

CAN MIGRATORY SMOLTS PRODUCED BY THE RESIDENT POPULATION OF
SASHIN LAKE BE USED AS A RESERVOIR TO BREED MIGRANTS
AND CONSERVE CREEK POPULATIONS?

by

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Submitted in partial fulfillment of the
requirements for Departmental Honors in
the Department of Biology
Texas Christian University
Fort Worth, Texas

May 2, 2022

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ABSTRACT

Migration can be broadly defined as the seasonal relocation of an animal to regions where conditions are alternately favorable or unfavorable, followed by a return to their place of origin. Migration as a phenomenon presents itself in various ways across a wide range of species, including the rainbow trout (*Oncorhynchus mykiss*). *O. mykiss* exhibit partial migratory behavior, in which some individuals in the population will choose to migrate, while others do not. As such, there are two varieties of *O. mykiss*: the non-migratory rainbow trout (also known as residents) and the migratory steelhead (also known as migrants). Anthropogenic effects have been adversely affecting *O. mykiss*, with more severe effects on the migratory steelhead populations. Therefore, conservation efforts have turned to residential rainbow trout populations to determine whether they could act as a reservoir to breed and replenish dwindling steelhead populations. The site for this study was the Sashin system, and although previous research has found that Sashin lake is able to produce migratory individuals, it had not yet been tested whether those individuals were able to complete their marine migrations and return to Sashin creek to spawn. Therefore, the primary objective of this study was to genotype returning steelhead samples to determine whether they originated from Sashin lake or Sashin stream, with the aim of identifying returning steelheads that had originated from the lake. DMAS-qPCR techniques were used to genotype fifteen samples at 8 previously identified loci containing SNPs between migrant and resident individuals. The results indicate that the steelhead produced by Sashin lake are able to successfully complete their marine migrations, and therefore could be used as a reservoir for conservation of the anadromous population.

Introduction

Migration can be broadly defined as the seasonal relocation of an animal to regions where conditions are alternately favorable or unfavorable, followed by a return to their place of origin (Dingle and Drake 2007). Migratory behavior has evolved in a wide variety of animal taxa, and in most cases is a strategy to maximize fitness in seasonal environments (Alerstam, Hedenstrom & Akesson 2003). Several factors, such as environmental conditions and genetics of the individual, can contribute to migratory behavior. Quantitative genetics analyses in various insect, bird, and fish species have shown that a significant portion of the phenotypic variance in migratory traits have a genetic basis, suggesting the trait is passed on from one generation to the next (Liedvogel et al. 2011). Although in some species, the ability and therefore propensity to migrate is determined by a single locus, this is incredibly rare (Dingle 1991). More often, the genetic component as to whether an individual within a population exhibits migratory behavior is polygenic and the relative contribution of each gene to the propensity to migrate is small (Dingle 1991). In many freshwater-resident fish populations, it has been demonstrated that the occurrence of migratory behavior has at least a partial genetic basis, and that the genetic “trigger” is likely influenced by both environmental and developmental factors, such as access to resources and progression into a new life cycle phase (Lucas and Baras 2001).

While many species are obligate migrants, some species exhibit partial migratory behavior, in which some members of a population will migrate to increase fitness, whereas others stay in their natal areas (Quinn and Myers 2004). *Oncorhynchus mykiss* is one such species that exhibits partial migratory behavior, wherein anadromous individuals - known as steelhead - undergo marine migrations, while resident individuals - known as rainbow trout - carry out their life

cycles in fresh water (Hendry et al. 2004). Juvenile *O. mykiss* that are to migrate undergo a process called smoltification, in which they adopt a silvery color and more streamlined shape in preparation for marine migration, as well as increased salinity tolerance and metabolism (Björnsson et al. 2011). Once they have undergone smoltification, they can then migrate out to the ocean to feed, and then return to their natal stream to breed (Hendry et al. 2004).

There are costs and benefits to both residential and anadromous life histories, the balance of which varies between different populations (Pavlov et al. 2008). Anadromous fish that migrate to the ocean may benefit from increased access to rich feeding grounds, allowing for them to be larger when they reach maturation and therefore greater fecundity (Kendall et al. 2014).

However, mortality rates of *O. mykiss* in the marine phase often exceed 90% due to the many obstacles faced during ocean migration, such as increased predation, which represents a great cost to migratory individuals (Thrower et al. 2004). In this way, resident populations benefit from an increased survival rate, and are more likely to have the opportunity to reproduce multiple times (Fleming and Reynolds 2004). However, since residents cannot take advantage of marine resources, they are smaller in size upon maturation and thus suffer from lower fecundity (Fleming and Reynolds 2004).

The environment can also influence which life history is more beneficial. Anthropogenic effects such as habitat loss, river fragmentation, and climate change have all been shown to adversely affect migratory salmonids (McClure et al. 2008; Keefer et al. 2008; Wade et al. 2013). For example, poor ocean conditions or increased difficulty of migration should decrease the benefits of anadromy (Hendry et al. 2004), while increased freshwater productivity (which could be a

result of climate change) benefits resident populations (Finstad and Hein 2012). This link with the environment makes climate change especially concerning. Many *O. mykiss* steelhead populations are declining because higher temperatures allow for increased productivity of natal streams. As natal streams approach optimal productivity, the fitness benefits an individual gains from maturing as a resident outweighs the risk of mortality that comes with marine migration (Benjamin et al. 2013). *O. mykiss* are designated as threatened in ten systems along the West Coast and endangered in one (National Oceanic and Atmospheric Administration, 2016). Whereas, resident rainbow trout are not experiencing the same level of decline, likely due to the fact that anthropogenic effects have less of an impact on their life history (Blair et al. 2013). Therefore, conservation efforts have turned to resident rainbow trout to see if they could be utilized as a reservoir to breed and replenish dwindling migrant populations (Thrower et al. 2004).

The Sashin system, located on Barnoff Island in Southeast Alaska, is an established study site for *O. mykiss*, and it is advantageous for use in this project for several reasons. The first of which is that Sashin lake and Sashin stream are separated by two barrier waterfalls (Thrower et al. 2004, Figure 1). This means that while juvenile lake trout can undergo smoltification and leave the lake by descending the two waterfalls, they are unable to return. Therefore, these individuals join the anadromous stream population, potentially replenishing its dwindling numbers. The Sashin system is also advantageous because there is a weir located at the junction of where Sashin stream lets out into the ocean (Thrower et al. 2004). This allows for researchers to sample out-migrating and returning anadromous fish as they are shunted through the weir (Thrower et al. 2004).

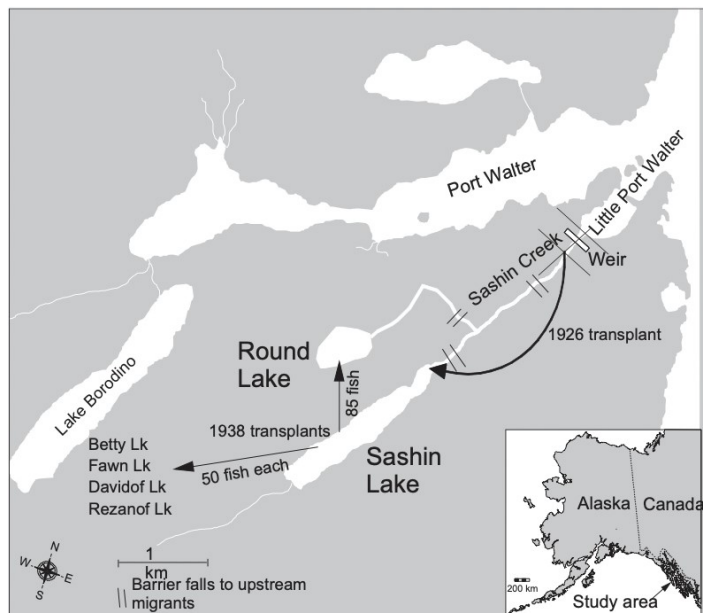


Figure 1: Map of the Sashin System, including Sashin Lake and Sashin Creek, as well as the locations of the barrier waterfalls and the weir (Thrower et al. 2004).

Recent data from the Hale Lab have confirmed that resident trout from Sashin Lake can produce migratory trout (Barfuss 2021), so now the question I aim to answer is whether the migratory trout produced by the lake can then return to the stream to breed following marine migration. The ecological implications of this would be to determine if resident trout could be used as a reservoir to breed migrant trout in struggling stream populations.

Methods

In order to answer the question of whether smolts produced by the resident population are returning to the stream, I genotyped samples from fifteen returning steelhead sampled in the year 2015. DNA was extracted from fin clips using Qiagen's DNeasy kit according to the manufacturer's protocol. Nanodrop was used to quantify the DNA and samples were then diluted to a standard concentration of 50.0 ng/ μ L for assay use.

DMAS-qPCR

Based on previously identified SNPs differentiating between adult fish in Sashin lake and Sashin stream in eight loci, double-mismatch allele-specific qPCR (DMAS-qPCR) techniques, as described by Lefever et al. (2019), were used to genotype the samples at each of the eight loci. DMAS-qPCR involves a common reverse primer and two forward primers, each specific to one of the SNP alleles: migrant or resident. The forward primers were designed so that the 3' end overlapped the SNP, and the nucleotide three base pairs prior to the 3' end of the primer was changed to a mismatch to allow for more clear discrimination between genotypes (Barfuss 2021). Therefore, the forward primer containing the correct SNP should be able to anneal to the sample DNA better than the other primer. SYBR Green reagent was used to detect fluorescence, and the better the primer annealed, the better the DNA would amplify in fewer cycles, and it would pass the fluorescence threshold quicker. Separate qPCR reactions were performed for each sample: one reaction using the "stream" forward primer, and the other using the "lake" forward primer. Therefore, when amplified using qPCR, the number of cycles required for the DNA sample in that reaction to pass a fluorescence threshold (C_q) can be compared between the two primer versions, and subsequently used to genotype the sample at that specific locus. When comparing the average C_q 's between the two reactions, homozygous individuals have a much lower average C_q for the SNP associated with their genotype, while heterozygous individuals have similar average C_q values for each of the primers. C_q values were set for each locus separately based on values measured from assays of samples with a known genotype (Barfuss 2021).

qPCR of samples was performed on an Applied Biosystems StepOnePlus Real-Time PCR System using Applied Biosystems SYBR Select reagents. qPCR assay mixtures were made with a DNA concentration of 5.0 ng/ μ L, primer concentration of 0.5 ng/ μ L, and totaled to a volume of 10 μ L. Assays were performed in triplicate, and the C_q was averaged between the triplicates. In the cases where the standard deviation was greater than 0.5 and there existed an obvious outlier in the triplicates, it was removed from analysis. If there was not an obvious outlier, genotyping for that sample was repeated. The average C_q's of the "stream" forward primer were then plotted against the C_q's of the "lake" forward primer (Figure 2). It was expected that the graph would produce three distinct clusters, one for each genotype: homozygotes for the "lake" allele, homozygotes for the "stream" allele, and heterozygotes.

A Chi-Square Test of Independence and Hardy-Weinberg Test of Equilibrium was performed for each of the 8 loci. Of the 8 loci, 6 were found to be in Hardy-Weinberg Equilibrium, with p-values for the 6 non-significant loci ranging from 0.072173 to 0.666942 (Table 1).

Next, the individuals were analyzed at each of the 8 loci to see if they agreed in order to categorize each fish. This was accomplished by taking the genotype at each locus for each sample, and then compiled them to see how many of the 8 loci agreed with each other within that sample (Figure 3). Individuals that were genotyped as stream homozygotes at 6 or more loci were assigned as originating from the stream, while individuals that were genotypes as lake homozygotes at 6 or more loci were assigned as originating from the lake. Individuals with fewer than 6 loci in agreement for either the lake or stream genotypes were designated as admixed.

Finally, the samples were sex-typed using *OmyY1*, which is a marker for the male-specific portion of the Y-chromosome (Brunelli et al. 2008). The sexing PCR protocol followed Brunelli et al. 2008, except annealing conditions were 60°C for 50 seconds. Then 3 µL of PCR product was run on a 1.0% agarose gel and stained with Gel Red for viewing under UV light. Sex was then assigned based on whether a band was produced, in which samples that produced a band were assigned as male and samples that had no band were assigned as females. This information was then used to categorize the returning steelhead based on sex in addition to how many of the loci within an individual agreed (Figure 4).

Results

Upon testing for Hardy-Weinberg equilibrium, all but two of the eight loci studied were found to be in equilibrium (Table 1). Meanwhile, in the two loci that had significant p-values and were outside of Hardy-Weinberg equilibrium, there was an observed a lack of heterozygosity.

Table 1: Allele frequencies and Hardy-Weinberg Test of Equilibrium statistics for SNPs used to genotype and categorize returning steelhead.

Locus	S/S	S/L	L/L	Observed Heterozygosity	Test for Significance
Chr1	6	5	4	0.333	not significant; p=0.213
Chr20	6	4	5	0.267	not significant; p=0.072
TSC22	13	2	0	0.133	not significant; p=0.783
PAAL	6	4	5	0.267	not significant; p=0.072
GAL-R1	8	2	5	0.133	significant; p=0.005
GCOAD	7	3	5	0.200	significant; p=0.022
Puromycin	5	8	2	0.533	not significant; p=0.667
Methyl-26	12	3	0	0.200	not significant; p=0.667

Eight fluorescence threshold plots, one for each locus, were constructed (Figure 2). Six of the eight fluorescence threshold plots obtained from DMAS qPCR show the expected three distinct clusters, each of which is representative of one of three genotypes: stream homozygote, lake homozygote, or heterozygote. Two of the eight plots, however, only had two clusters: the stream homozygote and the heterozygote, and entirely lacked the presence of a lake homozygote cluster. Therefore, none of the samples tested were homozygous for the lake allele at either locus. This is relatively consistent with results seen in Barfuss (2021), as only one out of forty-five smolts assayed from 2019 were homozygous for the lake allele at the Methyl-26 locus, and only three out of forty-five were homozygous for the lake allele at the TSC-22 locus.

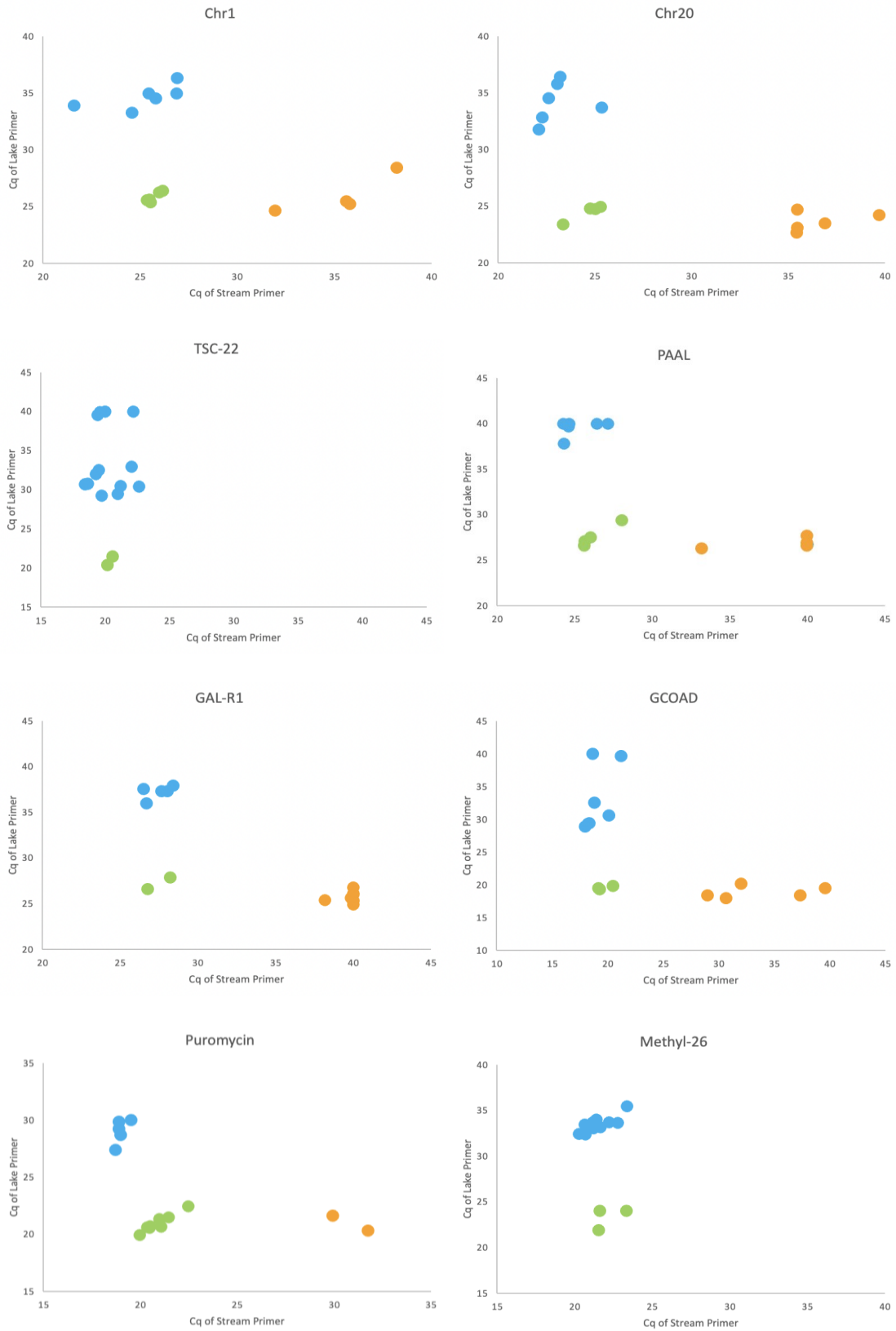


Figure 2: Discrimination plots obtained from DMAS-qPCR of returning steelhead.

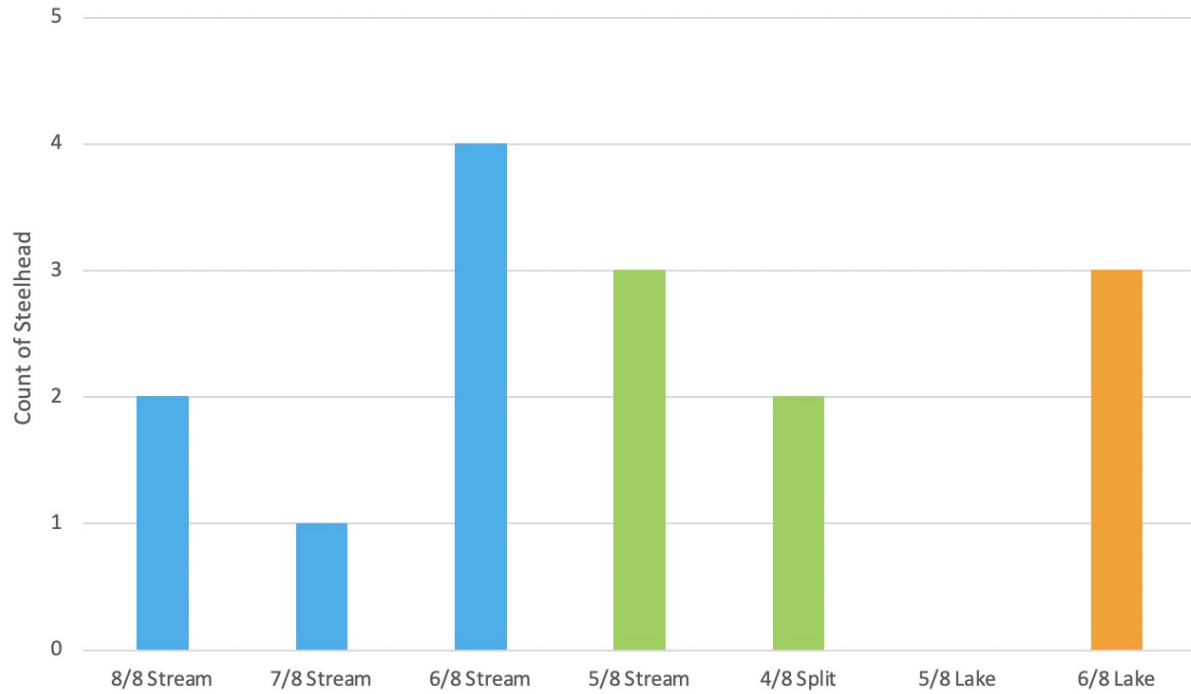


Figure 3: Count of populations assigned to returning steelhead based on DMAS-qPCR genotyping of identified SNPs.

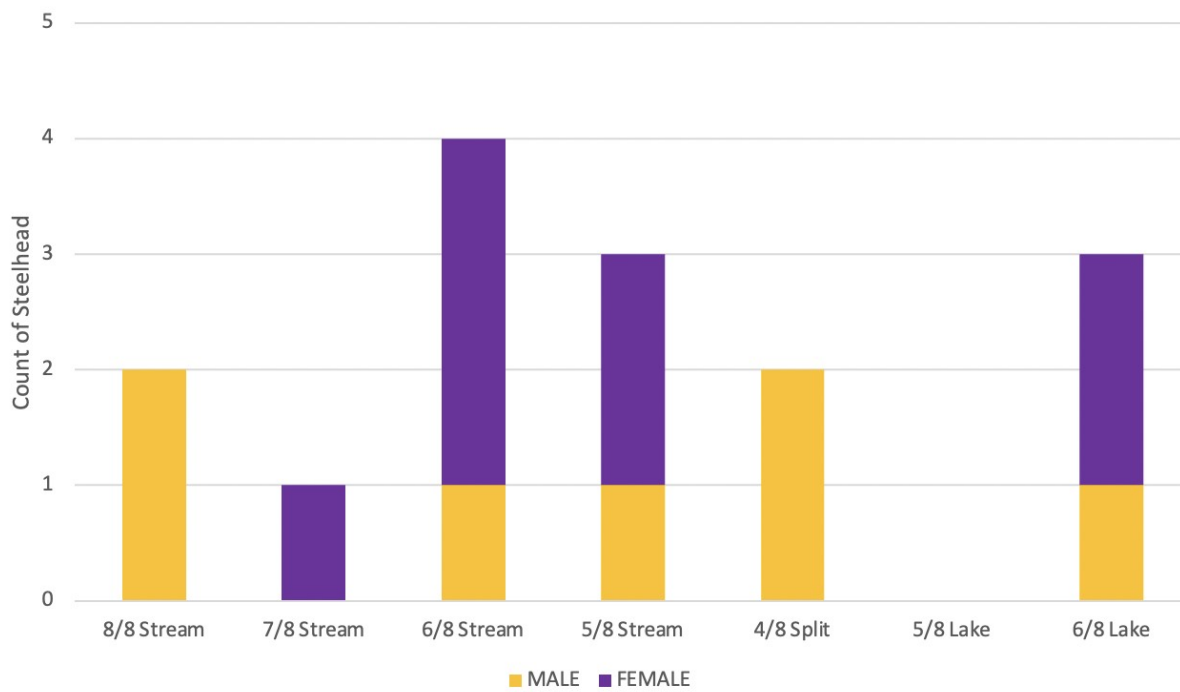


Figure 4: Count of populations assigned to returning steelhead based on DMAS-qPCR genotyping of identified SNPs, categorized by sex.

Of the fifteen returning steelhead assayed in this study, 47% were genotyped as originating from Sashin stream, 20% from Sashin lake, and 33% were admixed (Figure 3). The returning steelhead were then sex-typed, and 53% of them were females, while the remaining 47% were males (Figure 4). Furthermore, 67% of the returning steelhead that originated from Sashin lake were female, with the remaining 33% being male (Figure 4).

Discussion

The results generated herein support the conclusion that the outmigrating smolts being produced by Sashin lake (Barfuss 2021) are able to successfully complete their marine migrations and return to Sashin stream to spawn. Based on these results, there are several other studies that could be conducted to further investigate the ability of lake-originating steelhead to replenish struggling anadromous populations. One of which is to investigate whether the fitness – i.e., fecundity - of lake-originating steelhead differs from that of steelhead that originated from the stream. Because the goal of this study is to determine whether the lake-originating steelhead can reproduce in the stream to conserve stream populations, we would want to see that lake-originating steelhead have comparable fecundity to the stream-originating steelhead.

Additionally, it is surprising that the percentage of returning male steelhead and returning female steelhead are almost equivalent, considering female trout usually have a higher tendency to migrate than their male counterparts (Ohms et al. 2014). This is because there is a known positive correlation between size and fecundity, and that female reproductive success is usually dependent on gamete production, rather than access to mates (Schill et al. 2010; Quinn et al. 2011). Therefore, females gain a greater fitness benefit to migrating than males, as fecundity is

more tied to body size for females than males. However, additional studies looking at returning steelhead from other years found more females than males (Sachdeva 2022). It is possible that the returning steelhead in 2015 had an unusual sex ratio bias towards males, or that additional samples would have confirmed female bias. Further studies should be conducted to analyze whether this trend is present in other years, as fluctuations in the environment are in part known to influence migration patterns in *O. mykiss* and could therefore be altering the sex ratio of migratory trout.

It is also interesting that two of the loci I tested were outside of Hardy-Weinberg equilibrium. In both of those loci, a lack of heterozygosity was observed. While this could be due to incorrect genotyping of the samples, or some form of selection operating on the two loci, it is unlikely given that the loci have previously been used in two other experiments conducted by the Hale lab where no such significance outside of Hardy-Weinberg equilibrium was observed (Barfuss 2021; Sachdeva 2022). It is most likely that this lack of heterozygosity is due to the small sample size I assayed. Therefore, further experiments should be conducted with a greater number of samples from 2015 to see if the two loci are still out of Hardy-Weinberg equilibrium in the same year, despite a larger sample size.

The most important conclusion drawn from this data is that lake-originating steelhead can successfully complete their marine migrations, which subsequently means that Sashin lake could potentially be used as a reservoir to breed and replenish the dwindling anadromous population. This is an important aim of conservation studies, because while it has been proven that Sashin lake can produce outmigrating smolts (Barfuss 2021), no data had been generated regarding

whether said trout are able to successfully complete their marine migrations, or if they are dying out at sea and never return to breed. That said, there are many ways to further explore the conservational potential of Sashin lake and to further our understanding of the *O. mykiss* migration patterns within the Sashin system. Sachdeva (2022) found that 33.3% of returning steelhead from 2019 were lake originating, but in 2020, only 7.7% of returning steelhead originated from the lake. Those percentages are both much higher and much lower, respectively, than the 20% of lake originating returning steelhead I observed in 2015. This indicates that there has not been an upward or downward trend in the amount of returning steelhead produced by the lake over time. However, both Sachdeva (2022) and this study had relatively small sample sizes, which could explain the variation in lake originating steelhead percentages in different years. Therefore, additional studies should be conducted with larger sample sizes to determine whether the percentages are representative of the population. Furthermore, additional studies could explore what the percentages are in other years to determine whether there is a consistent trend in the percentage of returning steelhead that originate from the lake. And if there is a lot of variation between the years, then additional studies could analyze whether external factors that fluctuate (such as the environment) are playing an obvious role.

The data generated herein supports the conclusion that Sashin lake is able to produce steelhead that successfully complete their marine migrations. Therefore, this information indicates that Sashin lake could potentially serve as a reservoir to breed and replenish the dwindling anadromous population in the creek and can be used to contribute to the conservation of *O. mykiss*.

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