measured, and marked using a hole-punch in the caudal fin. Only Brook Trout (wild and M_{YY}) were marked and all other species were released after recovery from anesthesia. During the recapture pass, captured Brook Trout were anesthetized, identified as either wild or M_{YY} fish, measured to the nearest mm, checked for marks and tags, and released as above.

Data from the two mark-recapture survey transects in each stream were combined to estimate fish abundance and capture efficiency. The Fisheries Analysis+ software package (Montana Fish, Wildlife and Parks 2004) was used to estimate trout abundance, using the modified Peterson estimator. Separate abundance estimates were calculated for the smallest size groups possible (usually 25 mm), having at least three recaptured fish per group in order to satisfy model assumptions. We assumed that there was 1) no mortality of marked fish between marking and recapture passes, 2) no movement of marked or unmarked fish out of the survey

transect between the marking and recapture run, and 3) no difference in capture probability between wild and M_{YY} Brook Trout of a given size. Abundance estimates for each group (wild and M_{YY} Brook Trout) were calculated from the proportion of catch comprised by each group in each size class during the recapture run (Meyer and High 2011). Abundance estimates for wild and M_{YY} Brook Trout within the treatment reach (N_{Tx}) were extrapolated from the survey transects to the rest of the treatment reach to estimate total abundance.

M_{YY} Brook Trout mortality

Single-pass backpack electrofishing surveys were conducted 300 m above and below each treatment reach to help characterize emigration of M_{YY} Brook Trout out of their treatment reaches. Emigration rates (A') were estimated as:

$$A' = (n/R)/p,$$

where n was the number of M_{YY} Brook Trout emigrants from the treatment reach, R was the number of M_{YY} Brook Trout released into the treatment reach, and p was the capture efficiency for M_{YY} Brook Trout in the mark-recapture surveys. We calculated 90% confidence intervals (CIs) around the proportion of M_{YY} Brook Trout that emigrated out of the treatment reach in each stream in 2014, following Fleiss (1981). Because emigrants may have moved more than 300 m outside of the stocking reach, our characterization of emigration should clearly be regarded as minimum estimates of emigration

Unadjusted mortality (A) of M_{YY} Brook Trout was estimated by:

$$A = 1 - \left(\frac{N_{Tx}}{X}\right),$$

where N_{Tx} is the abundance of M_{YY} Brook Trout estimated within the treatment reach in October and X is the number released in June of 2014. Apparent mortality (A_{adj}) was calculated by subtracting the emigration rate (A') from the unadjusted mortality estimate (A).

Wild Brook Trout sex ratios

During removals in June 2014, dissections were conducted to phenotypically estimate sex ratios on 145 and 82 wild adult Brook Trout in Bear and Wildhorse creeks, respectively. To genetically estimate sex ratios for wild Brook Trout populations, approximately 100 tissue samples were collected from wild Brook Trout fry (<90mm) in each study stream in October of 2014. Tissue samples were clipped from the caudal fin and were preserved in vials filled with 100% ethyl alcohol. 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: CCCACCACCACTCCTAAG; UsdYMod-Forward (modified from Angles et al. 2004): CCCAGCACTSTTTTCTTRTCTCA, Reverse: CTTAAAACYACTCCACCCTCCAT; 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.

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

Spawn timing and percent maturity

We retained 100 M_{YY} Brook Trout in a circular tank at Mackay Hatchery through November 6 to estimate peak ripeness timing and maturation rate. From October 9 through November 6 2014, 20 Brook Trout per week were examined by squeezing the abdomen to express milt, then dissected to confirm they were males and characterize maturity of the gonads. Males were classified as immature if testes were opaque and threadlike and as mature if testes were large and milky white.

Genetic detection of first-generation offspring from M_{YY} Brook Trout

Approximately 100 tissue samples were collected from Brook Trout fry (<90 mm) at each study stream in October 2015. Tissue samples were collected, stored, and analyzed in the same manner as in 2014.

DNA was extracted from all samples using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland). All samples were screened with 240 single nucleotide polymorphic (SNP) loci identified and developed from Restriction Site Associated DNA sequencing. Genotyping followed Genotyping in Thousands Sequencing (GTseq) protocols developed by Campbell et al. (2015). Genotype data was initially formatted in program GENALEX 6.5, and Shannon’s Mutual Information Index (^SHUA) was calculated for each locus. The 100 loci that exhibited the highest pairwise ^SHUA differentiation between wild and M_{YY} Brook Trout were chosen for final analyses. Observed heterozygosity for all sample groups and pairwise genetic differentiation (F_{ST}) between wild and M_{YY} Brook Trout were estimated in program GENELEX. The power of genetic assignment tests depends largely on levels of genetic differentiation between populations, with high statistical certainty observed (99.9%) with $F_{ST} \geq 0.15$ (Manel et al. 2002).

Putative first generation (F_1) offspring from M_{YY} Brook Trout were identified using program STRUCTURE version 2.3.3 (Pritchard et al. 2000). STRUCTURE was used to estimate individual membership coefficients assuming an admixture model, uncorrelated allele frequencies, and no population priors. Under the admixture model, STRUCTURE estimates a membership coefficient (Q), which represents the portion of an individual’s genome that assigns to a predetermined number of K clusters. We assumed that $K = 2$, corresponded to

either wild or M_{YY} Brook Trout. A burn-in of 10,000 steps followed by 10,000 additional Markov Chain Monte Carlo iterations was performed. For comparison purposes, we created simulated F_1 offspring between known M_{YY} Brook Trout and wild individuals using functions in program Excel. For each study population, 10 simulated offspring genotypes were created by crossing 5 M_{YY} “parents” with 5 wild “parents” from each study stream. Offspring genotypes were created using functions in program Excel to randomly pick alleles at each locus and pass them to “offspring”. The expectation was that simulated F_1 offspring would have approximately equal probability of membership to each cluster ($K \approx 0.5$). The admixture proportions observed in the simulated F_1 offspring were used as criteria to assign juveniles as F_1 offspring between M_{YY} and treatment stream females.

RESULTS

Removals

For the full treatment in Bear and Wildhorse creeks, electrofishing resulted in the removal of 1,026 and 210 wild Brook Trout, respectively (Figure 2). In Bear Creek, wild Brook Trout that were removed averaged 145 mm TL (range 33-290), whereas in Wildhorse Creek, removed fish averaged 140 mm TL (range 68-254).

Abundance

Estimated abundance of wild Brook Trout was significantly higher in Bear Creek (3,254) relative to the other three treatment streams (average 1,671; Figure 3). Abundance was lowest in Cherry and Iron Bog creeks. In all study streams combined, we estimated 226 M_{YY} Brook Trout and 8,266 wild Brook Trout (>100 mm); therefore M_{YY} fish comprised 2.7% of the spawning Brook Trout population. Capture efficiencies averaged 56% (range 43-74%) for all size classes and at all four treatment streams for wild Brook Trout (Table 3). Average capture efficiency across all treatment streams was 83% for M_{YY} Brook Trout (range 75-100%).

Compared to the fall abundance estimates, and assuming no mortality in wild fish from June to October, M_{YY} fish were stocked at an average of 27% of the wild Brook Trout population across all study streams. Stocking rates ranged from 15% at Bear Creek to 40% at Iron Bog Creek and were similar at Cherry (30%) and Wildhorse (25%) creeks. The removals in June constituted a 33% suppression of the wild population in Bear Creek and an 11% suppression in Wildhorse Creek.

M_{YY} Brook Trout mortality

Emigration of M_{YY} Brook Trout out of the treatment reach and into adjacent stream reaches was rare, averaging only 1.2% and ranged from 0.2% in Wildhorse Creek to 2.4% in Bear Creek (Table 2). Apparent mortality of M_{YY} fish was high, averaging 87.4%, and ranged from a low of 76.6% in Bear Creek to 91.3% in Wildhorse Creek (Figure 4).

Angler exploitation rates varied across all treatment streams. Bear and Cherry creeks had zero estimated angler exploitation (Table 2). Exploitation of M_{YY} Brook Trout was moderate in Wildhorse Creek ($27.5 \pm 16.0\%$) with Iron Bog Creek only slightly lower ($22.0 \pm 14.4\%$). No catch and release of M_{YY} fish was documented via tag returns.

Wild Brook Trout sex ratios

Dissections of adult-sized wild Brook Trout, to estimate phenotypic sex ratios for the wild population, identified 82 (56.6%) males in Bear Creek and 34 (38.2%) males in Wildhorse Creek. To genetically estimate fry sex ratios, an average of 104 tissue samples (range 92-123) were collected from Brook Trout (<90 mm) in each of the six study streams (Table 4). Analyses of the Brook Trout genetic sex marker estimated that males were most strongly favored in Bear Creek (54%), and least favored in Iron Bog and Alder creeks (42%). We also collected tissue samples from approximately 25 wild adult male and approximately 25 wild adult female Brook Trout from each of the four treatment streams to validate the accuracy of the sex marker (Table 6). Concordance between phenotypic sex ratios and genetic sex ratios was high at each population and averaged 97.5% over all sexes and populations (Table 7).

Spawn timing and percent maturity

A high percentage of M_{YY} Brook Trout retained at Mackay Hatchery matured (75-85%; Table 5), though a small percentage (13%) did not express milt (Table 5). In 2014, peak spawn timing for this subsample of fish was estimated to be October 16-30.

Genetic detection of first-generation offspring from M_{YY} Brook Trout

In 2015, we collected tissue samples from an average of 103 (range 100-115) Brook Trout fry from each of the six study streams for genetic assignment tests at each population (Table 6). Expected heterozygosity (H_E) using the 100 SNP loci was generally high and similar among control and treatment wild Brook Trout populations, ranging from 0.36 in Alder Creek and E.F. Big Lost River sample groups to 0.42 in Bear Creek sample groups. The sample of M_{YY} Brook trout exhibited lower H_E (0.21). Genetic differentiation as measured by F_{ST} among Wild and M_{YY} Brook Trout Brook Trout was very large averaging 0.31 (range 0.23-0.34).

Wild individuals from study streams collected prior to stocking and M_{YY} Brook Trout were classified into their respective clusters with high membership coefficients ($Q > 0.95$). Simulated F_1 offspring assigned roughly equally to both clusters with an average membership coefficient ranging from 0.488 to 0.525. As expected, no individuals with genotypes indicating F_1 offspring were detected in the two control streams (Alder Creek and E.F. Big Lost River); however, some M_{YY} offspring were identified in each of the treatment streams. Fourteen probable F_1 M_{YY} offspring were identified in all treatment streams combined (Table 6). The highest number of F_1 offspring were observed in Wildhorse Creek ($n=5$), followed by Cherry Creek ($n=4$), Bear Creek ($n=3$), and Iron Bog Creek ($n=2$). All 14 individuals identified as probable F_1 offspring were genetic XY males.

DISCUSSION

We estimated that the number of M_{YY} Brook Trout that survived to spawn comprised about 2.7% of all spawning adults in study streams in 2014. Genetic assignment tests showed that M_{YY} offspring comprised about 3.7% of the progeny at all study streams. Using the phenotypic sex ratios at full treatment streams, we estimated the abundance of wild males spawning in October. Therefore, we estimated that at both Bear and Wildhorse creeks, 5.6% of spawning males were M_{YY} fish. If 5.6% M_{YY} fish produced 3.2% and 5.0% of the offspring at Bear and Wildhorse creeks respectively, they contributed 57% and 90% as well as their wild counterparts (>100 mm). Because wild adult abundance was estimated for all fish larger than

100 mm, our estimated proportion of spawning M_{YY} fish might be negatively biased if a low proportion of male Brook Trout between 100-149 mm matured (Meyer et al. 2006). We did not estimate size at maturity for wild fish, but if we assume most males are sexually mature at 150 mm (Meyer et al. 2006), and limit the abundance estimate to fish greater than 150 mm, then M_{YY} fish comprised 8.4% and 11.4% of all spawning male Brook Trout at Bear and Wildhorse creeks respectively (data not shown). Then, perhaps M_{YY} fish contributed only 38% and 44% as well as their wild counterparts at Bear and Wildhorse creeks, respectively. Therefore, M_{YY} fish contributed between 38-57% as well as wild males in Bear Creek, and between 44-90% as well as wild males in Wildhorse Creek. The difference between these two ranges is a result of the disparity in stocking rates at these two study streams.

Apparent mortality was high for hatchery M_{YY} Brook Trout but was similar to other catchable-sized hatchery trout stocked in streams (e.g., Miller 1952; Hochachka and Sinclair 1962; Bettinger and Bettoli 2002; High and Meyer 2009). Hatchery trout survival can be augmented through the reduction of wild fish abundance (Miller 1958). In our study, survival of M_{YY} fish was apparently improved by the suppression of wild Brook Trout abundance in Bear Creek (33% suppression) but not Wildhorse Creek (11% suppression). Higher stream discharge and lower ambient conductivity, which measures the water's ability to transmit electricity (Reynolds and Kolz 2012), both contributed to reduced electrofishing capture efficiency during June suppression efforts. Fall capture efficiencies were very high for M_{YY} Brook Trout in all streams, likely because of their larger size. During future removals, if such high flow and low conductivity conditions are identified, multiple efforts could be conducted to remove more fish to ensure that competition is substantially reduced.

M_{YY} Brook Trout released in these streams had a competitive size advantage over the resident wild trout, which may also have reduced their mortality (Hochachka and Sinclair 1962; Xu et al. 2010). Larger M_{YY} Brook Trout may also be more effective at shifting the sex ratio of a population. During agonistic behavior between hatchery and wild trout, size has been identified as an advantage (Petrosky and Bjornn 1984), so larger M_{YY} Brook Trout could perhaps be more effective spawners than the smaller wild Brook Trout. Considering the low proportion of progeny identified as M_{YY} offspring, we assumed they did not detectably alter the sex ratio of the wild population, so sex ratios were not evaluated in 2015. Genetic assignment tests were used here to identify low levels of reproduction because this was a short-term study. However, if multiple years of stocking were planned, monitoring the sex ratios using the genetic sex marker from fry is likely the most cost efficient tool for monitoring treatment progress.

Our estimates of emigration were considered minimum rates because we did not search the entire stream for migrants. Apparent mortality estimates were largely influenced by the fall abundance estimate, which depended in part on the accuracy of the emigration estimate. High and Meyer (2009) found 95% of hatchery Rainbow Trout released in an Idaho river to be within three kilometers of the release point; however, their study river was several times as large as our study streams. Data from another study, in a smaller Idaho stream more comparable to our study streams, also showed that a low percentage (6%) of hatchery trout move more than a few hundred meters (Heimer et al. 1985). Both of these studies suggest low rates of emigration out of the release area, which is substantiated by other research within Brook Trout's native range (e.g., Carlson and Letcher 2003; Hoxmeier and Dieterman 2013), which collectively suggests that emigration had little effect on our estimate of apparent mortality.

It was reasonable to assume that M_{YY} Brook Trout spawn timing was in synchrony with the wild population because streamside necropsies in October identified similar gonadal development between wild and M_{YY} Brook Trout and both male types readily expressed milt.

Similarly, peak spawn timing in M_{YY} fish retained at the hatchery was concurrent with observed spawn timing of wild Brook Trout at study streams. The estimated percent of M_{YY} fish that matured at the hatchery was considered a minimum estimate because they were lethally sampled for necropsy and inspection of gonad size. Fish that were not mature during earlier necropsies might have matured later, and none of the M_{YY} fish at the hatchery were in the presence of female Brook Trout to help encourage maturation. Maturation rates should continue to be monitored for several brood years of M_{YY} Brook Trout to validate the assumption that hatchery and wild Brook Trout have equal probabilities of spawning with females in the wild. For better estimates of maturity rates in the future, investigations should delay dissections until the end of spawning in early November to make sure that fish maturing later are identified, and maturity rates of M_{YY} Brook Trout should be monitored in the wild, in the presences of females.

Angler exploitation of M_{YY} Brook Trout occurred in half of our treatment streams. The treatment reaches in Iron Bog and Wildhorse creeks were both located near (<1 km) Forest Service campgrounds, though no angler exploitation data were available for these two streams *a priori*. Angler exploitation from only these two streams highlights the similarities between stream sets.

We are uncertain why concordance between phenotypic and genotypic sex was not 100% at Wildhorse and Iron Bog creeks. It is possible that there were errors in determining or recording phenotypic sex; however we are confident in results provided by the genetic sex marker. In 2016, the sex marker was validated in a nearby stream where phenotypic and genotypic sex were in 100% concordance for 1,181 Brook Trout (Schill et al. in preparation).

To our knowledge this experiment represents the first time that hatchery produced M_{YY} fish of any species have been released into the wild and documented to have persisted long enough to have spawned successfully with wild fish. This represents a major advancement toward using M_{YY} Brook Trout to eradicate unwanted Brook Trout in streams. Further evaluations are warranted to determine if this management approach to eradicating non-native Brook Trout populations is effective at shifting the sex ratio to the extent that a wild population of Brook Trout can be eradicated.

RECOMMENDATIONS

1. Implement a full-scale M_{YY} Brook Trout study with the intention of collapsing wild Brook Trout populations in multiple lakes and streams in Idaho. Study design should evaluate whether stocking fingerlings or catchable-sized M_{YY} Brook Trout are more effective at skewing sex ratios in wild populations over time, and whether concurrent suppression of wild fish accelerates sex ratio changes.
2. Validate the accuracy of the genetic sex marker test by phenotypically determining the sex of additional wild adult Brook Trout of each sex in additional streams and comparing results between phenotypic and genetic sex determinations.
3. Estimate percent maturity of M_{YY} Brook Trout at Mackay Hatchery and delay dissection of immature fish until early November. Estimate percent maturity of catchable-sized M_{YY} fish in the presence of females at a study stream.

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LITERATURE CITED

- Bahls, P. 1992. The status of fish populations and management of high mountain lakes in the western United States. *Northwest Science* 66:3.
- Benjamin, J. R., J. B. Dunham, and M. R. Dare. 2007. Invasion by nonnative Brook Trout in Panther Creek, Idaho: roles of local habitat quality, biotic resistance, and connectivity to source habitats. *Transactions of the American Fisheries Society* 136:875-888.
- Bettinger, J. M., and P. W. Bettoli. 2002. Fate, dispersal, and persistence of recently stocked and resident Rainbow Trout in a Tennessee tailwater. *North American Journal of Fisheries Management* 22:425-432.
- Bettles, C. M., J. VonBargen, and S. F. Young. 2005. Microsatellite DNA characterization of selected Bull Trout (*Salvelinus confluentus*) populations within the Pend Oreille River Basin. Unpublished WDFW Molecular Genetics Laboratory Report submitted to Joe Maroney, Kalispel Tribe.
- Billman, H. G., C. G. Kruse, S. St-Hilaire, T. M. Koel, J. L. Arnold, C. R. Peterson. 2012. Effects of rotenone on Columbia Spotted Frogs *Rana luteiventris* during field applications in lentic habitats of southwestern Montana. *North American Journal of Fisheries Management* 32:781-789.
- Britton, J. R., R. E. Gozlan, and G. H. Copp. 2011. Managing non-native fish in the environment. *Fish and Fisheries* 12.3:256-274.
- Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* 15:855-867.
- Carlson, S. M., and B. H. Letcher. 2003. Variation in Brook and Brown trout survival within and among seasons, species, and age classes. *Journal of Fish Biology* 63:780-794.
- Dunham, J. B., D. S. Pilliod, and M. K. Young. 2004. Assessing the consequences of nonnative trout in headwater ecosystems in western North America. *Fisheries* 29:18-26.
- Fisher, R. A., and J. H. Bennett. 1999. *The genetical theory of natural selection: a complete variorum edition*: Oxford University Press. Pages 121-145.
- Fleiss, J. L. 1981. *Statistical methods for rates and proportions*. Wiley, New York.
- Gamett, B. L. 2003. A summary: the origin of fishes in the Sinks Drainages of southeastern Idaho. Page 16 *in* Van Kirk, R. W., J. M. Capurso, and B. L. Gamett, editors. 2003. *The Sinks symposium: exploring the origin and management of fishes in the Sinks Drainages of southeastern Idaho*. Idaho Chapter American Fisheries Society, Boise.
- Gutierrez, J. B., and J. L. Teem. 2006. A model describing the effect of sex-reversed YY fish in an established wild population: The use of a Trojan Y chromosome to cause extinction of an introduced exotic species. *Journal of Theoretical Biology* 241:333-341.

- Hamilton, W. D. 1967. Extraordinary sex ratios: a sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science* 156:477-488.
- Hamilton, B. T., S. E. Moore, T. B. Williams, N. Darby, and M. R. Vinson. 2009. Comparative effects of rotenone and antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. *North American Journal of Fisheries Management* 29:1620-1635.
- Heimer, J. T., W. M. Frazier, and J. S. Griffith. 1985. Poststocking performance of catchable-size hatchery rainbow trout with and without pectoral fins. *North American Journal of Fisheries Management* 5:21-25.
- High, B., and K. A. Meyer. 2009. Survival and dispersal of hatchery triploid Rainbow Trout in an Idaho river. *North American Journal of Fisheries Management* 29:1797-1805.
- Hochachka, P. W., and A. C. Sinclair. 1962. Glycogen stores in trout tissues before and after stream planting. *Journal of the Fisheries Board of Canada*, 19:127-136.
- Hoxmeier, R. J. H., and D. J. Dieterman 2013. Seasonal movement, growth and survival of Brook Trout in sympatry with Brown Trout in Midwestern US streams. *Ecology of Freshwater Fish* 22:530-542.
- IDFG (Idaho Department of Fish and Game). 2007. Mountain Whitefish conservation and management plan for the Big Lost River Drainage, Idaho. Management Report. Volume 165-04. Idaho Department of Fish and Game, Boise.
- Koenig, M. K., K. A. Meyer, J. R. Kozfkay, J. M. Dupont, and E. B. Schriever. 2015. Evaluating the ability of Tiger Muskellunge to eradicate Brook Trout in Idaho Alpine Lakes. *North American Journal of Fisheries Management* 35:659-670.
- Manel, S., P. Berthier, and G. Luikart. 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology* 16:650-659.
- Meyer, K. A., J. A. Lamansky Jr., and D. J. Schill. 2006. Evaluation of an unsuccessful Brook Trout electrofishing removal project in a small Rocky Mountain stream. *North American Journal of Fisheries Management* 26:849-860.
- Meyer, K. A., and B. High. 2011. Accuracy of removal electrofishing estimates of trout abundance in Rocky Mountain streams. *North American Journal of Fisheries Management* 31:923-933.
- Meyer, K. A., and D. J. Schill. 2014. Use of a statewide angler tag reporting system to estimate rates of exploitation and total mortality for Idaho sport fisheries. *North American Journal of Fisheries Management* 34:1145-1158.
- Miller, R. B. 1952. Survival of hatchery-reared Cutthroat Trout in an Alberta stream. *Journal of the Fisheries Research Board of Canada* 15:27-45.
- Miller, R. B. 1954. Comparative survival of wild and hatchery-reared Cutthroat Trout in a stream. *Transactions of the American Fisheries Society* 83:120-130.

- Miller, R. B. 1958. The role of competition in the mortality of hatchery trout. *Journal of the Fisheries Research Board of Canada* 15:27-45.
- Montana Fish, Wildlife and Parks. 2004. *Fisheries Analysis +*, Version 1.0.8. Bozeman.
- Parshad, R. D. 2011. Long time behavior of a PDE model for invasive species control. *International Journal of Mathematical Analysis* 40:1991-2015.
- Petrosky, C. E., and T.C. Bjorn. 1984. Competition from catchables – a second look. Pages 63-68 in F. Richards and R.H. Hamre, editors. *Proceedings of the Wild Trout III Symposium at Yellowstone National Park*. Bozeman, Montana.
- Pritchard, J. K., M. A. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Rahel, F. J. 2000. Homogenization of fish faunas across the United States. *Science* 288:854-856.
- Reynolds, J. B., and A. L. Kolz. 2012. Electrofishing. Pages 305–361 in A. V. Zale, D. L. Parrish, and T. M. Sutton, editors. *Fisheries techniques*, 3rd edition. American Fisheries Society, Bethesda, Maryland.
- Schill, B. D., B. L. Gamett, M. R. Campbell, and N. Vu. *In Preparation*. An evaluation of phenotype-genotype concordance in two isolated Brook Trout populations.
- Schill, D. J., J. A. Heindel, M. R. Campbell, K. A. Meyer, and E. R. J. M. Mamer. 2016. Production of a YY male Brook Trout broodstock for potential eradication of undesired Brook Trout populations. *North American Journal of Aquaculture* 78:72-83.
- Schill, D. J., K. A. Meyer, and M. J. Hansen. In review. *Combining the stocking of YY Male Brook Trout with manual suppression for eradication of undesired Brook Trout populations*. *North American Journal of Fisheries Management* XX:XXX-XXX.
- Schuck, H. A. 1948. Survival of hatchery trout in streams and possible methods of improving the quality of hatchery trout. *Progressive Fish Culturist* 10:3-14.
- Shepard, B. B., L. M. Nelson, M. L. Taper, and A. V. Zale. 2014. Factors influencing successful eradication of nonnative Brook Trout from four small rocky mountain streams using electrofishing. *North American Journal of Fisheries Management* 34:988-997.
- Teem, J. L., and J. B. Gutierrez. 2010. A theoretical strategy for eradication of Asian carps using a Trojan Y chromosome to shift the sex ratio of the population. In *American Fisheries Society Symposium* Vol. 74.
- Thompson, P. D., and F. J. Rahel. 1996. Evaluation of depletion–removal electrofishing of Brook Trout in small Rocky Mountain streams. *North American Journal of Fisheries Management* 16:332-339.

- Thresher, R. E., K. Hayes, N. J. Bax, J. Teem, T. J. Benfey, and F. Gould. 2013. Genetic control of invasive fish: technological options and its role in integrated pest management. *Biological Invasions* DOI 10.1007/s10530-013-0477-0.
- Wedekind, C. 2012. Managing population sex ratios in conservation practice: how and why. *Topics in Conservation Biology* 81-96.
- Xu, C. L., B. H. Letcher, and K. H. Nislow. 2010. Size-dependent survival of Brook Trout *Salvelinus fontinalis* in summer: effects of water temperature and stream flow. *Journal of Fish Biology* 76:2342-2369.
- Yano, A., B. Nicol, E. Jouanno, E. Quillet, A. Fostier, R. Guyomard, and Y. Guiguen. 2013. The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evolutionary Applications* 6(3):486-496.

TABLES

Table 1. Coordinates (UTM) of the downstream boundary of the treatment reach (Tx) where M_{YY} Brook Trout were released in four study streams in 2014. Survey length and Tx length were used to estimate the proportion of the entire treatment reach surveyed for abundance.

Stream name	Treatment		Tx length (m)	Surveyed length (m)	Proportion surveyed
	downstream boundary (UTM)				
Bear Creek	12T 282491E	4839919N	1,931	600	0.31
Cherry Creek	12T 286076E	4845963N	2,414	600	0.25
Wildhorse Creek	11T 733569E	4854548N	2,575	600	0.23
Iron Bog Creek	12T 276435E	4836670N	2,414	600	0.25

Table 2. The number of M_{YY} Brook Trout released into four study streams in central Idaho in 2014. T-bar anchor-tagged fish were used to estimate angler exploitation rates and confidence intervals (CIs). The number of emigrants encountered during abundance surveys was used to estimate the emigration rate and CIs for M_{YY} Brook Trout out of each treatment reach.

Stream name	M_{YY} Brook Trout						
	Released	Tagged	Angler exploitation	90% CI	Emigrants	Emigration rate ^a	90% CI
Bear Creek	492	50	0.0%	0.0	9	2.4%	1.0
Cherry Creek	500	50	0.0%	0.0	2	0.5%	0.5
Wildhorse Creek	506	50	27.5%	16.0	1	0.2%	0.3
Iron Bog Creek	512	50	22.0%	14.4	9	1.8%	1.0

^a Minimum estimate of emigration out of the treatment reach

Table 3. Wild and M_{YY} Brook Trout marked and recaptured during abundance estimates, and capture efficiencies at four study streams in central Idaho during October 2014.

Stream name	Wild Brook Trout (≥ 100 mm)				M_{YY} Brook Trout		
	Removed	Marked	Recaptured	Capture efficiency (p)	Marked	Recaptured	Capture efficiency (p)
Bear Creek	1,026	445	182	47%	25	3	75%
Cherry Creek	0	250	115	62%	8	6	75%
Wildhorse Creek	210	201	78	43%	8	5	83%
Iron Bog Creek	0	223	106	74%	9	6	100%

Table 4. Proportion and 90% confidence intervals (CI) of wild Brook Trout fry (<90mm) from six study streams that were determined to be male using two genetic sex markers. These fry were the progeny of wild Brook Trout that spawned in the fall of 2013, before the introduction of M_{YY} Brook Trout.

Stream name	Designation	Brook Trout (n)	Male proportion	Lower CI	Upper CI
Bear Creek	Treatment	108	0.54	0.44	0.62
Cherry Creek	Treatment	92	0.51	0.41	0.60
Wild Horse Creek	Treatment	123	0.43	0.35	0.51
Iron Bog Creek	Treatment	102	0.42	0.34	0.51
Alder Creek	Control	100	0.42	0.33	0.51
East Fork Big	Control	89	0.44	0.35	0.53
Grand Total		614	0.46	0.37	0.55

Table 5. Examinations of M_{YY} Brook Trout at Mackay Hatchery from October 9 to November 6, 2014 to monitor spawn timing and percent maturation. Study fish were examined by squeezing to see if they expressed milt, and then dissected to characterize testes as mature or immature.

Exam date	Brook Trout examined	Squeezed		Dissected ^a		% Mature ^b
		Expressed milt	No milt	Mature testes	Immature testes	
10/09	20	5	15	2	1	25%
10/16	20	12	8	19	1	60%
10/23	20	17	3	17	3	85%
10/30	20	16	4	16	4	80%
11/06	20	15	5	16	4	75%
Total	100	65	35	58	13	

^a Only three fish were dissected on the first exam date.

^b Maturity estimated from fish that expressed milt.

Table 6. Sample group along with treatment level (full, partial, or control) and stream name. Sample size (n) and expected heterozygosity (H_E) is shown for each sample group, along with minimum, maximum, and average proportional membership (Q_1) observed. Juveniles collected in 2014 were sampled to determine starting sex ratios. Adults collected in 2015 were sampled to test phenotypic versus genetic sex.

Stream name	Treatment level	Sample group	Life stage	Sample year	n	H_E	Q_1 (Min)	Q_1 (Max)	Q_1 (Avg)
Alder Creek	Control	Alder	Juvenile	2015	92	0.36	0.001	0.137	0.015
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.973	0.999	0.996
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.265	0.679	0.488
Bear Creek	Full	Pre-treatment	Juvenile	2014	44	0.40	0.001	0.101	0.012
		Sex marker verification	Adult	2015	49	0.42	0.001	0.120	0.014
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.984	0.999	0.998
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.423	0.600	0.521
		Not detected as offspring	Juvenile	2015	92	0.42	0.001	0.110	0.011
		Detected as M_{YY} offspring	Juvenile	2015	3	N/A	0.456	0.554	0.509
Cherry Creek	Partial	Pre-treatment	Juvenile	2014	33	0.40	0.002	0.054	0.010
		Sex marker verification	Adult	2015	47	0.40	0.002	0.146	0.018
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.989	0.999	0.997
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.412	0.602	0.513
		Not detected as offspring	Juvenile	2015	89	0.40	0.002	0.326	0.014
		Detected as M_{YY} offspring	Juvenile	2015	4	N/A	0.417	0.552	0.500
East Fork Big Lost River	Control	E.F. Big Lost	Juvenile	2014	44	0.36	0.001	0.162	0.012
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.983	0.999	0.998
		E.F. Big Lost	Juvenile	2015	90	0.36	0.001	0.035	0.006
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.306	0.530	0.402
Iron Bog Creek	Partial	Iron Bog (pre-treatment)	Juvenile	2014	43	0.39	0.001	0.058	0.008
		Sex marker verification	Adult	2015	48	0.39	0.002	0.027	0.006
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.987	0.999	0.997
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.285	0.523	0.423
		Not M _{YY} offspring	Juvenile	2015	92	0.39	0.001	0.243	0.014
		M_{YY} offspring	Juvenile	2015	2	N/A	0.405	0.644	0.525
Wildhorse Creek	Full	Sex marker verification	Adult	2015	18	0.37	0.003	0.014	0.006
		M _{YY} Brook Trout	Adult	2015	69	0.21	0.986	0.999	0.996
		Simulated F ₁ offspring	N/A	N/A	10	N/A	0.251	0.606	0.421
		Not M _{YY} offspring	Juvenile	2015	95	0.36	0.002	0.197	0.014
		M_{YY} offspring	Juvenile	2015	5	N/A	0.383	0.605	0.490

Table 7. Concordance between phenotypic sex identification and genetic sex identification of adult wild Brook Trout collected from four study streams.

Stream name	Male (Pheno/Geno)	Concordance	Female (Pheno/Geno)	Concordance
Bear Creek	25/26	0.96	25/25	1.00
Cherry Creek	23/23	1.00	26/26	1.00
Wildhorse Creek	25/25	1.00	25/27	0.93
Iron Bog Creek	23/26	0.88	24/25	0.96

FIGURES

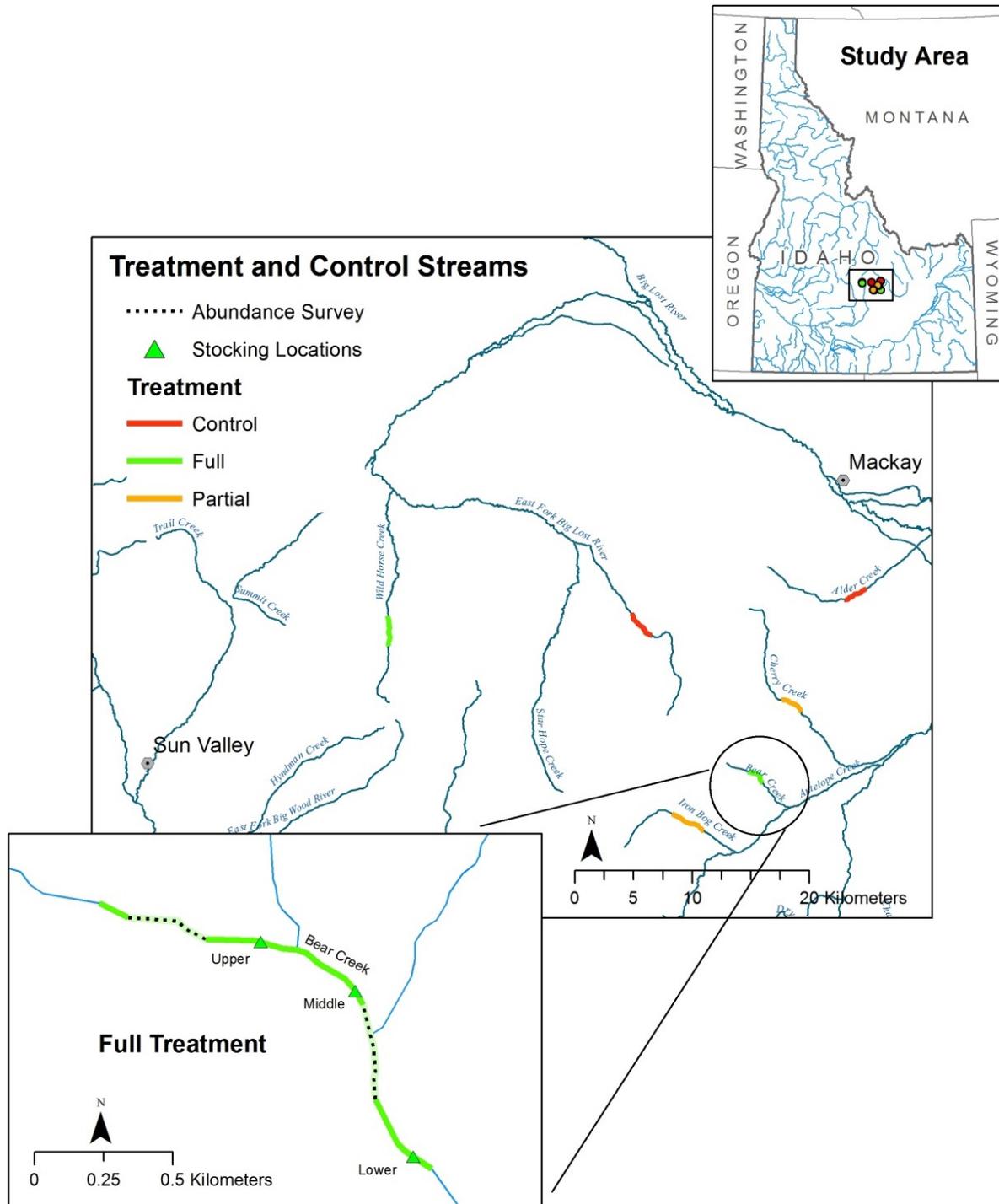


Figure 1. Study streams in the Big Lost River drainage where M_{YY} Brook Trout were released in 2014. Green areas indicate treatment reaches where electrofishing removals were conducted before M_{YY} Brook Trout were released. Yellow areas indicate treatment reaches where M_{YY} Brook Trout were stocked without removals. Red areas were control streams used to monitor sex ratios. Abundance surveys and tissue sampling for genetic analyses were conducted within each treatment reach as in the example shown with dashed lines.

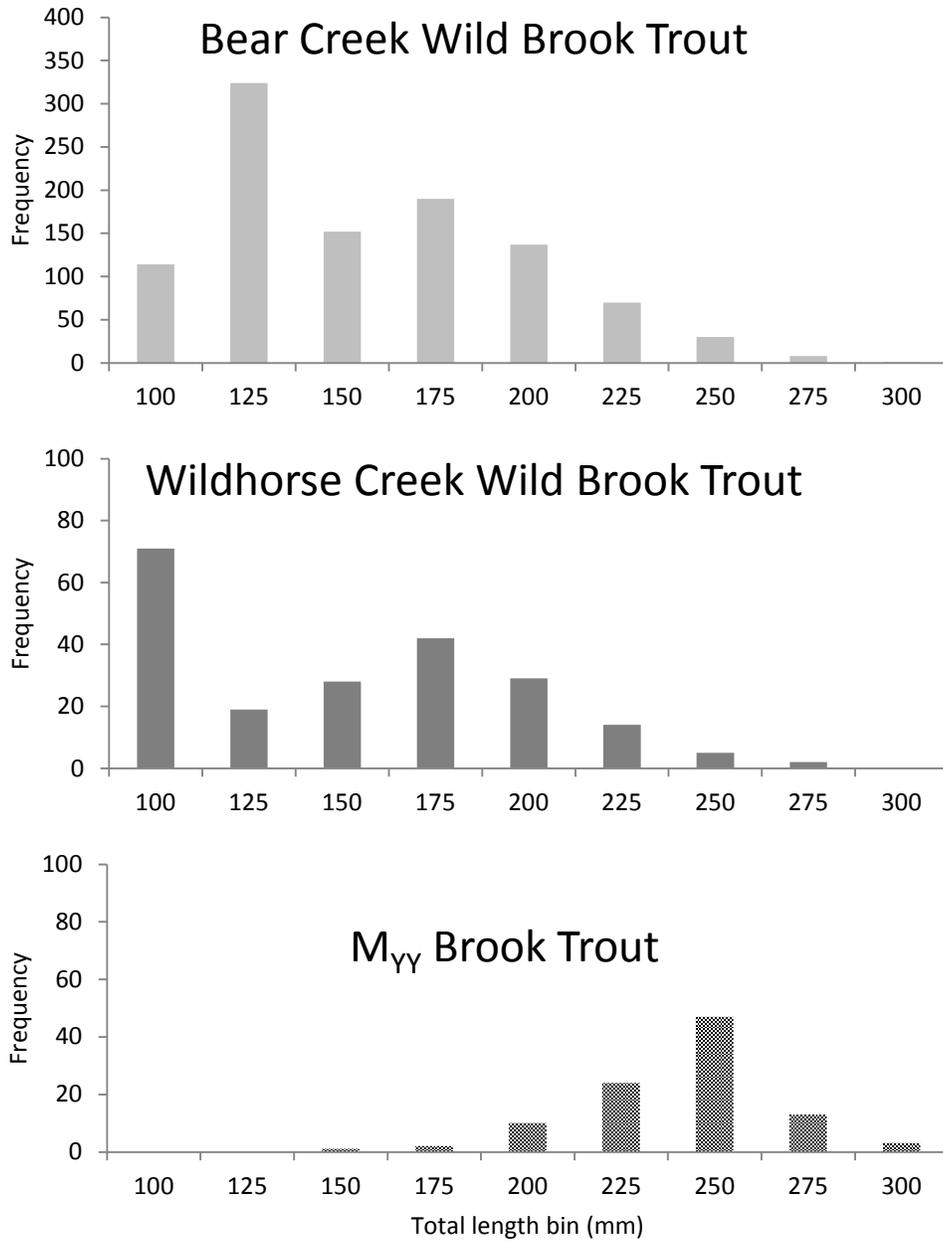


Figure 2. Length-frequency histograms of wild Brook Trout removed from Wildhorse and Bear Creeks in 2014; and length-frequency of catchable-sized M_{YY} Brook Trout released into these streams at the end of June 2014.

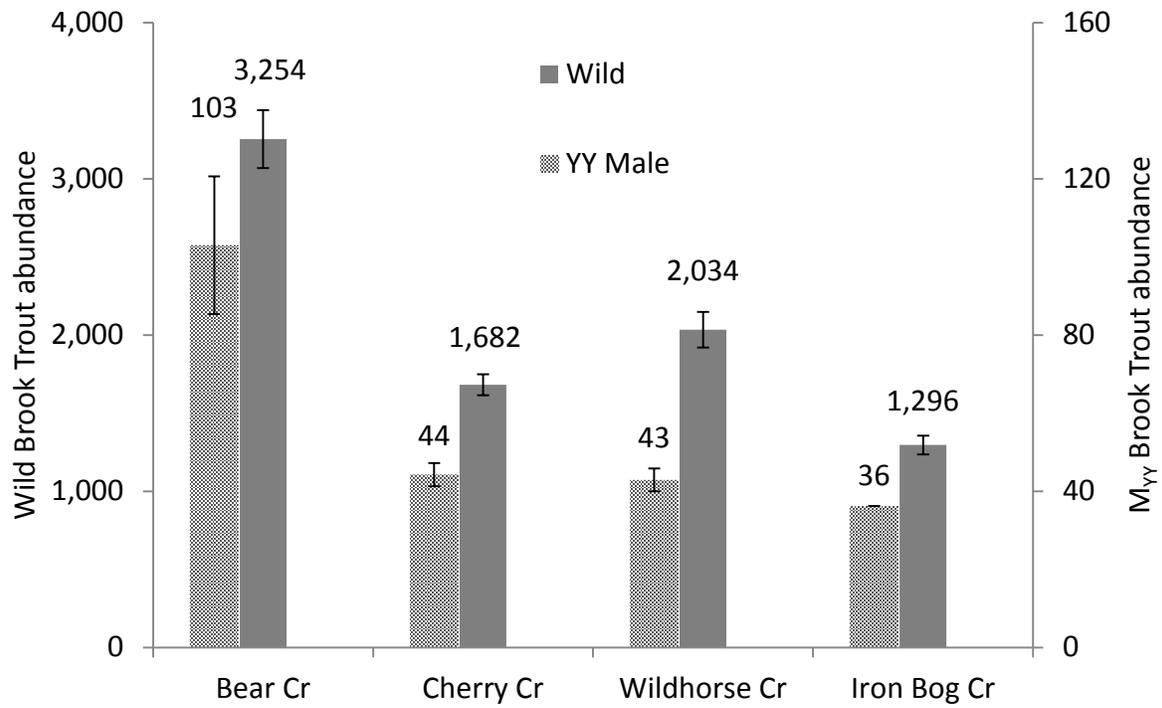


Figure 3. Estimated abundance of wild and M_{YY} Brook Trout at treatment reaches in four streams in early October 2014. Error bars are 90% confidence intervals.

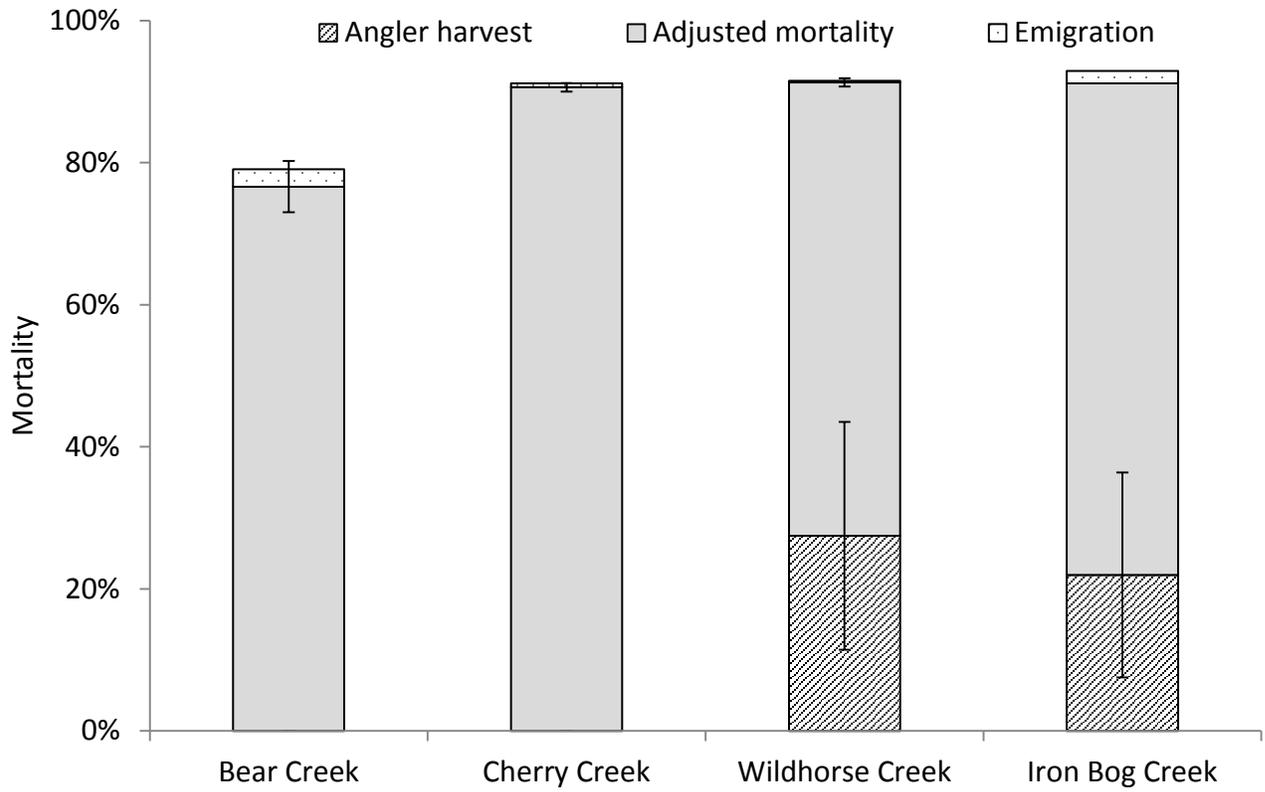


Figure 4. Summer mortality (June 26-October 17) of M_{YY} Brook Trout in four study streams in central Idaho in 2014. Adjusted mortality is increased by adding a minimum estimate of M_{YY} Brook Trout emigration outside of each treatment reach. Error bars are 90% confidence intervals.

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