FISHERY RESEARCH

WILD TROUT EVALUATIONS: M$_{YY}$ BROOK TROUT FIELD EVALUATIONS 2019

Report Period January 1, 2019 to December 31, 2019

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M_{YY} BROOK TROUT FIELD EVALUATIONS 2019

Non-native Brook Trout *Salvelinus fontinalis* were introduced throughout western North America in the early 1900s, resulting in widespread self-sustaining non-native 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. YY male Brook trout are 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, all offspring are males. This can potentially be used to shift the sex ratio of the wild population toward males, potentially reaching a point where no females remain in the population to reproduce, thus eliminating the population. We stocked fingerling (mean = 125 mm; range = 71–173 mm) M_{YY} Brook Trout in three streams and four lakes in 2019, and catchable (mean = 263 mm; range = 177–384 mm) M_{YY} Brook Trout in two streams and two lakes in 2019 to attempt to eradicate wild Brook Trout in these study systems. Prior to stocking, we suppressed wild Brook Trout via mechanical removal in two streams and two lakes to potentially increase survival of stocked M_{YY} Brook Trout, and therefore decrease the time to eradication. Suppression via mechanical removal in 2019 was 52% in Dry Creek, 28% in Pike's Fork Creek, 28% in Martin Lake, and 37% in Seafoam Lake. Sex ratios of wild Brook Trout were estimated for all five streams in which M_{YY} were present and in two additional control Brook Trout streams that were not stocked with M_{YY} Brook Trout. This long-term study is scheduled to be completed in 2026.

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INTRODUCTION

Brook Trout *Salvelinus fontinalis* were originally introduced outside their native range into waters of the western United States as early as 1872 by the California Fish Commission (MacCrimmon and Campbell 1969), and they 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 attempted to suppress or eliminate Brook Trout populations outside of their native range (reviewed in Dunham et al. 2004). There are several methods fisheries managers use to eradicate non-native fish (e.g., piscicides, mechanical removal, and introducing sterile predators). Managers have used piscicides with some success (Gresswell 1991; Lee 2001; Lentsch et al. 2001; Hepworth et al. 2002). However, piscicides may 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 to physically remove Brook Trout from streams (e.g., Thompson and Rahel 1996; Meyer et al. 2006; Shepard et al. 2014), but it has been questioned whether stream electrofishing removal alone can cause meaningful progress in Brook Trout eradication at the landscape scale (Meyer et al. 2006; Schill et al. 2017). Sterile predatory fish were introduced in alpine lakes and successfully eradicated Brook Trout in less than one-half of the lakes where the strategy was used (Koenig et al. 2015). The mixed success of these methods identifies the need for an additional method for non-native fish eradication.

One method, suggested decades ago for eradicating undesirable fish populations, is shifting the population sex ratio toward all males (Hamilton 1967). In this scenario, shifting the sex ratio over time could be accomplished by annual introductions of hatchery produced male fish with a YY genotype (M_{YY}), eventually resulting in population eradication by eliminating females (Gutierrez and Teem 2006; Teem and Gutierrez 2010). To create a M_{YY} brood stock, XY males are feminized by exposing them to estrogen (Teem and Gutierrez 2010). 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}. 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 of their progeny are M_{YY}. These M_{YY} progeny can then be stocked into wild fish populations 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 to eradicate a non-native fish species has not been tested in the wild (Wedekind 2012; Wedekind 2018).

In wild Brook Trout populations, sex ratios 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 16% 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. 2018a). Hatchery trout encounter many challenges upon being stocked into the wild, and often exhibit low survival, especially in streams (e.g., Miller 1952; Bettinger and Bettoli 2002; High and Meyer 2009). Low survival of hatchery trout in streams is largely attributed to the stress associated with adjusting to natural stream flows and competition with resident fish (Schuck 1948; Miller 1954; Miller 1958; Hochachka and Sinclair 1962). Though rarely evaluated, past studies suggested that 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 combining M_{YY} stocking with suppression of wild fish may decrease the time-to-eradication in wild Brook Trout populations.

Post-release survival of hatchery trout is also affected by size-at-release. Adult hatchery trout of catchable-size (avg. 220 mm), hereafter referred to as catchables, return to creel 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, reduced vulnerability to post-release predation, and reduced competition with wild fish. Catchables are also immediately vulnerable to anglers upon release, whereas fingerlings must survive and grow for months or perhaps more than a year before they grow to be vulnerable to anglers. Most work comparing survival between catchables and fingerlings has focused on overall return-to-creel, but the difference in short-term survival between fingerlings and catchables is unknown. The difference in survival between fingerlings and catchables is of particular interest in the case of M_{YY} fish because, the objective is to maximize abundance of mature Myy on the spawning grounds. However, it is unclear whether this is best achieved by stocking higher numbers of fingerling Myy or lower numbers of catchable Myy.

The Idaho Department of Fish and Game (IDFG) produced a YY Brook Trout broodstock that subsequently produced 20,000-30,000 M_{YY} Brook Trout for eventual stocking into the wild (Schill et al. 2016). Prior to large-scale stocking, survival and reproductive success of catchable M_{YY} Brook Trout in the wild were evaluated in Kennedy et al. (2018a), and this study indicated that M_{YY} Brook Trout could successfully survive and reproduce in the wild. Recent modelling suggests that annual stocking of M_{YY} Brook Trout into streams and alpine lakes can result in eradication of the wild population within 10 years if M_{YY} Brook Trout are stocked at a rate of 50% of the wild Brook Trout abundance (Schill et al. 2017). In model simulations, eradication occurred faster as suppression of the wild population increased. However, these models are theoretical and need to be tested on wild Brook Trout populations to validate predictions. For this study, the following objectives were outlined to guide our work:

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 streams and lakes.

2. Determine if suppressing the abundance of wild Brook Trout populations increases the survival 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 streams and lakes.

3. Determine if the use of M_{YY} is more effective at removing unwanted Brook Trout populations in lakes or in streams.

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 sizes at Mackay Hatchery in outdoor concrete raceways in 10-12°C single-use spring water until the time of release. However, as of 2019 production and rearing of M_{YY} Brook Trout has been shifted to Hayspur Fish Hatchery. All study fish were adipose clipped so they could be differentiated from wild fish after stocking. For this study, fingerlings and catchables were stocked at approximately 8 and 20 months after hatching, respectively.

Study streams and lakes were selected with self-sustaining Brook Trout populations comprising a large majority of the fish species composition (>80%). Each study stream had a total stream length of less than 10 km and had passage barriers which provided isolation from immigrating female Brook Trout (Figure 1; Table 1). Lakes were also chosen based on the presence of passage barriers which would prevent upstream immigration of Brook Trout (Figure 2; Table 2). Lakes varied in size from 1.8 hectares to 15.8 hectares. During 2015-2017, streams and lakes were assigned to one of two treatment levels (suppression and non-suppression) to evaluate fingerling and catchable stocking. At two of the streams and two of the lakes, we manually suppressed the wild Brook Trout population to improve survival and spawning success of stocked fish. Suppression was achieved by the removal of wild Brook Trout with backpack electrofishing in streams and gill nets and boat or raft electrofishing in lakes. Non-suppression streams and lakes were stocked with M_{YY} Brook Trout without the suppression of their wild counterparts. Two control streams and two control lakes were selected nearby to monitor the stochastic changes in wild Brook Trout populations in central Idaho. All treatment streams and 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. Sex ratios in each Brook Trout population will be assessed approximately every three years until the wild population is considered eradicated.

The first field evaluations of M_{YY} Brook Trout in streams began in 2016 and additional streams were included in 2017 (Table 1). Dry, Tripod, East Fork Clear, Alder, and Beaver creeks have been under evaluation since 2016, whereas Pike’s Fork and East Threemile creeks have been part of the evaluation since 2017. For a more complete discussion of previous study years for streams, see Kennedy et al. (2018c). Field evaluations of M_{YY} Brook Trout in Duck, Lloyds, Snowslide #4, and Upper Hazard lakes began in 2015, Black and Rainbow lakes were added in 2016, and Martin and Seafoam lakes have been part of the evaluation since 2017. For a more complete discussion of previous study years involving lake evaluations, see Kennedy et al. (2018b). Because of the three-year cycle associated with sampling, 2019 was a stream sampling year. Lakes were not surveyed in 2019, with the exception of Martin and Seafoam lakes which are annually surveyed. All lakes were stocked with M_{YY} in 2019, and sampling is scheduled to occur in 2021.

Stream surveys

Suppression of wild Brook Trout was conducted in Dry and Pike’s Fork creeks after snowmelt subsided (to maximize electrofishing capture efficiency) but prior to annual M_{YY} stocking. Before suppression, approximately 10 Brook Trout (≥100 mm) were marked with an upper caudal clip at each ½ km, 1-5 days prior to suppression so recaptured fish could be used to estimate abundance and capture efficiency. Single-pass electrofishing was conducted to capture fish over the entire study reach (range 3.9-9.1 km) and wild Brook Trout were removed. Electrofishing crews consisted of 2-3 people (depending on stream flow) with backpack electrofishers, and 1-3 people with nets and buckets (19 L). We used a pulsed-DC waveform typically operated at 50-60 Hz, 300-990 V, and a 1-6 ms pulse-width. During suppression, persons with backpack electrofishers covered all available habitats, moving methodically upstream in tandem. All wild Brook Trout captured were euthanized with a lethal dose of anesthetic. Data collected from captured fish included: species, total length (TL; mm), and any marks. Salmonids other than Brook Trout comprised less than 25% of the total catch among all study streams, were released unharmed, and were not included in further analyses.

At non-suppression streams (East Threemile, East Fork Clear, and Tripod creeks), wild Brook Trout abundance was estimated using multiple-pass depletion electrofishing during September at ten survey sites within each treatment reach. Survey sites were selected systematically from each stream by dividing the total treatment reach into ten equal sections. The downstream end of each section was then identified as a downstream boundary of a survey site. Block nets were installed on the upper and lower boundary of each survey site. Survey site length (approximately 50 m) was adjusted slightly as needed to utilize natural stream channel constraints. Electrofishing crew size and output settings were similar to those used during suppression efforts. If no salmonids were captured on the first pass, no more passes were made. If Brook Trout were captured on the first pass, a minimum of three electrofishing passes were conducted within the study reach, such that the last pass resulted in ≤50% of the wild Brook Trout capture as the prior pass for at least two consecutive passes. Captured fish were anesthetized, measured for length and inspected for marks as above, then placed in a bath of fresh water to recover from the anesthetic before being released back into the stream, but outside the survey site. Abundance will be estimated annually at suppression streams, and approximately every three years after the initial stocking in non-suppression streams.

Prior to M_{YY} stocking in each study stream we collected tissue samples from wild Brook Trout fry (<100 mm) to estimate genetic sex ratios and parentage of the Brook Trout populations. Sex-biased survival was anticipated in mature Brook Trout due to the stresses associated with spawning and size-selective harvest by anglers (McFadden 1961). Fry were assumed exempt from these biases so equal sex ratios for males and females were anticipated (Fisher and Bennett 1999). Tissue samples were clipped from the caudal fin and preserved on Whatman™ 3MM chromatography paper (Thermo Fisher Scientific, Inc., Pittsburgh, Pennsylvania)We sought a goal of 100 tissue samples from Brook Trout fry to characterize the sex ratio of each wild population. Fry were incidentally captured at non-suppression streams during the abundance estimates. Fry collections occurred at multiple locations over the entire treatment reach to minimize family effects (Whiteley et al. 2012). Additionally, 100 fry were sampled from each of the control streams (i.e., Alder and Beaver creeks; Table 1; Figure 1) to determine if the sex ratio was equal between males and females in a stream in which M_{YY} Brook Trout had not been introduced.

To evaluate presumed fish passage barriers, we collected Brook Trout via electrofishing downstream from the identified passage barrier. Passage barriers were a combination of natural and manmade structures depending on the waterbody. All salmonids captured were anaesthetized and measured for length as described above, and were given a maxillary clip on both sides of the mouth, then released near their point of capture. Over time, any maxillary-clipped fish captured upstream from the assumed passage barrier will help us assess the effectiveness of the barrier and the study population's demographic isolation.

Lake surveys

Only the two lakes that receive annual suppression (i.e., Martin and Seafoam lakes) were sampled in 2019. Sampling and suppression occurred in these two systems using gill nets and boat or raft electrofishing. Boat electrofishing was conducted in Martin Lake over two nights. Gill nets were also set each night to increase the number of wild Brook Trout that could be removed from the system. Suppression in Seafoam Lake consisted of similar protocols, but electrofishing was conducted via raft rather than boat. During electrofishing M_{YY} Brook Trout were identified based on adipose fin clips; these fish were marked with an additional upper caudal clip and then released so that a population estimate could be conducted via mark-recapture. In both systems all wild Brook Trout were removed. Data collected from captured fish included: species, total length (TL; mm), and any marks. Salmonids other than Brook Trout comprised less than 25% of the total catch among all study lakes, were released unharmed, and were not included in further analyses. Additionally, tissue samples from fry were collected in both systems to estimate sex ratios and parentage. Tissue samples were clipped from the caudal fin and preserved on Whatman™ 3MM chromatography paper (Thermo Fisher Scientific, Inc., Pittsburgh, Pennsylvania). A fish passage barrier assessment was also conducted at Seafoam Lake using the same methodology that was used in streams; Martin Lake has no inlet or outlet and is considered a closed system.

Abundance and survival

For mark-recapture surveys at each suppression stream and lake, survey data were pooled over the entire study area, then wild Brook Trout abundance and 95% confidence intervals (CIs) were estimated using the modified Peterson estimator in the Fisheries Analysis+ software package (Montana Fish, Wildlife, and Parks 2008). Due to small sample sizes (see below), wild Brook Trout abundance was calculated in Martin Lake by dividing the total catch of wild Brook Trout in the lake by the average capture efficiency (i.e., 0.29) from the prior two years of sampling. M_{YY} Brook Trout abundance was also estimated in Seafoam Lake using the modified Peterson estimator. After calculating M_{YY} abundance, the proportion of the catch made up of wild Brook Trout was calculated and then multiplied by the estimate of M_{YY} abundance in the system to calculate an estimate of wild Brook Trout abundance. To account for differences in capture efficiency among size classes, abundance was estimated separately for the smallest size groups that still allowed for at least three recaptured fish per size group in order to satisfy model assumptions. We assumed there was 1) no mortality of marked fish between marking and recapture passes, and 2) no movement of marked or unmarked fish out of the study reach between marking and recapture passes. For both wild and M_{YY} Brook Trout, estimates were summed for all size classes >100 mm to describe abundance for the entire study reach.

Wild Brook Trout abundance and CIs from depletion electrofishing surveys were estimated using the removal function from the FSA package (Ogle 2017) in statistical package R (R Core Team 2019). When no Brook Trout were captured on the second pass, total catch from the first pass was assumed equivalent to abundance. Abundance estimates were only made for fish ≥100 mm TL to maintain consistency with the mark-recapture surveys. Brook Trout abundance was then averaged across all 10 sites to determine the mean abundance per 50 m reach in the study stream. This average linear density was then multiplied by the length of the study reach to estimate total abundance in the study reach.

Survival of stocked M_{YY} in Pike's Fork Creek and the other study streams has previously been reported (see Kennedy et al. 2018c). However, apparent survival in Pike's Fork Creek has been 0% in previous years. Consequently, we were able to produce another survival estimate for M_{YY} Brook Trout in 2019. Survival of M_{YY} Brook Trout was estimated by dividing the number of M_{YY} Brook Trout captured during sampling by the total number of Brook Trout sampled to calculate the proportion of the catch that was made up of M_{YY} Brook Trout. The proportion of M_{YY} Brook Trout was then multiplied by the total Brook Trout abundance estimate in Pike's Fork creek to get an estimate of M_{YY} Brook Trout abundance. Survival was then estimated by dividing the M_{YY} Brook Trout abundance estimate by the number of stocked M_{YY} Brook Trout the previous August.

Stocking

Stocking M_{YY} Brook Trout was standardized to the month of August for most streams and lakes. However, due to logistical restraints Martin and Seafoam lakes were stocked during

September. All M_{YY} Brook Trout were stocked in a single event, so stocking densities described here are annual total stocking densities. Fingerling-sized trout are rarely stocked in Idaho streams due to their low survival and return-to-creel (Schuck 1948). Catchables are commonly stocked in Idaho streams, though the selected study streams are considerably smaller than most rivers IDFG stocks with trout. Silver Creek (tributary to the Middle Fork Payette River) was the most comparable in size to study streams described here, that was regularly stocked with hatchery trout by IDFG. Stocking densities ranged from 96-128 trout/km at Silver Creek. Therefore, we chose an a priori stocking density of catchable MYY Brook Trout at 125 fish/km.

Fingerling stocking rates were set at four times the stocking rate of catchables (i.e., 500 fingerlings/km) based on the ratio of juvenile fish to adult fish suggested in McFadden (1961; i.e., adult Brook Trout comprise 20% of the population). However, initial scouting trips to study streams identified major disparities in stream widths, to the extent that 500 fingerlings/km may have been detrimental to survival of stocked fish at very narrow streams. Therefore, at narrow streams (i.e., East Fork Clear and Tripod creeks; Table 1; Figure 1), we reduced stocking densities to 250 fingerlings/km.

Once collected, estimates of wild Brook Trout abundance were used to adjust *a priori* stocking densities. *A priori* stocking densities were adjusted to 50% of the wild Brook Trout abundance to test the modelling inputs and results described by Schill et al. (2017). Catchable stocking rates were adjusted to 50% of the wild Brook Trout abundance, and then divided by four. Subsequent stocking densities should be consistent to reduce bias when evaluating the rate of change in sex ratios, because a higher stocking rate of M_{YY} Brook Trout could result in a faster rate of change in sex ratios (Schill et al. 2017) and obscure our ability to detect a difference between treatment groups.

Stocking rates in alpine lakes were initially set based on the typical stocking rate of fry in alpine lakes used in Idaho of 500/ha. However, because fry are slightly smaller than fingerling M_{YY} Brook Trout that are stocked we slightly reduced the stocking density to 438/h for fingerling M_{YY} Brook Trout. To standardize the biomass being stocked, the stocking rate of catchables was adjusted to 1/5 the stocking rate of fingerlings (i.e., 88/ha) because preliminary testing indicated that fingerlings were approximately 1/5 the weight of catchables. Additionally, this stocking rate is supported by the fact that fingerling M_{YY} Brook Trout are typically immature at the time of stocking and catchables are typically mature and wild Brook Trout populations typically exhibit a 4:1 ratio of mature fish to immature fish (McFadden 1961; Meyer et al. 2006). Therefore, a catchable stocking rate of 1/5 the fingerling stocking rate makes sense from a biological standpoint as well as a biomass standpoint. These stocking rates will be used for the duration of the study.

Stocking fingerlings and catchables into streams near roads was usually completed using 19-L buckets from a 1-ton or ¾-ton hatchery tanker truck. Fish were counted into buckets with hatchery water, then carried to the river and released into a pool or other low-velocity stream section. At suppression streams, mark-recapture abundance estimates of wild fish every ½ km were used to inform how M_{YY} fish were distributed. Assuming stocked hatchery fish generally move downstream (High and Meyer 2009), M_{YY} Brook Trout were distributed at a higher density at the upstream extremities of each study reach and in reaches where electrofishing catch identified high abundances of wild fish. Where fish could move downstream over a passage barrier and out of the study reach, stocking did not occur for 1.0-1.5 km upstream. Hatchery trout generally exhibit minimal movement within the stream (Heimer et al. 1985; High and Meyer 2009), so we dispersed M_{YY} fish longitudinally throughout the entire stream except for the section of stream 0.0-1.5 km upstream of the barrier. To maximize the encounter rate of hatchery M_{YY} males with spawning females, we backpacked fish into headwater reaches or other roadless areas. For stocking in roadless sections, approximately 8 L of hatchery truck water (~12°C) was poured in a contractor-grade garbage bag inside of 19-L buckets. Then, fish were loaded into the garbage bag. An air stone and hose (connected to a Quiet-Bubbles® air pump) were inserted into the opening of the garbage bag, and then the bag was sealed. Fish loading densities and water displacement were calculated following Piper et al. (1982). To maintain fish health during transport, target fish loading densities were less than 3,392 g of fish/L. Depending on ambient temperatures, water temperature and dissolved oxygen were suitable for Brook Trout health for ≤45 minutes. At some locations, fish were transported in coolers on ATVs. Loading densities and water quality monitoring in coolers followed methods described above.

Fingerling and catchable M_{YY} Brook Trout were stocked into alpine lakes primarily by helicopter. Fish were counted and placed into a 208-L barrel filled with water. The helicopter flew to the designated lake and dumped the fish into the lake by tipping the bucket over. For some larger lakes, the fish were delivered in more than one trip to ensure appropriate loading densities. Because Martin and Seafoam lakes have road access, fish were stocked in these lakes directly from the hatchery truck.

Lengths and weights were measured from a subsample (*n* = 100) of fingerling and catchable M_{YY} Brook Trout immediately prior to loading the helicopter barrel or directly stocking from the truck.

Genetic sex ratios and reproductive success

During scheduled population monitoring in each stream or lake, tissue samples were collected from hatchery and wild fish to identify successful reproduction of M_{YY} Brook Trout in the wild and to monitor changes to the populations' sex ratio. Approximately 100 tissue samples were collected from wild Brook Trout fry (<100 mm) from each study system during July-September to estimate sex ratios and reproductive success. Tissue samples were clipped from the caudal fin and preserved on Whatman™ 3MM chromatography paper (Thermo Fisher Scientific, Inc., Pittsburgh, Pennsylvania).

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: 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 µL volume consisting of 0.50 µL of primer mix, 2.50 µL of Qiagen Master Mix (cat. 206143), 1.00 µL dH20, and 1.00 µL 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, 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 to differentiate sex in Brook Trout was previously validated by screening them on samples of known genetic sex (Schill et al. 2016). Gonadal tissue from 25 individuals of each sex from each study stream, whose phenotypic sex was identified in the field by dissection, was tested to validate the sex marker described above. Sex assignments from tissue samples were compare with the phenotype determined from dissections. We calculated 90% CIs around the estimated male proportions, following Fleiss (1981).

Genetic assignment evaluation

A 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 is impossible (e.g., due to cost and time limitations) to genetically sample all M_{YY} Brook Trout individually prior to release and when study designs require stocking thousands of M_{YY} Brook Trout into large lakes or rivers.

Several statistical software programs could be used to identify progeny from two different populations using GA methodologies. The program used to identify offspring of M_{YY} Brook Trout is called “Structure” (Pritchard et al 2000; Kennedy et al. 2018a). Structure used an admixture model that estimated a membership coefficient (*Q*), which represented the portion of an individual’s genotype that originated from a defined number of populations or genetic clusters (in the current study, two). This was accomplished prior to the introductions of M_{YY} Brook Trout by genetically screening samples collected from both the M_{YY} population used for stocking and from the receiving wild population fish. The expectation was that progeny from M_{YY} adults and wild adults had approximately equal probability of membership to each population (*Q* = 0.5).

Fry sampled during 2019 for sex ratio analysis were subjected to GA analysis to describe the origin of sampled fish as either progeny of wild or M_{YY} Brook Trout. Determining the origin of the sampled fry will allow us to describe relative spawning success of M_{YY} Brook Trout and the proportion of the offspring in the system produced by M_{YY} fish. The rate at which the composition of offspring produced solely by wild Brook Trout changes to offspring produced by M_{YY} Brook Trout (hatchery origin) will be assessed for both suppression and non-suppression streams, and for streams stocked with fingerlings versus catchables. The relative effectiveness of the various strategies can then be compared. All study streams and lakes will be sampled approximately every three years to monitor the change in the population from wild to hatchery origin.

RESULTS

Stream surveys

At Dry Creek, 5,386 Brook Trout were sampled, of which 2,651 were M_{YY} Brook Trout and 2,549 were wild fish, the latter being removed from the system. Estimated abundance of wild fish ≥100 mm was 4,935 fish (95% CI = 2,451-7,419) and abundance of M_{YY} fish ≥100 mm was 3,503 fish (95% CI = 2,705–4,301; Table 3), producing a suppression estimate of 52%. Length of wild

Brook Trout ≥100 mm averaged 184 mm (maximum = 336 mm), which was nearly identical to M_{YY} Brook Trout ≥100 mm, which also averaged 184 mm (maximum = 354 mm; Figure 3). No fish with maxillary clips were observed, suggesting the barrier is effective at preventing recolonization at Dry Creek. An additional 100 Brook Trout were maxillary clipped (mean = 216 mm; maximum = 316 mm) below the downstream barrier on the study reach to continue barrier evaluations in future years. In addition to Brook Trout, 193 Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri*, and two Tiger Trout *Salmo trutta* × *S. fontinalis* were captured.

At Pike's Fork Creek, 2,049 Brook Trout were captured, only eight of which were M_{YY}. All 2,041 of the wild Brook Trout captured were removed from the system. Estimated abundance of wild Brook Trout ≥100 mm was 7,247 (95% CI = 3,282–11,212; Table 3), and based on capture efficiency of wild fish, we estimated that the abundance of M_{YY} fish ≥100 mm was 28 fish. Suppression in Pike's Fork Creek was estimated to be 28%. Length of wild Brook Trout ≥100 mm averaged 145 mm (maximum = 303 mm) whereas M_{YY} Brook Trout ≥100 mm averaged 177 mm (maximum = 231 mm). Similar to Dry Creek, no fish with maxillary clips were detected above the barrier, and 139 new wild Brook Trout (mean = 150 mm; maximum = 284 mm) were maxillary clipped below the barrier for future barrier evaluation. Additionally, 812 Rainbow Trout *O. mykiss* and three Bull Trout *S. confluentus* were sampled in Pike's Fork Creek in 2019.

At East Threemile Creek, 384 Brook Trout were sampled from 10 50-m reaches, 38 of which were M_{YY} fish. Total abundance of wild Brook Trout ≥100 mm was estimated to be 5,814 fish (95% CI = 5,663–5,966; Table 3), compared to 643 M_{YY} fish (95% CI = 551-734). Length of wild Brook Trout in ≥100 mm averaged 141 mm (maximum = 249 mm) compared to an average of 267 mm (maximum = 302 mm) for M_{YY} fish. However, it is important to note that all sampling in non-suppression streams took place after stocking, which likely influenced the abundance and length structure of M_{YY} fish. No fish species other than Brook Trout were sampled in East Threemile Creek.

At East Fork Clear Creek, 70 Brook Trout ≥100 mm were sampled across 10 sites, 13 of which were M_{YY} Brook Trout. Total Brook Trout abundance in East Fork Clear Creek was estimated to be 449 wild fish (95% CI = 418–480) and 102 M_{YY} fish (95% CI = 80–125; Table 3). Fish length averaged 122 mm for wild Brook Trout (maximum = 186 mm) and 130 mm (maximum = 158 mm) for M_{YY} fish (Figure 4). Five Rainbow Trout were also sampled in East Fork Clear Creek.

At Tripod Creek, 461 Brook Trout ≥100 mm were sampled across 10 sites, 13 of which were M_{YY} Brook Trout. Total Brook Trout abundance in Tripod Creek was estimated to be 3,249 wild fish (95% CI 3,118–3,380) compared to 5,279 M_{YY} fish (95% CI = 5,042–5,534; Table 3). Fish length averaged 128 mm for wild Brook Trout (maximum = 206 mm) and 124 mm (maximum = 194 mm) for M_{YY} fish (Figure 4). An additional 132 Rainbow Trout were also sampled in Tripod Creek.

Lake surveys

At Martin Lake, 208 Brook Trout were sampled, 5 of which were M_{YY} Brook Trout and 203 were wild fish, the latter being removed from the system. Based on the mark-recapture survey, estimated abundance of M_{YY} Brook Trout ≥100 mm was 15 fish and abundance of wild Brook Trout ≥100 mm was 711 fish (Table 3). The Suppression rate was estimated to be 29% in Martin Lake. Length of wild Brook Trout ≥100 mm averaged 150 mm (maximum = 279 mm; Figure 5). Average length of M_{YY} Brook Trout ≥100 mm was greater (i.e., mean = 194 mm) than wild Brook

Trout ≥100 mm length; however, they achieved a smaller maximum length (i.e., maximum = 205 mm).

At Seafoam Lake, 219 Brook Trout were sampled, 68 of which were M_{YY} Brook Trout and 151 were wild fish, the latter being removed from the system. Estimated abundance of Brook Trout ≥100 mm was 591 fish (95% CI = 567 - 615). Given that 31% of the fish in Seafoam Lake were M_{YY} Brook Trout, the abundance of wild Brook Trout ≥100 mm in the lake was estimated to be 408 and the M_{YY} Brook Trout ≥100 mm abundance was estimated to be 183 fish (Table 3).The suppression rate of wild Brook Trout in the lake was estimated to be 37%. Length of wild Brook Trout ≥100 mm averaged 193 mm (maximum = 345 mm; Figure 5), which were similar to M_{YY} Brook Trout ≥100 mm, which averaged 207 (maximum = 274 mm). No fish were observed with maxillary clips, suggesting the barrier is effective. Additionally, 41 wild Brook Trout (mean = 134 mm; maximum = 246 mm) were marked with a maxillary clip below the barrier into Seafoam Lake to continue to assess the barrier.

Stocking

Fingerling M_{YY} Brook Trout were stocked into Dry Creek, Tripod Creek, East Fork Clear Creek, Martin Lake, Seafoam Lake, Duck Lake, and Lloyds Lake during 2019 (Table 4). Lengths and weights of stocked fish were similar across waterbodies. However, stocking rates varied considerably across waterbodies (i.e., 22%-288% of wild Brook Trout ≥100 mm abundance).

Catchable M_{YY} Brook Trout were stocked into Pike's Fork Creek, East Threemile Creek, Black Lake, and Rainbow Lake during 2019 (Table 4). Again, lengths and weights of stocked fish were similar across waterbodies. Stocking rates were also similar for the two waterbodies in which it could be calculated (i.e., Pike's Fork Creek and East Threemile Creek).

Survival

Survival was estimated for M_{YY} Brook Trout only in Pike's Fork Creek in 2019. Survival was estimated to be 3% for catchable M_{YY} Brook Trout between stocking in 2018 and sampling in 2019 (approximately 11 months).

Genetically determined sex ratios and reproductive success

Offspring produced by M_{YY} Brook Trout were detected in all study waters that were sampled for fry in 2019 (Table 5). Sex ratios were evaluated in study streams and Seafoam Lake in 2019. Sex ratios varied from 40% to 73% male in study streams. In Seafoam Lake, Alder Creek, and Beaver Creek Sex ratios were close to 50%.

Genetic assignment analyses indicated that the proportion of offspring produced by M_{YY} Brook Trout in stocked study streams varied from 2% to 29% (Table 5), and averaged 16% (SE = 6%) in streams stocked with fingerlings, 4% (SE = 3%) in streams stocked with catchables, and 10% (SE = 5%) across all streams. M_{YY} Brook Trout produced 3% of the offspring in the sample collected from Seafoam Lake.

DISCUSSION

Wild Brook Trout abundance has fluctuated greatly over the course of the current study. For example, wild Brook Trout abundance in 2019 in Tripod Creek was only 32% of the 2016 abundance (i.e., 10,225 wild Brook Trout). Conversely, wild Brook Trout abundance has increased by 188% in Pike's Fork Creek between 2018 (3,848 wild Brook Trout) and 2019. Because stocking rates were set based on the abundance of wild Brook Trout during the first year of sampling, in a given system, changes in wild fish abundance have caused the stocking rate to also fluctuate. Fluctuations in abundance have caused the stocking rate to become as high as 288% of the wild Brook Trout ≥100 mm abundance in some waterbodies (i.e., Seafoam Lake), and as low as 22% in other waterbodies (i.e., East Fork Clear Creek). However, despite the variability in wild fish abundance, stocking rates in each study water will remain constant for the remainder of the study.

Survival of catchable-sized M_{YY} Brook Trout stocked in Pike's Fork in summer 2017 — the first year of stocking in this stream — was 0% to the following summer of 2018. Consequently, a second estimate of M_{YY} Brook Trout survival was possible in Pike's Fork for fished stocked in summer 2018, which was estimated to be 3% to the following summer of 2019. While both estimates of survival are extremely low, as long as some M_{YY} fish successfully reproduce in the year they were stocked before they die, then sex ratios in Pike's Fork Creek may still skew heavily toward males. Indeed, 7% of fry sampled in 2019 were offspring of M_{YY} fish even though 11-month survival of M_{YY} fish was only 3%.

Sampling during 2019 indicated that sex ratios appear similar to baseline sex ratios for the majority of study waters (Kennedy et al. 2018c). Sex ratios have remained close to 50% in Pike's Fork Creek, East Threemile Creek, East Fork Clear Creek, Alder Creek, Beaver Creek, and Seafoam Lake over the course of the study. Conversely, the 2019 sex ratios in Dry Creek (28% female; SE = 4%) and Tripod Creek (27% female; SE = 4%) were skewed towards males compared to the time of initial sampling. The successful shift in sex ratio towards predominately male in Dry and Tripods Creeks could potentially be due to the use of fingerling-sized M_{YY} Brook Trout and the high stocking rates in both of these systems. Additionally, wild Brook Trout are suppressed annually in Dry Creek, which should increase the rate at which the sex ratio shifts towards males. The lack of a sex ratio shift in the other waterbodies could be due to a number of different reasons. For instance, the survival or fitness of M_{YY} Brook Trout could be lower than that of wild fish in these systems, which would increase the time to eradication in these systems (Schill et al. 2017). The stocking rates in these streams are also generally lower than the 50% stocking rate for M_{YY} Brook Trout recommended by Schill et al. (2017). However, despite the fact that Seafoam Lake has a stocking rate above 50% and receives suppression annually, the sex ratio has remained around 50% male. As mentioned above, this could be due to lower survival or fitness of M_{YY} Brook Trout in the wild, but this could also potentially be due to the fact that M_{YY} Brook Trout are predicted to take longer to be effective in lakes due to the shorter generational time in streams (Schill et al. 2017).

Based on the preliminary results, M_{YY} Brook Trout are surviving and reproducing in most streams and lakes where they are stocked, and there is evidence that they are beginning to skew the sex ratio in some study waters. However, this is only year five of a 12-year study. Therefore, these assertions are speculative and could change once the study has been completed.

RECOMMENDATIONS

1. Continue suppression efforts and stocking in all four study waters within the study design that are designated for annual suppression for the duration of the study.

2. Continue annual stocking of fingerling or catchable M_{YY} Brook Trout in remaining study waters until the effectiveness of the treatment has been determined using the current stocking numbers.

3. Continue to evaluate sex ratios and genetic assignment analyses in future reports approximately every three years to monitor reproductive success of M_{YY} fish.

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TABLES

Table 1. Study streams in central Idaho selected for M_{YY} Brook Trout evaluations including treatment levels, fish-size stocked, location (WGS84), and physical stream characteristics.

Stream name	Starting year	Treatment level	Stocked fish size	Reach length (km)	Avg. width (m)	Gradient (%)	Maximum elevation (m)	Latitude (dec. deg.)	Longitude (dec. deg.)
Dry Creek	2016	Suppression	Fingerling	6.5	5.2	1.5	2,377	44.12679	-113.56812
Pikes Fork Creek	2017	Suppression	Catchable	7.5	3.7	3.3	1,871	43.98315	-115.54843
East Threemile Creek	2017	Non-suppression	Catchable	6.5	2.7	5.3	2,320	44.39859	-112.08976
East Fork Clear Creek	2016	Non-suppression	Fingerling	3.9	2.1	5.7	1,827	44.47574	-115.83978
Tripod Creek	2016	Non-suppression	Fingerling	9.1	1.4	1.0	1,625	44.31776	-116.11995
Alder Creek	2016	Control	Control	2.4	4.9	3.2	2,000	43.82343	-113.60738
Beaver Creek	2016	Control	Control	4.0	2.4	2.2	1,650	43.98891	-115.60710

Table 2. Study lakes in central Idaho selected for MYY Brook Trout evaluations including treatment levels, size, and location.

Lake name	Starting year	Treatment	Stocked fish size	Surface area (ha)	Surface elevation (m)	Latitude	Longitude
Martin Lake	2017	Suppression	Fingerling	1.82	2,107	44.3032794	-115.26357350
Seafoam Lake	2017	Suppression	Fingerling	2.72	1,423	44.5076651	-115.12583244
Duck Lake	2015	Non-suppression	Fingerling	4.96	2,177	45.1145991	-116.15726311
Lloyds Lake	2015	Non-suppression	Fingerling	2.91	2,092	45.1929080	-116.16370556
Black Lake	2016	Non-suppression	Catchable	2.60	2,149	45.2453900	-116.19867000
Rainbow Lake	2016	Non-suppression	Catchable	8.78	2,175	45.2540600	-116.19663000
Snowslide Lake #4	2015	Control	n/a	4.86	2,188	44.9833739	-115.93431897
Upper Hazard Lake	2015	Control	n/a	15.84	2,265	45.1742372	-116.13500053

Table 3. Abundance of wild Brook Trout *Salvelinus fontinalis* and M_{YY} Brook Trout ≥100 mm sampled in study waters in Idaho during 2019. Estimates of abundance were calculated using either a mark-recapture survey (MR) or depletion electrofishing (DE). Also include are the 95% confidence estimates (CI) on abundance, the proportion of the population that is comprised of M_{YY} Brook Trout, the number of fish removed from the system when annual suppression was conducted, the suppression rate, and capture efficiency.

Waterbody	**Sample method**	**Wild Abundance**	**CI**	**M_{YY} Abundance**	**CI**	**M_{YY} Com-position**	**Number of fish removed**	**Sup-pression rate**	**Capture efficiency**
Dry Creek	MR	4,935	2,451–7,419	3,503	2,705–4,301	42%	2,549	52%	50%
Pike’s Fork Creek	MR	7,247	3,282–11,212	28	-	0%	2,041	28%	28%
East Threemile Creek	DE	5,814	5,663–5,966	643	551–734	10%	-	-	71%
East Fork Clear Creek	DE	449	418–480	102	80–125	19%	-	-	69%
Tripod Creek	DE	3,265	3,129–3,401	5,279	5,023–5,534	62%	-	-	73%
Martin Lake	MR	711	-	15	-	2%	203	29%	-
Seafoam Lake	MR	408	-	183	-	31%	151	37%	31%

Table 4. The number of M_{YY} Brook Trout *Salvelinus fontinalis* stocked into study waters in Idaho during 2019. Two sizes for M_{YY} Brook Trout were stocked into study waters. Fingerlings averaged 125 mm (SE = 1) and catchable fish averaged 263 mm (SE = 1). Additionally, the number of fish stocked divided by the total number of wild Brook Trout is included as an estimate of the stocking rate of M_{YY} Brook Trout compared to the wild Brook Trout population.

Waterbody	**Size**	**Stocking date**	**Number of fish stocked**	**Mean length (mm)**	**SE**	**Mean weight (g)**	**SE**	**Stocking rate**
Dry Creek	Fingerling	8/13/19	4,314	116	2	15	1	87%
Pike’s Fork Creek	Fingerling	8/22/19	716	263	3	198	6	10%
East Threemile Creek	Catchable	8/27/19	568	265	3	193	5	13%
East Fork Clear Creek	Fingerling	8/20/19	105	125	2	22	1	22%
Tripod Creek	Fingerling	8/20/19	6,900	125	2	22	1	233%
Martin Lake	Fingerling	9/20/19	788	-	-	-	-	110%
Seafoam Lake	Fingerling	9/27/19	1176	139	2	29	1	288%
Duck Lake	Fingerling	8/16/19	2,128	118	2	17	1	-
Lloyds Lake	Fingerling	8/16/19	1,246	118	2	17	1	-
Black Lake	Catchable	8/16/19	202	260	2	192	5	-
Rainbow Lake	Catchable	8/16/19	760	260	2	192	5	-

Table 5. Results of genetic sex ratio and genetic assignment analyses. Fry were sampled from study waters during 2019. Genetic sex and parental origin were then determined based on analysis of fin clips. Additionally, information on whether the system receives annual suppression to remove wild Brook Trout prior to stocking, and the size of the M_{YY} fish that are stocked into the system. Fingerlings averaged 125 mm (SE = 1) and catchable fish averaged 263 mm (SE = 1).

			Brook Trout fry sampled				**M_{YY} offspring (%)**		**Sex ration (% male)**	
Waterbody	**Stocking size**	**Treatment**	**Total**	**Wild Males**	**Wild Females**	**M_{YY}**	**Est.**	**SE**	**Est.**	**SE**
Dry Creek	Fingerling	Suppression	82	35	23	24	29	5	72	5
Pike’s Fork Creek	Catchable	Suppression	84	28	50	6	7	3	40	5
East Threemile Creek	Catchable	Non-Suppression	114	54	58	2	2	1	49	5
East Fork Clear Creek	Fingerling	Non-Suppression	110	41	41	28	25	4	63	5
Tripod Creek	Fingerling	Non-Suppression	59	22	34	3	5	3	43	5
Alder Creek	-	Control	89	49	40	0	0	0	55	5
Beaver Creek	-	Control	100	57	43	0	0	0	57	5
Martin Lake	Fingerling	Suppression	0	-	-	-	-	-	-	-
Seafoam Lake	Fingerling	Suppression	88	42	43	3	3	2	51	5

FIGURES

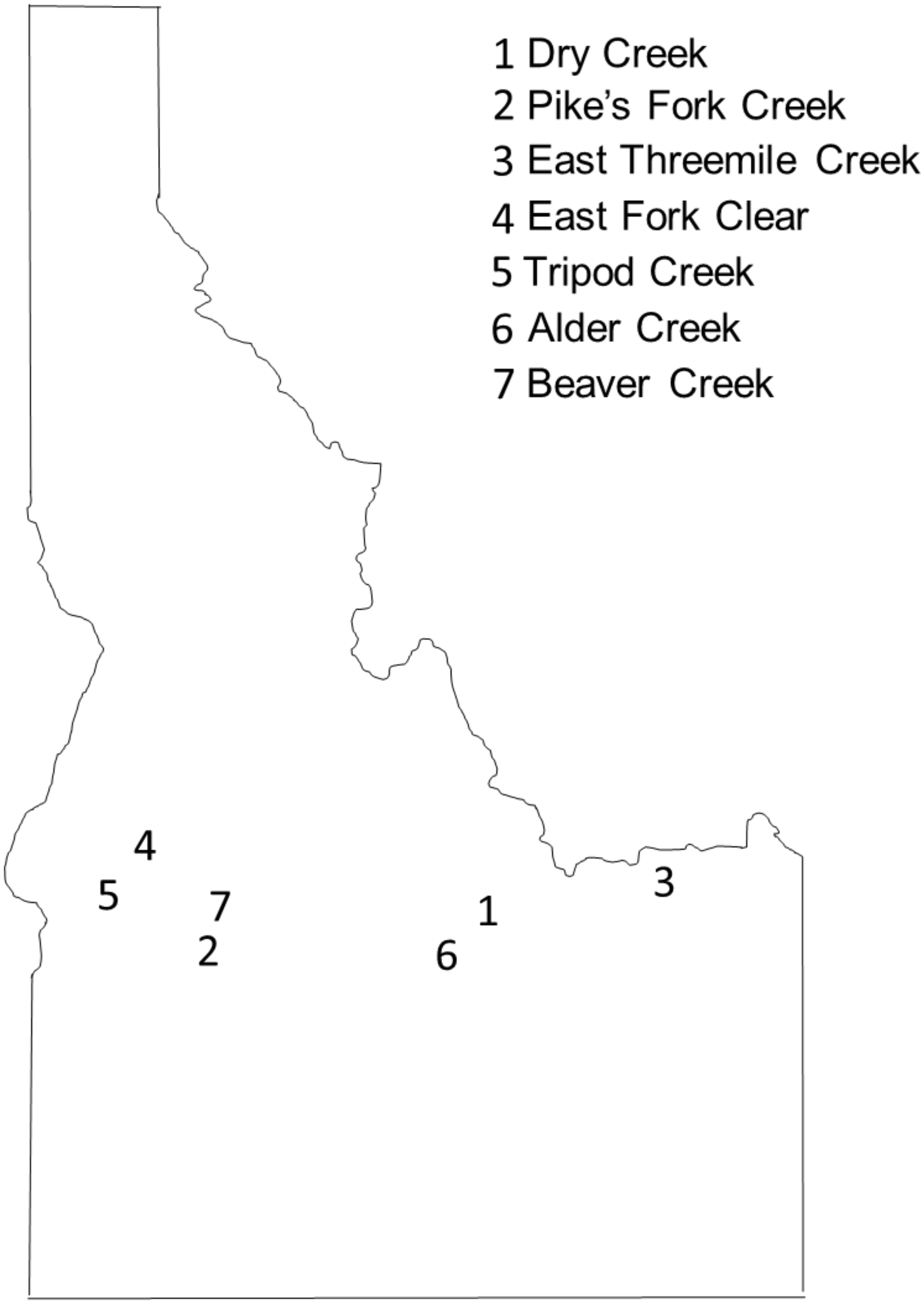

Figure 1. Locations of study streams for M_{YY} Brook Trout *Salvelinus fontinalis* field trials in Idaho.

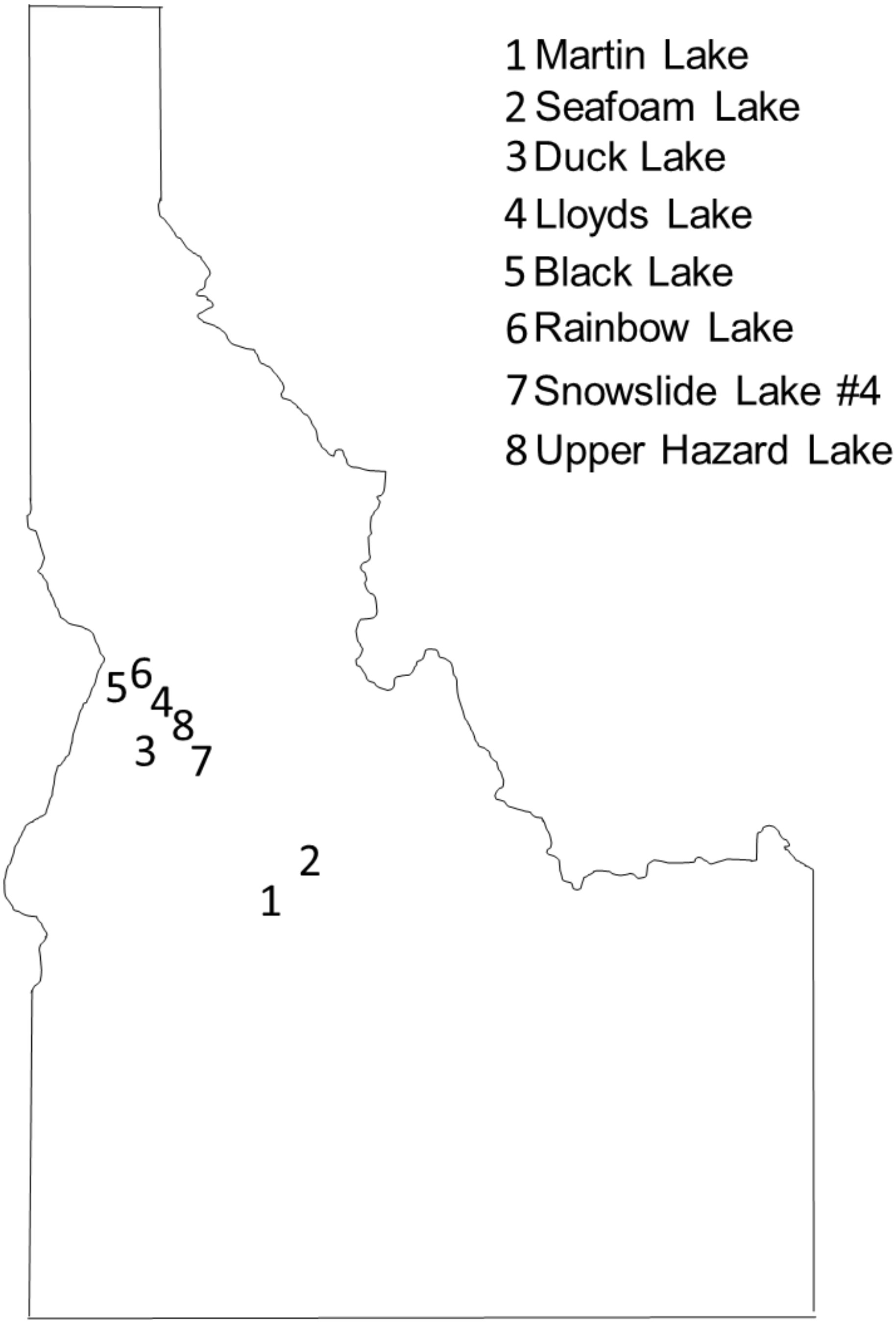

Figure 2. Locations of study lakes for M_{YY} Brook Trout *Salvelinus fontinalis* field trials in Idaho.

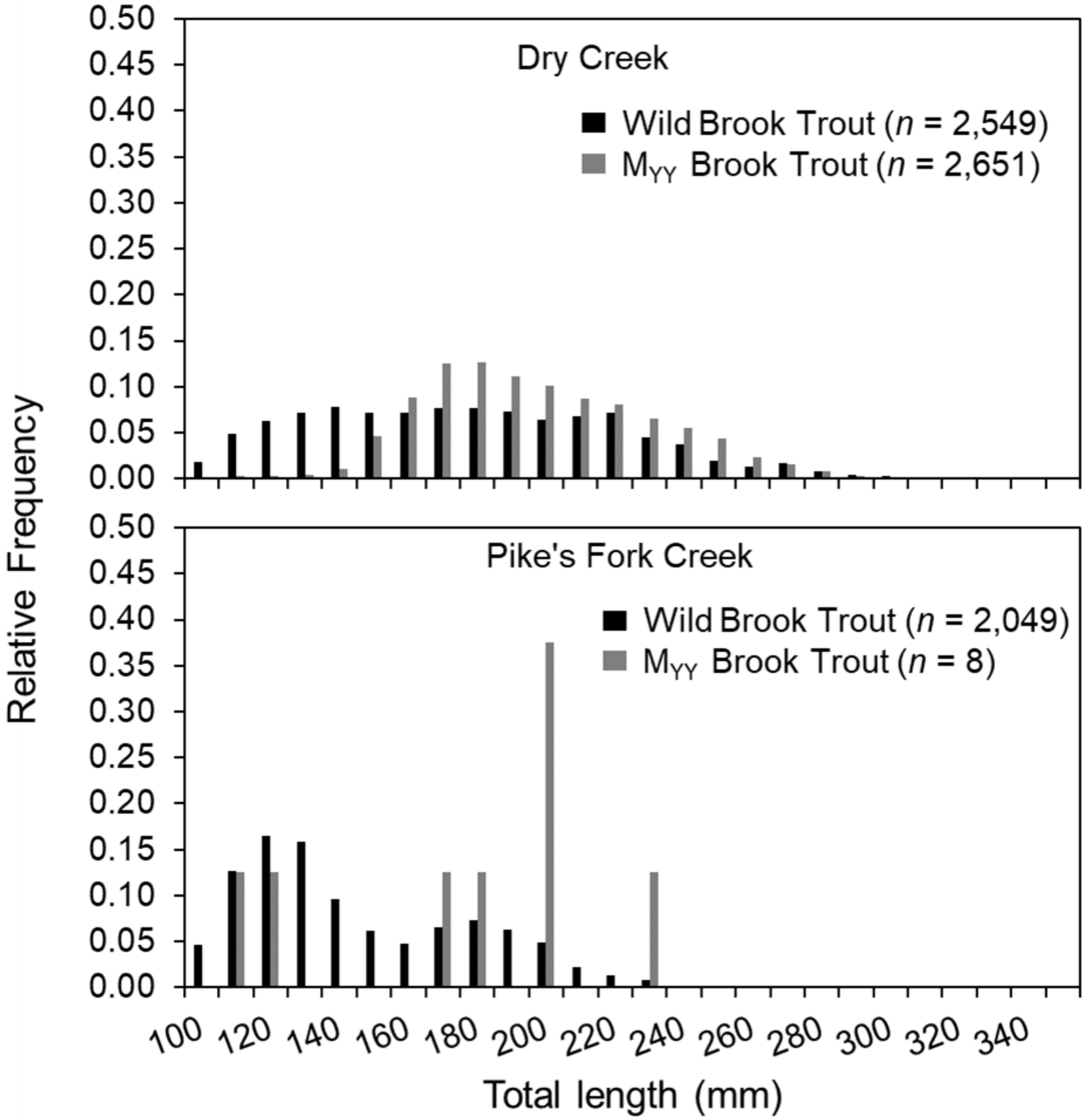

Figure 3. Length distributions of wild Brook Trout *Salvelinus fontinalis* and M_{YY} Brook Trout sampled in Dry Creek and Pike's Fork creeks, Idaho, during 2019.

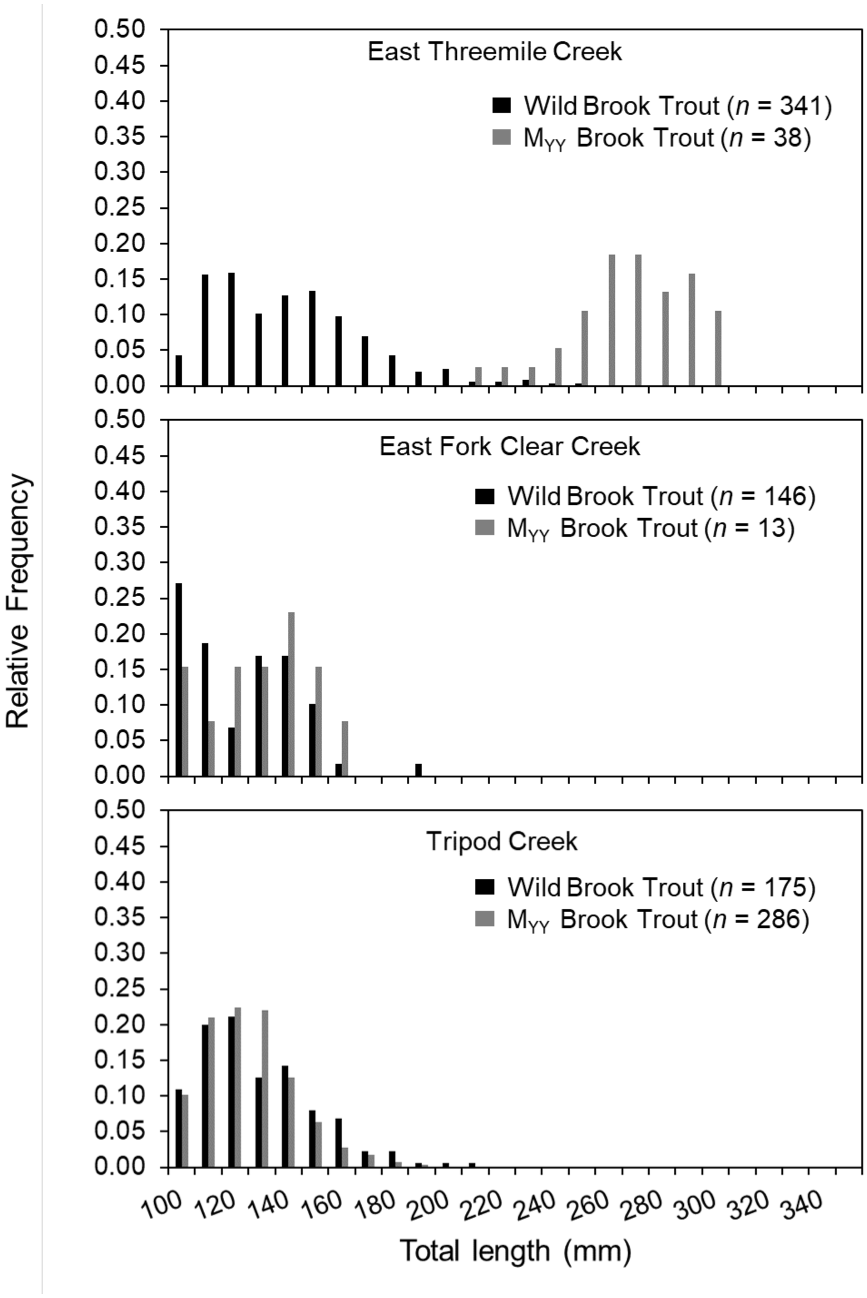

Figure 4. Length distributions of wild Brook Trout *Salvelinus fontinalis* and M_{YY} Brook Trout sampled in East Threemile, East Fork Clear, and Tripod creeks, Idaho, during 2019.

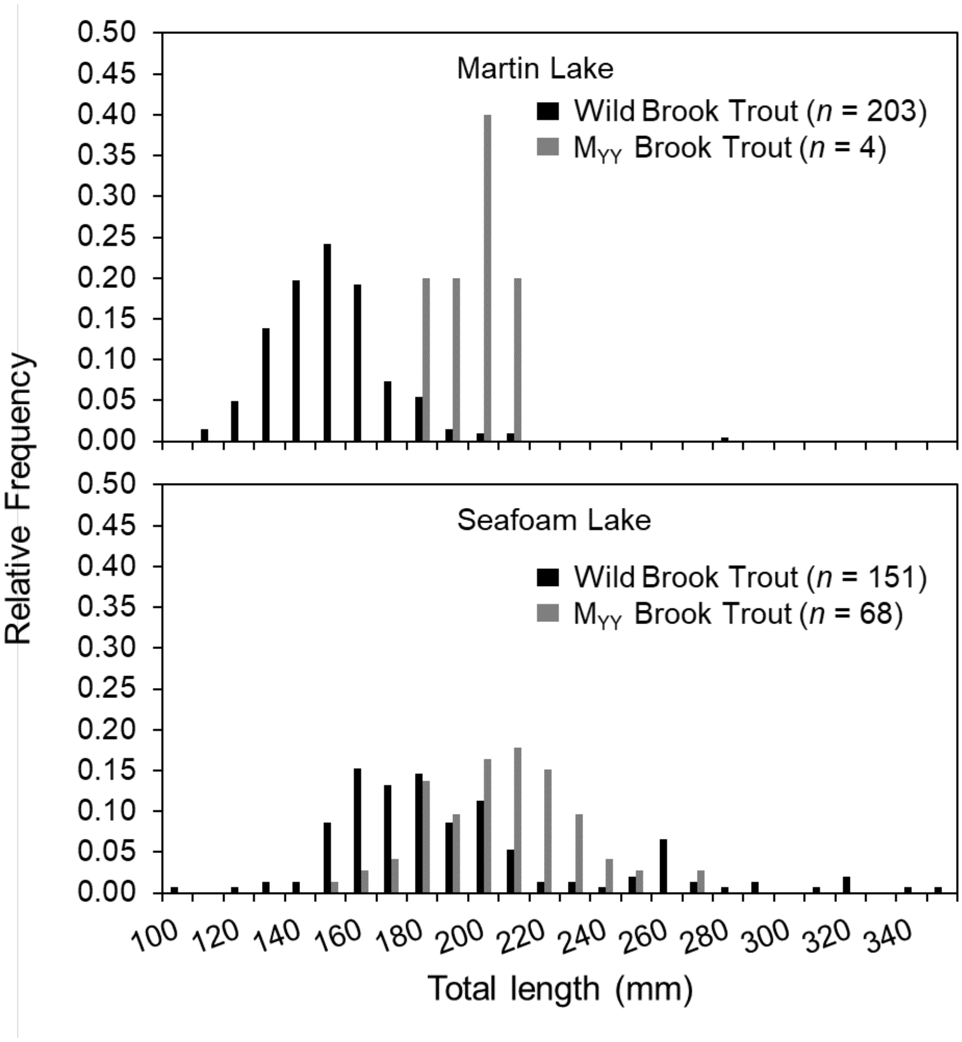

Figure 5. Length distributions of wild Brook Trout *Salvelinus fontinalis* and M_{YY} Brook Trout sampled in Martin and Seafoam lakes, Idaho, during 2019.

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