

Hybridization decreases native cutthroat trout reproductive fitness

William C. Rosenthal^{1,2}  | John M. Fennell^{3,4} | Elizabeth G. Mandeville^{1,4,5} | Jason C. Burckhardt⁶ | Annika W. Walters^{7,4,3,2} | Catherine E. Wagner^{1,2,8} 

¹Department of Botany, University of Wyoming, Laramie, Wyoming, USA

²Program in Ecology and Evolution, University of Wyoming, Laramie, Wyoming, USA

³Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming, USA

⁴Wyoming Cooperative Fish and Wildlife Research Unit, University of Wyoming, Laramie, Wyoming, USA

⁵Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

⁶Wyoming Game and Fish Department, Cheyenne, Wyoming, USA

⁷U.S. Geological Survey, Reston, Virginia, USA

⁸Biodiversity Institute, University of Wyoming, Laramie, Wyoming, USA

Correspondence

William C. Rosenthal, Department of Botany, University of Wyoming, Laramie, Wyoming, USA.

Email: will.rosenth@gmail.com

Funding information

Wyoming Game and Fish Department

Handling Editor: Josephine Pemberton

Abstract

Examining natural selection in wild populations is challenging, but crucial to understanding many ecological and evolutionary processes. Additionally, in hybridizing populations, natural selection may be an important determinant of the eventual outcome of hybridization. We characterized several components of relative fitness in hybridizing populations of Yellowstone cutthroat trout and rainbow trout in an effort to better understand the prolonged persistence of both parental species despite predictions of extirpation. Thousands of genomic loci enabled precise quantification of hybrid status in adult and subsequent juvenile generations; a subset of those data also identified parent–offspring relationships. We used linear models and simulations to assess the effects of ancestry on reproductive output and mate choice decisions. We found a relatively low number of late-stage (F3+) hybrids and an excess of F2 juveniles relative to the adult generation in one location, which suggests the presence of hybrid breakdown decreasing the fitness of F2+ hybrids later in life. Assessments of reproductive output showed that Yellowstone cutthroat trout are more likely to successfully reproduce and produce slightly more offspring than their rainbow trout and hybrid counterparts. Mate choice appeared to be largely random, though we did find statistical support for slight female preference for males of similar ancestry. Together, these results show that native Yellowstone cutthroat trout are able to outperform rainbow trout in terms of reproduction and suggest that management action to exclude rainbow trout from spawning locations may bolster the now-rare Yellowstone cutthroat trout.

KEYWORDS

conservation genetics, contemporary evolution, cutthroat trout, fish, genomics/proteomics, hybridization, natural selection

1 | INTRODUCTION

Characterizing natural selection in wild populations is a frequent, but often elusive, goal in evolutionary biology. The challenge in many systems lies in the difficulty of obtaining direct and comprehensive measurements of individual reproductive output and survival throughout the life cycle in most wild populations (Endler, 1986). Although exceptions where these measurements have been possible

are heralded as gold-standard examples (e.g., sticklebacks, *Phlox*, monkeyflowers, Darwin's finches, and guppies; McPhail, 1969; Levin & Kerster, 1968; Kiang & Libby, 1972; Grant et al., 1976; Endler, 1980; also reviewed in Clutton-Brock & Sheldon, 2010), in many more cases only partial information is attainable. Nevertheless, combining information on the quantifiable aspects of natural selection with information on current and past population status can provide key insights into the evolutionary dynamics of these systems (Endler, 1986).

Populations of hybridizing species are often seen as a way to understand the permeability of species boundaries (Barton & Hewitt, 1989; Harrison, 1993; Harrison & Larson, 2014; Ravinet et al., 2017), but they can also provide opportunity to effectively examine natural selection in action. Hybrids can exhibit traits that are intermediate to both parental species or entirely novel (Arnold, 1992; Ellstrand & Schierenbeck, 2000; Rieseberg et al., 1999), which, in turn, provides variation on which natural selection may act. Fitness differences may therefore have important consequences on the fate of hybrids and their parental species involved in these interactions. Natural selection can therefore act as an important driver of hybridization dynamics and influence the eventual outcome of hybridization (Abbott et al., 2013; Arnold, 1992, 1997; Arnold et al., 2012; Wolf et al., 2001).

One impetus to catalogue hybridization outcomes and decipher the drivers of those outcomes relates to human-mediated hybridization and subsequent decline of native species (Allendorf et al., 2011; Rhymer & Simberloff, 1996). Human-mediated hybridization can result from anthropogenic disturbance and species introductions, and results in the breakdown of reproductive barriers more frequently than naturally occurring instances of secondary contact (Brennan et al., 2014; Grabenstein & Taylor, 2018). Furthermore, the outcome of these hybridization events is difficult to predict; the extent of hybridization can vary even within the same pair of species across multiple contact zones (Mandeville et al., 2015), and is therefore likely dependent on environmental and historical context (Hatfield & Schluter, 1999). In-depth examination of species barriers in these systems may be especially useful for understanding the factors underlying variation in hybridization (Grabenstein & Taylor, 2018) and enable effective use of limited conservation resources.

Aquatic species may be especially vulnerable to human-mediated hybridization, as both anthropogenic disturbance (e.g., eutrophication) and non-native species introductions (e.g., fish stocking) are common in aquatic systems. In the United States alone, the number of fish species shared between states increased by an average of 15.4 after European colonization and displacement of native peoples (Rahel, 2000). Anthropogenic hybridization has led to drastic declines or extinction of aquatic species across the globe, including the endemic cichlids of Lake Victoria (Ogutu-Ohwayo, 1990; Seehausen et al., 1997; Witte et al., 1992); *Mercenaria* clams (Dillon & Manzi, 1989), *Cobitis* loaches (Kim & Yang, 1993), *Hyla* frogs (Dufresnes et al., 2015), *Ambystoma* salamanders (Fitzpatrick & Shaffer, 2007), whitefish (Vonlanthen et al., 2012) and *Cyprinodon* pupfish (Echelle & Connor, 1989). Similarly, the widely introduced (Crawford & Muir, 2008) rainbow trout (*Oncorhynchus mykiss*) hybridizes readily with cutthroat trout (*Oncorhynchus clarkii*) in western North America (Allendorf & Leary, 1988; Kovach et al., 2011; McKelvey et al., 2016; Ostberg et al., 2004). Loss of genetically unadmixed populations to hybridization with rainbow trout is the leading cause of decline for the Yellowstone cutthroat trout (*O. c. bouvieri*, YCT), which now occupies only 44% of its historical range (Al-Chokhachy et al., 2018).

The Yellowstone cutthroat trout population in the North Fork Shoshone River (Wyoming, USA) is one such population. In this system, the native Yellowstone cutthroat trout have been almost entirely replaced by hybrids and rainbow trout after the introduction of rainbow trout 110 years ago (Kruse et al., 2000; Mandeville et al., 2019; Nordberg et al., 2021). However, Mandeville et al. (2019) utilized genomic data to identify the persistence of unadmixed Yellowstone cutthroat trout individuals despite predictions of their extirpation two decades ago (Kruse et al., 2000).

In this study, we used genomic data from two generations of trout to precisely quantify hybrid status, estimate differences in reproductive output and characterize mate choice decisions among Yellowstone cutthroat trout, rainbow trout, and their hybrids in the North Fork Shoshone River. To do this, we use a variety of statistical approaches including Bayesian linear modelling and simulations. We then use these conclusions in conjunction with historical stocking data and population status assessments to infer possible explanations for the decline and prolonged persistence of Yellowstone cutthroat trout in this system. Additionally, this study shows the utility of genomic data for measuring some components of fitness in wild populations and presents methods that could enable similar fitness estimates in many non-model organism systems. Our findings provide context for conservation actions that may be effective for preserving an imperilled native fish and add to the growing body of research examining the fitness effects of interspecific and intraspecific hybridization on wild salmonids (Christie et al., 2014; Kovach et al., 2015; Muhlfeld et al., 2009; Shedd et al., 2022).

2 | MATERIALS AND METHODS

2.1 | System background and sampling

The North Fork Shoshone River runs for approximately 60 miles from the Absaroka mountains in NW Wyoming, USA and ends in Buffalo Bill Reservoir (NW Wyoming, USA). The river's hydrology is dominated by snowmelt runoff, and therefore has its highest discharge in late spring. Yellowstone cutthroat trout are native to the drainage but have experienced declines since the 1960s (Nordberg et al., 2021), which have been largely attributed to the introduction of rainbow trout in the early 1900s. Both rainbow trout and Yellowstone cutthroat trout were heavily stocked into the drainage during the 1900s, but Yellowstone cutthroat trout were stocked for a much longer duration and in larger numbers (Nordberg et al., 2021). Previous studies have found low numbers of unadmixed Yellowstone cutthroat trout (Kruse et al., 2000; Mandeville et al., 2019) and have found no evidence of spatial segregation between Yellowstone cutthroat trout and rainbow trout into upstream and downstream reaches (Kruse et al., 2000). Yellowstone cutthroat trout, rainbow trout and their hybrids exhibit an adfluvial life history: adult fish migrate upstream from Buffalo Bill Reservoir and into the river's tributaries to spawn from April to July. The offspring of these adults migrate out of their

natal streams during the late summer and fall of the same year (Kent, 1984).

Sampling occurred in two tributaries of the North Fork Shoshone River in 2019 and 2020. We sampled adult trout from mid-April through early July during their spawning migrations. We sampled adults via a picket weir and hoop nets on Trout Creek and hoop nets on Middle Creek. The picket weir on Trout Creek failed during high stream discharge on May 27th 2019, after which only hoop nets were used. All sampling occurred within 500 m of the confluence of the tributary and the North Fork Shoshone River. We surgically implanted each adult with a 12.5 mm full-duplex Biomark Passive Integrated Transponder (PIT) tag, took tissue samples from the adipose fin of each adult and recorded each adult's total length and sex. We maintained submersible PIT-tag antenna systems at the confluence of both tributaries from mid-April until the end of September to detect adult out-migration dates. We sampled juveniles as they migrated out of their natal streams from late August through early October. We electrofished three reaches per tributary for 10 min using a Smith Root LR24 backpack electrofisher. We sampled juveniles eight times in Trout Creek in 2019 and four times from Middle Creek in 2020. Sampling effort was evenly distributed across sampling events and electrofishing reaches. Sampling juvenile fish over different reaches and times as they migrate out of the tributaries should minimize the risk of selectively sampling a small number of families. We took tissue samples from the caudal fin of each juvenile. All fish handling and sampling protocols were approved under IACUC protocol # 20190724AW00383-01 at the University of Wyoming.

2.2 | DNA extraction and sequencing

All sampled adults were selected for DNA extraction and subsequent sequencing. Juveniles were subsampled in a temporally and spatially balanced manner to provide sample sizes of $n = 572$ for Trout Creek in 2019 and $n = 153$ for Middle Creek in 2020. Genomic DNA was extracted from fin tissue using Qiagen DNeasy Blood and Tissue kits and a QIAcube robot according to the manufacturer's instructions (Qiagen, Inc.). We prepared 11 reduced complexity genomic libraries for high-throughput DNA sequencing using a genotyping-by-sequencing protocol (Parchman et al., 2012), multiplexing 192–248 individuals per library. DNA was initially fragmented using restriction enzymes EcoRI and MseI and fragments from each individual fish's DNA were ligated to a unique 8–10 base pair nucleotide barcode. Following the ligation of the identification barcodes, individual samples were multiplexed and amplified in two separate replicates by PCR. Prior to sequencing, each library was size selected using BluePippin (Sage Science) to retain only fragments 350–450 base pairs in length. Each library was sequenced on one Illumina HiSeq 4000 lane to produce approximately 1.5 billion 150 base pair single-end sequence reads. DNA sequencing of all 11 libraries was completed at the Genomics and Cell Characterization Core Facility at the University of Oregon.

2.3 | Sequence assembly and variant calling

After parsing barcodes to assign each read to an individual fish, we aligned reads from each individual fish to the rainbow trout genome (Pearse et al., 2019, accession GCA_002163495.1), using the bwa-mem algorithm (Li, 2013). We then identified variable genetic sites using samtools mpileup and bcftools (Danecek et al., 2021). We filtered the initial set of variants to include only SNPs (single nucleotide polymorphisms) with two alleles, quality scores greater than 19 and genotype quality over 9. We also applied filters using vcftools (Danecek et al., 2011) to retain only loci with data in at least 50% of individuals and loci with a minor allele frequency of 0.03 or greater. Individuals missing data for 70% or more of these retained sites were removed from the dataset. This and all following sequence processing were performed on the University of Wyoming's Teton Computing Cluster (Advanced Research Computing Center, 2018).

2.4 | Ancestry estimation and parent-offspring relationship assignment

To identify the ancestry of individual fish to detect potential hybrids, we used entropy, a hierarchical Bayesian model (Gompert et al., 2014; Shastry et al., 2021). For each individual fish, we estimated q , proportion of ancestry, and Q , interspecific ancestry (proportion of genetic sites with ancestry from both parental species) for a $k = 2$ model assuming two genetic clusters (i.e., Yellowstone cutthroat trout and rainbow trout). We ran three replicate chains, and assessed convergence of each chain through examination of trace plots. Due to large numbers of individuals and computation time limits, we divided the juveniles and adults from Trout Creek into seven groups, each of which contained 54 reference Yellowstone cutthroat trout individuals from previous sampling (Mandeville et al., 2019), to be run in parallel. The reference individuals consisted of unadmixed Yellowstone cutthroat trout previously sequenced in Mandeville et al. (2019) and hatchery strain Yellowstone cutthroat trout from the Wyoming Game and Fish Department fish hatchery system. Each group had three replicate chains of 60,000 steps with each 10th step recorded and the first 10,000 steps (1000 recorded steps) discarded as burn-in. We included juveniles and adults from Middle Creek in one group with three replicate chains of 30,000 steps with each 5th step recorded.

We used the bivariate relationship of q and Q to classify individuals into categories of hybrid crosses for descriptive purposes. Fish with a proportion of Yellowstone cutthroat trout ancestry (q) less than 0.1 were classified as rainbow trout and those with proportions of Yellowstone cutthroat trout ancestry >0.9 were classified as unadmixed Yellowstone cutthroat trout. All fish with proportions of Yellowstone cutthroat trout ancestry >0.1 and <0.9 were classified as hybrids. Hybrids were further broken down into F1, F2 and backcross (BC) classes using the combination of q and Q values. Note that we define F2 hybrids as offspring resulting from the mating of two F1 hybrids and backcross hybrids as the product of any mating

between a hybrid individual and an unadmixed member of a parental species. We only consider offspring from an $F2 \times F2$ mating to be $F3$ hybrids. $F1$ hybrids were defined as fish with q between 0.4 and 0.6 and $Q > 0.8$. $F2$ hybrids also had q between 0.4 and 0.6, but had Q estimates between 0.4 and 0.6. $F3$ hybrids would have q between 0.4 and 0.6 and Q estimates < 0.4 . Backcross rainbow trout were defined as $Q - 2q$ between -0.1 and 0.1 ; backcross Yellowstone cutthroat trout were defined by $Q + 2q$ between 1.9 and 2.1 . These cut-offs were the same as those used in previous work in this system (Mandeville et al., 2019) and derived from work on the entropy model evaluating realistic expectations for these values (e.g., Lindtke et al., 2014). These classes are broad by necessity due to the effects of substantial genetic variation in parental populations when sampling tens of thousands of loci. Variation within each parental species can cause truly unadmixed individuals to score as > 0.00 or < 1.00 . This can then propagate to deviations in hybrid individual admixture coefficients (e.g., $F1$ hybrids with $q \neq 0.5$).

Initial results appeared to show an increase in $F2$ individuals in juvenile generations relative to adult generations; to test for an excess of $F2$ individuals in each juvenile generation compared to its adult counterpart, we used one-tailed Fisher's exact tests with an alternative hypothesis of an odds ratio less than one. To assess how adult and juvenile generations differed from one another across the entire range of q , we used a resampling approach. We first converted the density histograms for each generation to continuous density distributions using the density function in R with a kernel bandwidth of 0.025 for Middle Creek and 0.0125 for Trout Creek. We used the biweight smoothing kernel for both locations and estimated densities at each 0.01 along the range of q . Before smoothing, we reflected the q distributions over 0 and 1 to minimize boundary effects. Boundary effects are an artefact that occurs when estimating kernel densities for distributions with strict cut-offs. The density estimation process is unaware of these cut-offs and, in turn, sharply decreases the estimated density near those bounds. We then resampled with replacement from the adult density distribution at each location using the same sample size as the juvenile generation from that location. We repeated this process 10,000 times for each location, and converted each resampling event's results into a density histogram with bin widths of 0.01. We then recorded the 0.025 and 0.975 quantiles for the density in each histogram bin and visually compared them to the smoothed density for the adult and juvenile generations.

We identified parent-offspring relationships between adult and juvenile trout collected at Trout Creek in 2019, as we expect the high number of sampled adults at this site to increase the likelihood of identifying within-sample parent-offspring relationships. We further filtered the SNP dataset used for entropy analyses such that only informative and high-quality genotype calls would be retained. Using VCFtools (Danecek et al., 2011), we only retained genotype calls with 5 or greater reads. Any sites with a missing data proportion greater than 10% or a minor allele frequency less than 0.01 were removed. Sites were further thinned to include only one site (randomly chosen) within each 10,000 bp window. We identified parent-offspring relationships using hiphop (Cockburn et al., 2021). Correct parent-offspring assignments were determined visually as

per the hiphop vignette. We used the resultant parent-offspring relationships to calculate the number of sampled offspring per adult and to document matings between sampled males and sampled females. To examine whether the sampling of adults may have biased the assignment of parent-offspring relationships, we constructed a logistic regression model that assessed the effect of juvenile q on the probability of one or more parents being sampled. We fit this model in a frequentist framework using the lme4 R package (Bates et al., 2015). To estimate the Trout Creek spawning adult population size, we used the close-kin mark-recapture approach presented in Prystupa et al. (2021).

2.5 | Reproductive output modelling

We modelled differential reproductive output between ancestry classes in a Bayesian framework via a zero-inflated negative binomial model. We constructed models for both male and female reproductive output. We used a zero-inflated model in an attempt to account for the different ways in which a trout could have zero recorded offspring; an individual could have no offspring recorded if they either produced no offspring or if any offspring produced were not sampled. We included the individual's length at time of entry, date of entry (Julian day) and proportion YCT (Yellowstone cutthroat trout) ancestry (q) as covariates, and centred and scaled the length and date variables. We designed minimally informative prior probability distributions, which we centred around frequentist coefficient estimates for models including the same variables. We sampled posterior probability distributions from both models with the No-U-Turn Hamiltonian Monte Carlo sampler implemented in STAN (Carpenter et al., 2017). We used the R package brms (Bürkner, 2017, 2018) to interface with STAN via R v4.0.3 (R Core Team, 2020). We sampled each posterior distribution with three independent chains. Each chain took 3000 samples from the posterior distribution, and we discarded the first 1000 samples as burn-in. We assessed model convergence by visually verifying that the trace plots showed well-mixed chains, and we used posterior predictive checks to determine that model predictions reasonably corresponded with the observed data.

Model selection was performed for several different versions of each model, and the models detailed here were those with the best fit as measured by WAIC (Watanabe, 2010). If models were within three WAIC units of one another, the simplest model was selected. The models presented here were those chosen by the model selection process, and other renditions tested can be found in the associated code (available on GitHub at https://github.com/will-rosenthal/NFS_trout_hybrids). Full model equations can be found in the supplementary materials.

| Zero-inflated negative binomial models for estimating number of offspring

a. Female trout

$$\text{Sterile} \sim \text{Intercept}_1 + \text{Date}_1 + \text{Date}_2^2 + q_1 \quad (1)$$

if $Sterile_i = 1$: $Reproutput_i = 0$

if $Sterile_i = 0$:

$$Reproutput \sim Intercept_2 + Date_2 + Date_2^2 + q_2 + length \quad (2)$$

Prior probability distributions

$$Intercept_1 \sim Normal(0, 1.5)$$

$$Date_1 \sim Normal(0, 0.75)$$

$$Date_1^2 \sim Normal(0, 0.5)$$

$$q_1 \sim Normal(0, 1.5)$$

$$Intercept_2 \sim Normal(-1.75, 10)$$

$$Date_2 \sim Normal(0.7684, 6)$$

$$Date_2^2 \sim Normal(0.223, 5)$$

$$q_2 \sim Normal(2.42, 10)$$

$$length \sim Normal(0.28, 5)$$

b. Male trout

$$Sterile \sim Intercept_1 + q_1 + length_1 \quad (3)$$

if $Sterile_i = 1$: $Reproutput_i = 0$

if $Sterile_i = 0$:

$$Reproutput \sim Intercept_2 + q_2 + length_2 \quad (4)$$

Prior probability distributions

$$Intercept_1 \sim Normal(0, 1.5)$$

$$q_1 \sim Normal(0, 1.5)$$

$$length_1 \sim Normal(0, 0.5)$$

$$Intercept_2 \sim Normal(-0.621, 10)$$

$$q_2 \sim Normal(3.771, 10)$$

$$length_2 \sim Normal(0.189, 5)$$

2.6 | Mate choice modelling

Before modelling mate choice decisions, we needed to estimate the number of days each adult trout stayed in the spawning tributary. We used the subset of individuals with a documented exit date (Figure S1) to build a model meant to estimate stay duration. Examination of the observed stay duration data showed a somewhat bimodal distribution, and we therefore used a mixture model to ensure that the potential for “long stayer” and “short stayer” individuals was appropriately represented in the model.

We included entry date in these models as the spawning season for trout is of a finite length (from approximately May through June), and therefore stay duration would likely be influenced by entry date. We included proportion YCT ancestry (q) to account for differences between species and their hybrids. Trout spawning ecology varies greatly between sexes, with males in Trout Creek typically staying in tributaries longer and arriving earlier than females, so we included sex as a categorical variable where 0 signified a male fish and 1 signified a female fish. We centred and scaled the date (Julian day) variable.

Similar to the reproductive output models, we centred prior probability distributions around frequentist coefficient estimates. However, we estimated the coefficients for each mixture family with separate models. We obtained mixture distribution 1 ($k = 1$) estimates from a negative binomial model fit with data from adult trout with stay durations less than 100 days. We obtained the mixture distribution 2 ($k = 2$) estimates from a Poisson model fit with data from adult trout with stay durations greater than 100 days. Graphical examination of stay duration data showed that 100 days would be an appropriate cut-off between “long-stayer” and “short-stayer” fish (Figure S1). We again sampled the posterior probability distributions using the No-U-Turn Hamiltonian Monte Carlo sampler implemented in STAN (Carpenter et al., 2017), and used the R package `brms` (Bürkner, 2017, 2018) to interface with STAN via R v4.0.3 (R Core Team, 2020). We sampled each posterior distribution with three independent chains. Each chain took 3000 samples from the posterior distribution, and we discarded the first 1000 samples as burn-in. We set the target acceptance rate to 0.8 and the maximum tree depth to 10.

Model selection was performed for several different versions of each model, and the models detailed here were those with the best fit as measured by WAIC (Watanabe, 2010). If models were within three WAIC units of one another, the simplest model was selected. The models presented here were those chosen by the model selection process, and other renditions tested can be found in the associated code (available on GitHub at https://github.com/will-roenthal/NFS_trout_hybrids). Full model equations can be found in the supplementary materials.

I Negative binomial Poisson mixture model for estimating stay duration

$$\text{Stayduration} \sim \text{Intercept} + \text{Sex} + q + \text{Date} \quad (5)$$

Prior probability distributions

$$\text{Intercept}_{\text{negbin}} \sim \text{Normal}(3.853, 0.5)$$

$$\text{Sex}_{\text{negbin}} \sim \text{Normal}(0, 1)$$

$$q_{\text{negbin}} \sim \text{Normal}(0, 1)$$

$$\text{Date}_{\text{negbin}} \sim \text{Normal}(0, 1)$$

$$\text{Intercept}_{\text{poisson}} \sim \text{Normal}(4.79, 0.15)$$

$$\text{Sex}_{\text{poisson}} \sim \text{Normal}(0, 0.5)$$

$$q_{\text{poisson}} \sim \text{Normal}(0, 0.5)$$

$$\text{Date}_{\text{poisson}} \sim \text{Normal}(0, 0.5)$$

We modelled mate choice from the female perspective, where each observation included data from a unique male–female pair that occupied (or was predicted to have occupied) the spawning tributary at the same time. If a female had a documented mating with a sampled male, we populated an observation for each male fish which that female potentially encountered. We automatically included any male fish the female was documented to have mated with and set a binary variable signifying that the male was chosen as a mate to 1. We included other males as observations for that female if their entry date and stay duration data indicated that they would have been in the tributary at the same time as that female. We drew missing stay duration data from both male and female individuals from the posterior probability distribution of model II using the `posterior_predict` in `brms`. To account for the stochasticity inherent in sampling from the posterior probability distribution and to ensure all uncertainty in that distribution was properly carried forward into the mate choice models, we imputed the missing stay duration data and subsequently fit the mate choice model 1000 times.

Each model included a predictor variable for the proportion Yellowstone cutthroat trout ancestry (q) for the focal male and a variable representing the difference in q between the focal male and focal female ($q \text{ dist.}$). We also included male body length, as females may be more likely to mate with a larger male. We centred and scaled all of these continuous variables. The model intercept was allowed to vary for each focal female, which would allow for any female-specific differences in mate choice decisions to be estimated.

As with models I and II, we centred the prior probability distributions for model III around the coefficient estimates from a frequentist Bernoulli model that was fit on observations based on one round of posterior-predicted stay duration data. Because we used only one round of posterior prediction to centre these prior distributions, we set the prior distribution standard deviations to be minimally

informative while still avoiding the asymptotic behaviour created by logit-transforming high values of a continuous predictor. We chose the standard deviation of the distribution from which σ_u was drawn from via prior-predictive simulations.

We sampled from the posterior probability distribution of model III using the No-U-Turn Hamiltonian Monte Carlo sampler implemented in STAN (Carpenter et al., 2017). Again, we used the R (R Core Team, 2020) package `brms` (Bürkner, 2017, 2018) to interface with STAN. We used three independent chains to sample from each posterior distribution. Each chain took 3000 samples from the posterior distribution, and we discarded the first 1000 samples as burn-in. We set the target acceptance rate to 0.93 and the maximum tree depth to 10 to avoid divergent transitions. As stated above, we fit the model 1000 times with observations generated using stay duration data drawn from the posterior probability distribution of model II. Full model equations are found in the supplementary materials.

I Mate choice model

$$\text{Chosen} \sim \text{Intercept} + (1|\text{Female}) + \text{Maleq} + \text{length} + q\text{distance} \quad (6)$$

Prior probability distributions.

$$\text{Intercept} \sim \text{Normal}(-4.77, 1.5).$$

$$\text{Maleq} \sim \text{Normal}(0.878, 1.7).$$

$$\text{Length} \sim \text{Normal}(0.156, 0.8).$$

$$q\text{distance} \sim \text{Normal}(-0.3637, 1.3).$$

Male proportion Yellowstone cutthroat ancestry could affect our inferred mate choice decisions if it is shown to increase the average number of offspring in our sample. To assess the potential for this effect to drive our mate choice model results, we use the following conceptual equation.

$$P(\text{mating detected}) = P(\text{chosen as mate}) * P(> 0 \text{ offspring in sample})$$

Our mate choice model can only estimate the effects of male proportion Yellowstone cutthroat trout ancestry on the probability of a mating being detected, as detecting a mating requires sampling at least one offspring from that male. Our model of male reproductive output can predict the probability of a male having greater than zero offspring in our sample. Therefore, by comparing the effect of male proportion Yellowstone cutthroat trout ancestry on the probability of having >0 offspring in our sample to the effect of male proportion Yellowstone cutthroat trout ancestry on the probability of a mating being detected between two fish, we can infer the relative role of proportion Yellowstone cutthroat trout ancestry on mate choice decisions. We calculated the overlap of effect size distributions for proportion Yellowstone cutthroat trout ancestry on the probability of a mating being detected and the probability of having >0 offspring in

our sample overlap. We did this by first calculating the probability of unadmixed rainbow trout and unadmixed Yellowstone cutthroat trout males each having >0 offspring in our sample via the posterior probability distribution for our male reproductive output model. Each of these probability distributions was logit scaled to put it on the same scale as the mate choice model coefficients, and the difference between the two was calculated (hereafter referred to as distribution 1). We then sampled from the posterior probability distributions for all 1000 mate choice model replicates to obtain a new posterior probability distribution for the effect of male proportion Yellowstone cutthroat trout ancestry on the probability of a mating being detected (hereafter referred to as distribution 2). To see whether the effects of Yellowstone cutthroat trout ancestry on male reproductive output could explain a considerable proportion of the effect of Yellowstone cutthroat trout ancestry on the probability of a mating being detected, we calculated the proportion of distribution 1 that was greater than or equal to the minimum of distribution 2.

2.7 | Mating simulations

Sampling of spawning adults in 2020 on Middle Creek did not yield sufficient sample sizes to carry out pedigree construction and attempt to compare patterns in reproductive output and mate choice between Middle Creek and Trout Creek. Instead, we used simulations to assess whether the juvenile generation sampled on Middle Creek in 2020 could be a reasonably likely product of random mating of the sampled adults. We constructed four versions of the simulation: one version ("uninformed random") chose mates for each sampled female randomly from the pool of male fish, and the remaining three chose mates for each sampled female based on the relative entry dates of that female and sampled males. We set the strength of preference for males that entered around the same time as the female using an intercept-only negative binomial model. We fit the model using data from the inferred matings on Trout Creek in 2019, and used the absolute value difference in male and female entry date (Julian date) as the response. Each female in the informed null simulation had a value drawn from the model-supported negative binomial distribution; if no male fish entered the tributary a number of days before or after the female equal to that value, we drew another value from the distribution. If multiple males entered the tributary on the dates chosen from the distribution, we selected the male chosen by the female in one of three ways: randomly ("informed random"), choosing the male with the closest q to that of the female ("informed assortative") or choosing the male with highest q ("informed YCT preference").

Once we chose mates for each female, we calculated juvenile q values for each male–female pair using a binomial distribution to account for the natural variation chromosome inheritance. For each parent, we drew the number of Yellowstone cutthroat trout chromosomes passed to the offspring from a binomial distribution with size 32 (corresponding to $2n = 64$ in Yellowstone cutthroat trout and some rainbow trout; Loudenslager & Thorgaard, 1979) and a probability equal to the parent's q . We divided the total number of

YCT chromosomes from both parents by 64 to get the offspring q . We repeated this process 30 times per pair to generate 30 juveniles. Once we generated juveniles for all pairs, we randomly selected 153 samples from the pool of juveniles without replacement to mirror the sampling of wild juveniles. From the simulated sampled juvenile generation, we calculated the proportion of the sample that was unadmixed Yellowstone cutthroat trout juveniles ($q > 0.9$). We ran each simulation 5000 times to generate distributions of the proportion of unadmixed Yellowstone cutthroat trout juveniles. We compared the observed proportion of unadmixed Yellowstone cutthroat trout juveniles to these distributions by calculating the quantile of each distribution at which the observed proportion fell.

3 | RESULTS

3.1 | DNA sequencing, sequence assembly and variant calling

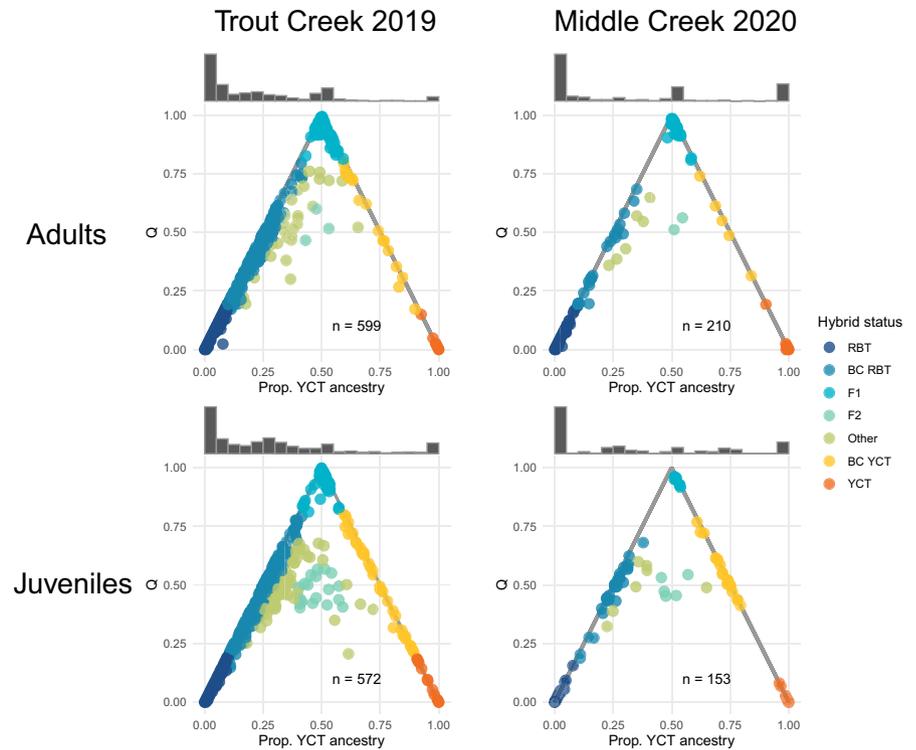
Sequencing resulted in an average of 1,103,100 reads per individual, and an average of 98.05% of those reads per individual were successfully mapped to the reference genome. In all, 133 individuals were removed from the dataset due to high amounts of missing data, leaving 1171 individuals from Trout Creek in 2019 (599 adults, 572 juveniles) and 473 individuals from Middle Creek in 2020 (210 adults, 263 juveniles). Of the Trout Creek adults, 403 were female (67.2%). Of the Middle Creek adults, 105 were female (50%). After filtering, 71,221 and 56,914 SNPs were retained in the 2019 and 2020 datasets, respectively. The further filtered set of variants used for pedigree inference on the 2019 samples contained 8389 SNPs.

3.2 | Ancestry estimation and pedigree construction

Entropy results had very low error estimates in both years. For individuals sampled in 2019, the average 95% credible interval width on q and Q estimates were 0.00652 and 0.01245, respectively. For individuals sampled in 2020, the average 95% credible interval widths were 0.00498 and 0.00961 for q and Q estimates, respectively. 5.20% of samples in 2019 were unadmixed YCT (7.16% of juveniles, 3.33% of adults). In 2020, 14.87% of all sampled fish were unadmixed YCT (13.85% of juveniles, 16.17% of adults) (Figure 1). Fisher's exact tests showed an excess of F2 juveniles relative to adults in Trout Creek (odds ratio = 0.1467, p -value = 0.0002) but not in Middle Creek (odds ratio = 0.2855, p -value = 0.1163). Resampling of the adult generations showed few differences between the adult and juvenile generations at each location that could not be reasonably attributed to sampling stochasticity (Figure S2).

Results from `hiphop` showed a break in `hot.dam` and `hot.sire` scores at eight mismatches (Figure S3). A similar break was seen at three mismatches for `hothiphop.parents` scores (Figure S4). Therefore, a parent–offspring relationship was considered correct if the `hot.dam` or `hot.sire` score was ≤ 8 . Additionally, an

FIGURE 1 Results from *entropy* for a model with $k = 2$. Each column of plots contains adult and juvenile individuals sampled from the same location in the same year, and each row of plots contains individuals of the same life stage collected from either location. Each plot displays all individuals of the given life stage collected at the given location in the given year; the x-axes show the proportion of Yellowstone cutthroat trout ancestry (q) for each individual, and the y-axes show the proportion of sampled loci with interspecific ancestry for that individual (Q). Histograms above each plot show the distribution of q within that plot [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



entire dam-sire-offspring trio was considered correct if the trio's *hothiphop.parents* score was ≤ 3 . In total, 218 juveniles were found to have at least one sampled parent and 109 of those juveniles had both parents sampled. The 109 juveniles with both parents sampled came from 62 unique male–female pairs, and the average absolute difference between the male and female q estimates within those pairs was 0.287. Adult fish had, on average, 0.279 sampled offspring. In all, 357 females had no sampled offspring (88%) (Figure S5). Among females with at least one sampled offspring, the average number of sampled offspring was 2.74. In all, 105 males had no sampled offspring (70%) (Figure S6). Among males with at least one sampled offspring, the average number of sampled offspring was 4.47. A plot of how q and date relate to reproductive output can be seen in Figure 3. The logistic regression showed a consistently positive effect of Yellowstone cutthroat trout ancestry (q) on the probability of a juvenile having a sampled parent (estimate of 6.46, p -value = <0.0001). Visual examination of observed matings showed that females of all ancestries did not show strong preference for males of a particular q , with the possible exception of intermediate q females favouring intermediate q males (Figure 2). Close-kin mark-recapture estimated the Trout Creek spawning adult population size to be 1928.8. This means that our adult sampling included approximately 28.6% of the spawning adult population.

3.3 | Reproductive output modelling

Reproductive output models converged on the posterior probability distribution as evidenced by Gelman–Rubin R^{\wedge} statistics, which were 1.0 for each estimated parameter for both male and female

reproductive output models (Figures S7 and S8). The effective sample size estimates for each parameter were also well over 1000. In the Bernoulli portion of both male and female models, all parameters had 95% credible intervals that overlapped zero (Tables 1 and 2). In the negative binomial portion of the model, the number of sampled offspring per female was predicted by the female's q (estimate of 2.72, lower 95% credible interval bound of 1.04, upper 95% credible interval bound of 4.60; Table 1). Male reproductive output was best predicted by male q (estimate of 3.12, lower 95% credible interval bound of 1.57, upper 95% credible interval bound of 4.78; Table 2). Models of both female and male reproductive output agreed that higher proportions of Yellowstone cutthroat trout ancestry would increase the number of offspring in our sample (Figure 3, though this effect was stronger for males). Posterior predictive checks revealed that the model could predict the data reasonably well, but would make some extremely high predictions with high associated error (Figures S9 and S10).

3.4 | Mate choice modelling

Each estimated parameter in model II had Gelman–Rubin R^{\wedge} statistics of 1.0 and effective sample size estimates well over 1000 (Figure S11). Posterior distribution checks show that model II captures the second peak in stay duration present in the data and predicts stay duration well for both males and females (Figure S12).

Replicates of model III also showed strong evidence of convergence. For each variable in each replicate of each model, no Gelman–Rubin R^{\wedge} statistic was below 0.99 and no effective sample size estimate was below 1000. Increasing the target acceptance

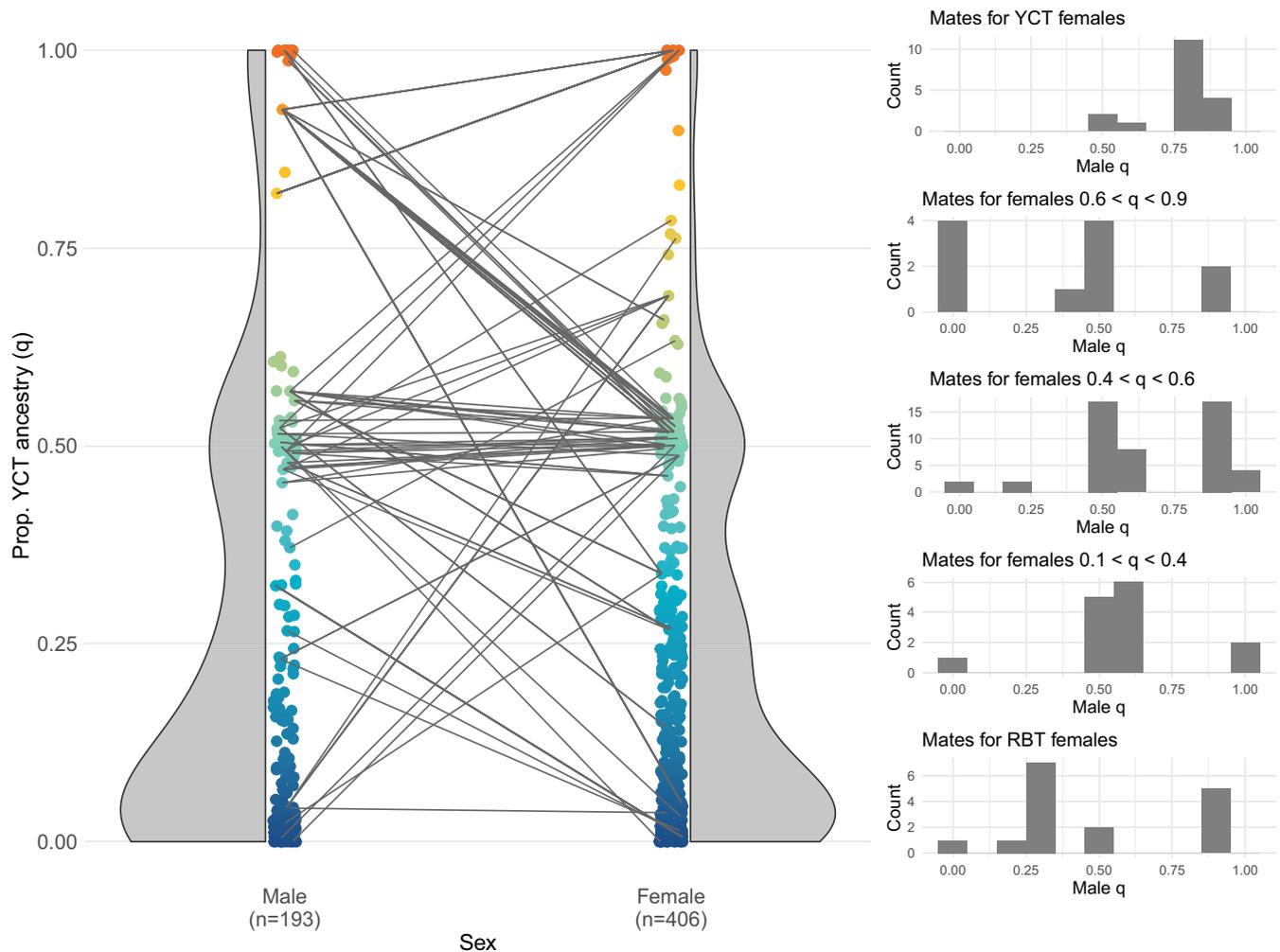


FIGURE 2 Plots detailing the inferred matings between sampled adult trout from Trout Creek. The half-violin plots show the density of male and female trout over the range of q . Each point represents an individual adult fish; the colour and y -axis position of the point represent that individual's q and lines between points represent an inferred mating. Histograms on the right of the figure show the distribution of mates for females in different bins of q [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/mec.16578)]

rate from the default 0.8 to 0.93 prevented the occurrence greater than one divergent transition in each model replicate. Examining the effects of including female identity as a random effect shows that the variability in the proportion of males chosen by each female is quite substantial, but the model's ability to partially pool data across strata reveals that this variation is in no small part due to variation in the number of males each female was exposed to.

Mate choice decisions by female trout were found to be predicted by the proportion Yellowstone cutthroat trout ancestry of the male in question, the difference in proportion Yellowstone cutthroat trout ancestry between the male and female (Figure 5), and the length of the male fish. While some 95% credible intervals for the estimate of the effects of male length did span zero, this occurred in only 2.4% of replicates (Table 3, Figure S13). The difference in q between the male and female (q dist.) was found to have a consistently negative effect on the male's probability of being chosen by the female. The average estimate across all replicates was -1.53 . Based on the mean estimate of 0.204 for the standard deviation of female-specific intercept deviations, the intra-class correlation (ICC) was estimated to be 0.012 (Nakagawa & Schielzeth, 2010). This low

ICC estimate suggests that there is little evidence for consistent individual differences in female mate choice decisions (i.e., personality). We therefore have no evidence for intrapopulation variability in female choosiness.

The effect Yellowstone cutthroat trout ancestry on reproductive output was found to partially explain the effects of Yellowstone cutthroat trout ancestry on mating detection. Over 36% of the distribution 1 estimates met or exceeded the minimum distribution 2 estimate (Figure S14). Please see the methods section for explanation of how these distributions were obtained.

3.5 | Mating simulations

Mating simulations showed that the observed proportion of Yellowstone cutthroat trout juveniles was most consistent with an intermediate strength of assortative mating or preference for Yellowstone cutthroat trout. The observed proportion of Yellowstone cutthroat trout juveniles from Middle Creek in 2020 fell at the 0.9986 quantile for the uninformed random mating simulation

TABLE 1 Results from the zero-inflated negative binomial model of female trout reproductive output

Parameter	Estimate	Lower 95% C.I. bound	Upper 95% C.I. bound
<i>Logistic model component</i>			
Intercept	0.13	-3.00	2.12
q	-1.33	-3.34	1.04
Date	-0.44	-1.50	0.83
Date ²	-0.02	-1.50	0.83
<i>Negative binomial model component</i>			
Intercept	-0.93	-2.26	0.62
q^*	2.72	1.04	4.60
Date	0.13	-0.88	1.04
Date ²	-0.97	-2.23	0.23
Length	0.10	-0.31	0.50

Notes: Asterisks indicate parameters with 95% credible intervals that do not overlap zero. The variable q ranges from 0 (unadmixed rainbow trout) to 1 (unadmixed Yellowstone cutthroat trout). The date and length covariates were centred and scaled. The logistic portion of the model assigns individuals to an “obligate zero offspring” class, and therefore a negative coefficient means a decreasing probability of being assigned to that class as the covariate increases.

TABLE 2 Results from the zero-inflated negative binomial model of male trout reproductive output

Parameter	Estimate	Lower 95% C.I. bound	Upper 95% C.I. bound
<i>Logistic model component</i>			
Intercept	-0.85	-3.28	0.67
q	-0.84	-3.04	1.37
Length	-0.46	-1.09	0.32
<i>Negative binomial model component</i>			
Intercept	-0.46	-1.23	0.37
q^*	3.12	1.57	4.78
Length	0.07	-0.41	0.55

Notes: Asterisks indicate parameters with 95% credible intervals that do not overlap zero. The variable q ranges from 0 (unadmixed rainbow trout) to 1 (unadmixed Yellowstone cutthroat trout). The date and length covariates were centred and scaled. The logistic portion of the model assigns individuals to an “obligate zero offspring” class, and therefore a negative coefficient means a decreasing probability of being assigned to that class as the covariate increases.

distribution. For the informed random mating simulation, the observed proportion fell at the distribution's 0.9102 quantile. The observed proportion fell at the 0.1476 and 0.1396 quantiles of the informed assortative mating and informed Yellowstone cutthroat trout preference simulation distributions, respectively (Figure 6).

4 | DISCUSSION

Natural selection is an important determinant of hybridization outcomes, and studying how it acts on hybrid individuals and their

parental species can provide information on the permeability of species barriers and inform conservation decisions. We used ecological data in conjunction with genome-wide genetic data to measure the efficacy of reproductive output and mate choice as partial barriers to hybridization between Yellowstone cutthroat trout and rainbow trout. We found that Yellowstone cutthroat trout ancestry was associated with a higher average number of offspring produced (Figure 4). Analysis of mating patterns showed slight female preference for similar males though matings between individuals of different ancestry backgrounds were common (Figure 2). Comparison of adult and juvenile generation ancestry distributions showed an over-representation of F2 individuals in the juvenile Trout Creek population relative to their parents (Figure 1). In conjunction with previous work identifying a dearth of F2+ hybrids in the population as a whole (Mandeville et al., 2019), we suggest that decreased fitness of F2+ hybrids may be affecting hybridization dynamics in this system. Overall, our results suggest that continued Yellowstone cutthroat trout persistence despite rampant hybridization is facilitated by outperforming rainbow trout and hybrids during reproduction, and decreased fitness in later generation hybrids.

The methods used in this study are likely applicable to many other systems studying fitness in wild populations. Leveraging molecular tools to infer mating between individuals and assess reproductive output represents a significant advancement in understanding evolution in wild populations, especially because reliably observing individual behaviour, reproduction and survival in situ is difficult or impossible for many species of interest. The multipurpose nature of genomic data also means that the same dataset used for fitness and mate choice estimation could be used for more traditional analyses in the population genomic and phylogenetic realm. We would like to emphasize, however, that successfully identifying a sufficient number of within-sample matings and parent-offspring relationships requires sampling as much of the breeding adult population as possible and minimizing any biases in sampling from the offspring generation. We attempted to minimize bias when sampling adult and juvenile generations by sampling over broad time windows during the migrations of both generations. High discharge unfortunately made our picket weir inoperable in late May of 2019 while sampling Trout Creek, which means our adult sampling is significantly sparser after that date. If the relationship between ancestry and reproductive output changes drastically depending on adult migration date, our sampling would fail to detect that pattern. Our sampling (and subsequent subsampling) of juvenile trout at both locations was intentionally evenly spread throughout the juvenile migration period, with the hope that this would ensure capturing juveniles from diverse families. If the juvenile populations were dominated by a few very large families outmigrating over short time periods, we would not be able to detect the pattern.

4.1 | Ancestry distributions

Results from *entropy* found similar distributions of ancestry to those documented in Mandeville et al. (2019) (Figure 1): unadmixed

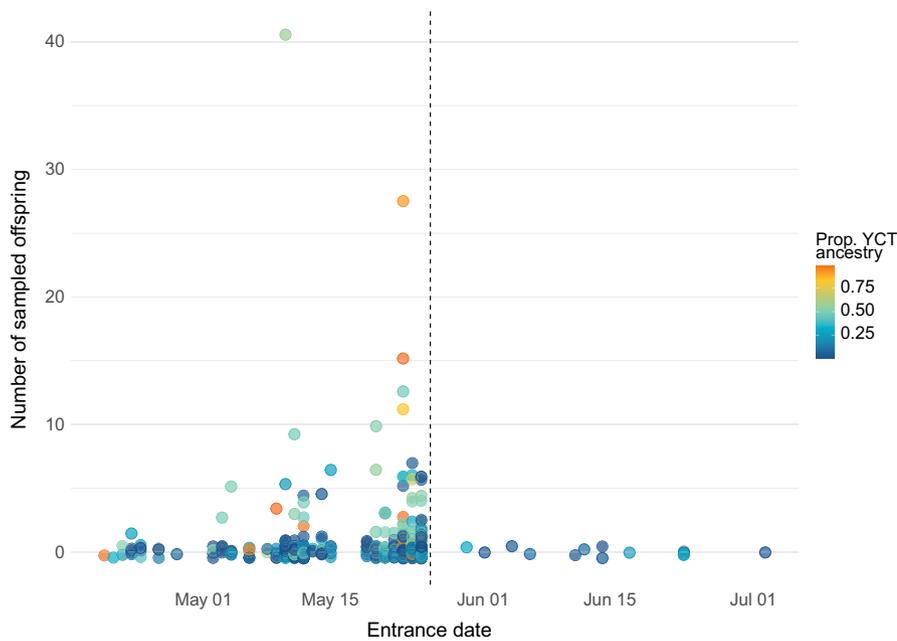


FIGURE 3 A plot showing variation in the number of sampled offspring per adult trout over time. All adults were sampled in Trout Creek in 2019. The dashed line represents the date of weir failure and peak discharge: All adults to the left of the line were sampled via weir and all adults to the right of the line were sampled via hoop nets. The x-axis shows the date each adult was sampled, and the y-axis shows the number of sampled juveniles that were determined to be the offspring of that adult. Each point is coloured by that adult's proportion Yellowstone cutthroat trout ancestry (q). Points are slightly jittered to mitigate overplotting [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Results from 1000 replicate logistic regression models of female trout mate choice

Parameter	Mean estimate	Mean lower 95% C.I. bound	Mean upper 95% C.I. bound
Intercept*	-4.866	-5.439	-4.320
Male q^*	3.575	2.697	4.468
q distance*	-1.535	-2.596	-0.509
Length*	0.366	0.081	0.667

Notes: Asterisks indicate parameters with mean 95% credible intervals that do not overlap zero. The variable q ranges from 0 (unadmixed rainbow trout) to 1 (unadmixed Yellowstone cutthroat trout). The length covariate was centred and scaled.

Yellowstone cutthroat trout individuals were rare and hybrids were more frequently the product of hybrid \times parental matings (backcross hybrids) than hybrid \times hybrid matings (F2 & F3 hybrids). Trout Creek had higher representation of rainbow trout and hybrids back-crossed with rainbow trout than Middle Creek, which is again consistent with the findings in Mandeville et al. (2019). Interestingly, results are roughly consistent even with the expanded temporal sampling included in this study (i.e., weekly sampling August–October in this study; one sampling event in September, Mandeville et al., 2019). Even with the relatively low number of F2 and F3 individuals overall, there are substantially more F2 hybrids in the Trout Creek juvenile generation than in the parental generation. While this might be expected given the number of inferred matings between male and female trout of intermediate q , it does raise questions about why F2 and F3+ hybrids are so infrequently seen within the system as adults. A relative lack of F2+ hybrid adults compared to simulations of hybridization in this river system was also documented by Mandeville et al. (2019).

If intermediate hybrids are mating with one another and producing detectable numbers of juveniles, why do we not see a larger

proportion of intermediate hybrids in the adult population? We propose that hybrid breakdown, or decreases in hybrid fitness at or after the F2 generation (Bateson, 1909; Dobzhansky, 1937; Muller, 1942) could be reducing the reproductive success or survival to maturity of F2 and F3 individuals. Consistent with this, none of the three sampled F2 adults from Trout Creek had any offspring detected (albeit 83% of all sampled adults did not have detected offspring). Though the rarity of F2 adults in this system limits the potential to statistically demonstrate this pattern, previous studies of hybridization between cutthroat and rainbow trout also support this claim. No study of hybridization between these species has documented the presence of F3 individuals (Baumsteiger et al., 2005; Bay et al., 2019; Boyer et al., 2008; Buehrens et al., 2013; Busack & Gall, 1981; Campbell et al., 2002; Campton & Utter, 1985; Corsi et al., 2013; Docker et al., 2003; Heath et al., 2010; Kovach et al., 2011, 2014; Kozfkay et al., 2007; Loxterman et al., 2014; Metcalf et al., 2008; Muhlfeld et al., 2009; Ostberg et al., 2004; Ostberg & Chase, 2012; Ostberg & Rodriguez, 2006; Pritchard et al., 2015; Rasmussen et al., 2010; Rubidge & Taylor, 2004; Strait et al., 2021; Weigel et al., 2003; Williams et al., 2007; Yau & Taylor, 2013), though this is partially due to the limitations of accurately identifying these hybrid classes with microsatellite markers or small numbers of SNPs. Detecting F3 individuals would also be more difficult if hybrid mate preferences lead to increased backcrossing rather than F2 \times F2 matings. Studies that identified non-backcrossed later generation hybrids only found relatively low numbers of F2 individuals (Bennett & Kershner, 2009; Gunnell et al., 2008; Young et al., 2001). A notable exception is Heim et al. (2020), which identified higher numbers of intermediate, non-F1 hybrids, though F3 individuals were still not identified and the assignment of traditional hybrid classes (F1, F2, etc.) was complicated by the general lack of unadmixed rainbow trout individuals in the system. Examination of unadmixed rainbow

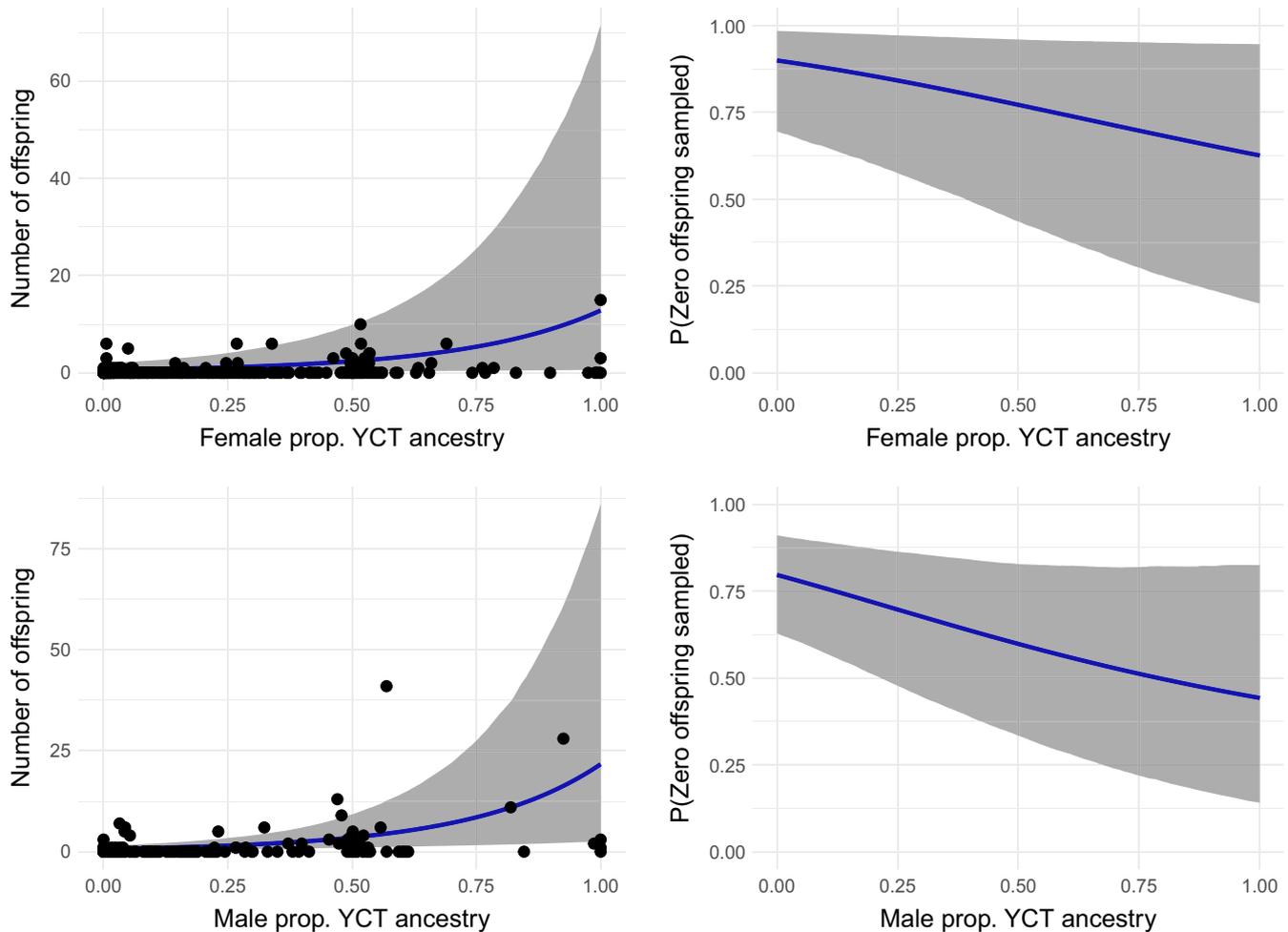


FIGURE 4 Predictions of female and male reproductive output, measured in both the average number of offspring produced (from the negative binomial model portion) and the probability of producing zero offspring (from both the logistic and negative binomial model portions), as a function of proportion Yellowstone cutthroat trout ancestry. Points in plots in the left column represent the observed number of offspring for each adult trout sampled on Trout Creek in 2019. Each line shows the mean prediction and the shaded area represents the 95% credible interval bounds of that prediction [Colour figure can be viewed at wileyonlinelibrary.com]

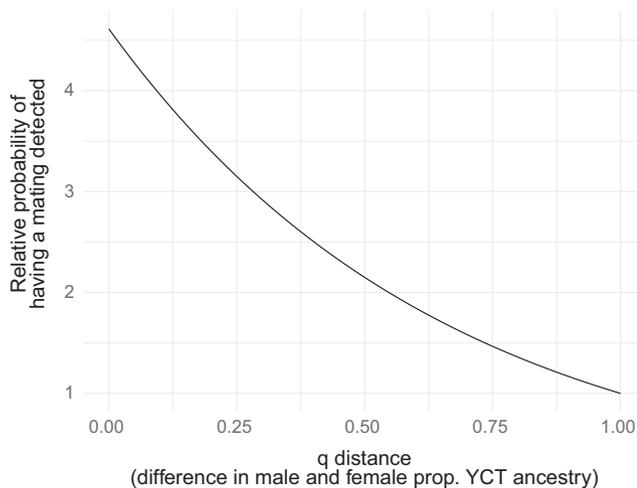


FIGURE 5 A plot showing the relationship between the relative probability of a mating being detected between a male and female fish as a function of the difference in their amount of Yellowstone cutthroat trout ancestry

trout, Yellowstone cutthroat trout and hybrid juveniles in a laboratory setting revealed no higher incidence of phenotypic abnormalities in F2 juveniles relative to the unadmixed parental species (Ostberg et al., 2011); in conjunction with our observation of F2 juveniles, these results suggest that selection against F2+ hybrid individuals is occurring at a later life stage. Selection against hybrids in later life stages has also been observed in *Populus* hybrids (Lindtke et al., 2014).

4.2 | Reproductive output and mating patterns

We detected a significant effect of individual ancestry on reproductive output as measured by both the number of offspring produced for both male and female trout. Our results are similar to that of Muhlfeld et al. (2009)'s westslope cutthroat trout work in that higher proportions of rainbow trout ancestry led to lower reproductive output. A notable departure from Muhlfeld et al. (2009)'s results was the lack of higher fitness of F1 hybrids relative to backcrossed

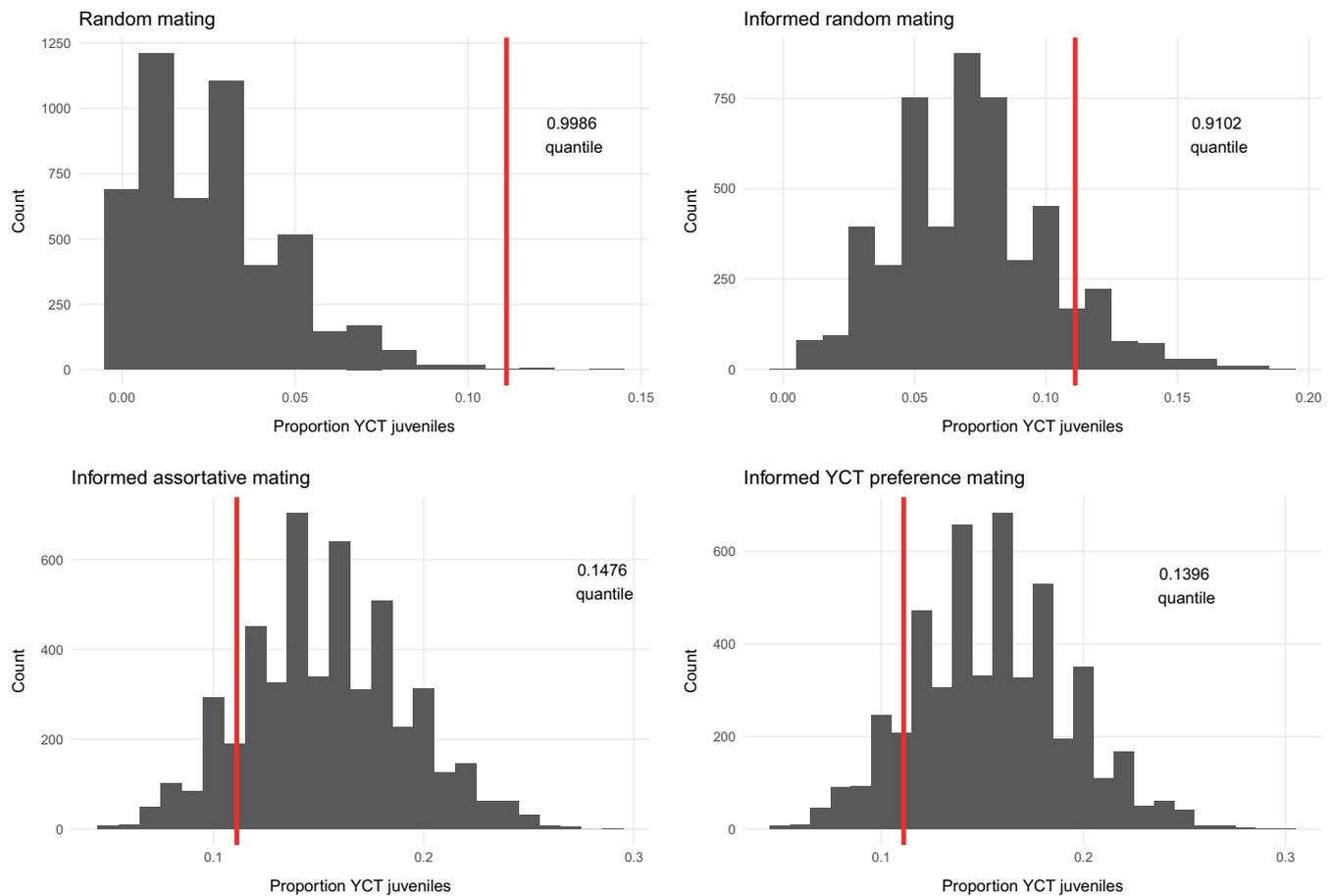


FIGURE 6 Histograms showing the distributions of proportion Yellowstone cutthroat trout juveniles from different simulations of mate choice. The red line in each plot shows the observed proportion of Yellowstone cutthroat trout juveniles from sampling of Middle Creek in 2020. The quantile of each distribution that the observed proportion falls at is displayed in each plot. Informed simulations utilize information on relative entry dates of males and females to choose mates, whereas the random simulation chooses mates entirely at random. Informed simulations differ from one another with how they decide between males with the same entry date. Each simulation was run 5000 times [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/mec.16578)]

individuals. In this river, hybrid reproductive success appears to be intermediate to that of either parental species. However, it is important to consider the differences in life history and sampling between this study and Muhlfeld et al. (2009). Muhlfeld et al. (2009) measured reproductive output via the number of age 2–3 offspring sampled, and was studying westslope cutthroat trout (*O. c. lewisi*) with a fluvial and adfluvial life histories—juveniles stay and grow in their natal tributary for several years before migrating to larger river systems. In contrast, the Yellowstone cutthroat trout studied here have an adfluvial life history where juveniles outmigrate from their natal tributaries in the fall after hatching in the spring. Thus, our fitness measurements capture substantially less duration of offspring survival than in the study design of Muhlfeld et al. (2009).

We did not find a significant relationship between adult length and the number of offspring sampled despite the documented relationship between female length and fecundity (Meyer et al., 2003); this could be because of the overall large size of female fish in this population (average 455 mm total length, Figure S15). Female fish of this size were at the limit of the female sizes examined in Meyer et al. (2003), and it could be that the variation

in female length within the Trout Creek population does not correspond to as much variation in fecundity as other portions of the size–fecundity curve.

We found a positive relationship between adult Yellowstone cutthroat trout ancestry and the number of offspring sampled (Figure 4). Although significant, this relationship is relatively small in magnitude, especially when compared to the relationship between the probability of having zero offspring sampled and ancestry. The larger effect of ancestry on the probability of having no offspring detected could result from introduced rainbow trout faring worse in streams with higher discharge during fry emergence (Fausch et al., 2001). Yellowstone cutthroat trout may delay spawning after entering the tributary until discharge decreases (DeRito et al., 2010; Henderson et al., 2000; Schmetterling, 2001), which may lower the chance of redd scouring by high currents (Elwood & Waters, 1969). Indeed, cutthroat trout appear to delay spawning relative to rainbow trout within the North Fork Shoshone River (Fennell et al., 2022). This mechanism has been previously implicated in limiting hybridization between cutthroat trout and rainbow trout (Muhlfeld et al., 2014), and could lead to greater reproductive fitness for individuals with

Yellowstone cutthroat trout ancestry in high gradient spawning tributaries like Trout Creek.

Initial examination of inferred matings from pedigree reconstruction shows little evidence for assortative mating by ancestry; matings between almost all possible combinations of ancestry classes were observed (Figure 2). However, by modelling mate choice decisions within the context in which they occur (i.e., comparing selected mates to the pool of potential mates for a given female), we demonstrate that females have a slight preference for males with ancestry more similar to their own. We did find evidence for female preference for males with higher proportions of Yellowstone cutthroat trout ancestry, but have been able to mathematically demonstrate that this effect can be partially attributed to the higher reproductive output of males with more Yellowstone cutthroat trout ancestry (Figure S14). Thus, although we were able to uncover ancestry-based predictors of mate choice, it is clear that these predictors do not represent hard-and-fast rules governing individual mating decisions. The results of this study clearly show that females of Yellowstone cutthroat trout, rainbow trout and hybrid ancestry are all capable of mating with males of any ancestry (Figure 2).

Another subspecies of cutthroat trout, the westslope cutthroat trout (*O. c. lewisi*), seems to show a stronger preference for assortative mating. McKelvey et al. (2016) reviewed the studies of westslope cutthroat trout x rainbow trout hybridization and found little evidence for random mating between individuals of different ancestries and observed few F1 individuals. One key difference in the interactions between these two cutthroat trout subspecies and rainbow trout relates to the fact that there is natural range overlap between westslope cutthroat trout and rainbow trout, whereas Yellowstone cutthroat trout and rainbow trout have no portions of natural overlap in their range (Behnke, 1992). Thus, historical interactions may have facilitated the evolution of assortative mating in westslope cutthroat trout-rainbow trout interactions (e.g., reinforcement; Ortiz-Barrientos et al., 2009), whereas there has never been opportunity for such interactions in Yellowstone cutthroat trout. Stronger assortative mating by ancestry in Westslope cutthroat trout may help them fare better than Yellowstone cutthroat trout as hybridization continues. Climate change appears to be weakening the potential mechanisms for post-zygotic selection against rainbow trout ancestry (Muhlfeld et al., 2014), and so assortative mating may be the only hope for unadmixed cutthroat trout to avoid extirpation via hybridization.

4.3 | Broader implications

The results presented here from Trout Creek demonstrate that Yellowstone cutthroat trout produce more offspring than rainbow trout or hybrid individuals. However, these findings are not immediately reconcilable with the system-wide pattern of Yellowstone cutthroat trout population declines. It is surprising to discover greater reproductive success in Yellowstone cutthroat trout given the precipitous decline of this species in the North Fork Shoshone

over the past 60 years (Nordberg et al., 2021). Variability in the reproductive success of Yellowstone cutthroat trout across tributaries of the North Fork Shoshone River could potentially explain this pattern; some spawning tributaries may produce a net positive number of Yellowstone cutthroat where others may act as a metapopulation sink. Mandeville et al. (2019) did find variability among tributaries in the amount of Yellowstone cutthroat trout ancestry present, but found that this variation was best explained by variables related to the number and location of fish stocked. Furthermore, simulations conducted by Mandeville et al. (2019) indicate that given random mating, we would not expect to see even the moderate number of Yellowstone cutthroat individuals given the number of generations of hybridization in this system. Supporting this, in the current study, the observed proportion of Yellowstone cutthroat trout juveniles in Middle Creek was best reproduced by an intermediate amount of non-random mating, and truly random mating was strongly rejected (Figure 6). Patterns of mate choice in Trout Creek also showed intermediate amounts of non-random mating. Because individuals with higher proportions of Yellowstone cutthroat trout ancestry produce more offspring but do not always mate with other high Yellowstone cutthroat trout ancestry individuals, hybridization may act as a demographic sink for Yellowstone cutthroat trout: every mating between a Yellowstone cutthroat trout and a hybrid or rainbow trout represents lost reproductive output. Therefore, the lack of completely assortative mating among Yellowstone cutthroat trout may have driven their decline as rainbow trout abundance increased, but the presence of some assortative mating may be enabling Yellowstone cutthroat trout persistence in some tributaries of the North Fork of the Shoshone River.

Our study focuses on reproductive success in a short window of the life cycle of these trout. We have not assessed fitness past the early juvenile stage, and it is possible that the decline of Yellowstone cutthroat trout in this river system is additionally driven by lower relative fitness of Yellowstone cutthroat trout compared to rainbow trout in the juvenile to adult life stages. Laboratory studies support this explanation; Yellowstone cutthroat trout juveniles exhibit slower growth in the presence of rainbow trout and hybrids compared to their growth when kept alone (Seiler & Keeley, 2009), and adult rainbow trout and hybrids have higher sustained swimming speed performance than Yellowstone cutthroat trout (Seiler & Keeley, 2007). Placed in the context of overwintering in a large lacustrine-like system like Buffalo Bill Reservoir, these factors may have large consequences for relative fitness of trout with rainbow trout versus cutthroat trout ancestry. Hybrids have performed consistently as intermediates to both parental species in the analyses presented here. If competitive advantages favour rainbow trout survival after out-migration from natal tributaries, the trade-off between reproductive output and surviving to maturity may have an optimum at some intermediate proportion of Yellowstone cutthroat ancestry. However, understanding these dynamics would require studies of juvenile growth and survival in Buffalo Bill Reservoir that take genetic ancestry into account. Analysis of juvenile growth and

survival could be accomplished via more traditional fisheries monitoring means, but would require the use of genomic data to reliably identify juvenile ancestry. More affordable genotyping methods, such as GT-seq (Campbell et al., 2015), would be an extremely effective tool for acquiring genomic data from sufficient numbers of individuals (Meek & Larson, 2019).

Overall, extirpation of parental species in this system appears to be mitigated by both pre-zygotic and post-zygotic barriers to reproduction. McKelvey et al. (2016) reviewed studies of cutthroat trout hybridization and found that only seven of 213 unique surveyed locations with rainbow trout x cutthroat trout hybridization had no parental species present, which suggests that despite likely variation in the causes of reproductive isolation (Hatfield & Schluter, 1999; Mandeville et al., 2015, 2017), enough potential for reproductive isolation exists to maintain both parental species. The degree of admixture and hybridization dynamics within each system will be uniquely shaped by the environmental context in which this species interaction occurs.

The success of Yellowstone cutthroat trout during reproduction suggests that in the North Fork Shoshone River, Yellowstone cutthroat trout might be capable of recovering with substantial management actions. However, the prezygotic reproductive barriers documented in this study are relatively weak. Limiting rainbow trout's access to spawning tributaries would enhance the probability of successful Yellowstone cutthroat trout reproduction. This strategy has been successful in Idaho, where weirs have been employed to selectively pass only Yellowstone cutthroat trout into spawning tributaries (High, 2010; McCormick & High, 2020). Stocking of Yellowstone cutthroat trout individuals may have bolstered the population in this system in the past, but may have also unintentionally introduced less-fit alleles and contributed to Yellowstone cutthroat trout decline (Nordberg et al., 2021). Increasing the number of Yellowstone cutthroat trout juveniles produced each year via rainbow trout exclusion may be a viable substitute that also does not risk replacing locally adapted alleles with those of hatchery origin.

AUTHORS' CONTRIBUTIONS

WCR, JMF, EGM, AWW and CEW designed the research. WCR, JMF and JCB performed the research. WCR analysed the data. All authors contributed to the writing of the paper.

ACKNOWLEDGEMENTS

This project was funded by the Wyoming Game and Fish Department, and we thank Mark Smith for support of the project. We thank Aaron Gloss and Hailey Barton for field assistance. CEW was partially supported by NSF Grant DEB-1556963. Computing was accomplished with an allocation from the University of Wyoming's Advanced Research Computing Center, on its Teton cluster. Research reported in this publication was partially supported by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Number P20GM103432. The content is the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Any use of trade, firm or product names is for descriptive purposes only and does not imply

endorsement by the U.S. Government. The authors thank Riley Jones and Alison Baas for their assistance in laboratory work. We thank the Wagner Lab members for discussions of this project and results, and Jeff Baldock for feedback and discussion. We thank Alex Buerkle for substantial input in discussion of the work and in feedback on the manuscript. The manuscript was also improved by comments from Josh Jahner.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Rosenthal, W. C., Fennell, J. M., Mandeville, E. G., Burckhardt, J. C., Walters, A. W., and Wagner, C. E. (2021). will-rosenthal/nfs_trout_hybrids: <https://doi.org/10.5281/zenodo.6812138>

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from our data being publicly available on the aforementioned databases. Additionally, this research has been included in a Masters of Science thesis made available to the Wyoming Game and Fish Department to inform management decisions.

ORCID

William C. Rosenthal  <https://orcid.org/0000-0003-2240-3970>

Catherine E. Wagner  <https://orcid.org/0000-0001-8585-6120>

REFERENCES

- Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C. A., Buggs, R., Butlin, R. K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S. H., Hermansen, J. S., Hewitt, G., Hudson, A. G., Jiggins, C., ... Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology*, 26(2), 229–246.
- Advanced Research Computing Center. (2018). *Teton computing environment*. University of Wyoming.
- Al-Chokhachy, R., Shepard, B. B., Burckhardt, J. C., Garren, D., Opitz, S., Koel, T. M., Nelson, L., & Gresswell, R. E. (2018). A portfolio framework for prioritizing conservation efforts for Yellowstone cutthroat trout populations. *Fisheries*, 43(10), 485–496.
- Allendorf, F. W., & Leary, R. F. (1988). Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology*, 2(2), 170–184.
- Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2011). The problems with hybrids: Setting conservation guidelines. *Trends in Ecology and Evolution*, 16(11), 613–622.
- Arnold, M. L. (1992). Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics*, 23(1), 237–261.
- Arnold, M. L. (1997). *Natural hybridization and evolution*. Oxford University Press on Demand.
- Arnold, M. L., Ballerini, E. S., & Brothers, A. N. (2012). Hybrid fitness, adaptation and evolutionary diversification: Lessons learned from Louisiana irises. *Heredity*, 108(3), 159–166.
- Barton, N. H., & Hewitt, G. M. (1989). Adaptation, speciation and hybrid zones. *Nature*, 341(6242), 497–503.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using [lme4]. *Journal of Statistical Software*, 67(1), 1–48.

- Bateson, W. (1909). *Heredity and variation in modern lights*. Darwin and Modern Science.
- Baumsteiger, J., Hankin, D., & Loudenslager, E. J. (2005). Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identifications. *Transactions of the American Fisheries Society*, 134(4), 829–840.
- Bay, R. A., Taylor, E. B., & Schluter, D. (2019). Parallel introgression and selection on introduced alleles in a native species. *Molecular Ecology*, 28(11), 2802–2813.
- Behnke, R. J. (1992). Native trout of western North America. *American Fisheries Society Monographs*, 6, i–xx+.
- Bennett, S. N., & Kershner, J. L. (2009). Levels of introgression in Westslope cutthroat trout populations nine years after changes to rainbow trout stocking programs in southeastern British Columbia. *North American Journal of Fisheries Management*, 29(5), 1271–1282.
- Boyer, M. C., Muhlfeld, C. C., & Allendorf, F. W. (2008). Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisii*). *Canadian Journal of Fisheries and Aquatic Sciences*, 65(4), 658–669.
- Brennan, A. C., Woodward, G., Seehausen, O., Muñoz-Fuentes, V., Moritz, C., Guelmami, A., Abbott, R. J., & Edelaar, P. (2014). Hybridization due to changing species distributions: Adding problems or solutions to conservation of biodiversity during global change? *Evolutionary Ecology Research*, 16(6), 475–491.
- Buehrens, T. W., Glasgow, J., Ostberg, C. O., & Quinn, T. P. (2013). Spatial segregation of spawning habitat limits hybridization between sympatric native steelhead and coastal cutthroat trout. *Transactions of the American Fisheries Society*, 142(1), 221–233.
- Bürkner, P. C. (2017). Brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, 80(1), 1–28.
- Bürkner, P. C. (2018). Advanced Bayesian multilevel modeling with the R package brms. *R Journal*, 10(1), 395–411.
- Busack, C. A., & Gall, G. A. (1981). Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). *Canadian Journal of Fisheries and Aquatic Sciences*, 38(8), 939–951.
- Campbell, M. R., Dillon, J., & Powell, M. S. (2002). Hybridization and introgression in a managed, native population of Yellowstone cutthroat trout: Genetic detection and management implications. *Transactions of the American Fisheries Society*, 131(3), 364–375.
- Campbell, N. R., Harmon, S. A., & Narum, S. R. (2015). Genotyping-in-thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources*, 15(4), 855–867.
- Campton, D. E., & Utter, F. M. (1985). Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 42(1), 110–119.
- Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M. A., Guo, J., Li, P., & Riddell, A. (2017). Stan: A probabilistic programming language. *Journal of Statistical Software*, 76(1), 1–32.
- Christie, M. R., Ford, M. J., & Blouin, M. S. (2014). On the reproductive success of early-generation hatchery fish in the wild. *Evolutionary Applications*, 7(8), 883–896.
- Clutton-Brock, T., & Sheldon, B. C. (2010). Individuals and populations: The role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology and Evolution*, 25(10), 562–573.
- Cockburn, A., Peñalba, J. V., Jaccoud, D., Kilian, A., Brouwer, L., Double, M. C., Margraf, N., Osmond, H. L., Kruuk, L. E., & van de Pol, M. (2021). HipHop: Improved paternity assignment among close relatives using a simple exclusion method for biallelic markers. *Molecular Ecology Resources*, 21(6), 1850–1865.
- Corsi, M. P., Eby, L. A., & Barfoot, C. A. (2013). Hybridization with rainbow trout alters life history traits of native westslope cutthroat trout. *Canadian Journal of Fisheries and Aquatic Sciences*, 70(6), 895–904.
- Crawford, S. S., & Muir, A. M. (2008). Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. *Reviews in Fish Biology and Fisheries*, 18(3), 313–344.
- Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158.
- Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, 10(2), 1–4.
- DeRito, J. N., Zale, A. V., & Shepard, B. B. (2010). Temporal reproductive separation of fluvial Yellowstone cutthroat trout from rainbow trout and hybrids in the Yellowstone River. *North American Journal of Fisheries Management*, 30(4), 866–886.
- Dillon, R. T., & Manzi, J. J. (1989). Genetics and shell morphology in a hybrid zone between the hard clams *Mercenaria mercenaria* and *M. campechiensis*. *Marine Biology*, 100(2), 217–222.
- Dobzhansky, T. (1937). Genetic nature of species differences. *The American Naturalist*, 71(735), 404–420.
- Docker, M. F., Dale, A., & Heath, D. D. (2003). Erosion of interspecific reproductive barriers resulting from hatchery supplementation of rainbow trout sympatric with cutthroat trout. *Molecular Ecology*, 12(12), 3515–3521.
- Dufresnes, C., Dubey, S., Ghali, K., Canestrelli, D., & Perrin, N. (2015). Introgressive hybridization of threatened European tree frogs (*Hyla arborea*) by introduced *H. intermedia* in Western Switzerland. *Conservation Genetics*, 16(6), 1507–1513.
- Echelle, A. A., & Connor, P. J. (1989). Rapid, geographically extensive genetic introgression after secondary contact between two pupfish species (Cyprinodon, Cyprinodontidae). *Evolution*, 43(4), 717–727.
- Ellstrand, N. C., & Schierenbeck, K. A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences*, 97(13), 7043–7050.
- Elwood, J. W., & Waters, T. F. (1969). Effects of floods on food consumption and production rates of a stream brook trout population. *Transactions of the American Fisheries Society*, 98(2), 253–262.
- Ender, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34(1), 76–91.
- Ender, J. A. (1986). *Natural selection in the wild*. Princeton University Press.
- Fausch, K. D., Taniguchi, Y., Nakano, S., Grossman, G. D., & Townsend, C. R. (2001). Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. *Ecological Applications*, 11(5), 1438–1455.
- Fennell, J. M., Rosenthal, W. C., Wagner, C. E., & Walters, A. W. (2022). Temporal segregation in spawning between native Yellowstone cutthroat trout and introduced rainbow trout. *Ecology of Freshwater Fish*, 1–13.
- Fitzpatrick, B. M., & Shaffer, H. B. (2007). Hybrid vigor between native and introduced salamanders raises new challenges for conservation. *Proceedings of the National Academy of Sciences*, 104(40), 15793–15798.
- Gompert, Z., Lucas, L. K., Buerkle, C. A., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2014). Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular Ecology*, 23(18), 4555–4573.
- Grabenstein, K. C., & Taylor, S. A. (2018). Breaking barriers: Causes, consequences, and experimental utility of human-mediated hybridization. *Trends in Ecology and Evolution*, 33(3), 198–212.
- Grant, P. R., Grant, B. R., Smith, J. N., Abbott, I. J., & Abbott, L. K. (1976). Darwin's finches: Population variation and natural selection. *Proceedings of the National Academy of Sciences*, 73(1), 257–261.
- Gunnell, K., Tada, M. K., Hawthorne, F. A., Keeley, E. R., & Ptacek, M. B. (2008). Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkii*

- bouvieri*) and introduced rainbow trout (*O. mykiss*) in the south fork of the Snake River watershed, Idaho. *Conservation Genetics*, 9(1), 49–64.
- Harrison, R. G. (1993). *Hybrid zones and the evolutionary process*. Oxford University Press on Demand.
- Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, 105(S1), 795–809.
- Hatfield, T., & Schluter, D. (1999). Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. *Evolution*, 53(3), 866–873.
- Heath, D., Bettles, C. M., & Roff, D. (2010). Environmental factors associated with reproductive barrier breakdown in sympatric trout populations on Vancouver Island. *Evolutionary Applications*, 3(1), 77–90.
- Heim, K. C., McMahon, T. E., Kalinowski, S. T., Ertel, B. D., & Koel, T. M. (2020). Abiotic conditions are unlikely to mediate hybridization between invasive rainbow trout and native yellowstone cutthroat trout in a high-elevation metapopulation. *Canadian Journal of Fisheries and Aquatic Sciences*, 77(9), 1433–1445.
- Henderson, R., Kershner, J. L., & Toline, C. A. (2000). Timing and location of spawning by nonnative wild rainbow trout and native cutthroat trout in the south fork Snake River, Idaho, with implications for hybridization. *North American Journal of Fisheries Management*, 20(Clancy 1988), 584–596.
- High, B. (2010). Yellowstone cutthroat trout conservation efforts on the south fork Snake River. In *Wild trout X: Conserving wild trout*. Wild Trout Symposium, Bozeman, Montana (pp. 275–284). Carol LoSapio.
- Kent, R. (1984). *Fisheries management investigations in the upper Shoshone River drainage, 1978–1982*. Wyoming Game and Fish Department. (August).
- Kiang, Y. T., & Libby, W. J. (1972). Maintenance of a lethal in a natural population of *Mimulus guttatus*. *The American Naturalist*, 106(949), 367.
- Kim, J. H. and Yang, S. Y. (1993). *Systematic studies of the genus Cobitis (Pisces: Cobitidae) in Korea IV*. The Korean Journal of Zoology. Introgressive Hybridization between Two Spined Loach Subspecies of the Genus Cobitis.
- Kovach, R. P., Eby, L. A., & Corsi, M. P. (2011). Hybridization between yellowstone cutthroat trout and rainbow trout in the upper snake river basin, Wyoming. *North American Journal of Fisheries Management*, 31(6), 1077–1087.
- Kovach, R. P., Joyce, J. E., Vulstek, S. C., Barrientos, E. M., Tallmon, D. A., & Tonn, W. (2014). Variable effects of climate and density on the juvenile ecology of two salmonids in an Alaskan lake. *Canadian Journal of Fisheries and Aquatic Sciences*, 71(6), 799–807.
- Kovach, R. P., Muhlfeld, C. C., Boyer, M. C., Lowe, W. H., Allendorf, F. W., & Luikart, G. (2015). Dispersal and selection mediate hybridization between a native and invasive species. *Proceedings of the Royal Society B: Biological Sciences*, 282(1799), 20142454.
- Kozfkay, C. C., Campbell, M. R., Yundt, S. P., Peterson, M. P., & Powell, M. S. (2007). Incidence of hybridization between naturally sympatric Westslope cutthroat trout and rainbow trout in the middle fork Salmon River drainage, Idaho. *Transactions of the American Fisheries Society*, 136(3), 624–638.
- Kruse, C. G., Hubert, W. A., & Rahel, F. J. (2000). Status of Yellowstone cutthroat trout in Wyoming Waters. *North American Journal of Fisheries Management*, 20(3), 693–705.
- Levin, D. A., & Kerster, H. W. (1968). Local gene dispersal in phlox. *Evolution*, 22(1), 130.
- Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM, 00(00), 1–3.
- Lindtke, D., Gompert, Z., Lexer, C., & Buerkle, C. A. (2014). Unexpected ancestry of *Populus* seedlings from a hybrid zone implies a large role for postzygotic selection in the maintenance of species. *Molecular Ecology*, 23(17), 4316–4330.
- Loudenslager, E. J., & Thorgaard, G. H. (1979). Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisii*) cutthroat trout. *Journal of the Fisheries Board of Canada*, 36(6), 630–635.
- Loxterman, J. L., Keeley, E. R., & Njoroge, Z. M. (2014). Evaluating the influence of stocking history and barriers to movement on the spatial extent of hybridization between westslope cutthroat trout and rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences*, 71(7), 1050–1058.
- Mandeville, E. G., Parchman, T. L., McDonald, D. B., & Buerkle, C. A. (2015). Highly variable reproductive isolation among pairs of *Catostomus* species. *Molecular Ecology*, 24(8), 1856–1872.
- Mandeville, E. G., Parchman, T. L., Thompson, K. G., Compton, R. I., Gelwicks, K. R., Song, S. J., & Buerkle, C. A. (2017). Inconsistent reproductive isolation revealed by interactions between *Catostomus* fish species. *Evolution Letters*, 1(5), 255–268.
- Mandeville, E. G., Walters, A. W., Nordberg, B. J., Higgins, K. H., Burckhardt, J. C., & Wagner, C. E. (2019). Variable hybridization outcomes in trout are predicted by historical fish stocking and environmental context. *Molecular Ecology*, 28(16), 3738–3755.
- McCormick, J. L., & High, B. (2020). Using an integrated population model to evaluate Yellowstone cutthroat trout responses to management actions. *Transactions of the American Fisheries Society*, 149(2), 135–146.
- McKelvey, K. S., Young, M. K., Wilcox, T. M., Bingham, D. M., Pilgrim, K. L., & Schwartz, M. K. (2016). Patterns of hybridization among cutthroat trout and rainbow trout in northern Rocky Mountain streams. *Ecology and Evolution*, 6(3), 688–706.
- McPhail, J. D. (1969). Predation and the evolution of a stickleback (*Gasterosteus*). *Journal of the Fisheries Research Board of Canada*, 26(12), 3183–3208.
- Meek, M. H., & Larson, W. A. (2019). The future is now: Amplicon sequencing and sequence capture usher in the conservation genomics era. *Molecular Ecology Resources*, 19, 795–803.
- Metcalfe, J. L., Siegle, M. R., & Martin, A. P. (2008). Hybridization dynamics between Colorado's native cutthroat trout and introduced rainbow trout. *Journal of Heredity*, 99(2), 149–156.
- Meyer, K. A., Schill, D. J., Elle, F. S., & Lamansky, J. A. (2003). Reproductive demographics and factors that influence length at sexual maturity of Yellowstone cutthroat trout in Idaho. *Transactions of the American Fisheries Society*, 132(2), 183–195.
- Muhlfeld, C. C., Kalinowski, S. T., McMahon, T. E., Taper, M. L., Painter, S., Leary, R. F., & Allendorf, F. W. (2009). Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters*, 5(3), 328–331.
- Muhlfeld, C. C., Kovach, R. P., Jones, L. A., Al-Chokhachy, R., Boyer, M. C., Leary, R. F., Lowe, W. H., Luikart, G., & Allendorf, F. W. (2014). Invasive hybridization in a threatened species is accelerated by climate change. *Nature Climate Change*, 4(7), 620–624.
- Muller, H. (1942). Isolating mechanisms, evolution, and temperature. *Biology Symposium*, 6, 71–125.
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: A practical guide for biologists. *Biological Reviews*, 85(4), 935–956.
- Nordberg, B. J., Mandeville, E. G., Walters, A. W., Burckhardt, J. C., & Wagner, C. E. (2021). Historical data provide important context for understanding declines in cutthroat trout. *North American Journal of Fisheries Management*, 41(3), 809–819.
- Ogutu-Ohwayo, R. (1990). The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes*, 27(2), 81–96.
- Ortiz-Barrientos, D., Grealy, A., & Nosil, P. (2009). The genetics and ecology of reinforcement: Implications for the evolution of prezygotic isolation in sympatry and beyond. *Annals of the New York Academy of Sciences*, 1168, 156–182.

- Ostberg, C. O., & Chase, D. M. (2012). Temporal genetic monitoring of hybridization between native westslope cutthroat trout and introduced rainbow trout in the Stehekin River, Washington. *Northwest Science*, 86(3), 198–211.
- Ostberg, C. O., Duda, J. J., Graham, J. H., Zhang, S., Haywood, K. P., Miller, B., & Lerud, T. L. (2011). Growth, morphology, and developmental instability of rainbow trout, yellowstone cutthroat trout, and four hybrid generations. *Transactions of the American Fisheries Society*, 140(2), 334–344.
- Ostberg, C. O., & Rodriguez, R. J. (2006). Hybridization and cytonuclear associations among native Westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River drainage, north cascades National Park. *Transactions of the American Fisheries Society*, 135(4), 924–942.
- Ostberg, C. O., Slatton, S. L., & Rodriguez, R. J. (2004). Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*) and interspecific hybrids. *Molecular Ecology*, 13(9), 2773–2788.
- Parchman, T. L., Gompert, Z., Mudge, J., Schilkey, F. D., Benkman, C. W., & Buerkle, C. A. (2012). Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, 21(12), 2991–3005.
- Pearse, D. E., Barson, N. J., Nome, T., Gao, G., Campbell, M. A., Abadía-Cardoso, A., Anderson, E. C., Rundio, D. E., Williams, T. H., Naish, K. A., Moen, T., Liu, S., Kent, M., Moser, M., Minkley, D. R., Rondeau, E. B., Briec, M. S., Sandve, S. R., Miller, M. R., ... Lien, S. (2019). Sex-dependent dominance maintains migration supergene in rainbow trout. *Nature Ecology and Evolution*, 3(12), 1731–1742.
- Pritchard, V. L., Garza, J. C., & Peacock, M. M. (2015). SNPs reveal previously undocumented non-native introgression within threatened trout populations. *Conservation Genetics*, 16(4), 1001–1006.
- Prystupa, S., McCracken, G. R., Perry, R., & Ruzzante, D. E. (2021). Population abundance in arctic grayling using genetics and close-kin mark-recapture. *Ecology and Evolution*, 11(9), 4763–4773.
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Rahel, F. J. (2000). Homogenization of fish faunas across the United States. *Science*, 288(5467), 854–856.
- Rasmussen, J. B., Robinson, M. D., & Heath, D. D. (2010). Ecological consequences of hybridization between native westslope cutthroat (*Oncorhynchus clarkii lewisii*) and introduced rainbow (*Oncorhynchus mykiss*) trout: Effects on life history and habitat use. *Canadian Journal of Fisheries and Aquatic Sciences*, 67(2), 357–370.
- Ravinet, M., Faria, R., Butlin, R. K., Galindo, J., Bierne, N., Rafajlović, M., Noor, M. A., Mehlig, B., & Westram, A. M. (2017). Interpreting the genomic landscape of speciation: A road map for finding barriers to gene flow. *Journal of Evolutionary Biology*, 30(8), 1450–1477.
- Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, 27(1), 83–109.
- Rieseberg, L. H., Archer, M. A., & Wayne, R. K. (1999). Transgressive segregation, adaptation and speciation. *Heredity*, 83(4), 363–372.
- Rubidge, E. M., & Taylor, E. B. (2004). Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarkii lewisii*) and introduced rainbow trout (*O. mykiss*). *Molecular Ecology*, 13(12), 3735–3749.
- Schmetterling, D. A. (2001). Seasonal movements of fluvial Westslope cutthroat trout in the Blackfoot River drainage, Montana. *North American Journal of Fisheries Management*, 21(3), 507–520.
- Seehausen, O., Van Alphen, J. J., & Witte, F. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, 277(5333), 1808–1811.
- Seiler, S. M., & Keeley, E. R. (2007). Morphological and swimming stamina differences between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), rainbow trout (*Oncorhynchus mykiss*), and their hybrids. *Canadian Journal of Fisheries and Aquatic Sciences*, 64(1), 127–135.
- Seiler, S. M., & Keeley, E. R. (2009). Competition between native and introduced salmonid fishes: Cutthroat trout have lower growth rate in the presence of cutthroat-rainbow trout hybrids. *Canadian Journal of Fisheries and Aquatic Sciences*, 66(1), 133–141.
- Shastri, V., Adams, P. E., Lindtke, D., Mandeville, E. G., Parchman, T. L., Gompert, Z., & Buerkle, C. A. (2021). Model-based genotype and ancestry estimation for potential hybrids with mixed-ploidy. *Molecular Ecology Resources*, 21(5), 1434–1451.
- Shedd, K. R., Lesca, E. A., Habicht, C., Knudsen, E. E., Dann, T. H., Hoyt, H. A., Prince, D. J., & Templin, W. D. (2022). Reduced relative fitness in hatchery-origin pink Salmon in two streams in Prince William sound, Alaska. *Evolutionary Applications*, 15(3), 429–446.
- Strait, J. T., Eby, L. A., Kovach, R. P., Muhlfeld, C. C., Boyer, M. C., Amish, S. J., Smith, S., Lowe, W. H., & Luikart, G. (2021). Hybridization alters growth and migratory life-history expression of native trout. *Evolutionary Applications*, 14(3), 821–833.
- Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiadere, C. R., & Seehausen, O. (2012). Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature*, 482(7385), 357–362.
- Watanabe, S. (2010). Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. *Journal of Machine Learning Research*, 11, 3571–3594.
- Weigel, D. E., Peterson, J. T., & Spruell, P. (2003). Introgressive hybridization between native cutthroat trout and introduced rainbow trout. *Ecological Applications*, 13(1), 38–50.
- Williams, I., Reeves, G. H., Graziano, S. L., & Nielsen, J. L. (2007). Genetic investigation of natural hybridization between rainbow and coastal cutthroat trout in the Copper River Delta, Alaska. *Transactions of the American Fisheries Society*, 136(4), 926–942.
- Witte, F., Goldschmidt, T., Wanink, J., Oijen, M. V., Goudswaard, K., Witte-maas, E., & Bouton, N. (1992). The destruction of an endemic species flock haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes*, 34(Wilson 1988), 1–28.
- Wolf, D. E., Takebayashi, N., & Rieseberg, L. H. (2001). Predicting the risk of extinction through hybridization. *Conservation Biology*, 15(4), 1039–1053.
- Yau, M. M., & Taylor, E. B. (2013). Environmental and anthropogenic correlates of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisii*) and introduced rainbow trout (*O. mykiss*). *Conservation Genetics*, 14(4), 885–900.
- Young, W. P., Ostberg, C. O., Keim, P., & Thorgaard, G. H. (2001). Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). *Molecular Ecology*, 10(4), 921–930.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rosenthal, W. C., Fennell, J. M., Mandeville, E. G., Burckhardt, J. C., Walters, A. W., & Wagner, C. E. (2022). Hybridization decreases native cutthroat trout reproductive fitness. *Molecular Ecology*, 31, 4224–4241. <https://doi.org/10.1111/mec.16578>