

## Production and Evaluation of YY-Male Brook Trout to Eradicate Nonnative Wild Brook Trout Populations

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**Abstract**—Nonnative Brook Trout *Salvelinus fontinalis* were introduced throughout western North America in the early 1900s, resulting in widespread self-sustaining populations that are difficult to eradicate and often threaten native salmonid populations. A novel approach for their eradication involves use of 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 could eventually drive the sex ratio of the wild population to 100% males, at which point the population would not be able to reproduce and would be eradicated. This study represents the first successful development of a  $F_{YY}$  and  $M_{YY}$  salmonid broodstock, which was produced in four years at relatively low cost. Field trials demonstrated that stocked hatchery  $M_{YY}$  Brook Trout survived and produced viable  $M_{YY}$  offspring in streams, although reproductive fitness appeared to have been lower than their wild conspecifics. Even if reduced fitness is the norm in both streams and alpine lakes, our population simulations suggest that eradication can be achieved in reasonable time periods under some  $M_{YY}$  stocking scenarios, especially when wild Brook Trout are simultaneously suppressed in the population.

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### Introduction

Brook Trout *Salvelinus fontinalis* have been artificially introduced in many lakes and streams outside their native range and continue to colonize new habitats in western North America. Nonnative Brook Trout populations have negatively impacted native salmonid populations through hybridization, competition, and predation. Thus, fisheries managers have worked to eliminate some exotic Brook Trout populations most commonly using piscicides and electrofishing (Gresswell 1991; Shepard et al. 2014). However, the former negatively impacts non-target aquatic fauna and the latter has resulted in mixed success. Complications with both of these methods points to a need for alternate or companion methods for eradicating nonnative fish.

Gutierrez and Teem (2006) suggested a novel approach that would shift the sex ratio of a wild fish population toward males by annually introducing

hatchery produced male fish with a YY genotype (known as “supermales” but herein referred to as  $M_{YY}$  fish). To create a  $M_{YY}$  brood stock, normal  $M_{XY}$  males are feminized by exposing them to estrogen. The resulting  $F_{XY}$  fish are crossed with normal  $M_{XY}$  males and one-quarter of the subsequent progeny are  $M_{YY}$  (Teem and Gutierrez 2010). By exposing half of the  $M_{YY}$  fish to estrogen, an  $M_{YY}$  and  $F_{YY}$  brood stock can be created, and all offspring are  $M_{YY}$ . Large numbers of these  $M_{YY}$  offspring can then be reared and stocked into wild fish populations to drive the sex ratio of the wild population to 100% males, theoretically resulting in population eradication (Gutierrez and Teem 2006). Such a stocking program has not been tested in the wild to eradicate a nonnative fish species, though monosex culture is commonly used in commercial hatcheries for artificial fish production (Schill et al. 2016).

Sex ratios in Brook Trout populations would only change under such a stocking program if the  $M_{YY}$  fish survive adequately and successfully reproduce after

stocking. In order to shift the sex ratio significantly toward eradication, stocking and suppression will have to be conducted annually for many years. Hatchery trout encounter many challenges upon their liberation into the wild, often leading to very low survival after stocking, especially in streams (Miller 1952). Competition with wild resident fish has been identified as a primary factor contributing to the low survival of hatchery trout in streams (Miller 1958), suggesting that suppression of wild fish prior to stocking hatchery  $M_{YY}$  fish could improve survival of the hatchery fish.

From 2008 to 2016 the Idaho Department of Fish and Game (IDFG) conducted a series of studies to (1) develop a  $M_{YY}$  hatchery Brook Trout broodstock, (2) evaluate post-release survival and reproductive success of  $M_{YY}$  Brook Trout, and (3) model how long it might take to eradicate undesirable Brook Trout populations in both streams and alpine lakes. Herein we briefly summarize the findings of these three studies.

## Methods

### $M_{YY}$ Brook Trout Broodstock Development

For further details on  $M_{YY}$  Brook Trout broodstock development, see Schill et al. (2016). Phase 1 involved the creation of feminized, genetically male fish ( $F_{XY}$ ). Normal fertilized Brook Trout eggs were hatched (winter 2008/2009) under typical hatchery operations. At swim-up, fry were split into two groups. One group was fed for 60 d with commercially-produced salmonid starter diets treated via spraying with  $17\beta$ -estradiol (hereafter estradiol) at a concentration of 20 mg steroid per kilogram diet; the other group was fed the same food without estradiol. A subsample of male fish from the untreated group was later used as standard  $M_{XY}$  breeders at the beginning of Phase 2 (Figure 1). Fish within each crossing were reared separately to ensure that no siblings were bred together in subsequent generations. At 309 d post-hatch (mean length of about 130 mm TL), all fish were PIT tagged in the body cavity for individual identification, and fin clipped for genetic sex identification.

Concurrently, a suite of microsatellite markers were screened for their ability to genetically determine Brook Trout sex. These efforts proved successful, and in the fall of 2010, a suite of markers were used to individually identify  $F_{XY}$  and  $F_{XX}$  fish; genotypic  $F_{XX}$  females were then culled. The remaining fish in the treated and untreated groups were then examined physically to determine maturation (Figure 1).

Although phenotypic sex could be determined in most fish via secondary sex characteristics, all genetic  $F_{XY}$  fish in the treatment groups were examined via a hand-held ultrasound system to identify egg producing fish, which were held separately until mature. All identified  $F_{XY}$  fish were either spawned in Phase 2 or were subsequently necropsied to evaluate the proportion successfully feminized.

Phase 2 of this effort involved the development of  $M_{YY}$  fish. In November 2010,  $F_{XY}$  fish were crossed with standard  $M_{XY}$  males. Developing eggs were separated by spawn-pairings and reared as in Phase 1. Standard genotypic crosses yielded 50%  $M_{XY}$ , 25%  $F_{XX}$ , and 25%  $M_{YY}$  fish. These progeny were split into treated and untreated family groups. The treatment groups were again fed estradiol-treated starter feed (as described above), whereas untreated groups were fed an identical untreated diet. Fish were reared by individual spawn-pairings until they were large enough for tagging (fall of 2011).

Genetic markers were again used to identify XX, XY, and YY fish in the raceway. When these fish reached maturity (October 2012), maturing  $F_{YY}$  and  $M_{YY}$  fish in the raceway were identified by combining both genetic marker information and observed phenotypic secondary sex characteristics (Figure 1). Once genotypes and phenotypes were available, all  $F_{XX}$ ,  $F_{XY}$ , and  $M_{XY}$  fish present in the raceway were culled, leaving only  $F_{YY}$  and  $M_{YY}$  fish (i.e., the  $M_{YY}$  broodstock). In Phase 3, these fish were spawned to produce  $M_{YY}$  fish for subsequent pilot field trials (see below) and to renew the  $M_{YY}$  broodstock.

### Field Trials of Survival and Successful Spawning

For further details on field evaluations, see Kennedy et al. (2016). Two treatment levels were implemented for this evaluation: (1) suppression of the wild Brook Trout via electrofishing removal prior to  $M_{YY}$  stocking to reduce potential competition (Wildhorse and Bear creeks); and, (2) no suppression with  $M_{YY}$  stocking only (Iron Bog and Cherry creeks). The study streams were selected for known Brook Trout populations and relatively narrow stream width so we could efficiently conduct backpack electrofishing removals in this pilot study. All  $M_{YY}$  fish were adipose fin clipped prior to stocking so they could be differentiated from wild Brook Trout in the stream.

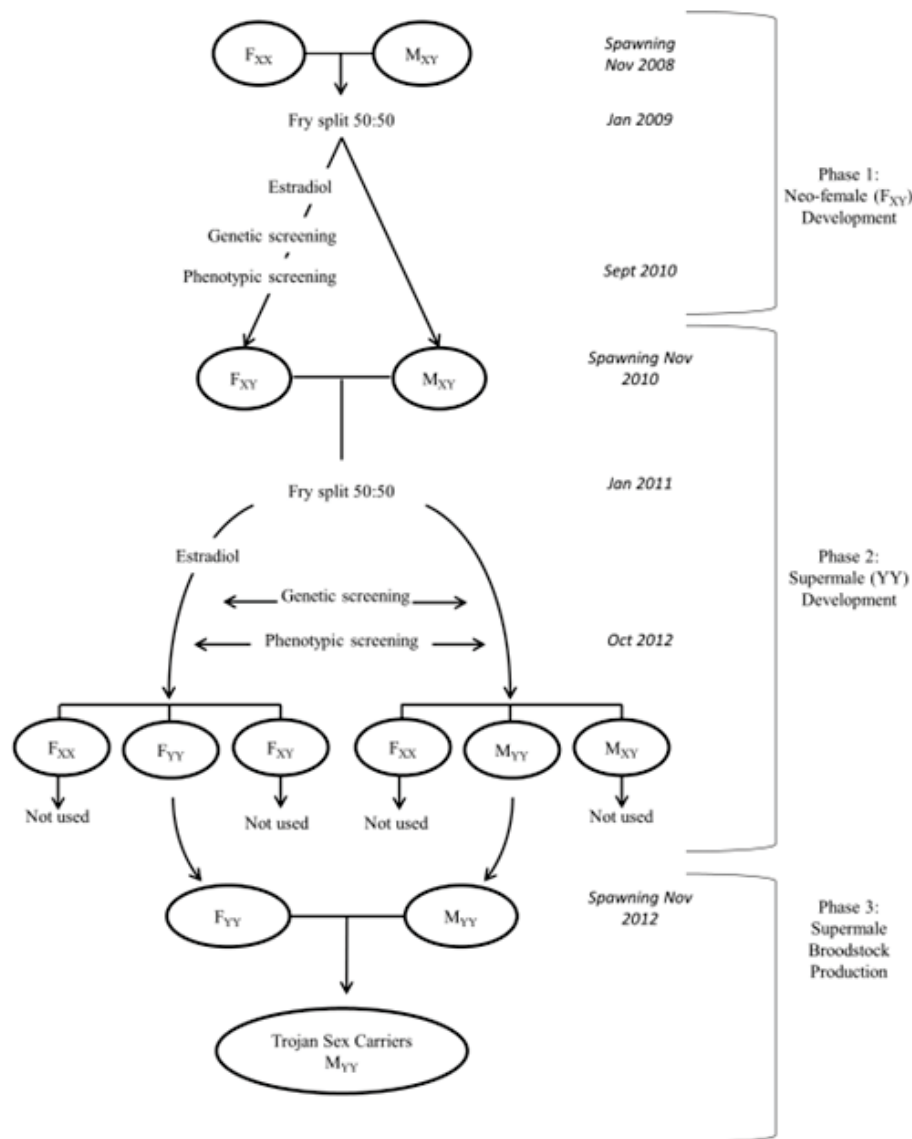


Figure 1. Schematic outlining general method of M<sub>YY</sub> Brook Trout production, 2008-2012.

In early June of 2014, At Bear and Wildhorse creeks, all wild Brook Trout captured via electrofishing were euthanized and measured to the nearest mm. In late June of 2014, approximately 500 M<sub>YY</sub> Brook Trout were evenly dispersed throughout the study reach of each study stream (Table 1).

In October of 2014, mark-recapture electrofishing was conducted in each study reach to estimate the abundance of adult wild ( $\geq 100$  mm) and M<sub>YY</sub> Brook Trout. Single-pass electrofishing was also conducted 300 m above and below each study reach to estimate post-stocking emigration of M<sub>YY</sub> Brook Trout out of the study reaches. Emigration rates were estimated

by dividing the proportion of captured emigrants from each study reach by the capture efficiency for M<sub>YY</sub> Brook Trout in the mark-recapture surveys. Unadjusted survival of M<sub>YY</sub> Brook Trout was estimated by dividing the abundance of M<sub>YY</sub> Brook Trout estimated within the study reach in October by the number originally stocked. Apparent survival was calculated by subtracting the emigration rate from the unadjusted survival estimate.

Angler exploitation of M<sub>YY</sub> trout after stocking was estimated following the methods of Meyer and Schill (2014). Approximately 10% were tagged prior to stocking using T-bar anchor tags. Anglers could

report tags through the IDFG phone system, website, regional offices, or by mail. We assumed that anglers reported 41% of these non-reward tags (K. Meyer, unpublished data). We calculated angler exploitation through four months after stocking.

Tissue samples were collected from  $M_{YY}$  Brook Trout at the hatchery and from wild Brook Trout at each stream. To determine if  $M_{YY}$  Brook Trout successfully reproduced in the wild, approximately 100 tissue samples were collected and genotyped from Brook Trout fry (< 90 mm) the following year (in Fall of 2015) at each study stream. All samples were screened with 240 single nucleotide polymorphic (SNP) loci. Genotyping followed protocols developed by Campbell et al. (2015). Putative first generation ( $F_1$ ) offspring from  $M_{YY}$  Brook Trout were identified using program STRUCTURE version 2.3.3 (Pritchard et al. 2000) to estimate individual membership coefficients ( $Q$ ). A total of 50,000 Markov Chain Monte Carlo samples were drawn after discarding the first 10,000 iterations. 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. The admixture proportions observed in the simulated  $F_1$  offspring were used as criteria to assign juveniles as  $F_1$  offspring from  $M_{YY}$  and wild females.

### Simulated Time to Eradicate Brook Trout Populations

For further details regarding eradication modeling, see Schill et al. (2017). Briefly, we constructed an age-structured stochastic model to simulate effects of a range of fishing mortality (imposed via manual suppression) and  $M_{YY}$  stocking rates on long-term viability of hypothetical wild Brook Trout populations. For stream evaluations, we parameterized the model to mimic the Brook Trout population in Hunt Creek, Michigan, during 1949–1962 (McFadden et al. 1967), because population demographics were similar to introduced Brook Trout in western North America (e.g., Meyer et al. 2006). The population growth rate ( $R$ ) in each year was treated as a function of year-specific total abundance and an assumed carrying capacity ( $K$ ) of 10,000 total fish of all ages, based on a reasonable density of 1,000 total Brook Trout per stream km (Meyer et al. 2006) and a hypothetical

stream length of 10 km. The maximum population growth rate ( $R_{max}$ ) for simulated populations was set at the highest net reproduction rate estimated for the Brook Trout population in Hunt Creek.

Simulated fishery management actions included a range of suppression rates (via electrofishing removals) and  $M_{YY}$  stocking rates, both conducted annually. In practice, fish stocked in streams would be adipose fin-clipped to distinguish them from wild fish during electrofishing, so they could be released. Wild fish suppression was simulated for three rates (25, 50, and 75% of the population), in conjunction with relative age-specific selectivity to backpack electrofishing gear derived from typical recapture rates of marked fish in Idaho streams. Stocking of  $M_{YY}$  fingerlings was incorporated into the models at three proportions (10, 25, and 50%) of the expected number of age-0 Brook Trout (6,640 fish) present at the simulated  $K$  and average age-specific survival rates of the simulated population. Fitness (survival and reproductive success) was initially assumed to be the same for stocked  $M_{YY}$  Brook Trout as for their wild counterparts. To evaluate less than optimal fitness of stocked  $M_{YY}$  fish relative to wild fish, we also ran simulations assuming that stocked fish were only 20% as fit as wild fish.

Modeling of alpine lake populations was the same as for streams except that parameter values were set to mimic abundance and survival of Brook Trout in Idaho alpine lakes (M. Koenig, IDFG, unpublished data). Population  $K$  was set at 3,500 fish of all ages. Suppression and  $M_{YY}$  stocking levels were the same as for streams, but suppression would require use of lethal overnight gill netting in alpine lakes, thus stocked  $M_{YY}$  fish were subjected to the same suppression in years after stocking as wild fish.

For each water type and combination of suppression and stocking rates, 1,000 iterations of the model were run for a 50-year period. Time to eradication for each combination of removal and stocking rate was represented as the year that total abundance of all age groups declined to zero for all simulations.

We also modeled years to eradication in streams and alpine lakes across a wider range of stocking rates than established above. For these simulations, we modeled suppression rates of 0, 25, and 50%, assumed that  $M_{YY}$  fish were as fit as wild fish, and varied stocking rates from 0 to 100% in 10% increments.

Assuming that  $M_{YY}$  fish are only 20% as fit as wild fish, results from these simulations equated to stocking rates of 0 to 500% in the poor fitness scenario.

## Results

### YY Brook Trout Broodstock Development

In Phase 1, sex reversal of genetically male ( $M_{XY}$ ) Brook Trout to  $F_{XY}$  females did not prove difficult. Necropsies performed on putative  $F_{XY}$  females genetically identified from the estradiol treatment group (but not needed for YY broodstock production) indicated a near 100% success in full feminization to  $F_{XY}$  females, as only one individual intersex fish was observed. Thus for all treated XY Brook Trout reared to maturity, 223 of 224 fish or 99.6% had fully-formed, functional ovaries and were considered phenotypic females.

During Phase 2, use of both genetic sex markers and phenotypic screening of pre-spawners, 51 maturing feminized  $F_{YY}$  fish and 49 maturing  $M_{YY}$  fish were preliminarily identified in October 2012. In Phase 3, of the 19  $F_{YY} \times M_{YY}$  crosses made, six failed to produce viable progeny while the remaining crosses yielded more than enough viable  $M_{YY}$  green eggs for future YY broodstock and for the field experiment described below.

### Field Trials of Survival and Successful Spawning

In June of 2014, a total of 2,010 MYY Brook Trout catchables produced in Phase 3 above were stocked in the study streams (Table 1). Prior to stocking, we removed 1,026 wild Brook Trout from Bear Creek

and 210 from Wildhorse Creek. By October, across all streams, we estimated 226 MYY fish and 8,266 wild Brook Trout (>100 mm) remained in the study reaches; therefore, MYY fish comprised 2.7% of the spawning Brook Trout population (wild and hatchery). Capture efficiencies averaged 57% (range 43-74%) for wild Brook Trout and 83% (range 75-100%) for MYY fish. Considering our abundance estimates and assuming no mortality of wild fish from June to October, MYY fish were presumably stocked at an average of 27% (range = 15-40%) of the wild Brook Trout population across all study streams, and the removals would have constituted a 24% suppression of wild fish in Bear Creek and a 9% suppression in Wildhorse Creek.

Emigration of  $M_{YY}$  Brook Trout out of the study reach and into adjacent stream reaches averaged 1.2% (Table 1). Angler exploitation was nil in Bear and Cherry creeks and averaged 24.8% in Wildhorse and Iron Bog creeks. Survival averaged 16.1% at suppression streams and 9.1% at non-suppression streams. In 2015, 14 individuals with genotypes indicating  $F_1 M_{YY}$  offspring were detected in all study streams combined, and  $M_{YY}$  offspring were detected in each of the study streams (Table 2). All 14 individuals identified as  $F_1$  offspring were genetic XY males.

### Simulated Time to Eradicate Brook Trout Populations

Simulations of the time required to eradicate Brook Trout populations using  $M_{YY}$  stocking revealed that if stocked  $M_{YY}$  fish survive and reproduce as well as wild males in streams, Brook Trout could be eradicated using several combinations of  $M_{YY}$  stocking and electrofishing suppression. For example, time to

**Table 1. Estimates (Est.) of and 90% confidence intervals (CI) for the abundance of wild Brook Trout (>100mm) and the abundance, exploitation, emigration, and survival of  $M_{YY}$  Brook Trout stocked in four study streams in central Idaho.**

Creek	Treatment	Wild Brook Trout				$M_{YY}$ Brook Trout								
		October abundance			Stocked in June	October abundance			Angler exploitation (%)		Emigration (%)		Apparent survival (%)	
		Est.	CI	p (%)		Est.	CI	p (%)	Est.	CI	Est.	CI	Est.	CI
Bear	Suppressed	3,254	185	47	492	103	18	75	0.0	0.0	2.4	1.0	23.4	3.6
Wildhorse	Suppressed	2,034	114	43	506	43	3	83	27.5	16.0	0.2	0.3	8.7	0.6
Cherry	Not suppressed	1,682	67	62	500	44	3	75	0.0	0.0	0.5	0.5	9.4	0.6
Iron Bog	Not suppressed	1,296	60	74	512	36	0	100	22.0	14.4	1.8	1.0	8.8	0.0

**Table 2. Sample group along with treatment level (suppression or non-suppression) and stream name. Sample size is shown for each sample group, along with minimum, maximum, and average proportional membership observed.**

Creek	Treatment level	Sample group	Sample size	Proportional membership		
				Min	Max	Avg.
Bear	Suppressed	$M_{YY}$ offspring	3	0.456	0.554	0.509
Wildhorse	Suppressed	$M_{YY}$ offspring	5	0.383	0.605	0.490
Cherry	Not suppressed	$M_{YY}$ offspring	4	0.417	0.552	0.500
Iron Bog	Not suppressed	$M_{YY}$ offspring	2	0.405	0.644	0.525

**Table 3. Predicted years to eradication and 95% lower (LCI) and upper (UCI) confidence intervals for Brook Trout in hypothetical streams and alpine lakes in Idaho subjected to a range of selective electrofishing (streams) and non-selective gill-netting (lakes) suppression rates and  $M_{YY}$  stocking rates. Predictions assumed  $M_{YY}$  fitness (survival and reproductive success) was equivalent to wild males (good survival) and 20% of wild males (poor survival).**

Suppression rate	Stocking rate	Streams						Alpine lakes					
		Good survival			Poor survival			Good survival			Poor survival		
		Years	LCI	UCI	Years	LCI	UCI	Years	LCI	UCI	Years	LCI	UCI
0%	10%	>50	>50	>50	>50	>50	>50	>50	1	>50	>50	1	>50
	25%	>50	9	>50	>50	>50	>50	23	1	25	>50	1	>50
	50%	12	3	12	>50	>50	>50	8	1	8	>50	1	>50
25%	10%	>50	13	>50	>50	>50	>50	>50	1	>50	>50	1	>50
	25%	13	4	14	>50	>50	>50	14	1	15	>50	1	>50
	50%	6	3	7	>50	14	>50	8	1	8	>50	1	>50
50%	10%	13	5	14	>50	15	>50	20	1	23	>50	1	>50
	25%	6	3	7	26	8	28	10	1	10	>50	1	>50
	50%	4	2	4	12	5	15	7	1	8	18	1	21
75%	10%	6	4	6	10	6	11	11	1	13	25	1	30
	25%	4	2	4	7	4	8	7	1	8	16	1	19
	50%	4	2	4	6	4	6	6	1	7	11	1	12

eradication was 12–13 years for 10% stocking and 50% electrofishing suppression, 25% stocking and 25% suppression, or 50% stocking and 0% suppression (Figure 2, Table 3). Similarly, time to eradication was as little as 6 years for 10% stocking and 75% electrofishing suppression, 25% stocking and 50% suppression, or 50% stocking and 25% suppression. If stocked  $M_{YY}$  fish are only one-fifth as fit as wild males in streams, Brook Trout could be eradicated only by using high rates of stocking, with or without concurrent electrofishing suppression.

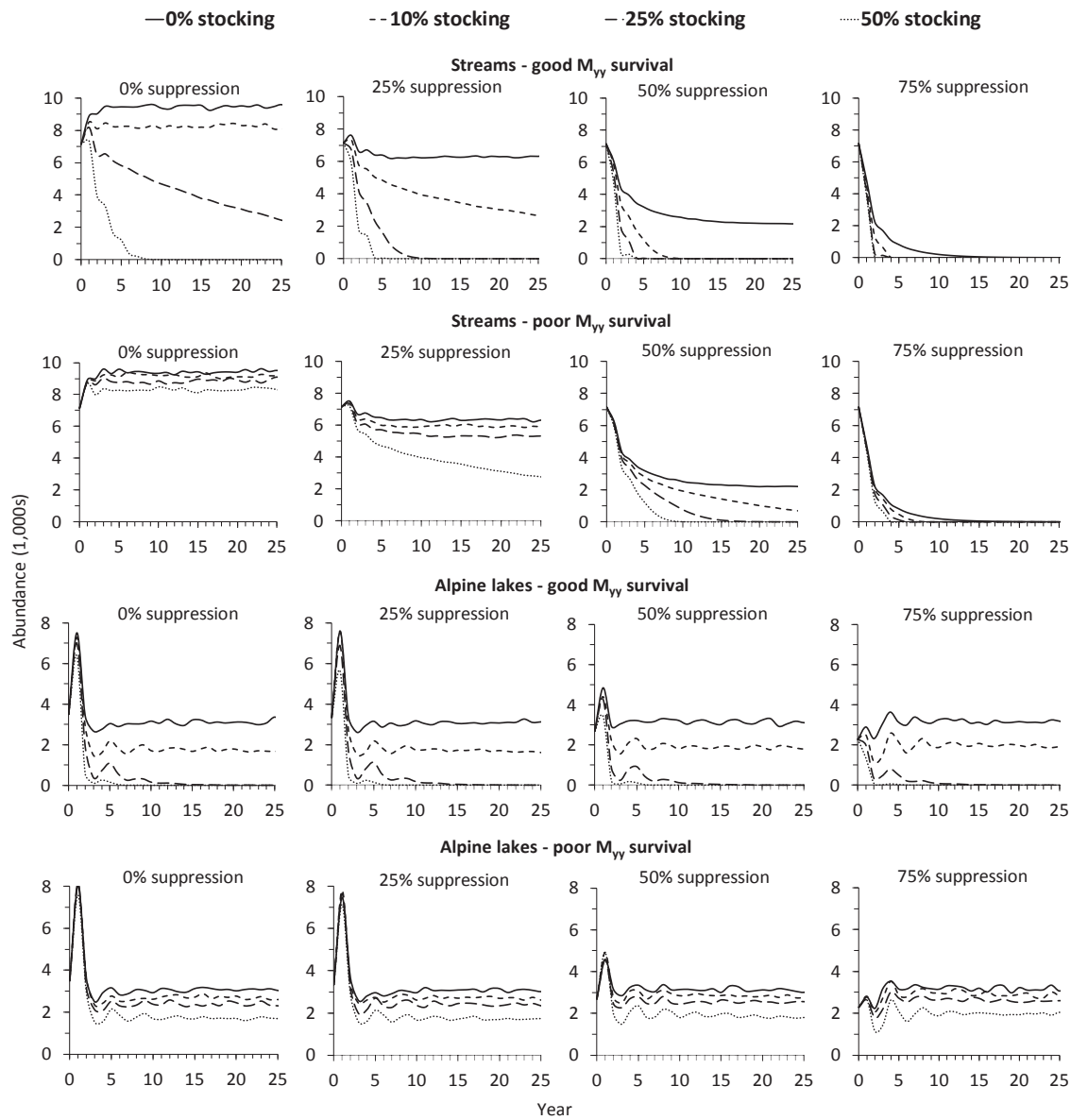
In alpine lakes, if  $M_{YY}$  fish survive and reproduce as well as wild males, Brook Trout could be eradicated using several combinations of  $M_{YY}$  stocking and gill

net suppression, but time to eradication was longer than in streams. For example, time to eradication was 8–11 years at 10% stocking and 75% gill netting suppression, 25% stocking and 50% suppression, and 50% stocking and 25% suppression (Figure 2, Table 3). Population eradication was achievable in 10 years or less only at a stocking rate of 50% or greater (regardless of the suppression rate), or a stocking rate of 25% and a suppression rate of 50% or greater. If  $M_{YY}$  fish are one-fifth as fit as wild males in alpine lakes, Brook Trout could only be eradicated by using very high rates of stocking and gill net suppression. For example, Brook Trout were only eradicated by a suppression rate of 75%, regardless of stocking

rate, or when both stocking rate and suppression rate were 50% or greater. Eradication was not achievable within 10 years in alpine lakes at any of the initial combinations of suppression and stocking rates when we assumed that stocked  $M_{YY}$  fish were 80% less fit than wild fish.

Across a broader range of potential stocking rates, suppression rate influenced years to eradication for hypothetical Brook Trout populations more dramatically in streams than in alpine lakes. For

example, reducing suppression in streams from 50% to 25% would require more than a doubling of the stocking rate to maintain a 10-year eradication time frame, whereas in alpine lakes, the same reduction in suppression would require only a 40% increase in stocking rate to maintain a 10-year eradication time frame (Figure 3). Assuming that  $M_{YY}$  fish are as fit as wild males, any stocking rate greater than 49% in alpine lakes or 60% in streams achieved eradication in 10 years or less, regardless of the suppression rate.



**Figure 2. Simulated abundance of Brook Trout in hypothetical streams and alpine lakes in Idaho subjected to a range of suppression and MYY stocking rates, assuming MYY survival was equivalent to wild males (good survival) and 20% of wild males (poor survival).**

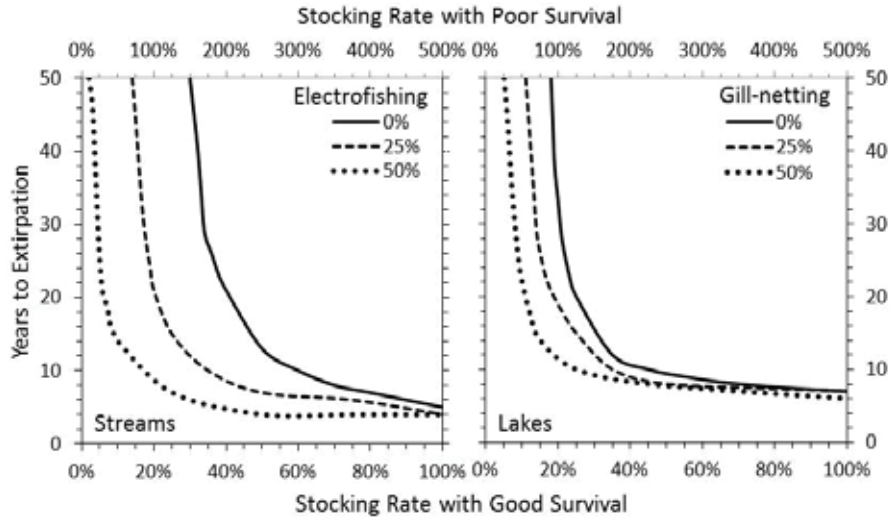


Figure 3. Predicted years to eradication of Brook Trout in hypothetical streams and alpine lakes in Idaho subjected to a range of suppression and MYT stocking rates, assuming MYT fitness (survival and reproductive success) was equivalent to wild male fitness (good survival) and 20% of wild male fitness (poor survival).

## Discussion

### YY Brook Trout Broodstock Development

Although the process we used to produce a  $M_{YY}$  Brook Trout broodstock sounds complex, monosex production of numerous species has been accomplished for decades in commercial aquaculture. In our case, nearly all of the elapsed time from project initiation (Fall of 2008) to successful  $F_{YY}$  and  $M_{YY}$  broodstock spawning (Fall of 2012) was to allow fish to mature between production phases. Labor was primarily focused in 2- to 3-d spawning periods at the end of each production phase and for PIT-tagging and fin clipping. Total costs to develop the broodstock, including genetic testing, feed, and labor, were probably less than US \$30,000, in part because a sex-linked genetic sequence had presumably already been identified and thus the costs of developing a working sex marker were minimal. The availability of such sex markers has been identified as a crucial step in developing a  $M_{YY}$  program (Cotton and Wedekind 2007).

### Field Trials of Survival and Successful Spawning

Across all streams combined, the  $M_{YY}$  Brook Trout we stocked comprised an estimated 2.7% of

all spawning adults in 2014 and they produced an estimated 3.7% of the progeny that year. While these findings demonstrate that hatchery  $M_{YY}$  Brook Trout can successfully compete reproductively with wild male conspecifics in streams, these numbers do not indicate that  $M_{YY}$  fish were more prolific, since only a portion of the wild Brook Trout were mature males. Considering the estimated sex ratios at Bear and Wildhorse creeks (data not shown), and assuming that male Brook Trout in Idaho streams become sexually mature at about 150 mm, and limiting abundance estimates to fish greater than 150 mm for these two streams, we estimate that  $M_{YY}$  fish comprised about 10% of all spawning male Brook Trout at Bear and Wildhorse creeks combined, and produced 4% of the progeny, suggesting that while  $M_{YY}$  Brook Trout are reproductively capable, they are not as fit as their wild conspecifics.

Apparent survival was low for hatchery  $M_{YY}$  Brook Trout but was similar to results from other studies of catchable-sized hatchery trout stocked in streams (e.g., Miller 1952). Hatchery trout survival can be increased by reducing wild fish abundance (Miller 1958), and our study showed some evidence of improved survival of  $M_{YY}$  fish in suppression streams. Post-release survival was reduced only slightly by emigration and exploitation. When considering an  $M_{YY}$



stocking program to eradicate undesirable fish, one question worth evaluating is which size of hatchery fish to stock. Rearing fish to fry and fingerling size is much less expensive and requires much less hatchery rearing space, but post-release survival is typically much lower, and the fish must survive at least 1-2 years to reach maturity before they can spawn with wild fish.

### Simulated Time to Eradicate Brook Trout Populations

Our simulations suggest that stocking  $M_{YY}$  fish could eradicate wild nonnative fish populations in reasonable timeframes, whereas previous studies have generally suggested that many decades would be required to eradicate populations (Gutierrez and Teem 2006; Teem and Gutierrez 2010). Such slow responses are unlikely to be acceptable to fishery managers or the public, and may partly explain why management interest in a  $M_{YY}$  approach has been limited to date. Responses predicted by previous studies were slower than our predictions for several reasons. First, previous studies have generally modeled low (4–7%) stocking rates of reproductively competent adults, whereas we modeled fingerling stocking rates as high as 100% of the existing wild fish. Such stocking rates are certainly feasible for many undesirable Brook Trout populations in western North America, and our results demonstrate the importance of stocking at higher rates to quickly eradicate populations. Second, Brook Trout have a shorter generation time than most other nonnative fish species, thereby making them more vulnerable to sex-skewing eradication methods. Third, no prior  $M_{YY}$  simulation study has included concurrent manual suppression, which our results suggest can greatly speed the eradication process.

Our simulations suggest longer time to eradication and higher stocking rates would be needed to eradicate Brook Trout in alpine lakes compared to streams, in part because Brook Trout mature later and live longer in lakes than in streams, which slows the demographic input of successful  $M_{YY}$  spawning in the wild population. Also, lethal overnight gill netting removes some  $M_{YY}$  fish from lakes that are not killed with selective electrofishing removals in streams. A likely reason for the relative insensitivity of our alpine lake results to suppression above 40% was use of non-selective gill nets, because stocked  $M_{YY}$  fish would also be killed at the same high rates as wild males.

Although fitness of stocked fingerling  $M_{YY}$  Brook Trout may be lower than that of wild fish, such results are not a certainty in all instances. For example, suppression of wild Brook Trout may have increased survival of stocked  $M_{YY}$  catchables in our study streams. In alpine lakes, stocked  $M_{YY}$  fingerlings may benefit from increased age-0 survival and an associated recruitment pulse that was consistently found in California alpine lakes following sustained wild Brook Trout removal via gill netting (Hall 1991).

### Conclusion

To our knowledge, this effort represents the first successful construction of a  $F_{YY}$  and  $M_{YY}$  salmonid broodstock, which IDFG staff produced in 4 years and with minimal cost. Our field trials demonstrated that stocked hatchery  $M_{YY}$  Brook Trout survived and produced viable  $M_{YY}$  offspring in streams, although fitness appeared to have been lower than their wild conspecifics. Even if reduced fitness is to be expected in both streams and alpine lakes, our population simulations suggest that eradication can be achieved in reasonable time periods under some stocking and wild suppression scenarios. Based on these findings, IDFG initiated (in 2016) a broad-scale evaluation (eight alpine lakes and eight streams in Idaho) to determine the potential for an  $M_{YY}$  stocking program to eradicate undesirable wild Brook Trout populations; both fingerling and catchable-sized  $M_{YY}$  Brook Trout are being evaluated.

Public acceptance will be an important issue with such a program, although for several reasons we believe this should not pose a major challenge. First, no fish destined for human consumption have been treated with hormones. Second, the method is specific to the target exotic species, not native species, so there is little to no possibility of direct ecological collateral damage. Perhaps most importantly in regard to public acceptance, a  $M_{YY}$  fish is not a genetically modified food organism (GMO) since no new genetic material is infused into released fish. For this reason, we suggest that an  $M_{YY}$  program is the least likely of various “genetic” approaches for exotic fish suppression to generate public controversy (Thresher et al. 2013). Finally, it is also worth noting that the amount of hormone released into the aquatic environment for development of the existing YY broodstock is inconsequential (Schill et al. 2016).

This work may represent a major advancement toward eradicating undesirable nonnative Brook Trout populations where they threaten native species or provide inconsequential fisheries for anglers. While our field trials demonstrated successful  $M_{YY}$  reproduction, further evaluations are needed to determine if this program is effective at actually shifting the sex ratio to the extent that a wild population of Brook Trout can be eradicated. If so, stocking  $M_{YY}$  fish could become an effective and economic method of eliminating nonnative Brook Trout populations across western North America.

In an effort to present a brief synthesis of IDFG's YY Male Brook Trout work, this written document briefly summarizes three separate aspects of the program. The reader is referred to more detailed written summaries for more specifics (Kennedy et al. 2016; Schill et al. 2016; Schill 2017).

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