

Variable hybridization outcomes in trout are predicted by historical fish stocking and environmental context

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Abstract

Hybridization can profoundly affect the genomic composition and phenotypes of closely related species, and provides an opportunity to identify mechanisms that maintain reproductive isolation between species. Recent evidence suggests that hybridization outcomes within a species pair can vary across locations. However, we still do not know how variable outcomes of hybridization are across geographic replicates, and what mechanisms drive that variation. In this study, we described hybridization outcomes across 27 locations in the North Fork Shoshone River basin (Wyoming, USA) where native Yellowstone cutthroat trout and introduced rainbow trout co-occur. We used genomic data and hierarchical Bayesian models to precisely identify ancestry of hybrid individuals. Hybridization outcomes varied across locations. In some locations, only rainbow trout and advanced backcrossed hybrids towards rainbow trout were present, while trout in other locations had a broader range of ancestry, including both parental species and first-generation hybrids. Later-generation intermediate hybrids were rare relative to backcrossed hybrids and rainbow trout individuals. Using an individual-based simulation, we found that outcomes of hybridization in the North Fork Shoshone River basin deviate substantially from what we would expect under null expectations of random mating and no selection against hybrids. Since this deviation implies that some mechanisms of reproductive isolation function to maintain parental taxa and a diversity of hybrid types, we then modelled hybridization outcomes as a function of environmental variables and stocking history that are likely to affect prezygotic barriers to hybridization. Variables associated with history of fish stocking were the strongest predictors of hybridization outcomes, followed by environmental variables that might affect overlap in spawning time and location.

KEYWORDS

ancestry, hybridization, *Oncorhynchus*, reproductive isolation, simulation

1 | INTRODUCTION

Hybridization has the potential to substantially alter the evolutionary trajectory of species, and outcomes of hybridization also reflect underlying mechanisms of reproductive isolation between

species. While hybridization has often been studied to better understand primary divergence and speciation (Barton & Hewitt, 1985; Harrison & Larson, 2014; Ravinet et al., 2017), hybrid zones reflecting secondary contact between divergent species can reveal how isolation between close relatives is maintained in

sympatry (Wagner & Mandeville, 2017). When species hybridize following a species introduction or anthropogenic disturbance, hybridization has the potential to threaten biodiversity. Negative effects of hybridization on native species can include genetic or demographic swamping (Buerkle, Wolf, & Rieseberg, 2003; Grabenstein & Taylor, 2018; Rhymer & Simberloff, 1996; Todesco et al., 2016; Wolf, Takebayashi, & Rieseberg, 2001), potentially leading to extinction or local extirpation of the native species. Hybrids can also compete with parental species, and in some cases have higher fitness (Fitzpatrick & Shaffer, 2007; Rieseberg, Archer, & Wayne, 1999). Taken together, the potential for both ecological and genetic effects on native species has led conservation practitioners to be deeply alarmed about the prospect of interspecific hybridization with non-native species (Allendorf, Leary, Spruell, & Wenburg, 2001). However, it is far from straightforward to predict outcomes of hybridization, due to the potential for geographic variation in hybridization outcomes (Mandeville et al., 2017), incomplete understanding of the relative fitnesses of hybrids and parental species (Arnold, Ballerini, & Brothers, 2012), and the potential for environmental context to influence outcomes of hybridization (Hatfield & Schluter, 1999).

It is therefore important to precisely quantify variation in outcomes of hybridization, and to connect variable hybridization outcomes more specifically to mechanisms that cause variation in realized reproductive isolation (Gompert, Mandeville, & Buerkle, 2017). Geographic variation in outcomes of hybridization remains incompletely characterized, despite recent evidence that hybridization outcomes and mechanisms of reproductive isolation can vary across locations where a pair of species interacts (Buerkle & Rieseberg, 2001; Gompert et al., 2014; Good, Handel, & Nachman, 2008; Haselhorst & Buerkle, 2013; Mandeville et al., 2017; Nolte, Gompert, & Buerkle, 2009; Sweigart, Mason, & Willis, 2007; Teeter et al., 2010; Vines et al., 2003). In some cases, incidence of hybridization varies (e.g., Mandeville et al., 2017), while in other cases the genetic ancestry of hybrids varies, reflecting different numbers of generations of hybridization and different degrees of backcrossing to parental species (Gompert et al., 2014; Haselhorst & Buerkle, 2013; Mandeville et al., 2017) or different patterns of locus-specific introgression (Nolte et al., 2009; Teeter et al., 2010). Although it is now apparent that hybridization often varies across locations, there are several limitations common to recent studies. Some studies showing geographic variation in hybridization and introgression explore only a few locations (e.g., Mandeville, Parchman, McDonald, & Buerkle, 2015; Nolte et al., 2009; Vines et al., 2003), while others incorporate samples from many locations, but sampling is inconsistent or opportunistic due to the difficulties of sampling across the range of widespread species (e.g., Mandeville et al., 2017). In other cases, studies with excellent geographic sampling have relied on genetic data from few loci due to logistical constraints or desire for compatibility with historical data (Muhlfeld et al., 2017, 2014; Young et al., 2016). Methods that use thousands of loci sampled across the genome permit fundamentally different kinds of analyses and inferences than studies

with only handfuls of loci (Gompert et al., 2017; Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013), and represent a new opportunity to better understand hybridization.

In addition to limitations stemming from sampling, few studies have conclusively identified causes of variation in hybridization. This is a difficult undertaking, but is a necessary next step for truly understanding variation in reproductive isolation between species (Gompert et al., 2017). Hybridization in river-dwelling fishes presents a unique opportunity for understanding variation in hybridization because the structure of fish populations is constrained by the geography of river drainages, and spawning preferences often lead to multiple, distinct spawning zones across relatively small spatial scales. Therefore, it is possible to observe spatially consolidated, replicated outcomes of hybridization in these systems. This geographic constraint and local subdivision of populations in turn makes it more feasible to investigate associations between hybridization outcomes and environmental variables (as in Loxterman, Keeley, Njoroge, & Bradford, 2014; Muhlfeld et al., 2017; Young et al., 2016).

In this study, we used genomic data to describe variation in outcomes of hybridization across 27 tributaries in the North Fork Shoshone river basin (Wyoming, USA) where native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and non-native rainbow trout (*Oncorhynchus mykiss*) come into contact. Our goals were to quantify variation in outcomes of hybridization across locations, and to connect patterns of variation explicitly to environmental and ecological attributes of tributaries.

Rainbow trout have been widely introduced in the US (Halverson, 2008, 2010), and hybridization with rainbow trout has long been known to pose a threat to cutthroat trout subspecies (Allendorf & Leary, 1988; Kovach, Eby, & Corsi, 2011; Muhlfeld et al., 2009, 2014; Ostberg et al., 2011; Ostberg, Hauser, Pritchard, Garza, & Ka, 2013; Seiler & Keeley, 2007). The North Fork Shoshone River basin has experienced extensive stocking of salmonids over the past century, including rainbow trout and Yellowstone cutthroat trout. Yellowstone cutthroat trout without hybrid ancestry are now rare in this basin and throughout the historic range of the taxon (Endicott et al., 2016; Gresswell, 2011; Kruse, Hubert, & Rahel, 2000). Stocking of rainbow trout in the North Fork Shoshone River basin ended in the mid-20th century and was primarily constrained to Buffalo Bill reservoir. Stocking of Yellowstone cutthroat ended in the late 20th century in most locations (Nordberg, B. J., Mandeville, E. G., Walters, A. W., Burckhardt, J. C., & Wagner, C. E., in preparation), continuing until 2009 in the headwaters of a single drainage (Trout Creek). Despite extensive hybridization and long-term presence of rainbow trout, some unhybridized Yellowstone cutthroat trout still persist in the North Fork Shoshone river basin, defying predictions of extirpation via hybridization 20 years ago (Kruse et al., 2000).

Although hybridization dynamics in westslope cutthroat trout have been extensively studied (e.g., Allendorf & Leary, 1988, 1988; Muhlfeld et al., 2009, 2014, 2017; Bennett, Olson, Kershner, & Corbett, 2010; Hohenlohe et al., 2013; Loxterman et al., 2014; Kovach et al., 2015; Young et al., 2016), relatively little work has focused on Yellowstone cutthroat trout hybridization (but see Kovach

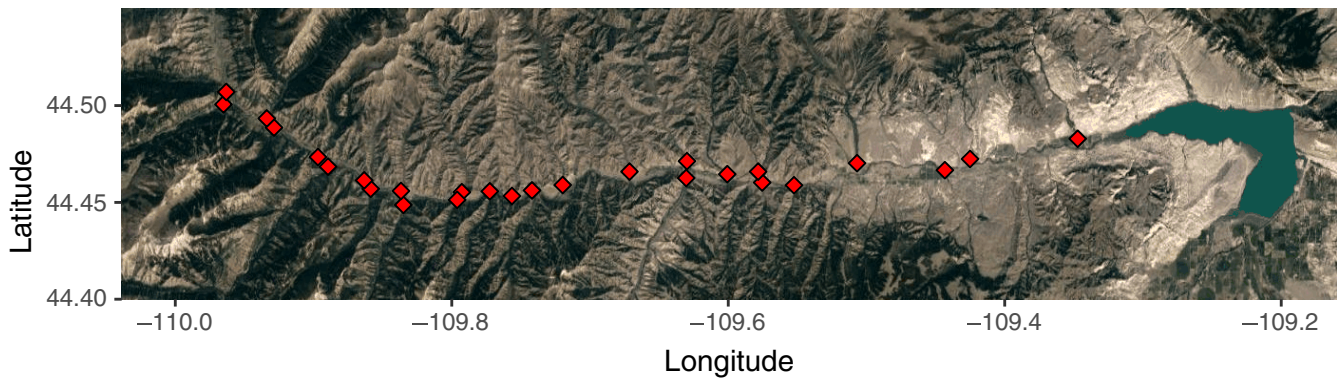


FIGURE 1 Sampling locations for this study. We sampled juvenile trout from 26 tributaries of the North Fork Shoshone River, plus the main stem of the North Fork Shoshone at the upstream limit of our sampling. In each tributary, 2–3 reaches were sampled (summarized by a single point in this map). The North Fork Shoshone River flows down out of the Absaroka Range (left) into Buffalo Bill Reservoir (right). Trout migrate upstream from the reservoir and lower river in the spring to spawn in tributaries. Fish sampled for genetic analysis comprised single cohort of fish, sampled in their natal tributaries prior to outmigration [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2011; Ostberg et al., 2013, and others). It is an open question to what extent hybridization dynamics are consistent or variable across hybridizing pairs of species or subspecies within a genus. Unlike with westslope cutthroat systems, upstream refugia for native trout are not known to exist in the North Fork Shoshone River basin (based on extensive longitudinal sampling of streams; Kruse et al., 2000), suggesting that mechanisms of reproductive isolation might differ for these Yellowstone cutthroat trout compared to westslope cutthroat trout. Identifying patterns of hybridization and connecting hybridization outcomes with stocking history and environmental data will yield a more complete understanding of how and to what extent reproductive isolation between Yellowstone cutthroat and rainbow trout is maintained in the North Fork Shoshone river basin.

2 | MATERIALS AND METHODS

2.1 | Sampling and library preparation

We sampled 26 tributaries of the North Fork Shoshone River and the main stem of the North Fork Shoshone at the upstream limit of our sampling in September 2016 (Figure 1). We sampled in the fall because of the specifics of the migratory life history of trout in this drainage. Yellowstone cutthroat trout and rainbow trout in the North Fork Shoshone River drainage use the downstream portion of the river and Buffalo Bill Reservoir in the fall and winter. Adult trout migrate into North Fork Shoshone tributaries in the spring (May–June) to spawn. Emergence occurs in mid- to late summer with most juvenile trout outmigrating in the fall (Kent, 1984). By sampling out-migrating juveniles in the fall, we sampled a single cohort of fish in their natal tributaries.

We sampled all individuals using backpack electrofishing, and sampled 2–3 reaches in each tributary. Reaches were separated by >200 m. For each reach, we sampled upstream for 10 min, capturing all fish observed in each reach. We processed fish in random order, and took fin clips for genetic analysis from the first 20 fish processed in each reach, for a total of 40–60 individuals

sampled for each tributary (sampling conducted under IACUC permit 20160803NW00248-02 from University of Wyoming). Sampling was conducted without respect to phenotype (i.e., without attempting to identify juvenile *Oncorhynchus* to species), and individuals selected for genetic analysis represent a random sample from the reaches surveyed. We stored fin clips in 95% ethanol between sampling and DNA extraction.

In addition to juvenile individuals sampled from wild populations in the North Fork Shoshone River basin, we also included reference individuals from known nonadmixed populations. These reference individuals came from three fish hatchery broodstocks maintained by the Wyoming Game and Fish Department, as well as one isolated population of Yellowstone cutthroat trout from outside the study region. Hatchery reference individuals included Yellowstone cutthroat trout from the Tensleep Hatchery, rainbow trout from the Story Hatchery, and Snake River cutthroat trout from the Auburn Hatchery. Yellowstone cutthroat from an isolated wild population in the Wind River mountains (Wyoming, USA) were also included as reference individuals.

To prepare genomic libraries for high throughput DNA sequencing, we first extracted DNA from fin tissue using Qiagen DNeasy Blood & Tissue kits according to the manufacturer's instructions, using a QIAcube robot (Qiagen, Inc.). We then prepared reduced-complexity genomic libraries according to protocols in Parchman et al. (2012), similar to previous sequencing projects involving fish with similarly sized genomes (Mandeville et al., 2015, 2017; Underwood, Mandeville, & Walters, 2016; Walters et al., 2017). This genotyping-by-sequencing method involves first fragmenting DNA using restriction enzymes (EcoRI and MseI), then ligating unique 8–10 base pair (bp) nucleotide barcodes to the DNA of each individual fish. Once barcoded, individual samples were multiplexed and amplified by PCR. For this project, we pooled 192 individuals per library for lanes 1–6, and 153 individuals in the seventh lane. Each library was sequenced on one Illumina HiSeq lane. Prior to sequencing, genomic libraries were size-selected using BluePippin (Sage Science) to retain fragments 350–450 bp in length. DNA sequencing of all seven

libraries (1,305 individual fish) on Illumina HiSeq 4000 (1 × 150) was completed at the University of Texas Genome Sequencing and Analysis Facility, Austin, Texas.

We used the Mount Moran high performance computing cluster at the University of Wyoming (Advanced Research Computing Center, 2012) for all bioinformatics processing of data and subsequent analyses. We filtered raw data to remove common contaminants, including *E.coli*, PhiX (which is used as a control in Illumina sequencing), and leftover primers, barcodes, and adaptors from the library preparation process. We then assigned reads to individual fish by parsing barcodes and removed barcode sequence from the retained sequences using a custom perl script. After parsing barcodes, we aligned reads to the Atlantic salmon genome (ICSASG_v2; Lien et al., 2016) using `bwa mem` (version 0.7.15; Li, 2013; Li & Durbin, 2009). We chose to use the Atlantic salmon genome because it is a high quality reference genome with chromosome-level assemblies, which makes overassembling paralogous loci together less likely. Additionally, we chose this reference to avoid alignment bias that might result from using the rainbow trout genome - i.e., to avoid a scenario where Yellowstone cutthroat individuals might have lower alignment rates than rainbow trout individuals. Following alignment, we excluded individual fish with <10,000 reads aligned to the reference genome (19 individuals). All subsequent analyses were done with the remaining 1,286 individuals with greater than or equal to 10,000 reads aligned.

We then identified variant sites using `samtools` and `bcftools` (version 1.3.1; Li, 2011). Rather than calling genotypes at these loci, we retained genotypes as genotype likelihoods that reflect differences in information obtained across loci. We filtered these variants using `vcftools` (version 0.1.14; Danecek et al., 2011) to acquire a set of well-supported SNPs for downstream analyses. To retain a single nucleotide variant, we required that at least 60% of individuals had data at that genomic site. We retained only SNPs where the minor allele frequency was >5%. To avoid paralogous loci, we excluded any sites with >2 alternative alleles. We also plotted per-locus coverage and verified that the distribution is unimodal, suggesting that our retained loci are unlikely to include paralogs. We retained genotype data for 12,666 SNPs in genotype likelihood format, to facilitate analyses carrying through genotype uncertainty in a Bayesian framework, allowing for better quantification of uncertainty in higher-level parameter estimates (Li, 2011; Pasaniuc et al., 2012). This probabilistic genotyping approach allowed us to retain more of the data, including loci with relatively low coverage or individuals with more missing data, because we quantified and incorporated uncertainty about genotypes in our estimates of ancestry of individuals. By pooling information across many loci and individuals, we were able to accurately estimate ancestry of individuals despite uncertainty at some loci for some individuals (Buerkle & Gompert, 2013).

Although panels of putatively diagnostic loci are frequently used for the assessment of hybrid ancestry in trout populations, we opted to use a genotyping-by-sequencing approach that includes a cross-section of all SNPs identified, rather than focusing on putatively diagnostic loci. We made this choice for several reasons. While putatively

diagnostic loci are usually sufficient for simply identifying ancestry of an individual, there are some drawbacks to using diagnostic loci to study hybridization as an evolutionary process, and population genetic processes in wild populations in general. First, diagnostic SNPs are defined relative to reference individuals, so intraspecific variation in parental species might cause misleading inferences if a SNP is not actually fixed in all populations of that species. This is particularly likely to be problematic in species with large and highly structured geographic ranges, as is the case for Yellowstone cutthroat trout. Second, SNPs that are sharply differentiated between parental taxa are more likely to be under selection or associated with reproductive isolation (Gompert & Buerkle, 2010, 2011a), and might therefore introgress unusually relative to the overall ancestry of an individual. Indeed, this property of unusual introgression patterns is the concept behind methods which seek to detect loci under selection in hybrid zones, such as `introgress`, `bgc`, and other genomic cline methods (Gompert & Buerkle, 2010, 2011b). Finally, the use of genotyping-by-sequencing methods allowed us to gather genotype data from a larger number of SNPs across the genome. The number of loci needed to identify a backcrossed individual also scales with the number of generations of hybridization that have occurred (McFarlane & Pemberton, 2019), which in this study system might be >25 generations in some locations. For all of these reasons, we chose to sample all SNPs, including those that are potentially variable in both species, for quantifying genome-wide ancestry of hybrid individuals (see also Gompert et al., 2017).

2.2 | Quantifying genomic outcomes of hybridization

To identify ancestry of individual fish, we ran `entropy` to estimate proportion of ancestry in each parental species for each individual. `entropy` is a hierarchical Bayesian model that uses Markov Chain Monte Carlo to converge on estimates of population genetic parameters such as proportion of ancestry in a cluster, and incorporates estimates of uncertainty surrounding these parameters (Gompert et al., 2014). This model requires specification of number of genetic clusters, but does not incorporate a priori assumptions about ancestry of individuals (similar to `STRUCTURE`; Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard, 2003). We ran three chains for the $k = 2$ model for 100,000 steps, discarded the first 80,000 steps as burnin, and retained every tenth sample from the posterior distribution. To confirm proper mixing and convergence of parameter estimates, we extracted posterior samples using the `rhdf5` package in `R` (Fischer & Pau, 2017), then plotted a subset of chains in `R` and visually assessed convergence and mixing of parameter estimates. From this model output, we generated estimates for q , proportion of ancestry, and Q , interspecific ancestry. Interspecific ancestry (Q) is the proportion of loci in an individual's genome that are estimated to have ancestry from two parental species.

The bivariate relationship of proportion of ancestry in each cluster (q) and interspecific ancestry (Q) allowed us to assign individuals to hybrid classes representing different histories of hybridization (e.g., F_1 ,

F_2 , backcrossed hybrids, and later-generation recombinant hybrids; as in Gompert et al., 2014, Lindtke, Gompert, Lexer, & Buerkle, 2014, Mandeville et al., 2017). Yellowstone cutthroat individuals were identified as having $q > 0.9$ and $Q \leq 0.25$. Similarly, rainbow trout individuals were identified as having $q < 0.1$ and $Q \leq 0.25$. Individuals were designated F_1 hybrids if they had q estimates 0.4–0.6 and Q estimates > 0.8 . F_2 hybrids were designated as having q 0.4–0.6 (the same as F_1 hybrids) but Q 0.4–0.6, consistent with reduced interspecific ancestry due to recombination. We described backcrosses to both parental species using an equation quantifying the proximity of an individual to the expected line between parental species at (0,0) or (1,0) and F_1 hybrids at (0.5,1). Backcrosses to rainbow trout were defined as individuals with $-0.1 < Q - 2q < 0.1$; backcrosses to Yellowstone cutthroat were defined as individuals with $1.9 < Q + 2q < 2.1$. Finally, hybrid individuals were classified as "Other" if they did not meet any of these conditions for specific hybrid classes, but were intermediate between parental species.

We chose these criteria to bin individuals into categories based on two factors: q and Q values for reference individuals in this data set representing parental species, and also on applications of the `entropy` model to other data sets, including simulated data sets (Gompert et al., 2014; Lindtke et al., 2014; Mandeville et al., 2017). Both of these lines of evidence suggest that it is reasonable to expect among-individual variation in q and Q within a hybrid class. This variation likely stems from several sources. First, there is likely stochastic variation in the exact proportion of the genome coming from each parental species within each individual. This variation might result from variation in outcomes of recombination, and potentially as a result of selection acting on some subset of ancestry combinations. Intraspecific variation in parental species can also affect q and Q estimates; Q estimates in particular are known to be depressed when there is intraspecific variation in parental species (Mandeville et al., 2017) or when parental species are closely related (see simulations in Lindtke et al., 2014). In these trout, it is likely that individuals with $< 10\%$ ancestry in one cluster are a product of variable intraspecific ancestry in parental species due to historical stocking of both rainbow and cutthroat trout from multiple sources. For some individuals, model uncertainty might also contribute to individuals having $< 10\%$ ancestry in a cluster, but since credible intervals around ancestry estimates were small (mean 95% credible interval for q was 0.0218; mean 95% credible interval for Q was 0.0421), variable intraspecific ancestry is a more plausible explanation.

We quantified hybridization outcomes for each river using five different statistics, calculated from `entropy` results. For each river, we quantified (a) proportion Yellowstone cutthroat ancestry, (b) proportion Yellowstone cutthroat individuals, (c) proportion rainbow trout individuals, (d) proportion hybrid individuals, and (e) range of backcrossing. Range of backcrossing was defined as the 95% quantiles q for individuals designated as hybrids in a stream. This summary statistic captures whether backcrossing occurs symmetrically to both parental species or is more constrained (i.e., backcrossing to only one parental species, or hybrids restricted to intermediate proportions of ancestry; as in Mandeville et al., 2017).

2.2.1 | Simulations

To better understand how our results relate to specific possible evolutionary scenarios for past hybridization in the North Fork Shoshone River basin, we constructed an individual-based simulation in R to model hybridization outcomes in terms of the summary statistics (proportion of ancestry and interspecific ancestry) that we generated for empirical data using `entropy`. For our null model, we assumed random mating, no immigration, and no selection against any genetic category, including hybrids. For each generation, parents were randomly selected for the next generation, and proportion of ancestry (q) and interspecific ancestry (Q) were calculated for each offspring based on parental q and Q . This makes simulated outcomes of hybridization directly comparable to empirical estimates of ancestry from `entropy`.

We started with a population of 1,000 individuals split between two parental species, and varied the starting proportions of parental species, including starting populations with 50%, 75%, and 90% individuals from Species 1. Since all stocking of both parental species in the North Fork Shoshone ended by 2000 in most tributaries (with the exception of stocking of Yellowstone cutthroat in the headwaters of Trout Creek until 2009; Nordberg B. J. et al., in preparation), and fish were sampled for genetic analysis in 2016, we present simulation results for five generations and 10 generations of hybridization without stocking of additional individuals from parental species, which is comparable to the known history of stocking in the North Fork Shoshone River basin (assuming generation time of 3 years, which is a conservative estimate; Behnke, 1992). We also relaxed the assumption of no immigration of parental species after the onset of hybridization in a subset of simulations; see Supporting Information for additional details.

2.3 | Environmental and ecological correlates of hybridization

To identify potential environmental dependence in genomic outcomes of hybridization, we modeled hybridization outcomes as a function of environmental and historical stocking variables. We considered several response variables, including proportion Yellowstone cutthroat trout ancestry, proportion Yellowstone cutthroat individuals, proportion rainbow trout individuals, proportion hybrid individuals, and extent of backcrossing. Each of these variables captures a different aspect of hybridization dynamics, and they are not necessarily correlated in a predictable way in all systems (Mandeville et al., 2017). In the North Fork Shoshone River tributaries, proportion Yellowstone cutthroat (both ancestry and individuals) was strongly positively correlated with both proportion hybrid individuals and extent of backcrossing. These four variables were strongly negatively correlated with proportion rainbow trout individuals. We used three of these response variables for modelling the effects of environmental and ecological predictor variables on hybridization dynamics, because different factors might predict the persistence of Yellowstone cutthroat, the propensity to hybridize, and to what extent hybrids

TABLE 1 Predictor variables for outcomes of hybridization fall into major categories according to our hypotheses about what mechanisms drive variable outcomes of hybridization in the North Fork Shoshone River Basin

Category	Predictor variables	Hypothesized mechanism
Environmental variables	Temperature, Basin size, Elevation, Aspect, Canopy, Slope, Precipitation	Environmental variables influence temporal or spatial overlap in spawning, affecting opportunity for hybridization
Stocking history	Number of Yellowstone cutthroat stocked, Number of rainbow trout stocked, Distance from core rainbow stocking location (Buffalo Bill Reservoir), Duration of stocking	Relative abundance of parental species could affect probability of hybridization versus probability of conspecific reproduction
Disturbance metrics	Proximity to road (binary, indicating same side of river as road), Total number of fish stocked from all species	Disturbance can promote hybridization by bringing species into contact or changing relative fitnesses

backcross to parental species. Our response variables were therefore proportion Yellowstone cutthroat ancestry, proportion hybrid individuals, and extent of backcrossing.

We selected predictor variables according to our hypotheses about what mechanisms might influence hybridization outcomes. Predictor variables fell into three categories: environmental variables, stocking history variables, and disturbance metrics (Table 1; similar to Yau & Taylor, 2013). Environmental variables are likely to affect hybridization outcomes by causing variation in the temporal or spatial overlap of spawning for parental species. For example, in some systems, Yellowstone cutthroat trout are known to spawn several weeks later than rainbow trout, suggesting that temporal asynchrony in spawning could be an isolating mechanism that might allow local persistence of native Yellowstone cutthroat (DeRito, Zale, & Shepard, 2010; Henderson, Kershner, & Toline, 2000). Temperature has previously been shown to be important in mediating hybridization between westslope cutthroat and rainbow trout (Muhlfeld et al., 2017, 2014; Yau & Taylor, 2013; Young et al., 2016). Environmental variables included were mean modelled August stream temperature, precipitation, basin size, slope, canopy cover, elevation, and aspect. We acquired data for all of these variables except aspect from the NorWeST Stream Temperature Regional Database (Isaak et al., 2017), which uses historical temperature data and climate models to project current and future water temperatures. We selected the modelled point closest to our fish sampling location, and checked these points visually by plotting both sampling locations and points for modelled data on a shapefile of streams in the North Fork Shoshone River basin.

We also included predictor variables related to stocking history. Since stocking history is likely to have directly affected ratios of Yellowstone cutthroat to rainbow trout in the North Fork Shoshone River basin, and therefore the probability of interspecific hybridization, we used historical stocking data to generate predictor variables for our analysis of hybridization. Prior to this study, stocking records earlier than 1980 had not been digitized. We compiled all available fish stocking data for North Fork Shoshone River between 1905 and the present (Table S2; this data set is described in more detail in Nordberg B. J. et al., in preparation). From these data, we calculated the total number of Yellowstone cutthroat and rainbow trout stocked in each tributary, and the duration of stocking at each location. Because far more rainbow trout were stocked in Buffalo Bill

Reservoir than into the North Fork Shoshone River and its tributaries directly, we also calculated distance from each sampling site to the confluence of the North Fork Shoshone River with Buffalo Bill Reservoir, using NHDPlusV2 river shapefiles for this basin (National Hydrography Data set; McKay et al., 2012) and the `riverdist` package in R (Tyers, 2017), and used this distance as a predictor variable in our models. Previous studies of introduced rainbow trout suggest that many individuals remain close to their stocking location, but a subset disperse (Bennett et al., 2010; Cresswell & Williams, 1981) and several studies suggest that distance from stocking locations of introduced rainbow trout can influence hybridization outcomes with cutthroat trout, or use indices of propagule pressure that include distance to quantify rainbow trout stocking (Bennett et al., 2010; Gunnell, Tada, Hawthorne, Keeley, & Ptacek, 2008; Muhlfeld et al., 2017).

Finally, predictor variables also included two disturbance metrics. Ecological disturbances can promote hybridization (reviewed in Grabenstein & Taylor, 2018), and variation in disturbance intensity could generate variation in hybridization outcomes. The North Fork Shoshone River basin is relatively undisturbed, as it lies almost entirely in Shoshone National Forest or Yellowstone National Park, and headwater portions of many tributary streams are in Wilderness areas. Therefore, we only considered two metrics of disturbance: proximity to the road that parallels the North Fork Shoshone main stem (quantified as a binary variable indicating whether a tributary is on the same side of the river as the road), and total number of fish stocked into a drainage (all species stocked, not just cutthroat and rainbow trout).

We used two methods to account for multicollinearity in predictors. First, we used a stepwise variance inflation factor calculation (`vif` in the `car` package in R; Fox & Weisberg, 2011) to remove predictors with severe multicollinearity that might negatively affect model inference. We sequentially removed the predictor with highest variance inflation factor until all remaining predictors had variance inflation factors of less than or equal to 10 (as suggested by Marquardt, 1970), removing first elevation and then total number of fish stocked. One predictor, distance from Buffalo Bill Reservoir, had variance inflation factor equal to 10. We also used partial correlations (`ppcor` in R; Kim, 2015) to examine which predictors were correlated after removing the effect of all other predictor variables. This analysis also supported removing elevation and total number of fish stocked. Since distance from Buffalo Bill Reservoir did not have a high partial correlation with remaining predictors following

removal of elevation and total number of fish stocked, we retained this variable in our final set of predictor variables.

We then conducted AICc-based model-averaging in a generalized linear modelling framework to rank relative importance of predictor variables associated with genomic outcomes of contact between Yellowstone cutthroat and rainbow trout in tributaries of the North Fork Shoshone River (Burnham & Anderson, 2002). Specifically, we used the `MuMIn` package in R to calculate AICc for generalized linear models using all possible additive combinations of our 11 predictor variables. We then calculated the relative importance (RI) of each predictor variable as the sum of AICc weights of models in which that predictor variable was included. Relative importance (RI) scales from 0 to 1, with 0 indicating the variable had little influence on improving model fit and 1 indicating that the variable had very strong influence on improving model fit. In addition to calculating relative importance for each predictor variable, we also identified whether there was a positive or negative relationship between that predictor and the response variable. We also examined the top five models as ranked by AICc, and identified which predictors were significant across multiple models within the best-supported subset.

3 | RESULTS

DNA sequencing resulted in 2,047,579,520 reads 150 base pairs in length, representing seven lanes of sequencing on the Illumina HiSeq 4000 platform (1×150). 1,219,638,920 of the reads matched to barcoded individual fish. Following alignment to the Atlantic salmon genome (*Salmo salar*; Lien et al., 2016) we retained 1,106,351,121 reads (90.7% of barcoded reads). We identified variable sites in the genome for 1,286 individuals, and retained 12,666 well-supported SNPs, which we then used in all subsequent analyses. Mean coverage at these retained SNPs was approximately six reads per locus per individual.

3.1 | Quantifying genomic outcomes of hybridization

We quantified outcomes of hybridization in each tributary using genome-wide analyses. We used a hierarchical Bayesian model, `entropy`, to quantify ancestry of each individual fish (Gompert et al., 2014) by estimating both proportion of ancestry from each parental

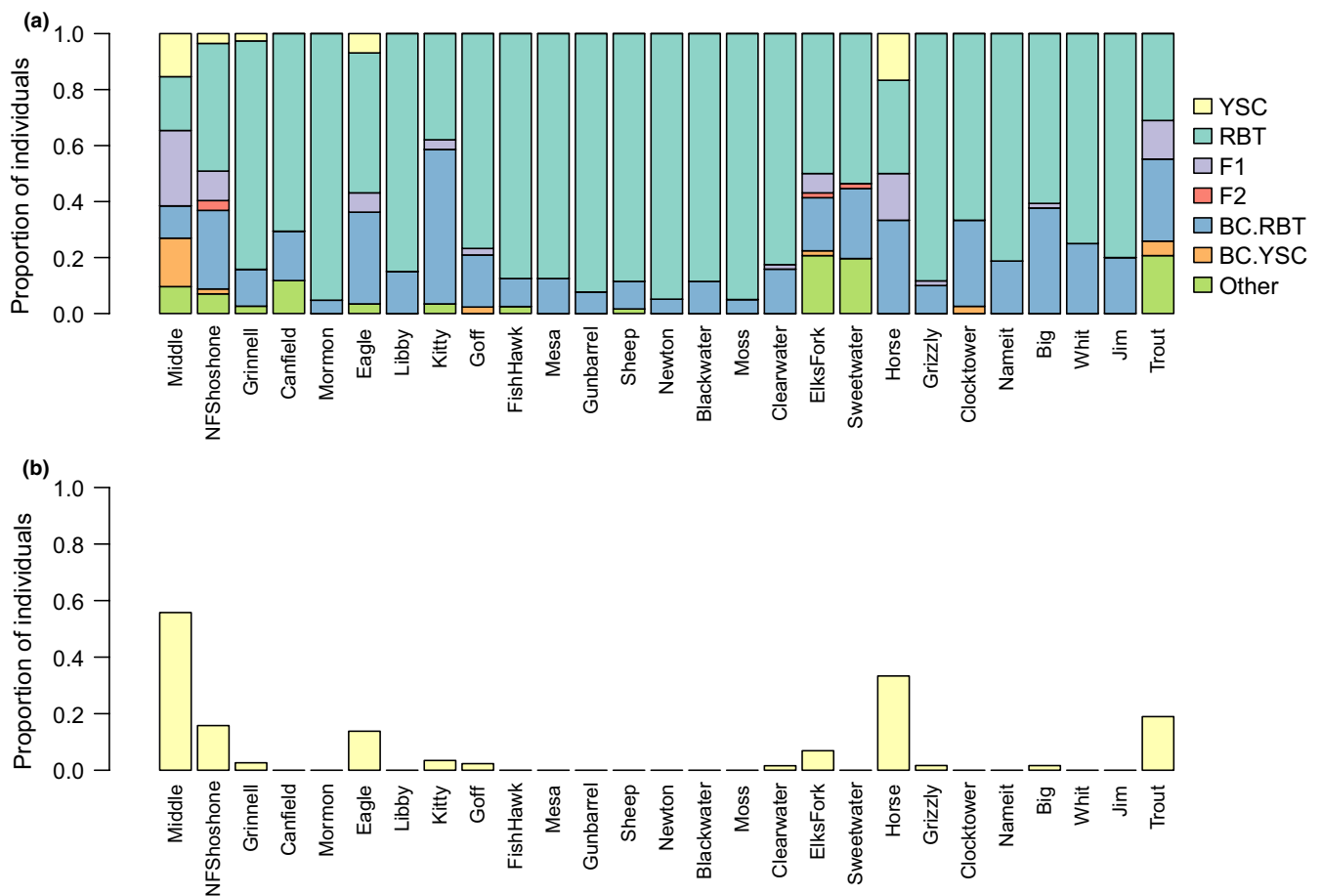


FIGURE 2 (a) Distribution of parental species and hybrids varied across locations, shown in upstream (left) to downstream (right) order. Individuals sampled included Yellowstone cutthroat trout (YSC), rainbow trout (RBT), first generation hybrids (F1), second generation hybrids (F2), backcrosses towards both parental species (BC.RBT, BC.YSC), and additional unclassified hybrids (Other). (b) Proportion of juvenile *Oncorhynchus* sampled that had at least one Yellowstone cutthroat parent. Although juvenile Yellowstone cutthroat were rare, we were able to infer adult Yellowstone cutthroat spawners from the distribution of hybrid ancestry [Colour figure can be viewed at wileyonlinelibrary.com]

source and interspecific ancestry. These two metrics allowed us to distinguish among histories of hybridization that produced an individual fish, and distinguish first generation, backcrossed, and later generation recombinant hybrids (Figures 2 and 3). Estimates for q and Q for each individual fish were quite precise (mean 95% credible

interval for q was 0.0218; mean 95% credible interval for Q was 0.0421).

Our results indicate that nonhybridized juvenile Yellowstone cutthroat trout were very rare in the areas we sampled in the North Fork Shoshone river drainage, comprising <2% of sampled juveniles. We

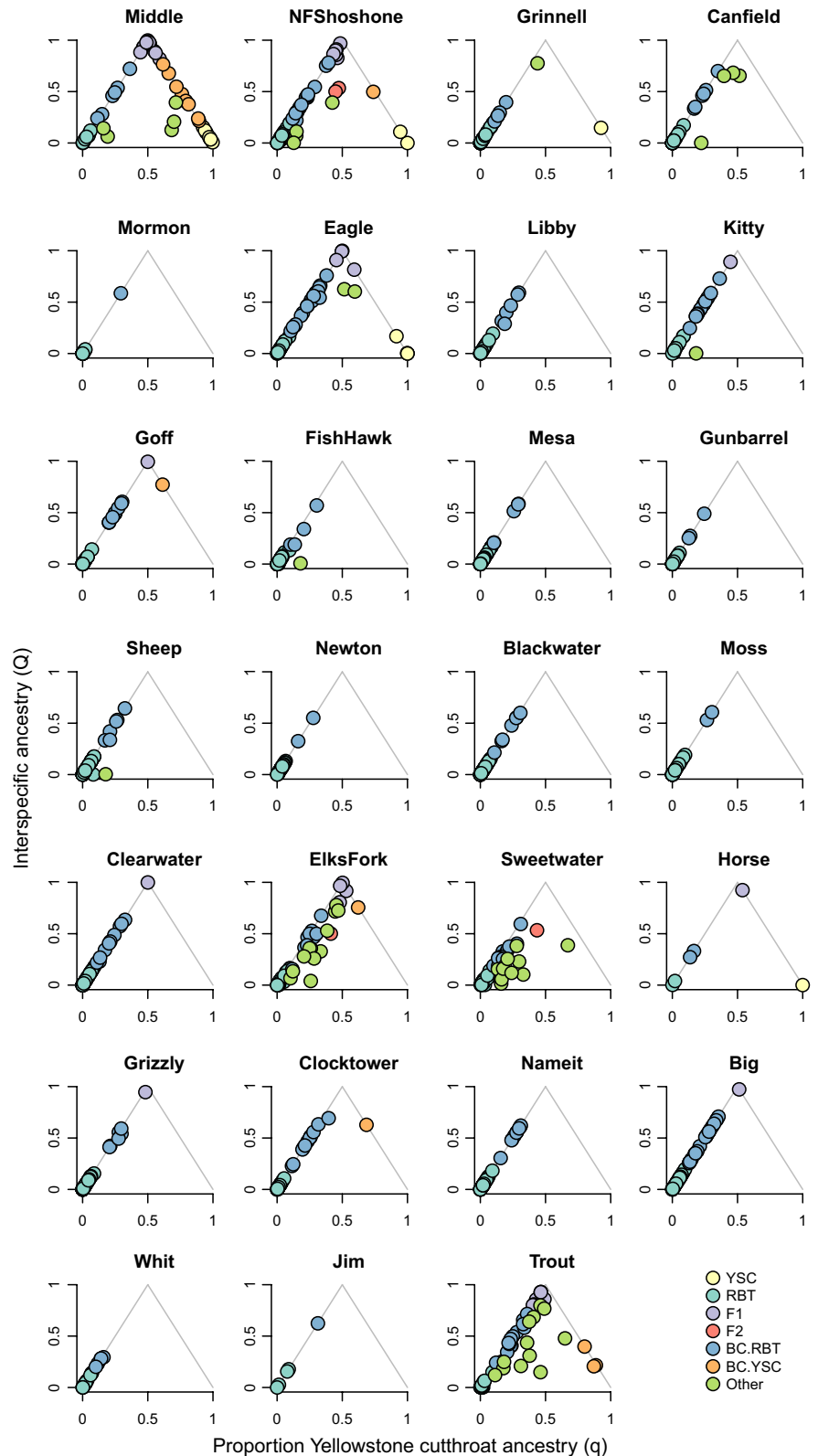


FIGURE 3 Hybridization outcomes varied among tributaries (ordered upstream-downstream) where we sampled juvenile trout. In a few locations (e.g., Middle Creek), both parental species, first generation hybrids, and backcrosses were sampled; in other locations (e.g., Blackwater Creek) only rainbow trout and backcrosses to rainbow trout were sampled. Abbreviations are the same as those used in Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

sampled Yellowstone cutthroat juveniles in only five out of 27 locations (Figure 2a), although we can infer that adult Yellowstone cutthroat used at least 11 of the tributaries due to the presence of hybrids that had at least one Yellowstone cutthroat parent (F_1 hybrids and advanced backcrosses towards Yellowstone cutthroat; see Figure 2b). Most juveniles sampled were either rainbow trout or hybrid individuals. In 13 tributaries, non-native rainbow trout comprised >80% of sampled individuals, and all of the remaining individuals were hybrids.

Hybridization outcomes varied among the 27 tributaries we sampled (Figures 2 and 3). The majority of hybrids we sampled (65% of hybrids; 19% of sampled juveniles) were backcrosses towards rainbow trout, and we sampled backcrosses towards rainbow trout at all locations. In some tributaries, we also sampled F_1 hybrids (11 locations), backcrosses to Yellowstone cutthroat trout (six locations), and F_2 or unidentified later-generation recombinant hybrids (11 locations).

3.1.1 | Simulations

We used individual-based simulations to explore expectations for genomic outcomes of hybridization under a set of null expectations. These simulations assumed random mating, no selection against any genetic category of hybrids or parental species, and no immigration of additional parental individuals after the onset of hybridization. These results were informative for comparisons against empirical data to infer potential explanations for variation in hybridization. Results of simulations (Figure 4; Figures S2–S6) suggested that under most scenarios we explored, including scenarios where starting populations of parental species were skewed to be 90% one species, hybridization with random mating and no selection against hybrids led to loss of parental taxa and convergence of hybrid individuals on very homogeneous genetic ancestry within 10 generations with a starting population of 1,000 individuals. In contrast, individuals from at least one parental species were present in each location sampled in the North Fork Shoshone River, and both summary statistics of hybrid ancestry (q and Q , proportion of ancestry and interspecific ancestry) were variable among the hybrid individuals at each sampling location. Later-generation intermediate hybrids (F_2 and beyond) were also less common in the North Fork Shoshone drainage than we would expect if there was no assortative mating or selection against hybrids. Similarly, individuals with at least one parent from a parental species - those individuals along the sides of the triangle in Figure 4 - were over-represented in the North Fork Shoshone relative to expectations generated by our simulations. Taken together, these features of our simulation results suggest that in the North Fork Shoshone River drainage, some mechanism or mechanisms of reproductive isolation operate to explain this deviation from neutral expectations.

3.2 | Environmental and ecological correlates of hybridization

We modeled hybridization outcomes as a function of environmental variables, stocking history, and disturbance metrics (Table 1)

to identify factors that contribute to the variation we observed in hybridization outcomes (Figures 2 and 3) and that might influence reproductive isolation between Yellowstone cutthroat and rainbow trout. We used a model averaging approach to rank predictors using relative importance (Burnham & Anderson, 2002). For proportion Yellowstone cutthroat ancestry (Figure 5a), total number of cutthroat trout stocked was the single most important predictor variable, and was the only significant predictor that was included in at least four of the top five models as ranked by AICc. Drainages where more Yellowstone cutthroat were stocked currently have a higher proportion of Yellowstone cutthroat alleles, suggesting that stocking has bolstered Yellowstone cutthroat populations. Only number of Yellowstone cutthroat trout stocked was a significant predictor in all of the five top models ranked by AICc.

For proportion hybrid individuals (Figure 5b), the three leading predictors ranked by relative importance were distance from Buffalo Bill Reservoir (negative relationship), total number of Yellowstone cutthroat trout stocked (positive relationship), and precipitation (positive relationship), and all three of these predictors were significant in at least four out of five of the top models ranked by AICc. Extent of backcrossing was positively predicted by two variables that were significant in at least four of the top five models, namely number of Yellowstone cutthroat stocked and mean August temperature (Figure 5c). The subset of highest-ranked models for each response variable predicted a substantial portion of the variance in the response variable (R^2 values .41–.63; see Tables S2–S4).

4 | DISCUSSION

When closely related species come into contact and hybridize, outcomes are often variable across replicate zones of contact (Gompert et al., 2017), as we observed in the study of Yellowstone cutthroat \times rainbow trout hybridization in the North Fork Shoshone River basin. While recent work has identified variation in genomic outcomes of hybridization in diverse systems (including plants, fish, mammals, and insects; Gompert et al., 2014; Haselhorst & Buerkle, 2013; Lepais et al., 2009; Mandeville et al., 2017; Nolte et al., 2009; Teeter et al., 2010), the causes and evolutionary consequences of variable hybridization remain poorly understood in most systems. Identifying why outcomes of hybridization vary across instances of contact is crucial for progress in understanding mechanisms of reproductive isolation between species, and how variable reproductive isolation is across the range of an interaction between a pair of species (Gompert et al., 2017). Better mechanistic understanding of reproductive isolation is also crucial for predicting outcomes of hybridization in study systems where hybridization poses a threat to the persistence of native species (Todesco et al., 2016).

In this study, we observed variable outcomes of hybridization across the 27 locations we sampled where Yellowstone cutthroat trout interact with rainbow trout (Figure 3). The distribution of hybrids in different classes (i.e., first-generation vs. backcrossed

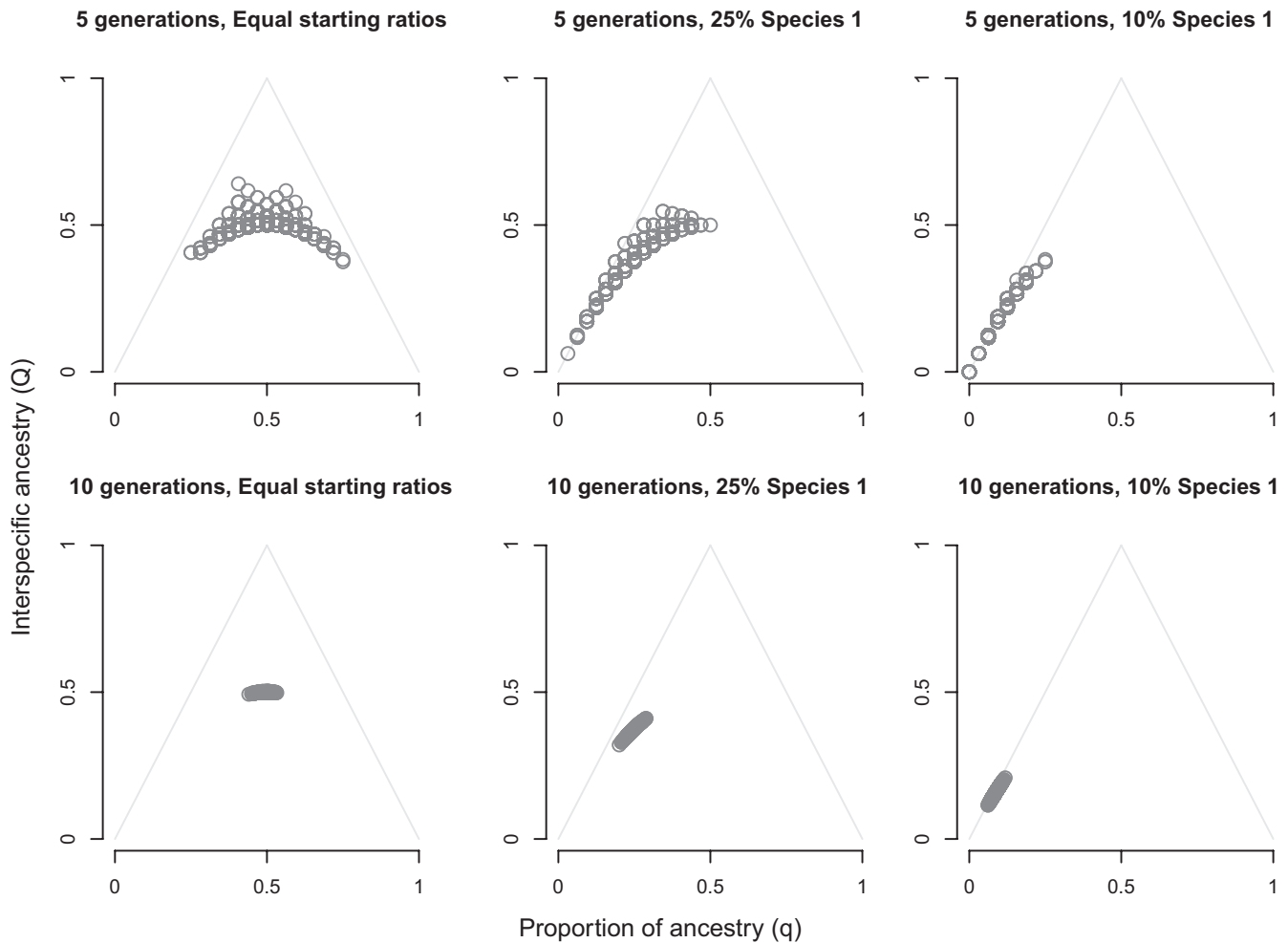


FIGURE 4 Results of simulations after 5 (top row) and 10 generations (bottom row) of hybridization for a population of 1,000 individuals, assuming random mating, no selection against hybrids or either parental species, and no immigration of new individuals. These results suggest that the fish we sampled in the North Fork Shoshone (Figure 3) include more individuals from parental species than we would expect given the number of generations of hybridization we know have occurred post-stocking. Simulations also suggest that we see fewer intermediate later generation hybrids (F_2 and later) than we would expect under null expectations of random mating and no selection

or recombinant hybrids) varied across locations (Figures 2 and 3), and in most places backcrosses towards rainbow trout were far more common than either intermediate hybrids or backcrosses towards Yellowstone cutthroat, suggesting asymmetry in backcrossing and introgression (Figure 3). Spatial heterogeneity in hybridization is consistent with other studies of river-dwelling fishes, including hybridization in *Catostomus* suckers (Mandeville et al., 2015, 2017), between sculpin species (Nolte et al., 2009), and between westslope cutthroat and rainbow trout (Loxterman et al., 2014; Muhlfeld et al., 2017; Yau & Taylor, 2013; Young et al., 2016). However the patterns we observed are distinct in several ways, in particular from observations in other cutthroat trout systems. In this study, juvenile Yellowstone cutthroat trout, the native species, were only present in a subset of locations. This contrasts with recent work on westslope cutthroat trout, which finds high relative abundances of native cutthroat trout despite ongoing introgressive hybridization with rainbow trout in some locations (Mckelvey et al., 2016; Young et al., 2016), although westslope

cutthroat are rare relative to hybrids and rainbow trout in many locations too. Additionally, Yellowstone cutthroat \times rainbow trout hybrids were sampled at all locations, in contrast with studies that have shown variation in presence of hybridization across locations (e.g., Mandeville et al., 2017; Muhlfeld et al., 2017). Spatial ubiquity of hybridization in the North Fork Shoshone River basin confirmed that rainbow and Yellowstone cutthroat trout lack complete reproductive isolation in this part of the introduced range of rainbow trout. However, simulation results also suggested that some mechanisms of reproductive isolation might maintain an overrepresentation of parental species and an underrepresentation of later-generation intermediate hybrids relative to null expectations. Overrepresentation of parental species relative to null expectations is also clear from our estimates of how many individuals have Yellowstone cutthroat parents (Figure 2), potentially suggesting some degree of assortative mating. We therefore explored relationships between potential predictors of prezygotic isolation and hybridization outcomes.

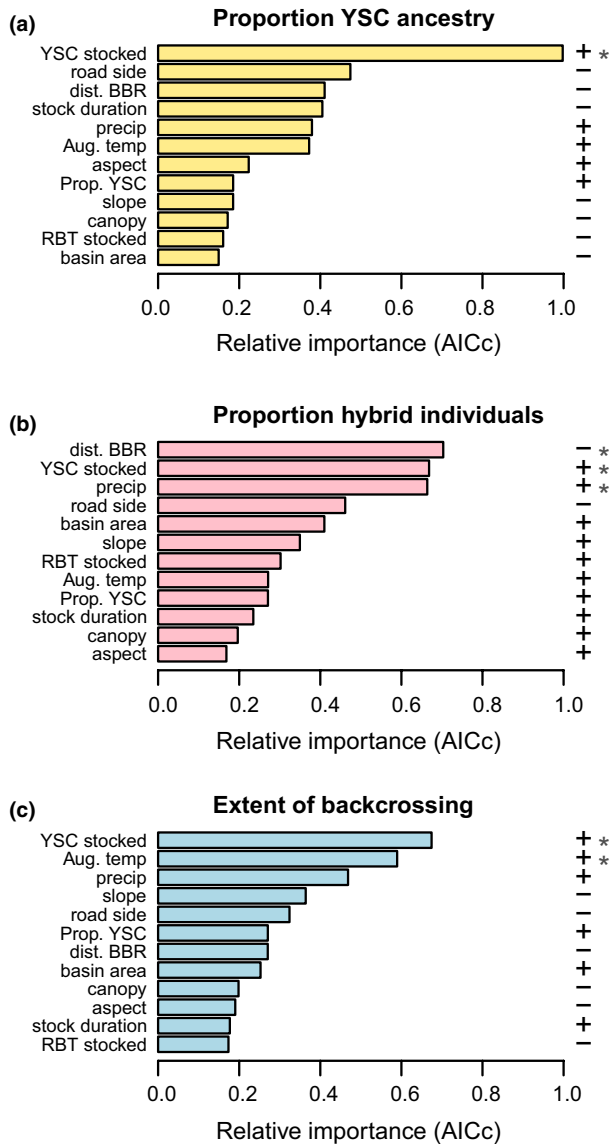


FIGURE 5 Model results identified different variables as most important for proportion Yellowstone cutthroat ancestry (a), proportion hybrid individuals in a tributary (b), and extent of backcrossing (c). Signs of relationships between predictors and an individual response variable are shown to the right of the figure. Predictors marked with red asterisks are those that were significant in at least four out of the five top ranked models by AICc. BBR, Buffalo Bill Reservoir; RBT, rainbow trout; YSC, Yellowstone cutthroat trout [Colour figure can be viewed at wileyonlinelibrary.com]

4.1 | Why do hybridization outcomes vary?

Linking variation in hybridization to specific mechanisms of reproductive isolation is essential for improving our knowledge of the nature of species boundaries (Gompert et al., 2017; Harrison & Larson, 2014), but understanding of the causal mechanisms of reproductive isolation is still limited for most species pairs. Many studies of hybridization and reproductive isolation have focused on barrier loci involved in intrinsic genetic components of reproductive isolation (Cutter, 2012; Payseur, 2010; Ravinet et al., 2017), and a few studies

have identified variation in specific genetic mechanisms of reproductive isolation (Good et al., 2008; Kozłowska, Ahmad, Jahesh, & Cutter, 2012; Larson et al., 2018; Sweigart et al., 2007; Vyskocilová, Prazanová, & Piálek, 2009). However, hybridization is known to be environment-dependent in some systems (Taylor & Donald McPhail, 2000; Vines et al., 2003), and it is likely that prezygotic isolation imposed by features of the environment substantially influences outcomes of hybridization in many species pairs. Based on the results of our simulations of hybridization, and the disparity between simulation results and empirical results (Figures 3 and 4), it is likely that in the North Fork Shoshone river drainage either postzygotic selection acts to constrain which categories of hybrids survive, or that prezygotic mechanisms produce some degree of assortative mating.

Another possibility is that there is ongoing immigration from parental species. Given that both Yellowstone cutthroat and rainbow trout in this system have an adfluvial life history (Kent, 1984) - moving upstream from Buffalo Bill Reservoir annually to spawn, then returning to the reservoir - this would not be immigration in the typical sense, but rather a shift in migratory dynamics to favour different spawning tributaries. This scenario is possible, but we have no specific evidence regarding whether such a shift is occurring. Since this river system is bounded at its downstream extent by a dam at the outflow of Buffalo Bill Reservoir, individuals cannot easily enter the North Fork Shoshone river and its tributaries from more distant populations. The addition of more individuals by stocking is also unlikely. No rainbow trout have been stocked in this system since the 1960s, although there is precedent for a lag in long distance dispersal following rainbow trout stocking (Bower & Aitken, 2008). Yellowstone cutthroat stocking also ended in nearly all locations by the year 2000 (one drainage, Trout Creek, continued to have some stocking in a headwater lake until 2009). Simulations also suggest that immigration alone is unlikely to explain the dynamics we observed in the North Fork Shoshone River.

We modeled hybridization outcomes as a function of features of the environment and history of fish stocking. The rationale for selecting environmental predictor variables was that environmental features are likely to affect spatial and temporal overlap of spawning for rainbow and Yellowstone cutthroat trout. Although spatial separation of spawning locations appears not to be a driving factor in the North Fork Shoshone River basin (Kruse, 1998), temporal separation of spawning might be very important. In other river basins Yellowstone cutthroat and rainbow trout spawning times differ by up to a month, with hybrids spawning at a more similar time to rainbow trout (DeRito et al., 2010; Henderson et al., 2000). Stocking history was included in our set of predictor variables because stocking has likely altered ratios of parental species, therefore potentially influencing the probability of hybridization.

For all three hybridization response variables that we used in our models, stocking variables were most strongly predictive of outcomes of contact between Yellowstone cutthroat trout and rainbow trout (Figure 5). The best predictor of the proportion of cutthroat ancestry at a site was the total number of Yellowstone cutthroat trout stocked in that location over the past century. For proportion

hybrid individuals, two out of the three best predictor variables were related to stocking. The farther upstream a sampling location was from Buffalo Bill Reservoir, the primary location that rainbow trout were stocked in this system, the lower the proportion of hybrids in a stream was (Figure 5b). These results suggest that hybridization dynamics have been influenced by stocking programs of Yellowstone cutthroat trout and rainbow trout in this river system, and that the persistence of Yellowstone cutthroat trout in the face of population increases of rainbow trout (Kruse, 1998, Nordberg B. J. et al., in preparation) may have been facilitated by the continued stocking of Yellowstone cutthroat trout until approximately the year 2000, long after the cessation of rainbow trout stocking in the 1960s.

The patterns of hybridization we have observed in this study are consistent with studies of westslope cutthroat × rainbow trout hybridization in the sense that both this study and studies of westslope cutthroat identify proximity to rainbow trout introduction sites, temperature, and precipitation as important predictors of hybridization outcomes (Loxterman et al., 2014; Muhlfeld et al., 2014, 2017; Yau & Taylor, 2013; Young et al., 2016). For both cutthroat subspecies, hybridization is predicted by warmer stream temperatures, although our results showed that increased temperature was more strongly predictive of backcrossing than with the proportion of hybrid individuals in a location (Figure 5). However, studies of westslope cutthroat hybridization identify cold water temperatures as a mechanism that allows westslope cutthroat to persist (Yau & Taylor, 2013; Young et al., 2016), although cold water temperatures might not entirely prevent hybridization (Muhlfeld et al., 2017). In contrast, our study suggests that increased Yellowstone cutthroat ancestry in the form of more symmetrical backcrossing to both rainbow trout and Yellowstone cutthroat trout is associated with warmer temperatures (Figure 5). It also does not appear that Yellowstone cutthroat trout in this system occupy headwater refugia as westslope cutthroat do (Kruse et al., 2000; Paul & Post, 2001), so spatial components of reproductive isolation might differ from the westslope cutthroat trout system, although we note that our sampling locations were not located in headwaters. It is unclear how thermal tolerance and thermal drivers of spawning vary among cutthroat subspecies (Bear, McMahon, & Zale, 2007), although these subspecies have generally been considered similar enough to combine data across cutthroat subspecies (e.g., Mandeville, Rahel, Patterson, & Walters, 2019). It is also likely that relationships between hybridization and temperature also depend on relative fitness and growth rates of Yellowstone cutthroat, rainbow, and hybrid trout (Al-Chokhachy, Alder, Hostetler, Gresswell, & Shepard, 2013; Ostberg et al., 2013; Seiler & Keeley, 2009), and that the exact effect of temperature on hybridization will be complex and context-dependent.

Most prior work with westslope cutthroat has relied on fewer genetic markers than we used here (but see Hohenlohe et al., 2013; Kovach et al., 2016) and has relied more on pre-selected highly differentiated SNPs. Additionally, previous studies have typically focused on maximizing and quantifying accuracy in admixture proportions (Hohenlohe et al., 2013), but not necessarily estimating the combination of ancestry at specific loci (i.e., our *Q* parameter), which

would allow differentiation between an F_1 hybrid and later-generation hybrids with the same admixture proportion. It is therefore somewhat difficult to parse whether the patterns of hybridization we have observed - overrepresentation of backcrosses, underrepresentation of later-generation intermediate hybrids - are consistent across cutthroat subspecies when they hybridize with rainbow trout. This would be an interesting avenue for future comparison. We note that the use of smaller sets of diagnostic SNPs for previous studies might have provided narrower estimates of ancestry because smaller amounts of admixture are not detectable with smaller sets of SNPs. A challenge going forward will be distinguishing between small amounts of admixture and variation in parental species, and figuring out what level of admixture is relevant for conservation. Identifying small amounts of admixture correctly is potentially extremely important, given that a study that directly quantified cutthroat trout fitness in the wild suggested that all levels of admixture detectable with a total of ten diagnostic loci were detrimental to fitness in westslope cutthroat trout hybrids (Muhlfeld et al., 2009). However, while this study offered compelling and alarming evidence of the potential fitness consequences of hybridization, we also argue that this number of fixed SNPs might not accurately characterize ancestry of all hybrid individuals, and that there is likely to be significant variation in phenotype, including fitness, among hybrid individuals (Gompert et al., 2017). We believe there is still much work to be done to understand how hybridization affects fitness, both in this system and more generally. Indeed, recent studies of locus-specific hybridization in cutthroat trout provide a more complicated picture - one recent study in Montana suggests that rainbow trout alleles are resistant to introgression and therefore likely to be disadvantageous to fitness (Kovach et al., 2016), while another recent study suggests that in western Canada, some rainbow trout alleles are present at high frequency in hybrids across multiple contact zones, potentially suggesting fitness advantages (Bay, Taylor, & Schluter, 2019).

We emphasize that hybridization may proceed differently across different subspecies of cutthroat trout, and that one contribution of this study is identifying similarities and differences between this system and others. One difference we observed is that numbers of native cutthroat in the North Fork Shoshone appear to be much lower than those in some studied locations within the westslope cutthroat trout range, especially in headwaters (McKelvey et al., 2016; Young et al., 2016). However, there do not appear to be upstream refuges for Yellowstone cutthroat in the North Fork Shoshone River system, unlike scenarios commonly encountered for westslope cutthroat (Kruse, 1998; Muhlfeld et al., 2014; Paul & Post, 2001; Young et al., 2016). Additionally, hatchery-derived westslope cutthroat have not generally been stocked in numbers as large as the number of Yellowstone cutthroat stocked into the North Fork Shoshone River (Muhlfeld et al., 2017, Nordberg B. J. et al., in preparation). In contrast with studies of westslope cutthroat trout hybridization, the stocking variables that best predict Yellowstone cutthroat × rainbow trout hybridization in the North Fork Shoshone River basin are related to stocking of cutthroat trout, not stocking of rainbow trout. There are also life history differences between Yellowstone cutthroat trout in

this system and westslope cutthroat trout. In westslope cutthroats, juveniles often outmigrate as year-old fish the spring after spawning (Behnke, 1992), although there is significant variation across locations (Downs, White, & Shepard, 1997). In contrast, outmigration in Yellowstone cutthroat in the North Fork Shoshone happens in the fall of the same year in which they are born in the summer. This life history difference may have important implications for selection in the first 1–2 years of life, which may create differences in the viability and fitness of juveniles, particularly hybrid juveniles, in these systems (Muhlfeld et al., 2009). Indeed, in other salmonids, an age 0 outmigration is associated with low juvenile survival and minimal contribution to the adult population (as in bull trout; Downs, Horan, Morgan-Harris, & Jakubowski, 2006). This contrast underscores the need to consider cutthroat trout subspecies individually, and take into account population differences in life history, when considering conservation status and management strategies. It is clear that one cutthroat trout subspecies cannot be equated with all cutthroat trout subspecies because of important life history differences like those highlighted above.

These results suggest some additional factors to bear in mind when considering cutthroat trout conservation. First, it might be important in some studies of fish populations to account for stocking of not just non-native species, but native species as well. Any stocked population will differ genetically from the original wild populations, and the long-term stability of these stocked quasi-native populations is unclear (Létourneau et al., 2017). Replacement of native populations with stocked populations of the same species might have serious implications for the conservation of native fish species if fitness or life history traits in hatchery-derived fish are substantially different from the original native populations. Specifically in this case, it is unclear to what extent the Yellowstone cutthroat trout that persist in the North Fork Shoshone are derived from stocked ancestors, or are derived from a combination of stocked ancestors and the original native populations in the system, and if there are fitness differences among fish with these different ancestries. Preliminary analyses indicate that Yellowstone cutthroat trout in the North Fork Shoshone River are very similar genetically to current Yellowstone cutthroat broodstock maintained in the Tensleep Hatchery in Tensleep, Wyoming. Although this could indicate origins of the Tensleep broodstock from populations closely related to the North Fork Shoshone River populations, we cannot distinguish between this and a predominantly hatchery-derived origin for current North Fork Shoshone Yellowstone cutthroat trout. Range-wide genetic sampling of Yellowstone cutthroat trout is needed to understand whether the genetic variation present in this system likely represents populations that were originally there, or more closely resembles genetic variation in hatchery stocks used during the stocking program of the past 100 years.

Another consideration that our work brings to light is that stocking that occurred outside of the focal area - namely, intensive stocking of rainbow trout in Buffalo Bill reservoir, downstream of the tributaries we sampled - was relevant to outcomes

of hybridization in upstream populations. In this case, it was not just stocking within the focal tributaries of this study that mattered, but also stocking downstream and adoption of a migratory life history in the North Fork Shoshone River trout populations, particularly for rainbow trout. Our examination of historical stocking records revealed that very few rainbow trout were stocked into the North Fork Shoshone River basin upstream of Buffalo Bill Reservoir (Nordberg B. J. et al., in preparation), which leads us to believe that rainbow trout currently in the tributaries of the North Fork Shoshone are likely descendants of individuals originally stocked into the reservoir. Our finding that distance from Buffalo Bill Reservoir is negatively associated with proportion hybrid individuals is therefore in agreement with previous studies have shown that propagule pressure, a metric of the intensity of stocking that includes distance from introduction location, can drive hybridization outcomes (Bennett et al., 2010; Loxterman et al., 2014; Muhlfeld et al., 2017; Yau & Taylor, 2013).

4.2 | Consequences of variable hybridization

Our observation that hybridization outcomes vary across locations for trout species in the North Fork Shoshone River, and are jointly predicted by both environmental conditions and history of fish stocking, has implications for our broader understanding of the origins and maintenance of reproductive isolation. As we study more replicate instances of interspecific hybridization, it is increasingly clear that the extent of reproductive isolation between a pair of species can vary spatially (Gompert et al., 2014; Haselhorst & Buerkle, 2013; Mandeville et al., 2017; Nolte et al., 2009; Teeter et al., 2010). This means that reproductive isolation is itself variable, not uniform across the range of an interaction between a pair of species. Variation in reproductive isolation is potentially a result of complex and polymorphic genetic and phenotypic mechanisms that maintain reproductive isolation between species (Cutter, 2012). Intrinsic genetic mechanisms of reproductive isolation like hybrid sterility are known to vary in model organisms (Kozłowska et al., 2012; Larson et al., 2018; Sweigart et al., 2007), but few studies of hybridization in the wild in non-model organisms have included enough replicate locations to robustly identify links to environmental or ecological components of reproductive isolation. Our study of Yellowstone cutthroat × rainbow trout hybridization demonstrates that hybridization dynamics can vary even on a relatively small spatial scale, and are potentially dependent on both historical ecological events (stocking) and abiotic environmental variables, although additional work is required to identify mechanisms of reproductive isolation more precisely.

Variation in outcomes of hybridization also poses a significant challenge for conservation of native species threatened by hybridization (Allendorf et al., 2001; Todesco et al., 2016; Grabenstein & Taylor, 2018). Historically, understanding threats to native species by hybridization has been complicated by the uncertain conservation value of hybridized populations (Allendorf et al., 2001; Allendorf et al., 2004), as well as ambiguity in the legal status of

hybrids with ancestry from threatened or endangered species, especially in the U.S. under the Endangered Species Act (Knudsen et al., 2005; Lind-Riehl, Mayer, Wellstead, & Gailing, 2016). While we do not advocate specific conservation actions based on this work at present, especially for individuals with 1%–10% ancestry from rainbow trout, our work suggests that a broader view of hybridization as a conservation challenge might be warranted. Hybridization is often considered as a species-level threat to genetic integrity of native species (Grabenstein & Taylor, 2018; Rhymer & Simberloff, 1996; Todesco et al., 2016; Wolf et al., 2001), but it is likely that the threat posed by hybridization varies across the range of locations where a pair of species comes into contact. This is intuitive, as we know that when closely related species come into contact where they are both native, they often hybridize infrequently (e.g., McDonald, Parchman, Bower, Hubert, & Rahel, 2008; Trotter, 1989), even if the same species hybridize extensively when brought together in locations where they are not historically sympatric. For conservation, heterogeneity in outcomes of hybridization poses a quandary: on the one hand, variation makes managing for persistence of the native species more complicated, as it might be imprudent to apply a single, common strategy across the shared geographic range of hybridizing native and introduced species. However, variation in outcomes of hybridization also presents the opportunity to prioritize conservation efforts where there are strongholds of native species to protect, and also to identify the conditions associated with persistence of native species and attempt to recreate these conditions in other locations.

5 | CONCLUSIONS

Describing variation in hybridization outcomes, and understanding the corresponding mechanisms of reproductive isolation, is crucial for better understanding geographic dimensions of reproductive isolation, and how the efficacy of isolating mechanisms can vary across space and potentially through time. In this study, we identified substantial variation in outcomes of hybridization, which is biologically relevant because it potentially reflects differences in overlap of spawning timing, spawning location, or fitness of hybrids across locations. We modeled this variation as a function of environmental variables and stocking, and were also able to identify a strong influence of stocking history on outcomes of hybridization. This result emphasizes that we need to consider the history of populations and how historical events might shape modern-day distributions of biodiversity. Along with stocking history, our work identified environmental variables as important predictors of hybridization, implying a potential interaction between the history of stocking and effects of current environmental conditions on spawning and hybridization. Additional field studies are needed to identify specific links between these predictors and hybridization outcomes, and quantify relative fitness of parental species and hybrids. In this system, and likely other systems too, multiple mechanisms interacted to determine the efficacy of reproductive isolation between species, with

the potential for extensive variation across geographic locations. Beyond the implications of this work for our understanding of the evolution of reproductive isolation, we also identify challenges and opportunities for conservation going forward. While heterogeneity makes managing for persistence of native fish species more challenging from a policy standpoint, it also suggests that some populations might have greater potential for conservation success due to variation in hybridization dynamics.

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AUTHOR CONTRIBUTIONS

E.G.M., A.W.W., J.C.B., and C.E.W. planned research and designed sampling strategy. J.C.B. coordinated field sampling and contributed expertise in the system. K.H. extracted DNA and assisted E.G.M. with genomic library preparation. E.G.M. and C.E.W. planned and executed genomic analyses. E.G.M. conducted the simulation study. B.J.N. compiled and analyzed historical fish stocking data. E.G.M., A.W.W., and C.E.W. modelled environmental and ecological correlates of hybridization. All authors contributed to writing and revising the manuscript.

DATA AVAILABILITY STATEMENT

Data and scripts used for analysis are available in the Data Dryad repository (DOI: <https://doi.org/10.5061/dryad.6s7d02q>).

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SUPPORTING INFORMATION

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

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