U.S. Fish and Wildlife Service
Columbia River Fish and Wildlife Conservation Office

YY Male Brook Trout Stocking and Population Monitoring in Tyee Springs 2022-2023

2022-2023 Progress Report

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On the cover is an image of a field crew electrofishing for Brook Trout in Tyee Springs habitat Unit #3. Photo credit: Jennifer Poirier.

Disclaimers

The correct citation for this report is:

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2022 – 2023 PROGRESS REPORT

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Abstract

A population of nonnative Brook Trout currently resides in Tyee Springs, the primary water source for Carson National Fish Hatchery. This population has been a hatchery management concern and past attempts at eradicating the Brook Trout population have been unsuccessful. In 2018, the Columbia River Fish and Wildlife Conservation Office, Abernathy Fish Technology Center and Carson National Fish Hatchery initiated a long-term project using YY male Brook Trout (MYY) as a tool to eradicate nonnative Brook Trout in Tyee Springs. This method involves producing male Brook Trout with two Y-chromosomes which are released into the population targeted for eradication. Offspring of MYY and resident females (XX) are all male (XY), so over time, the population becomes skewed toward a single sex, leading to extirpation of the target population. Brook Trout population suppression and MYY stocking in Tyee Springs began in 2020. Concurrent with these efforts, genetic samples and other biological information were collected from MYY and the resident Brook Trout population to measure the progress of the project. In 2022, we removed a total of 1,827 resident Brook Trout and in 2023 we removed 1,405 fish. Capture efficiencies of Brook Trout have decreased annually since 2020, indicating fish removal efforts may be reducing the resident population. A total of 1,992 age-0 MYY Brook Trout were stocked into Tyee Springs in 2022 and 2,002 age-0 MYY were stocked in 2023. All fish were adipose clipped and PIT tagged prior to release. A total of four (0.2%) MYY stocked in 2021 were recaptured during fish removals in 2022 and 24 (1.2%) MYY stocked in 2022 were recaptured in 2023.

The higher post-release survival in 2023 may be attributable to using an improved acclimation strategy prior to stocking. The average fecundity estimate in 2023 was 708 eggs per mature female Brook Trout (n=76). This was higher than that observed for native and nonnative Brook Trout populations in other streams. Genetic assignment tests conducted by Abernathy FTC identified conclusive evidence of MYY offspring in Tyee Springs. The percentage of young-of-year tissue samples identified as MYY-resident hybrids was 22% in 2022 (n=81) and 13% in 2023 (n=53). These results confirm that MYY stocked into Tyee Springs have spawned successfully and produced viable offspring. Annual sex ratio estimates from field collections indicate that the resident Brook Trout population is skewing to male. In 2022, we determined the phenotypic sex of 684 Brook Trout and 43% were female. In 2023, 770 fish were examined and 40% were female. Based on these results and what we have recently learned about MYY survival and female fecundity, we plan to revise Brook Trout abundance estimates and refine the Tyee Springs population model to attain a more accurate estimate of when eradication of Brook Trout might occur in Tyee Springs.

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Introduction

An established population of nonnative Brook Trout resides in Tyee Springs directly upstream from Carson National Fish Hatchery (NFH) in southwestern Washington. Brook Trout are one of the most prevalent nonnative fish in the western United States (Benjamin et al. 2013; Shade and Bonar 2005). Once a population becomes established, complete eradication is often difficult to achieve (e.g., Koenig et al. 2015; Meyer et al. 2006; Thompson and Rahel 1996). Traditional methods to eradicate invasive or unwanted fish include the use of fish toxicants (piscicides), targeted harvest (e.g., angling, gill nets), physical removal (e.g., electrofishing), or biological control (e.g., introduction of predators or pathogens). Tyee Springs is considered a poor candidate for piscicide treatment because of the proximity of the hatchery and presence of upwelling springs that may dilute or reduce the effectiveness of the chemical. Physical removal efforts (i.e., electrofishing) periodically implemented over nearly a decade have also proven ineffective in Tyee Springs because aquatic plant density and pockets of deep water provide refuge for fish to escape capture (USFWS 2004).

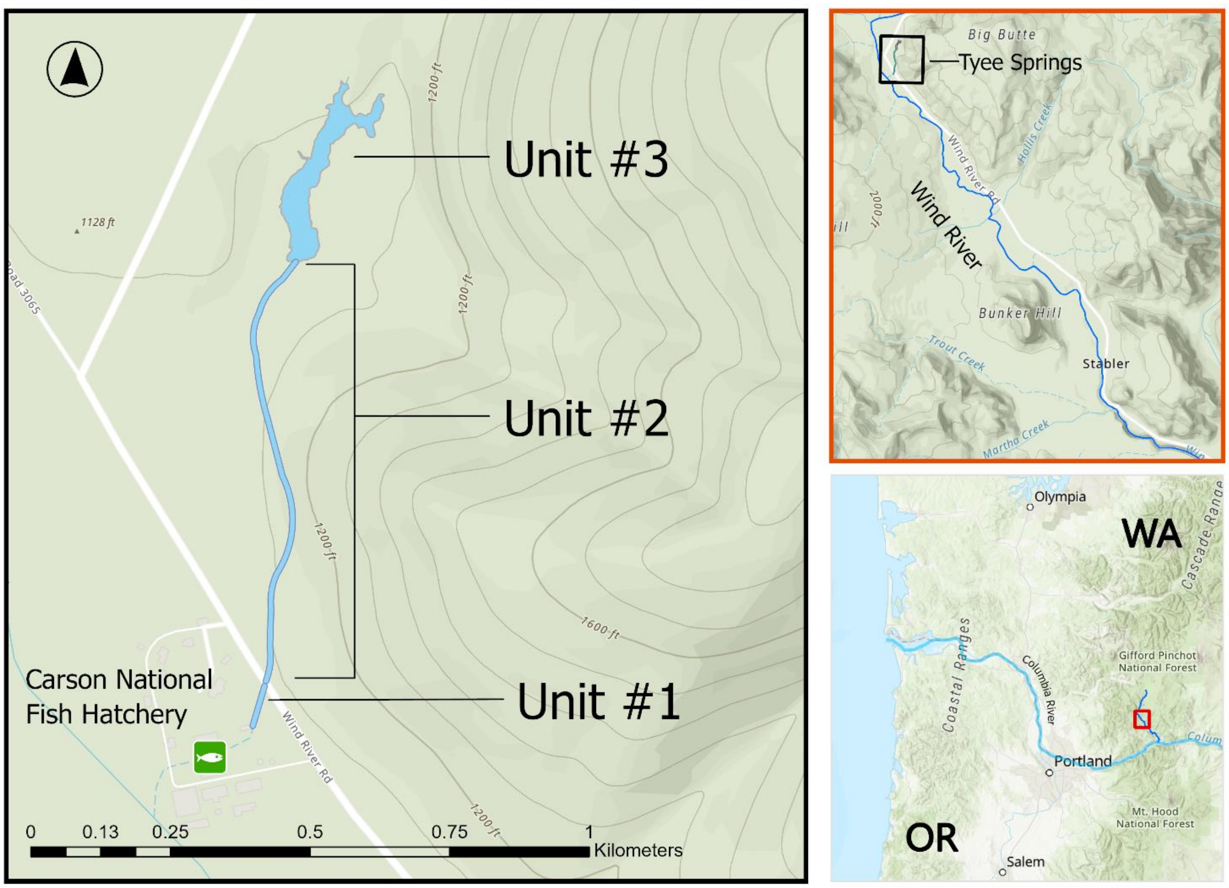
An alternative biological control strategy that could eradicate Brook Trout in Tyee Springs is the Trojan Y Chromosome approach. This method involves producing male Brook Trout with two Y-chromosomes (YY) and releasing them into the population targeted for eradication (Shill et al. 2016). Offspring of YY males (hereafter MYY) and resident females (XX) are all male (XY), so over time, the population becomes skewed toward a single sex (i.e., all males), theoretically leading to extirpation of the target population due to reproductive failure. The Idaho Department of Fish and Game has developed a broodstock of MYY Brook Trout for experimental use and field stocking trials are currently underway in six western states with encouraging results (Miller et al. 2024; Armstrong et al. 2022; Kennedy et al. 2018).

In 2018 the Columbia River Fish and Wildlife Conservation Office (FWCO), Abernathy Fish Technology Center (FTC) and Carson NFH initiated a collaborative proof-of-concept study to assess the feasibility of stocking MYY Brook Trout to eradicate nonnative Brook Trout in Tyee Springs (Poirier et al. 2020). In 2020 we initiated the implementation phase of the Tyee Springs MYY Brook Trout project with the goal of suppressing approximately 50% of the resident Brook Trout population annually and stocking up to 3,000 MYY Brook Trout into Tyee Springs prior to the spawning season (Davis and Poirier 2023). Concurrent with stocking and removal efforts, we have collected genetic samples from stocked MYY and resident Brook Trout to evaluate the spawning contribution of YY males. We also collect information from MYY and resident Brook Trout populations during annual fish removal efforts to measure the progress of MYY stocking efforts in Tyee Springs and evaluate the overall effectiveness of the MYY technique as a biological control strategy. This report presents results of fish suppression and MYY stocking efforts conducted in 2022 and 2023 including a summary of Brook Trout capture efficiency, female fecundity estimates, male to female sex ratios and an analysis of genetic samples collected from 2018-2023.

Methods

Study Area

Tyee Springs is a 0.7 km long spring-fed stream located within the Wind River watershed, approximately 19 km north of the town of Carson, Washington in Skamania County (Figure 1). Carson NFH, which began operation in 1937, was constructed to utilize Tyee Springs as its primary water source, providing year-round flow at a fairly constant 6.6°C. Water from Tyee Springs enters the hatchery through an intake grate, flows through the facility and exits via the adult ladder and bypass channel before entering the Wind River at RKM 29. The hatchery acts as a complete passage barrier to fish attempting to enter Tyee Springs, but a bypass channel provided some opportunity for fish to exit the springs for roughly six months of the year (Davis and Poirier 2023). In 2022, Washington Department of Fish and Wildlife (Yakima screen shop) fabricated and installed an incline belt screen in the bypass channel that effectively blocked passage of fish that may be flushed into the bypass channel when it is open.



Oregon State Parks, WA State Parks GIS, Esri, TomTom, Garmin, FAO, NOAA, USGS, Bureau of Land Management, EPA, NPS, USFWS, Sources: Esri, TomTom, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community, Esri, USGS

Figure 1 Map of Tyee Springs and three habitat units in relation to the Wind and Columbia rivers.

Historically Carson NFH reared and released fall Chinook salmon and various trout species (including Brook Trout), before shifting to focus on spring Chinook salmon in 1981 (USFWS 2002). Although hatchery releases of Brook Trout were discontinued in 1964, a naturally reproducing population still exists in Tyee Springs. Semi-regular attempts to suppress the Brook Trout population in Tyee Springs that occurred from 1999 to 2009 did not result in the extirpation of the unwanted population (USFWS 2004). Brook Trout and sculpin are the only fish species that inhabit Tyee Springs today.

Tyee Springs is comprised of three distinct habitat segments, referred to as Units #1, #2, and #3 (Figure 1; Appendix). Unit #1 is a roughly 50 meter long, deepwater channel that starts at the hatchery intake screen and terminates at the downstream end of the Wind River Road culvert. Unit #2 is the longest habitat segment and is characterized as a meandering stream stretching roughly 550 meters upstream from the Wind River Road culvert to Unit #3. Unit #3 is the uppermost segment of Tyee Springs characterized as a spring-fed pond roughly 230 meters in length.

Tyee Springs Population Suppression

In 2022 and 2023, Brook Trout removals occurred two days per week, during the months of June, July, and August. We utilized up to three methods of capture that varied dependent on available staff and targeted habitat. Two different Smith-Root electrofishing tote barges referred to as B1 (older aluminum barge with stationary anode dropper) and B2 (newer plastic barge with cathode plate and two handheld anode poles), were utilized in all three habitat units. Unit B1 was used exclusively in the 50 meter deepwater channel (Unit #1) immediately upstream of the hatchery intake screen. One or two backpack electrofishers (model LR-24) were used in wadeable depths (<1.2 m) and backwater areas within Units #2 and #3, and two gillnets were employed in Unit #3 to passively capture Brook Trout overnight between consecutive sampling days. Each gillnet targeted different sized Brook Trout with 1/2 in. and 3/4 in. square mesh sizes in total lengths of 50 ft., and 100 ft., respectively.

All resident Brook Trout captured during fish removal efforts were euthanized prior to sampling using a high concentration of carbon dioxide (CO^2) so they could be donated safely as food fish. Captured fish were measured to the nearest millimeter in fork length, weighed (g), scanned for PIT tags, and inspected externally for adipose clips, deformities, wounds or parasites. A random subsample of Brook Trout >100 mm in length were also cut open and inspected internally to determine the phenotypic sex. Approximately 100 tissue samples (i.e., fin clips) were collected from resident Brook Trout (>120 mm in length) each year to establish a genetic baseline of the resident population. Tissue samples were also collected from 400 young-of-year Brook Trout each year to determine if any fish were offspring of M_{YY} Brook Trout. Collection of tissue samples was dispersed spatially and temporally throughout the field season.

Captured Brook Trout with a PIT tag and adipose clip were presumed to be M_{YY} fish. All M_{YY} fish were scanned to record the PIT tag number, measured, weighed, and returned to the point of capture (Unit #1 only), or to the headwaters of Tyee Springs in Unit #3.

M_{YY} Stocking

All M_{YY} stocked into Tyee Springs originated from Hayspur State Fish Hatchery operated by Idaho Fish and Game. Hayspur staff annually produce M_{YY} Brook Trout embryos in the fall which are subsequently transported to various projects, agencies and states for the purpose of research. Eyed Brook Trout eggs were transferred from Hayspur Hatchery to Spokane State Hatchery in December where they were incubated, hatched and reared until their release. Approximately one week prior to stocking, all M_{YY} were given an adipose fin clip and implanted with a 12 mm FDX PIT tag for monitoring purposes. Fish stocked in 2022 were from brood year 2021 and fish stocked in 2023 were from brood year 2022.

Monitoring

Post stocking mortalities

Carson NFH staff monitored hatchery intake screens for M_{YY} mortalities in the following weeks after 2022 and 2023 stocking events. Post-stocking mortalities were collected and frozen so that they could be enumerated and scanned for PIT tags at a later date.

Brook Trout Capture Efficiency

Both Smith-Root electrofishing tote barges and backpack electrofishers were equipped with a mechanical counter that measured operational runtime in seconds. Barge and backpack counters were reset daily and for each habitat unit. E-fisher counter seconds along with Brook Trout capture totals were used to calculate Catch-per-unit effort (CPUE) as a measure of capture efficiency (Murphy and Willis, 1996). CPUE was calculated by dividing the total number of Brook Trout captured by electrofisher seconds for every sampling day, habitat unit, and capture method.

Fecundity

During the fish removal and data collection process in 2023, eighty resident Brook Trout identified as sexually mature females were collected across seven days in the month of July (07/06 – 07/26/2023). Females were stored separately on ice and taken to Carson NFH each day of collection to evaluate fecundity. Individual females were measured (fork length in mm) and weighed (g). Both egg sacs were removed and weighed (g) together, then a subsample of 25 individual eggs from each egg sac was weighed (Figure 2). Four fish less than 130 mm in length were rejected from the fecundity analysis due to their eggs being too immature and delicate to enumerate. Thus, only fish greater than 130 mm were taken for fecundity after the second collection day.

Data collected from each female was used to calculate two fecundity estimates (F) where n represented the number of eggs in each subsample, M was the total weight of both egg sacs and g was the total weight of a 25-egg subsample (Smalås, Amundsen and Knudsen, 2017).

$$F = \frac{\eta M}{g}$$

The mean of both fecundity estimates (F) for every fish was used for the final fecundity estimate.

A negative binomial (aka poisson-gamma) regression was used to model the number of eggs (fecundity) per resident female given its length (x_i). A log likelihood function was constructed as following:

$$Y_i \mid x_i, \alpha, m, \beta \ \sim Negativebinomial(e^{\alpha + m x_i}, e^{\beta})$$

Model parameters were estimated using a Bayesian framework and the MCMCpack package (Martin et al. 2011) within the R programming environment (R Core Team, 2023). The posterior was sampled with two chains iterating 1,000,000 times using a random walk Metropolis algorithm with a 10,000 burn-in period.

Figure 2. Images of weighing all eggs from both egg sacs (left) and subsample of 25 individual eggs from a single egg sac (right).

Genetic Assignment Tests

State Hatchery (2022, 2023), and adult resident Brook Trout at Tyee Springs (2018-2023), to assess the genetic variation between the two groups (M_{YY} vs resident). Tissue samples were also collected from young-of-year Brook Trout in Tyee Springs (2021-2023) to determine whether any M_{YY} Brook Trout successfully reproduced with resident females. Tissue samples were clipped from the adipose, ventral, or caudal fin and preserved in vials with 100% ethyl alcohol. Vials were stored at room temperature (~21°C) until they were sent to Abernathy FTC for analysis.

A Genotyping in Thousands by Sequencing (GTseq) panel of 245 single nucleotide polymorphism (SNP) loci was amplified for all individuals (Campbell et al. 2015; Kennedy et al. 2018). These data were then filtered to remove loci with more than 20% missing data and individuals with over 30% missing data using custom scripts. Loci fixed for a single allele in all samples were also discarded.

Genotypes for hatchery-origin M_{YY} Brook Trout were compared to pre-treatment (i.e., 2018 and 2019) reference data derived from resident Brook Trout collected in Tyee Springs. This was accomplished by performing a principal components analysis (PCA) on allele frequency data to examine interannual variation among M_{YY} cohorts and contrast them with Tyee Springs Brook Trout. Pairwise F_{ST} values (Weir and Cockerham 1984) were calculated to quantify genetic differentiation among sample groups. Both analyses were performed in R v4.2.2 (R Core Team 2022) using the *hierfstat* v0.5 package (Goudet 2005).

We tested for hybridization between two genetically differentiated groups to identify offspring of M_{YY} individuals (i.e., F1 hybrids). Genotype data were evaluated using the Bayesian method NewHybrids v2.0 (Anderson and Thompson 2002). NewHybrids was executed using 100,000 Markov chain Monte Carlo (MCMC) generations of burn-in, followed by 200,000 generations of data collection. The Jeffreys prior for allele frequencies and mixing proportion was applied, and reference samples (i.e., 2018-2019 Tyee Springs Brook Trout and 2019-2023 M_{YY} Brook Trout) were treated as known-ancestry individuals by using the 'z' option to assign ancestry priors. NewHybrids calculated assignment probabilities for individuals to each of three possible ancestry classes (wild-origin, M_{YY}, or F1 hybrid).

Results

Tyee Springs Population Suppression

2022

A total of 1,827 resident Brook Trout were removed over twelve days from 06/07/22 to 08/03/22. Most fish removed from Tyee Springs (96%) were captured in Unit #2 and #3 (Table 1). Only 80 fish were captured in Unit #1 across two recapture events. The tote barge outperformed all other capture methods and was effective in all habitat units (Table 1; Figure. 3).

Five Brook Trout were captured and released due to having PIT tags. Four fish were presumably M_{YY} fish (based on the PIT tag code) and one individual originated from a 2019 tagging event during the feasibility portion of this study (Poirier et al. 2020). This fish was tagged on 05/21/2019 with a fork length of 99 mm and was recaptured on 07/06/2022 at 201 mm in length. The four M_{YY} fish were originally stocked in Tyee Springs in August 2021 and ranged from 184 mm to 221 mm in length. M_{YY} fish were captured throughout every habitat unit using all capture methods. One M_{YY} individual was captured on two separate occasions in Unit #1 with the electrofishing tote barge (06/22/22 and 07/19/22).

Table 1. Number of Brook Trout removed by date, habitat unit and capture method in 2022. TB = tote barge (both units combined); EF = backpack electrofisher (both units combined); GN = gillnets (both gillnets combined).

	Unit #1			**Unit #2**			**Unit #3**				
Date	EF	TB	**Sum**	EF	TB	**Sum**	EF	TB	GN	**Sum**	Total
06/07/2022	0	0	**0**	0	70	**70**	0	55	0	**55**	125
06/08/2022	0	0	**0**	0	88	**88**	0	106	0	**106**	194
06/22/2022	0	54	**54**	0	0	**0**	0	0	0	**0**	54
06/28/2022	0	0	**0**	0	99	**99**	47	62	0	**109**	208
06/29/2022	0	0	**0**	71	40	**111**	8	63	4	**75**	186
07/05/2022	0	0	**0**	0	56	**56**	0	71	0	**71**	127
07/06/2022	0	0	**0**	0	73	**73**	0	83	20	**103**	176
07/12/2022	0	0	**0**	0	68	**68**	0	0	0	**0**	68
07/13/2022	0	0	**0**	74	0	**74**	0	104	15	**119**	193
07/19/2022	0	26	**26**	49	0	**49**	0	105	0	**105**	180
08/02/2022	0	0	**0**	84	0	**84**	0	96	0	**96**	180
08/03/2022	0	0	**0**	56	0	**56**	0	70	10	**80**	136
Total	**0**	80	**80**	334	494	**828**	55	815	49	**919**	**1827**

Cells with zeros indicate no effort for that day, unit and sampling method.

The median fork length of Brook Trout removed from Tyee Springs in 2022 was 103 mm, with a range of 22 to 256 mm (Figure 3). Fish captured in the deepwater channel near the hatchery intake screen (Unit #1) were larger on average than the other habitat units. The widest range of lengths were captured in Unit #3 with the electrofishing tote barge (Figure 3). These length data were multimodal, with young-of-year fork lengths ranging from 22 mm to approximately 72 mm with a median of 45 mm. Length distributions from other age classes suffered from too much overlap to confidently identify, but likely consisted of ages one through three, based on analyses conducted in 2020 (Figure 4; Poirier et al. 2020).

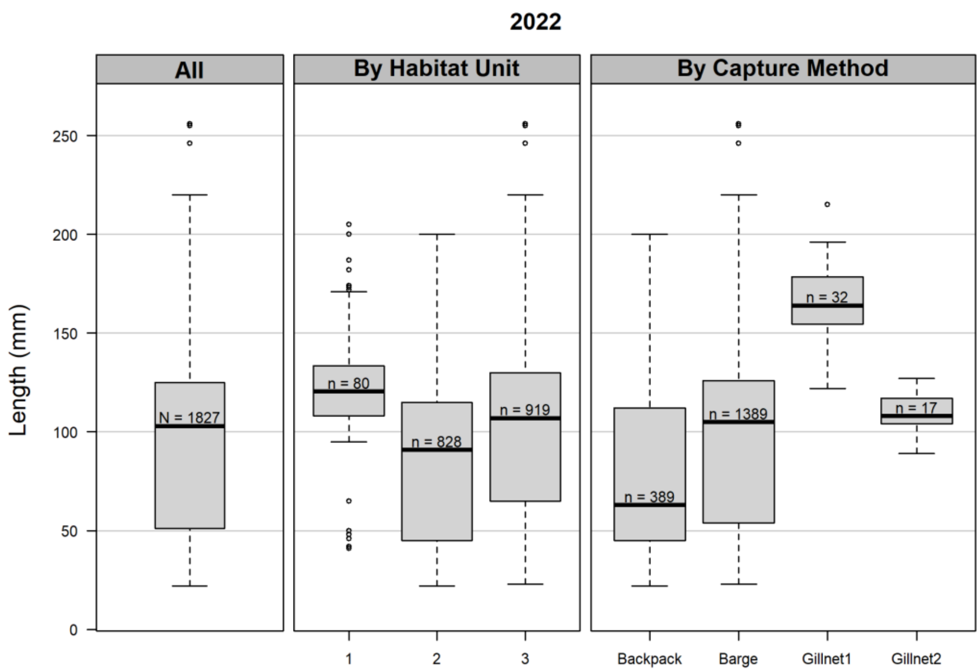


Figure 3. Boxplots of Brook Trout fork lengths from Tyee Springs removal efforts in 2022, all habitat and capture methods combined (left plot), by habitat unit (middle plot) and by capture method (right plot). Boxes indicate the interquartile ranges (25% – 75%), whiskers indicate the total range (sans outliers), and circles indicate outliers (observations greater than 1.5 times the interquartile range).

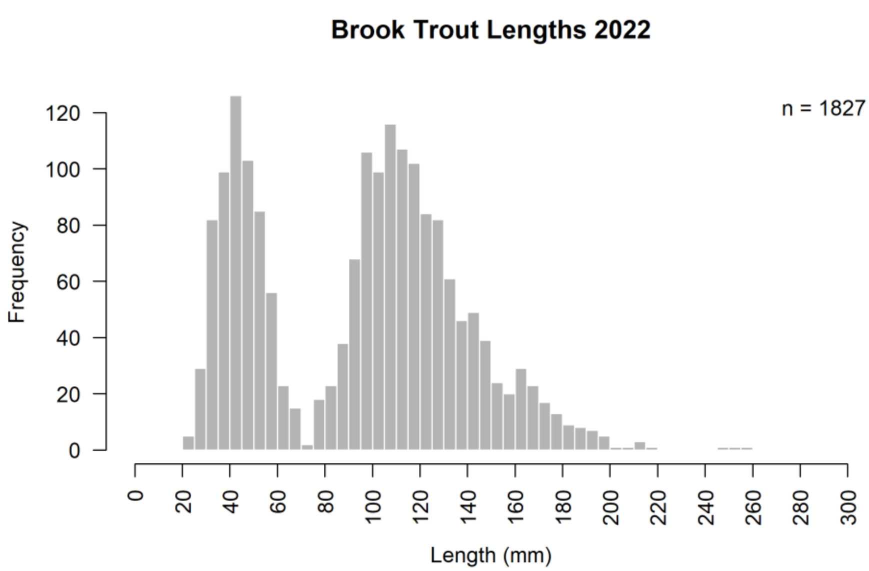


Figure 4. Length frequency histogram of all Brook Trout removed from Tyee Springs (n = 1827) in 2022 from June 06 to August 03.

2023

Brook Trout removal efforts in 2023 occurred over ten days during June and July. A total of 1,405 Brook Trout were removed during this sampling period. The majority of Brook Trout (93%) were captured with the electrofishing tote barge, which was the most effective capture method in all habitat units (Table 2). The backpack electrofisher was used less frequently in 2023, as the tote barge has proven to be more effective.

Twenty-four PIT tagged M_{YY} Brook Trout were captured and released during the 2023 removal period. All M_{YY} fish were captured using the tote barge and originated from the previous year's 2022 stocking event. Twelve M_{YY} fish were recaptured a single time, nine were recaptured twice, and three were recaptured three times.

Table 2. Number of Brook Trout removed by date, habitat unit and capture method in 2023. TB = tote barge (both units combined); EF = backpack electrofisher (both units combined); GN = gillnets (both gillnets combined).

	Unit #1			**Unit #2**			**Unit #3**				
	EF	TB	**Sum**	EF	TB	**Sum**	EF	TB	Gillnet	**Sum**	Total
06/27/2023	0	29	**29**	7	40	**47**	27	0	0	**27**	103
06/28/2023	0	0	**0**	0	0	**0**	0	146	24	**170**	170
07/05/2023	0	0	**0**	0	52	**52**	0	53	0	**53**	105
07/06/2023	0	0	**0**	0	184	**184**	0	28	6	**34**	218
07/11/2023	0	20	**20**	17	73	**90**	0	0	0	**0**	110
07/12/2023	0	0	**0**	0	0	**0**	0	121	2	**123**	123
07/18/2023	0	0	**0**	0	158	**158**	0	0	0	**0**	158
07/19/2023	0	0	**0**	0	0	**0**	0	151	6	**157**	157
07/25/2023	0	0	**0**	0	127	**127**	0	0	0	**0**	127
07/26/2023	0	0	**0**	0	0	**0**	0	125	9	**134**	134
Total	0	49	**49**	24	634	**658**	27	624	47	**698**	**1405**

The median fork length of Brook Trout captured in 2023 was 106 mm, ranging from 19 to 242 mm (Figure 5; Figure 6). The effect of capture method and habitat unit on fish length were similar to results observed in previous years (see 2022 above).

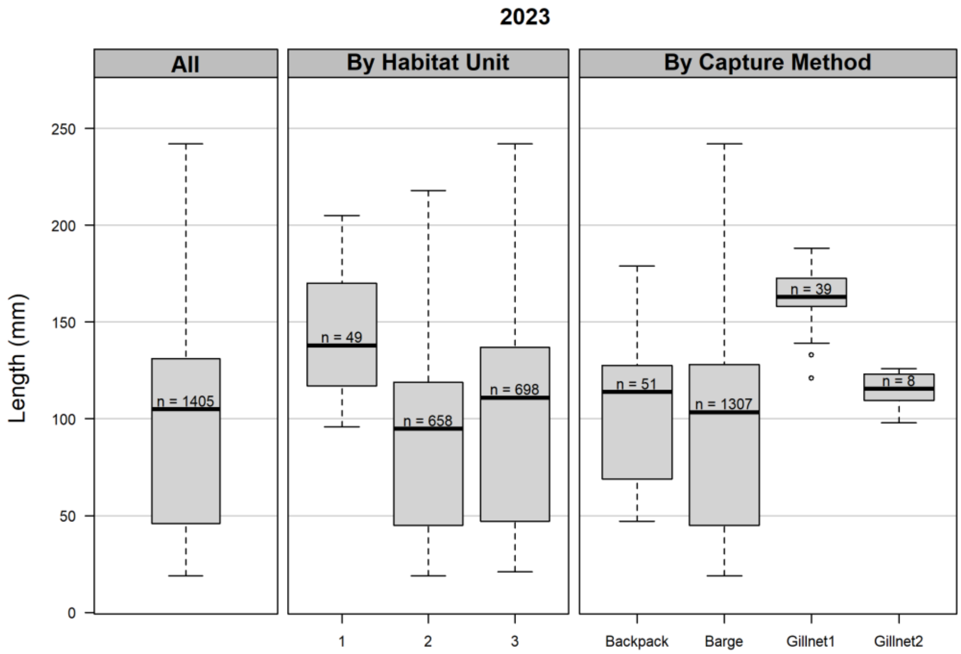


Figure 5. Boxplots of Brook Trout fork lengths from Tyee Springs removal efforts in 2023, all habitat and capture methods combined (left plot), by habitat unit (middle plot) and by capture method (right plot). Boxes indicate the interquartile ranges (25% – 75%), whiskers indicate the total range (sans outliers), and circles indicate outliers (observations greater than 1.5 times the interquartile range).

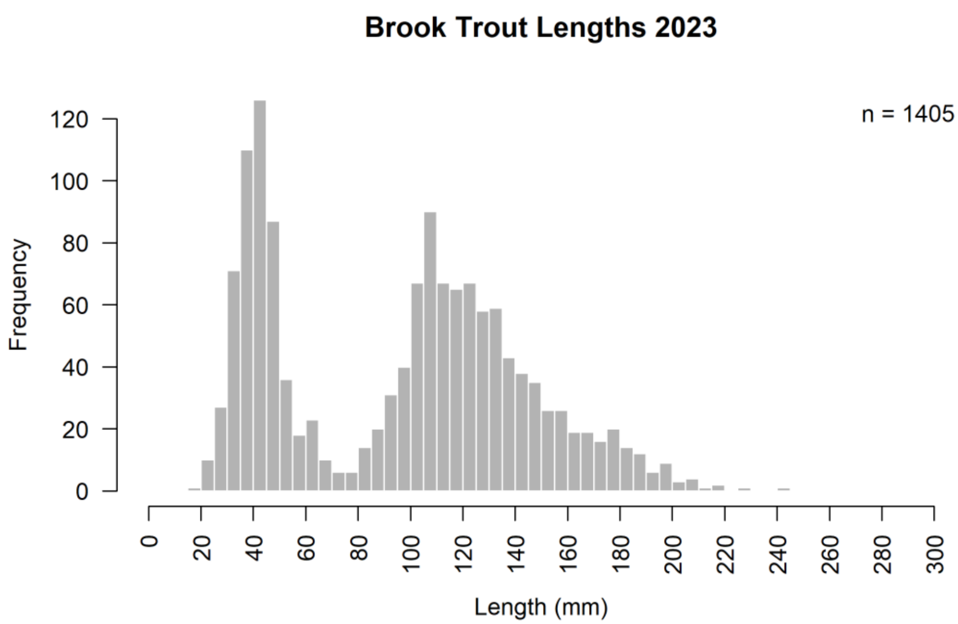


Figure 6. Length frequency histogram of all Brook Trout removed from Tyee Springs (n = 1,405) in 2023 from June 27 to July 26.

M_{YY} Stocking

2022

A total of 1,992 M_{YY} Brook Trout were stocked in Tyee Springs Unit #3 on 08/09/2022. All individuals were both adipose clipped and PIT tagged and average length in millimeters was 138 ± 16.6 (mean ± SD) at the time of tagging (Figure 7). We chose to stock all Brook Trout into the Unit #3 headwaters in 2022 after observing high post-stocking mortality among fish groups stocked lower in the system in previous years (Davis and Poirier 2023).

2023

A total of 2,002 M_{YY} Brook Trout were stocked in Tyee Springs Unit #3 on 08/08/2023. All individuals were both adipose clipped and PIT tagged and average length in millimeters was 124 ± 20 (median ± IQR) at the time of tagging (Figure 7).

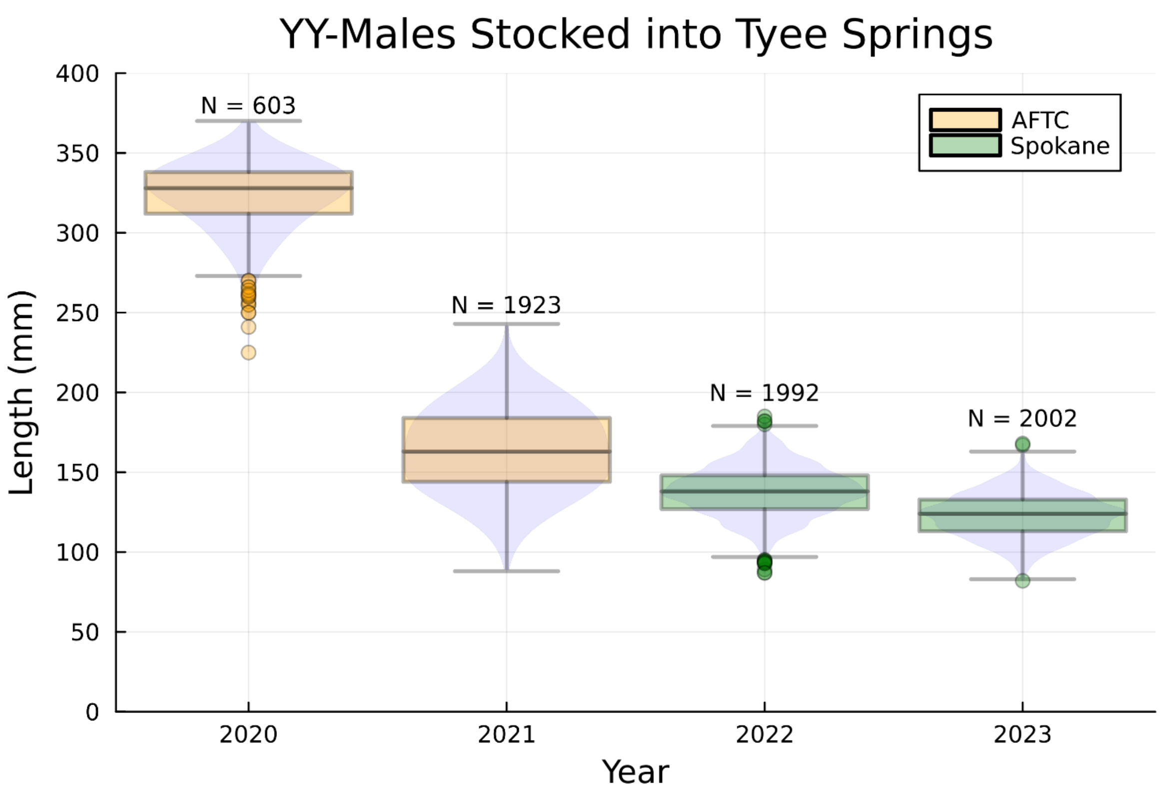


Figure 7. Boxplot of fork lengths of M_{YY} fish stocked in Tyee Springs 2020-2023. M_{YY} fish stocked in 2020 and 2021 were reared at Abernathy FTC and fish stocked in 2022 and 2023 were reared at Spokane State Hatchery. Boxes indicate the interquartile ranges (IQR) (25% – 75%), whiskers indicate the total range and circles indicate outliers (observations greater than 1.5 times the interquartile range).

Monitoring

Post-stocking mortalities

In 2022, Carson NFH staff collected a total of 48 M_{YY} Brook Trout mortalities (2.4%) and in 2023 hatchery staff collected approximately 74 M_{YY} mortalities (3.7%). All mortalities were recovered within two days of stocking at the hatchery intake grate (Unit #1). Fork lengths of mortalities were representative of the entire stocked population.

Additional post-stocking mortality likely occurred in 2022 and 2023, but due to predation, scavenging, location and/or timing, the fish were never recovered and the total number of mortalities is unknown (Davis and Poirier 2023).

Brook Trout Capture Efficiency

In general, Brook Trout capture efficiency has decreased annually since the project's start in 2020 (Figure 8). Though, there were a few outliers of increased CPUE, these events typically occurred during the first week of fish removals when abundance was higher (compared to subsequent days) and fish were not yet wary of the sample gear. Overall, tote barge B1 was the most efficient capture method used in Tyee Springs (Figure 9), but in 2023, we discontinued its use in Units #2 and #3 due to its older design and safety concerns. Tote barge B1 is still used exclusively in Unit #1 as we net fish and operate the barge from the shoreline, so there are no safety concerns. The backpack electrofisher was the least efficient method for capturing Brook Trout in Tyee Springs (Figure 9). Despite its lowered efficiency, we found it to be a valuable tool to sample areas that were too difficult for the tote barge to access such as small side channels, narrow inlets and shallow pools.

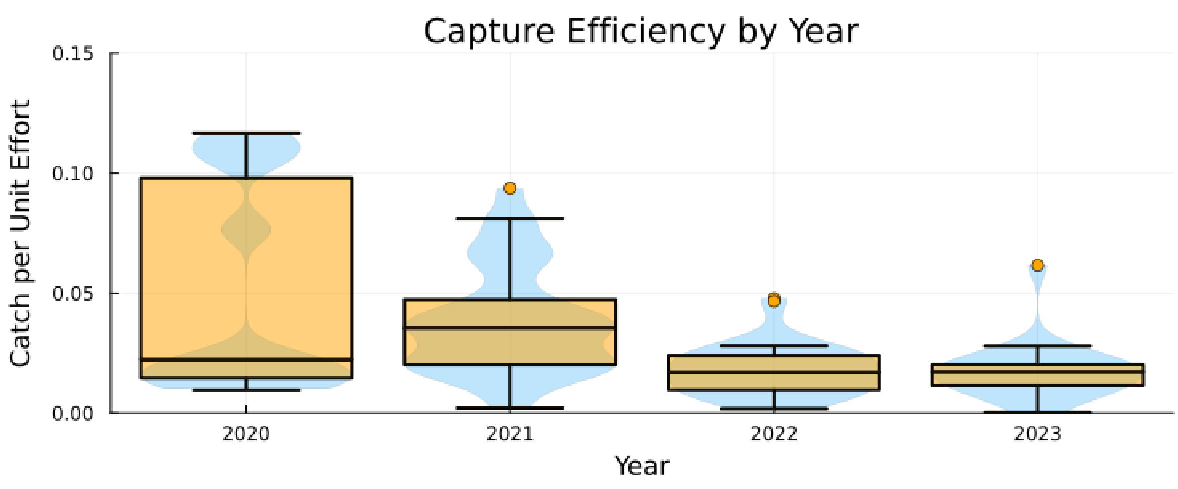


Figure 8. Boxplot of Brook Trout Catch per Unit Effort for years 2020-2024, all capture methods combined. Boxes indicate the interquartile ranges (25% – 75%), whiskers indicate the total range, circles indicate outliers (observations greater than 1.5 times the interquartile range) and blue shaded area

represents the data distribution.

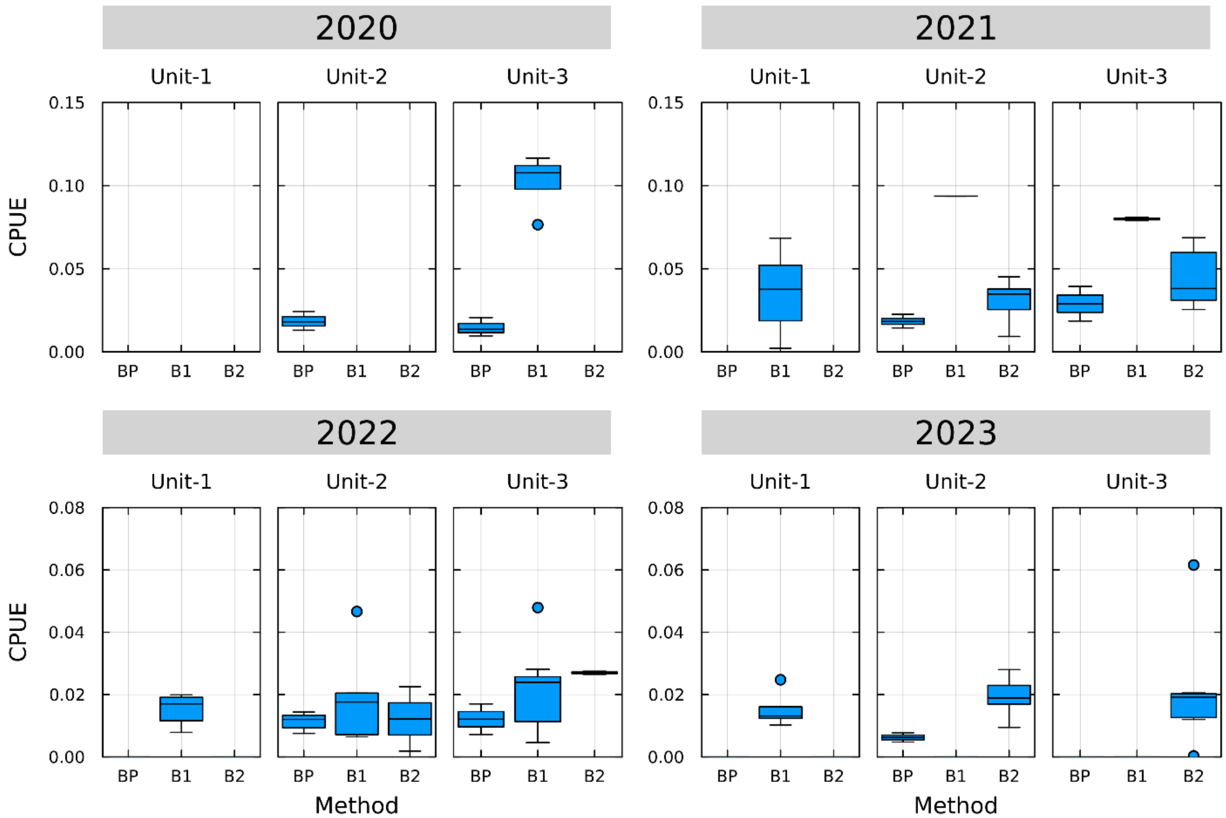


Figure 9. Boxplots of Brook Trout Catch Per Unit Effort by fishing method and habitat unit for years 2020 (upper left), 2021 (upper right), 2022 (lower left) and 2023 (lower right).Boxes indicate the interquartile ranges (25% – 75%), whiskers indicate the total range and circles indicate outliers (observations greater than 1.5 times the interquartile range). BP = Back Pack E-fisher, B1 = Barge1 and B2 = Barge2.

Fecundity

The average fecundity estimate in 2023 was 708 ± 284 (mean ± SD, n = 76) eggs per mature female Brook Trout. The average fork length of sampled females was 169 mm ± 24 mm (mean ± SD, n = 76). We found that fish length positively affected fecundity with an r^2 value of 0.43 (Figure. 10). We estimated the Negative-binomial intercept parameter α to be 4.508, the slope coefficient 0.012 and the dispersion parameter β 2.696 (Table 3). Given the model, we would assume Tyee Springs females at 169 mm (the mean length of our data) to have a mean fecundity value of 680 eggs with 95% credible intervals of [640.55, 722.16] (Figure 10).

Table 3. Fecundity (Negative-binomial) model output. Mean values in first column are parameter estimates for the Brook Trout fecundity model. These parameters along with length (mm) values (x_i) can be used to estimate fecundity (e.g., when $x = 140,\ e^{a+mx} \approx 486.87$).

	Mean	SD	Naïve SE	Time-series SE	2.5%	25%	50%	75%	97.5%
a	4.508	0.221	2.13e-04	7.89e-04	4.072	4.360	4.509	4.657	4.941
m	0.012	0.001	1.30e-06	4.64e-06	0.009	0.011	0.012	0.013	0.014
β	2.696	0.168	1.68e-04	5.92e-04	2.354	2.586	2.701	2.811	3.014

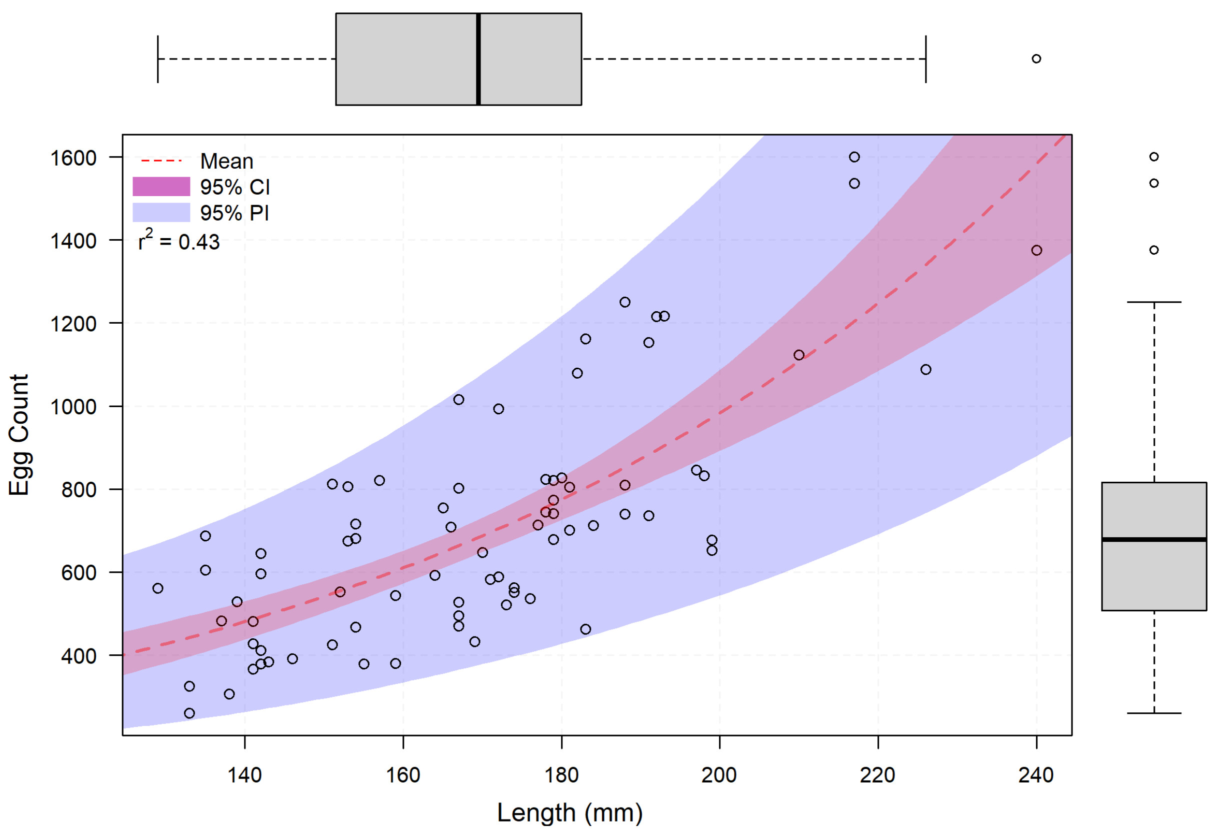


Figure 10. Negative-Binomial model of predicted mean egg count (dashed red line) as a function of Brook Trout fork length.Pink band indicates 95% credible intervals and purple band indicates 95% prediction intervals. n = 76.

Genetic Assignment Tests

Overall, 218 of 240 autosomal loci (90.8%) were retained after application of missing data filters. Eighteen loci were discarded because they exceeded the 20% missing data threshold, and four additional loci were removed because they were fixed for a single allele in all samples. Nearly all individuals

(2,275 of 2,313; 98.4%) were retained for analysis. Our filtering parameters resulted in a final dataset with low missing data per individual (Mean = 1.9%; Median = 1.4%; Standard Deviation = 2.2%).

Principal component 1 (PC1; Figure 11), which explained 20.71% of overall variance, separated Tyee Springs Brook Trout from all M_{YY} cohorts. Genetic differentiation among M_{YY} cohorts was evident on PC2, which explained 3.4% of overall variance. Genetic differentiation between Tyee Springs and the M_{YY} Brook Trout (mean F_{ST} = 0.213; SD = 0.012) was greater than the differentiation among M_{YY} cohorts (mean F_{ST} = 0.037; SD = 0.028). Together, these analyses indicated that sufficient genetic differentiation existed between the hatchery-origin M_{YY} Brook Trout and wild-origin Tyee Springs Brook Trout to accurately identify offspring of these two groups through hybrid analysis.

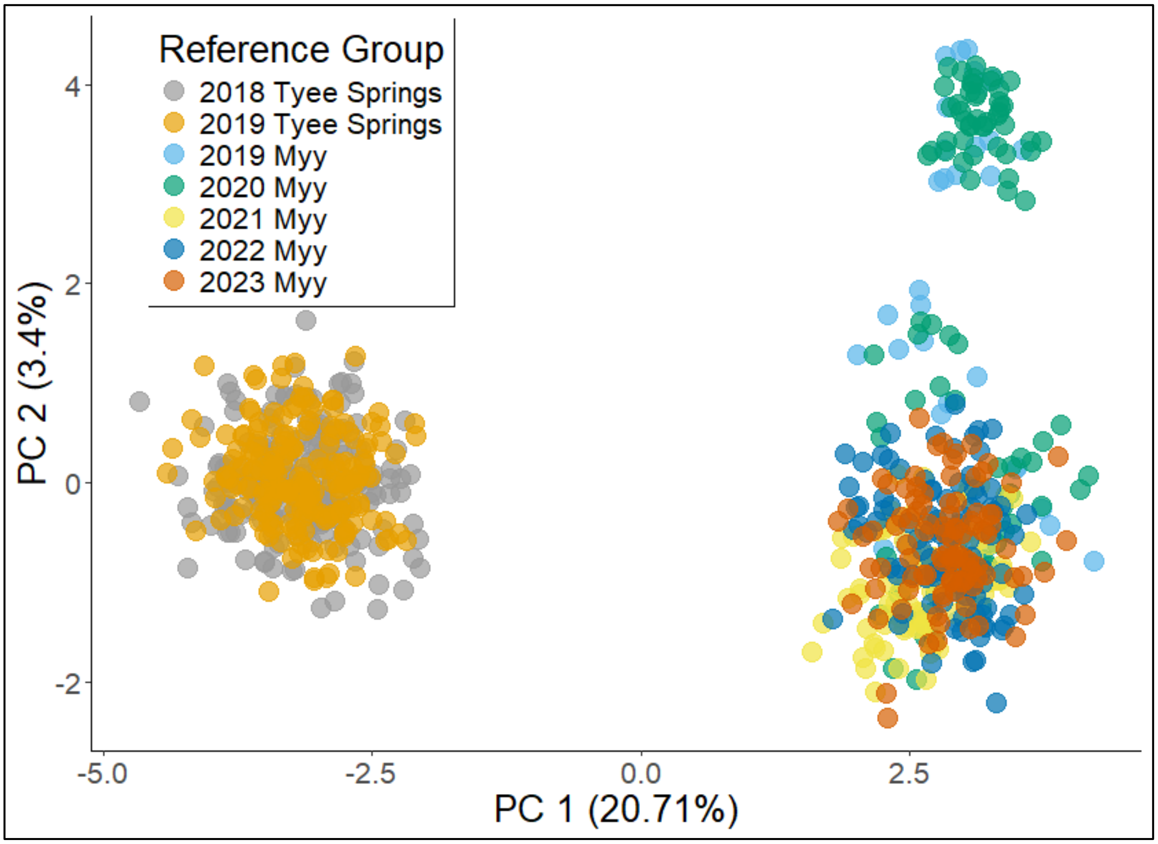


Figure 11. Principal components analysis (PCA) plot of M_{YY} Brook Trout hatchery cohorts (2019 through 2023) compared to pre-introduction wild-origin reference data for Tyee Springs (2018 through 2019).Axis labels indicate the percent variance explained by each principal component (PC).

YY male offspring (F1 hybrids) were first detected among the 2021 young-of-year cohort (N = 1; 0.28%). They were detected again in 2022 (N = 80; 21.11%) and 2023 (N = 40; 9.98%) (Table 4; Figure 12). F1 hybrids were detected only among age1+ fish in 2022 (N = 1; 0.91%) and 2023 (N = 13; 13.00%). All F1 hybrids were assigned with high confidence (Bayesian posterior probability ≥ 0.991).

Table 4. The number of Brook Trout hybrids (progeny of M_{YY}) detected from tissue samples collected in Tyee Springs 2021-2023. All Brook Trout from Tyee Springs are further subdivided by age class (YOY = young of year; Age 1+ = all fish >1 year of age). 'Tissue Samples' indicate the number of individuals from which genetic samples were taken per year, excluding genotypes that did not pass data quality standards.

	2021		**2022**		**2023**	
Life stage	YOY	Age 1+	YOY	Age 1+	YOY	Age 1+
Tissue Samples	361	101	379	106	401	100
F1 Hybrids	1	0	80	1	40	13
% F1	**0.28**	**0**	**21.11**	**0.94**	**9.98**	**13.00**

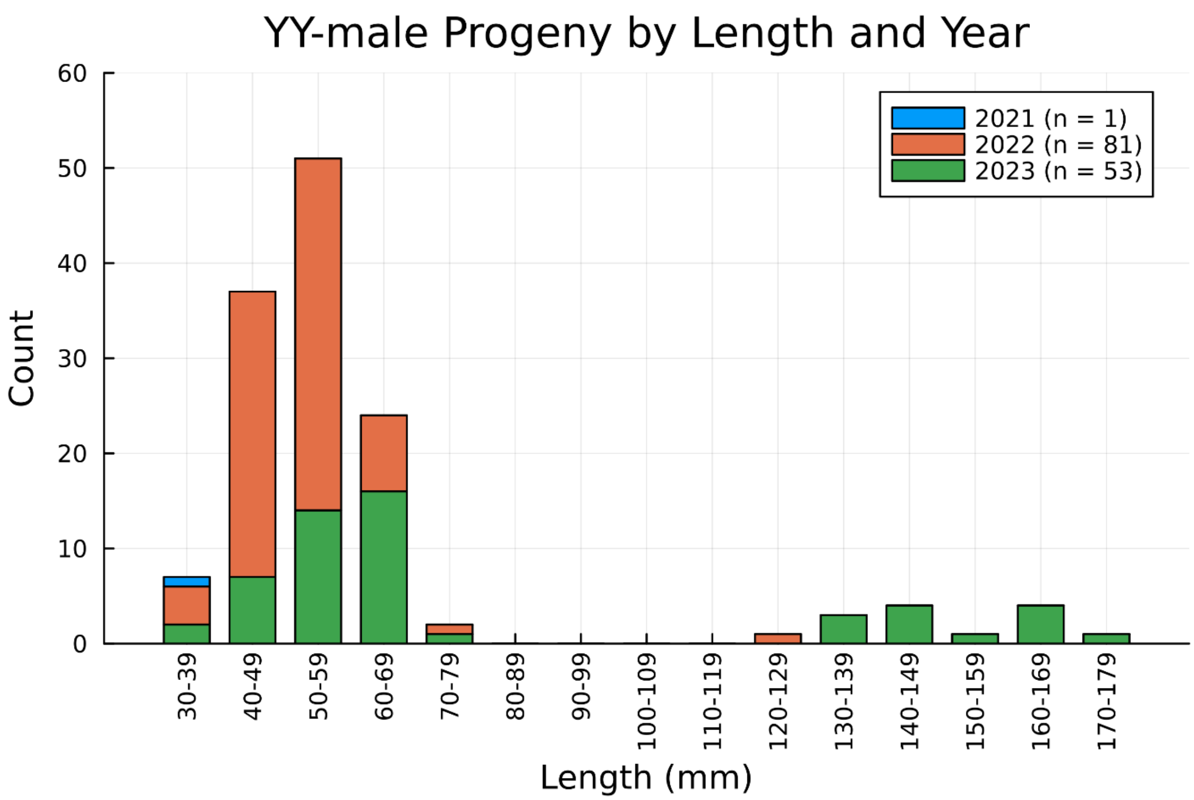


Figure 12. Barplot of Tyee Springs young-of-year tissue samples identified as M_{YY} progeny out of 1,139 samples.Bars are color coded to indicate the year the genetic samples were taken.

Sex Ratio

Sex ratios of Brook Trout observed during fish removal efforts are slowly shifting to male. In 2021, 59% of all Brook Trout (>100mm) sampled for phenotypic sex (n=384) were female (Figure 13). In 2022, 43% were observed to be female (n = 682). In 2023, 40% of sampled fish (n=770) were female and 60%

were male (Figure 13). The shift in sex ratio observed in 2022 can be attributed to one-year-old offspring from the 2020 M_{YY} stocking. 2022 was the first year we had the potential to observe a sex ratio shift since progeny from the first 2020 stocking event would be too small and immature in 2021. For this reason, we also evaluated the sex ratio of Brook Trout believed to be one-year-old fish based on length (100-150 mm) as these fish represent the progeny of cohorts stocked two years prior (Figure 13).

In addition to genetic assignment tests, Abernathy FTC also conducted sex genotyping of genetic samples. The genetic results were in 96.7% concurrence with field observations (n = 307). Ten samples (3.3%) that were classified as male in the field, were female based on genetic analysis. These samples were collected over a period of two days (07/13/2022, n = 5 and 06/28/2023, n = 5) and were likely misidentified due to sampler error. Four of these errors occurred for fish less than 100 mm in length. While developing eggs can still be observed for Brook Trout at this life stage, we have found it more difficult. As a result, we have adjusted our sampling protocol and only select fish 100 mm or greater for visual sex identification. It is important to note that sex ratio sampling error will always be biased to more males than females. This is because determining sex in the field is driven by observing either the presence or absence of eggs.

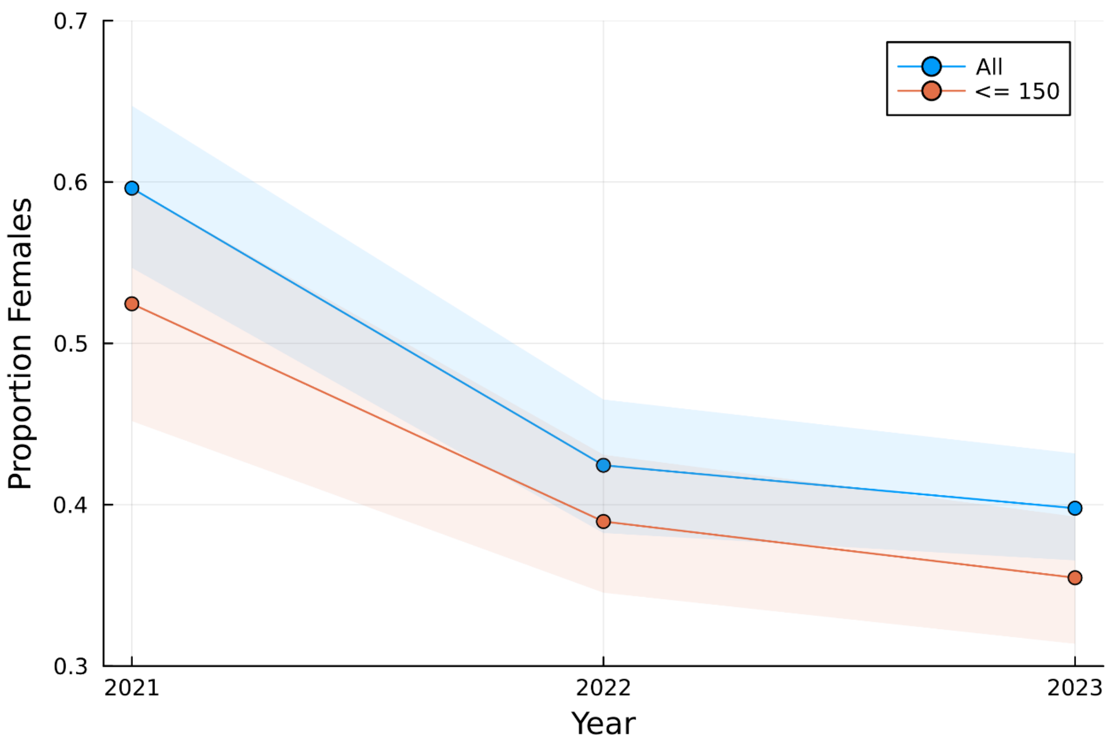


Figure 13. Proportion of females captured during fish removals in Tyee Springs, 2021-2023. The blue line represents all females greater than 100mm in length and the red line represents females greater than 100mm but less than 150mm in length.

Discussion

Multiple sources of evidence suggest that M_{YY} stocked into Tyee Springs have successfully spawned with resident females and sex ratios of the resident population are shifting towards more males. Genetic assignment tests conducted by Abernathy FTC identified conclusive evidence of M_{YY} offspring in Tyee Springs (Figure 12). M_{YY} stocking efforts began in 2020 and a single M_{YY}-resident hybrid was detected in 2021 among the 362 young-of-year samples that were analyzed. A second one-year-old sized Brook Trout was found to be an M_{YY} hybrid in 2022, suggesting it was also progeny from the 2020 stocked cohort (Figure 12). While these numbers are low, only 600 M_{YY} Brook Trout were stocked in 2020. The number of M_{YY} offspring detected in 2022 (n=81) and 2023 (n=53) was much larger and can likely be attributed to higher stocking levels (Figure 7). These results confirm that an unknown number of M_{YY} stocked into Tyee Springs have spawned successfully each year and produced viable offspring. It is important to note that the number of M_{YY} offspring is certainly higher since we only collect tissue samples from 400 young-of-year Brook Trout each year.

Annual sex ratio estimates from field observations also show evidence that the resident brook trout population is slowly skewing to male (Figure 13). In 2022, we determined the phenotypic sex of 684 randomly selected brook trout ranging in size from 53 to 256 mm. Of the total, 43% were female. In 2023, 770 fish ranging in size from 58 to 242 mm were examined and 40% were female (Figure 13). These results suggest that the male progeny of stocked M_{YY} brook trout is skewing the sex ratio towards more males each year. Genetic sex assignment tests performed by Abernathy FTC revealed that the most visual sex identifications conducted in the field (96.6%) agreed with genetic results.

Catch-per-unit-effort (CPUE) is assumed to be proportional to stock density and a good indicator to changes in species abundance (Murphy and Willis, 1996). Brook Trout CPUE has gone down each year of the project (Figure 8), suggesting that M_{YY} stocking and fish removal efforts are reducing the resident population. Field crews have removed approximately 6,520 Brook Trout from Tyee Springs since the start of the project. Fish removal efforts are showing positive results, with both the number of fish captured and the total number of days spent on removal efforts decreasing each year. For example, in 2021, we removed 2,314 Brook Trout in fourteen field days. In 2022, we removed 1,827 fish in twelve days and in 2023 we removed 1,405 fish in ten days. It has proven necessary to shorten the field season by two days each year to compensate for decreasing fish abundance. We anticipate this pattern will continue as the population continues to decline and fish become more dispersed, making it even harder to target and remove them effectively.

Overwinter survival of M_{YY} increased significantly in 2023. A total of 24 (1.2%) M_{YY} stocked in 2022 were recovered during fish removal efforts in 2023. It is unclear why survival in 2022/23 was so much higher than in previous stocking years (e.g., five fish in 2021, four fish in 2022), but it may be due to using an improved acclimation strategy. Beginning in 2022 we utilized a slow stepwise water mixing strategy prior to stocking. In brief, approximately one quarter of the water in the fish transport tank was removed and replaced with water from Tyee Springs. Fish were allowed to acclimate for approximately twenty minutes before repeating the process again. A total of five water exchanges were performed before stocking all fish at the headwaters of Tyee Springs in Unit #3. Though we still observed some post stocking mortality (2.4% of M_{YY} in 2022 and 3.7% in 2023), these levels were lower than those observed in 2020 (12.1%) and 2021 (5.2%) using a different acclimation strategy (Davis and Poirier 2023). We are hopeful this increased trend in M_{YY} overwinter survival will continue, eventually allowing us to estimate growth and recapture probabilities of M_{YY} using fish recaptured during annual fish removals.

Several M_{YY} have been recaptured multiple times in one season (e.g., nine fish were recaptured twice and three fish were recaptured three times in 2023), though we have yet to recover an M_{YY} fish two or more years after stocking. In contrast, 19 resident Brook Trout that were PIT tagged during the feasibility study in 2018 and 2019 were recaptured during fish removal efforts two years later (30% of all feasibility study recaptures) and a single resident Brook Trout PIT tagged in 2019 was recovered in 2022 over three years later. These data indicate that resident Brook Trout have higher overwinter and long-term survival compared to hatchery M_{YY} in Tyee Springs. However, we are skeptical of the single resident fish recovered three years after tagging. This fish was supposedly tagged on 05/21/2019 with a fork length of 99 mm and was recaptured on 07/06/2022 at 201 mm in length, suggesting this fish was approximately four years old and grew only 100 mm over three years. While a slow growth rate is common in Brook Trout populations inhabiting cold headwater streams (Xu et al. 2010; Letcher et al. 2022), there are limited records of stream dwelling populations surviving beyond three to four years of age (Ficke et al. 2009; Kennedy et al. 2003). We also found that a growth rate of 100 mm over three years does not concur with growth rate data from similar sized Brook Trout in Tyee Springs. It is possible that this fish survived to four years of age, but no other resident Brook Trout from the feasibility study were recaptured three years after tagging. There is also a small chance that this fish could have ingested a PIT tag shed by another fish, but this phenomenon has not been observed directly for Brook Trout to our knowledge (Hemeon et al. 2024; Peterson and Engle 2021).

There is limited information about female Brook Trout fecundity in the published literature (Ficke et al. 2009; Kennedy et al. 2003). Although our sample size was relatively small (n=76 females), we found that average female fecundity in Tyee Springs (708 eggs per female; Figure 10) was generally higher than that observed for native and nonnative Brook Trout populations in other streams (Davenport 2018; Ficke et al. 2009; Meyer et al. 2006). The factors influencing increased fecundity rates in Tyee Springs are unclear but may be associated with consistent low water temperatures (~6.6°C) leading to slower growth and later maturity or increased resource availability (e.g., food availability, spawning habitat) related to lower Brook Trout densities. Understanding Tyee Springs Brook Trout fecundity rates and their inherent variability will help to further refine the Tyee Springs population model used to estimate the number of years until eradication is expected to occur. This information might also be useful for other modeling efforts as Brook Trout fecundity information for populations west of the Rocky Mountains is sparse.

Given the overall progression of the project (e.g., genetic detection of M_{YY} offspring, male dominant sex ratio, fewer resident Brook Trout in Tyee Springs) we are satisfied with our current Brook Trout removal and M_{YY} stocking efforts. However, based the total number of fish removed from Tyee Springs over the last two years and what we have recently learned about M_{YY} survival and female fecundity, we would like to re-estimate the abundance of the resident Brook Trout population and run the population simulation model again to see if our eradication timeline has changed based on our work so far. At last calculation (2021), we estimated eradication of Brook Trout in Tyee Springs would potentially occur in 2033 (Davis and Poirier 2023). It is likely this estimate has changed given current removal efforts and improved M_{YY} survival. We will continue to implement an adaptive management approach by using annual fish removal of M_{YY} and resident Brook Trout demographic data to improve the population model and attain a more accurate estimate of when complete eradication of Brook Trout is likely to occur at Tyee Springs.

The use of MYY Brook Trout as a biological control strategy remains relatively new. Evaluation of the technique's effectiveness (or potential shortcomings) is important to inform the science and refine field implementation methods for both current and future applications. If the eradication of Brook Trout is successful in Tyee Springs, the Service will save money by eliminating the cost of maintaining Brook Trout exclusion screens at Carson NHF. Successful eradication will also remove the threat of inadvertently releasing Brook Trout into the Wind River and will eliminate a vector for BKD transmission to Chinook salmon in Carson NFH. This proof-of-concept evaluation can also help the Service and its partners determine if and how the MYY technique can be used to control nuisance Brook Trout populations in other locations and set the stage for evaluating whether the approach may be useful with other invasive fish species, like common carp, which have proven very difficult to control in places like Malheur National Wildlife Refuge.

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Appendix

Tyee Springs habitat Unit #1: hatchery intake channel (left), habitat Unit #2: free flowing stream (middle) and habitat Unit #3: headwaters of Tyee Springs (right).

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