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Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout

Clint C. Muhlfeld[1,2]* | Ryan P. Kovach[1]* | Robert Al-Chokhachy[3] | Stephen J. Amish[2] | Jeffrey L. Kershner[3] | Robb F. Leary[4] | Winsor H. Lowe[5] | Gordon Luikart[2] | Phil Matson[2] | David A. Schmetterling[4] | Bradley B. Shepard[6] | Peter A. H. Westley[7] | Diane Whited[2] | Andrew Whiteley[8] | Fred W. Allendorf[5]

[1]Northern Rocky Mountain Science Center, U.S. Geological Survey, West Glacier, MT, USA

[2]Flathead Lake Biological Station, Division of Biological Sciences, University of Montana, Polson, MT, USA

[3]Northern Rocky Mountain Science Center, U.S. Geological Survey, Bozeman, MT, USA

[4]Montana Fish, Wildlife and Parks, Missoula, MT, USA

[5]Division of Biological Sciences, University of Montana, Missoula, MT, USA

[6]B.B. Shepard & Associates, Livingston, MT, USA

[7]School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK, USA

[8]Wildlife Biology Program, Department of Ecosystem and Conservation Sciences, College of Forestry and Conservation, University of Montana, Missoula, MT, USA

Correspondence
Clint C. Muhlfeld, Northern Rocky Mountain Science Center, U.S. Geological Survey, Glacier National Park, West Glacier, MT, USA.
Email: cmuhlfeld@usgs.gov

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Abstract

Hybridization between invasive and native species, a significant threat to worldwide biodiversity, is predicted to increase due to climate-induced expansions of invasive species. Long-term research and monitoring are crucial for understanding the ecological and evolutionary processes that modulate the effects of invasive species. Using a large, multidecade genetics dataset (N = 582 sites, 12,878 individuals) with high-resolution climate predictions and extensive stocking records, we evaluate the spatiotemporal dynamics of hybridization between native cutthroat trout and invasive rainbow trout, the world's most widely introduced invasive fish, across the Northern Rocky Mountains of the United States. Historical effects of stocking and contemporary patterns of climatic variation were strongly related to the spread of hybridization across space and time. The probability of occurrence, extent of, and temporal changes in hybridization increased at sites in close proximity to historical stocking locations with greater rainbow trout propagule pressure, warmer water temperatures, and lower spring precipitation. Although locations with warmer water temperatures were more prone to hybridization, cold sites were not protected from invasion; 58% of hybridized sites had cold mean summer water temperatures (<11°C). Despite cessation of stocking over 40 years ago, hybridization increased over time at half (50%) of the locations with long-term data, the vast majority of which (74%) were initially nonhybridized, emphasizing the chronic, negative impacts of human-mediated hybridization. These results show that effects of climate change on biodiversity must be analyzed in the context of historical human impacts that set ecological and evolutionary trajectories.

KEYWORDS
biodiversity, climate change, cutthroat trout, hybridization, introgression, invasion, propagule pressure, translocation

*These authors contributed equally.

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1 | INTRODUCTION

Widespread species translocations and subsequent invasions have led to unprecedented reorganization of Earth's biota (Mooney & Cleland, 2001; Simberloff et al., 2013; Vitousek, Mooney, Lubchenco, & Melillo, 1997). Rates of human-mediated biological invasions are accelerating, causing declines and extirpation of native species, loss of ecosystem function and services, and ultimately biotic homogenization of floras and faunas (Lockwood & McKinney, 2001; McKinney & Lockwood, 1999; Olden, Poff, Douglas, Douglas, & Fausch, 2004; Rahel, 2000). Future climate change is predicted to facilitate biological invasions and exacerbate biodiversity loss, as species differentially track their climatic niches (Parmesan, 2006; Root et al., 2003; Walther et al., 2002). The impacts of climate change and invasive species are often considered independently, yet these key elements of global environmental change are occurring concomitantly and likely interacting in complex ways to affect future patterns of biodiversity (Rahel & Olden, 2008; Sorte et al., 2013). Our understanding of climate-mediated invasions is hindered by the paucity of long-term datasets covering large spatial scales. Space-for-time substitutions are commonly used to circumvent these data limitations (Blois, Zarnetske, Fitzpatrick, & Finnegan, 2013). However, this approach limits our ability to assess how environmental heterogeneity affects species invasions, and to test whether spatial patterns accurately reflect and predict temporal dynamics (Hellmann, Byers, Bierwagen, & Dukes, 2008). Long-term empirical data are needed to understand, predict, and mitigate the severe and chronic effects of species invasions (Strayer, Eviner, Jeschke, & Pace, 2006).

Hybridization and genetic introgression between native and invasive species (invasive hybridization) are a major evolutionary consequence of human-mediated biological invasions (Simberloff, 2014) that is responsible for the genomic extinction of many populations, lineages, and species (Allendorf & Leary, 1988; Rhymer & Simberloff, 1996). Invasive hybridization is especially problematic for fishes because there are limited pre- or postzygotic barriers to introgression among closely related taxa, and humans intentionally propagated and released staggering numbers of non-native fish over the previous century (Gozlan, Britton, Cowx, & Copp, 2010). This is true for salmonids in the genera *Oncorhynchus*, *Salmo*, and *Salvelinus*, where widespread introductions for sportfishing and harvest have resulted in extensive introgression among populations and species (Allendorf & Leary, 1988; Araguas, Sanz, Pla, & García-Marín, 2004; Escalante et al., 2014; Harbicht, Alshamlih, Wilson, & Fraser, 2014; Heath, Bettles, & Roff, 2010; Marie, Bernatchez, & Garant, 2012). Invasive hybridization in these taxa could be intensified by climate change, which is predicted to profoundly affect coldwater aquatic ecosystems by increasing stream temperatures, altering streamflow regimes, and increasing the frequency and severity of disturbance events, such as extreme drought and floods (Woodward, Perkins, & Brown, 2010), with substantial consequences for the spread of introduced salmonid species and their genes (Almodovar, Nicola, Ayllon, & Elvira, 2012; Comte & Grenouillet, 2013; Kelly, Whiteley, & Tallmon, 2010; Kovach, Muhlfeld, et al., 2016; Wenger et al., 2011).

Hybridization with non-native rainbow trout *Oncorhynchus mykiss*, the world's most widely introduced fish, threatens all cutthroat trout *Oncorhynchus clarkii* subspecies in western North America (Behnke, 1992). These species produce fertile offspring, and introgression often continues until the native cutthroat genomes are supplanted by hybrids (Allendorf & Leary, 1988). Despite strong selection against rainbow trout and their hybrids (Kovach et al., 2015; Kovach, Hand, et al., 2016; Muhlfeld, Kalinowski, et al., 2009), hybridization between these species is widespread in populations inhabiting a range of environmental conditions (Muhlfeld, McMahon, Boyer, & Gresswell, 2009; Rubidge & Taylor, 2005; Yau & Taylor, 2013). There is growing evidence that continuous immigration of dispersing hybrids from historical stocking locations is the primary vector for the spread of introgression (Bennett, Olson, Kershner, & Corbett, 2010; Boyer, Muhlfeld, & Allendorf, 2008; Kovach et al., 2015; Muhlfeld, McMahon, Belcer, & Kershner, 2009). Moreover, climate change could be promoting expansion of invasive hybridization in native trout through alterations in temperature and streamflow regimes in ways that are conducive to rainbow trout and hybrid dispersal, leading to increased sympatry with cutthroat trout (Muhlfeld et al., 2014). For example, relative to cutthroat trout, rainbow trout prefer warmer temperatures, lower spring flows, and earlier spring runoff and tolerate greater environmental disturbance (Bear, McMahon, & Zale, 2007; Fausch, Taniguchi, Nakano, Grossman, & Townsend, 2001; Hitt, Frissell, Muhlfeld, & Allendorf, 2003; Muhlfeld, McMahon, Belcer, et al., 2009; Muhlfeld, McMahon, Boyer, et al., 2009). High spring flows and peak pulses in the snow melt hydrograph caused by spring precipitation, which strongly influence the timing and magnitude of streamflow in the Northern Rockies (Pederson et al., 2011), can limit rainbow trout recruitment outside their native range because such flows can scour eggs from spawning nests or wash away newly emerged juveniles (Fausch et al., 2001; Muhlfeld et al., 2014). Therefore, climate-induced periods of reduced and earlier spring snow melt and precipitation (e.g., drought or decreases in orographic precipitation enhancement) may promote expansion of rainbow trout from source populations, threatening native trout genomes (Muhlfeld et al., 2014). A holistic understanding of the dynamics of invasion and hybridization requires explicitly addressing the interaction of climate and historical effects of stocking.

Here, we used an extensive long-term genetics monitoring dataset (Figure 1) to test the effects of climatic variation and past stocking on the spread of invasive hybridization between non-native rainbow trout and native westslope cutthroat trout *O. clarkii lewisi* throughout the Northern Rocky Mountains of the United States. This region (210,000 km^2) spans three major continental river drainages (the Columbia, Missouri, and South Saskatchewan River basins) in which these trout species evolved in allopatry. Concerns about the effects of introgressive hybridization on the region's iconic cutthroat trout motivated the development of genetic techniques and extensive monitoring over the past four decades (Figure 1), resulting in one of the most comprehensive genetic datasets for any fish species worldwide. In total, we combined genetic data from

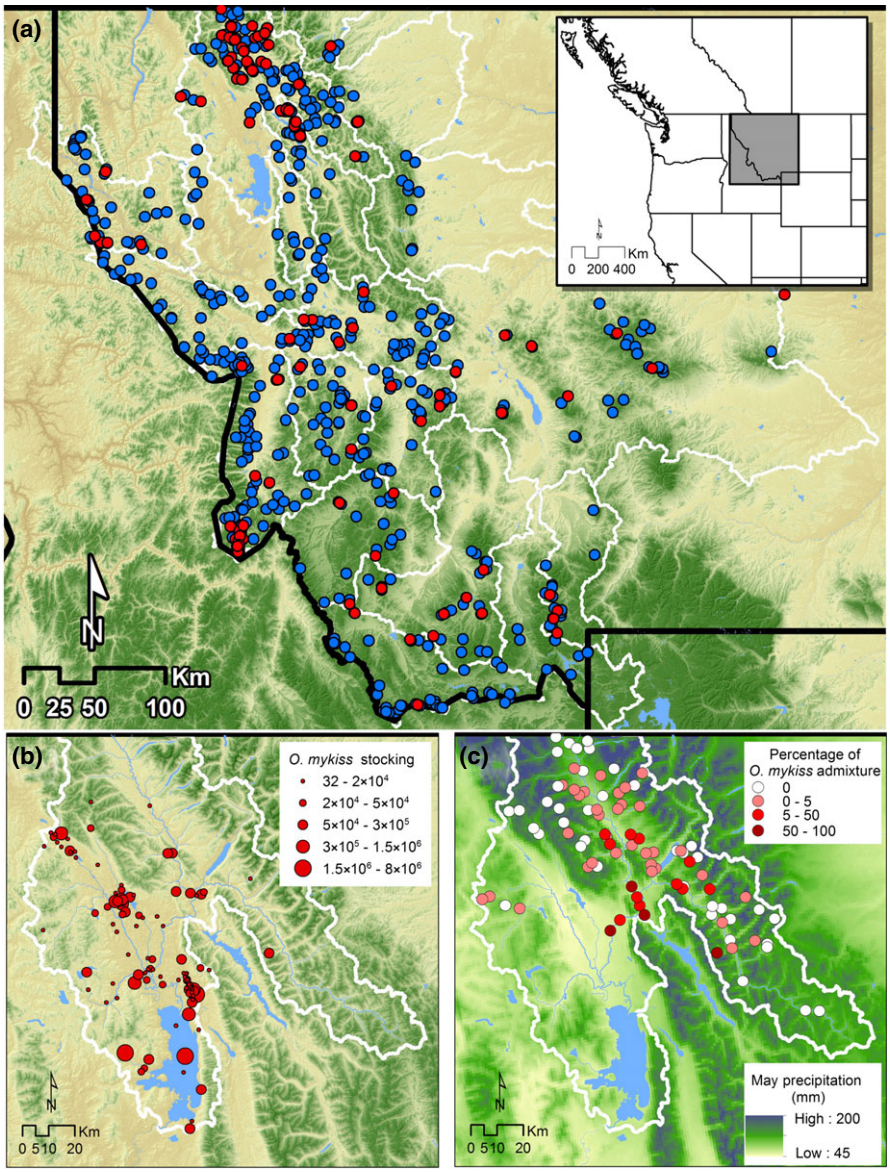

FIGURE 1 Study area of long-term genetic monitoring locations and non-native rainbow trout (*Oncorhynchus mykiss*) stocking sites in the Northern Rocky Mountains, USA. (a) Study domain and fish genetic collection sites for spatial (blue dots, $N = 582$) and temporal (red dots, $N = 98$) analyses. Boundaries of major river basins are shown in white; state boundaries (and U.S.–Canadian border) in black. (b) Subset of historical (1924–1980) rainbow trout stocking data ($N = 20{,}550{,}118$ total fish) at 109 sites in the Flathead River drainage, Montana; bubble plots show cumulative number of rainbow trout stocked at each site. (c) Spatial distribution of rainbow trout hybridization ($N = 89$ sites) in relation to average May precipitation (1993–2011) in the Flathead River drainage, Montana

12,878 individuals at 582 interconnected locations (98 with long-term repeat sampling) with high-resolution climate predictions and detailed historical stocking records for ~200 million introduced rainbow trout. We tested whether climatic variation (stream temperature, spring precipitation, timing of spring runoff), historical stocking, and stream habitat conditions influenced the occurrence, extent of, and temporal changes in non-native admixture across a large, complex landscape. Specifically, our objectives were to (1) identify biotic and abiotic factors associated with the occurrence and extent of hybridization across space, (2) quantify changes in hybridization over time, and (3) determine what ecological factors likely influenced the spread of hybridization over time. This dataset provides an unprecedented opportunity to quantify the interaction and synergistic effects of climate change and legacy introductions on patterns of invasive hybridization across a broad geographic region through time.

2 | MATERIALS AND METHODS

2.1 | Study area and genetic data

We used long-term genetic monitoring data collected over the range of westslope cutthroat trout in Montana, USA (Figure 1). Biologists collected fish or fish tissue samples from streams where westslope cutthroat trout historically occurred (Shepard, May, & Urie, 2005). Sample sites were not randomly selected, but were located where research and management information was needed to determine the genetic status (potential amount of genetic introgression) for populations of westslope cutthroat trout. Those sites that were resampled over time were often resampled because there was concern that rainbow trout could potentially disperse into locations occupied by westslope cutthroat trout and hybridize with them.

We only used data from those locations where at least 10 individuals were genotyped per site. The spatial dataset consisted of

recent samples (N = 582; 1990–2015) collected from sites within hydrologically connected river basins. The temporal dataset (N = 98; 1980–2015) included sites where time-series genetic sampling was conducted at least 5 years between initial (hereafter referred to as "historical") and repeated (hereafter "contemporary") sampling events (mean = 18 years; SD = 6) within hydrologically connected basins. We excluded data from river drainages where native redband rainbow trout *O. m. gairdneri* were naturally sympatric with westslope cutthroat trout (Kootenai River basin), because there are no diagnostic markers to distinguish native redband trout and non-native (coastal) rainbow trout genes (Muhlfeld et al., 2015).

All individual fish were genotyped at six species diagnostic allozyme loci (Allendorf & Leary, 1988), 13 indel and microsatellite diagnostic loci (Ostberg & Rodriguez, 2004), seven diagnostic microsatellite loci (Boyer et al., 2008; Muhlfeld, Kalinowski, et al., 2009), or 19 diagnostic SNP loci (Amish et al., 2012). A species diagnostic locus has nonoverlapping allele sizes or is fixed for alternate alleles in the two parental taxa. Individual trout could have zero, one, or two rainbow trout alleles at each locus. The proportion of rainbow trout alleles (pRBT) in each population sample was calculated as the number of rainbow trout alleles divided by the total number of alleles genotyped. Genetic analyses were conducted in the Conservation Genetics Laboratory at the University of Montana, Missoula, MT, USA.

Importantly, the additional diagnostic loci in more recent samples act to decrease sampling error around estimates of pRBT and slightly increase power to detect low levels of hybridization. With a sample size of 10 individuals (minimum sample size in the dataset), the power to detect 1% rainbow trout admixture with six diagnostic loci is 0.70, 0.75 with seven diagnostic loci, 0.87 with 13 diagnostic loci, and 0.98 with 19 diagnostic loci. With a sample size of 23 individuals (mean sample size in the dataset), the power to detect 1% rainbow trout admixture is 0.94 with six diagnostic loci, 0.96 with seven diagnostic loci, 0.99 with 13 diagnostic loci, and 0.99 with 19 diagnostic loci. Differences in number of diagnostic loci ultimately contribute to sampling variation in the spatial data, but could potentially bias results focused on temporal changes in pRBT (see Section 2.3 below).

2.2 | Predictor variables

Predictor variables for each sample location included mean August stream temperature (Isaak, Young, Nagel, Horan, & Groce, 2015), spring precipitation (Muhlfeld et al., 2014), road density (Hitt et al., 2003), propagule pressure (Bennett et al., 2010), center timing of flow (Wenger, Luce, Hamlet, Isaak, & Neville, 2010), slope (Carim, Eby, & Pierce, 2015), basin area (Muhlfeld, McMahon, Boyer, et al., 2009), and presence of potential intermittent barriers to fish movement (i.e., seasonal obstructions to fish passage during high flow or low flow periods; www.fwp.mt.gov/fishing/mFish/). We tested for additive and interactive effects between covariates. Covariates were obtained for each location using ArcGIS version 10.3 (Environmental Systems Research Institute, Redlands, CA, USA). Average summer (August) stream temperature conditions (1993–2011) were obtained from the NorWeST spatially explicit stream temperature database (www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html). Average April, May, and June precipitation (Muhlfeld et al., 2014) from 1993 to 2011 were calculated from daily precipitation surfaces (1 km) processed from National Aeronautics and Space Administration Daymet data. We ultimately used average precipitation for the month of May because it was found to have the strongest overall correlation with patterns of rainbow trout hybridization relative to other spring runoff months. Center timing of flow (day of the water year at which 50% of year's flow has occurred) was acquired from the Western U.S. Stream Flow Metric Dataset (average 1993–2011; Wenger et al., 2010). Stream temperature, precipitation, and center timing of flow were averaged across the baseline period (1993–2011). Extensive fish stocking records (1924–1980) and geographic information system (GIS) data were used to estimate a spatially explicit index of propagule pressure (propagule pressure index [PPI]; Bennett et al., 2010) exerted by introduced rainbow trout, which incorporates distance from stocking sites, total cumulative number of rainbow trout outplanted at each georeferenced stocking location (www.fwp.mt.gov/fishing/mFish/), and the existence of any barriers to movement. Specifically, PPI (herein referred to as propagule pressure) was derived for each sample location using the following formula:

$$\mathrm{PPI} = \mathrm{NoFish}[\exp(-0.05(\mathrm{StreamDist}))],$$

where PPI is the relative propagule pressure at each genetic sampling site, NoFish is the number of fish stocked at a stocking location (total over all years), −0.05 is the constant decay rate for straying fish, and StreamDist is the distance to each stocking site in kilometers. Stream distances were calculated using the National Hydrography Dataset (NHD) stream layer within GIS. Barriers that spanned the entire stream channel and were greater than 3 m (e.g., bedrock, cascades or waterfalls, dams, culverts, insufficient flow, and water diversions; www.fwp.mt.gov/fishing/mFish/) prevented fish movement between stocking sites and sample locations; over 70% of barriers were classified as dams or waterfalls. The basin area above each sample location was calculated using ESRI's watershed delineation tool (ESRI, 2015), which uses 30 m National Elevation Data within the continental United States. Slope was acquired from the NHD Plus Version 2 (NHDPlusV2) (McKay et al., 2012). Finally, we included "year" as a covariate to account for the fact that data were collected over several decades, during which time power to detect admixture increased (more molecular markers were used), monitoring strategies shifted to focus on locations where pure cutthroat trout may be present (see above), and dynamics of hybridization may have changed (Hitt et al., 2003; Muhlfeld et al., 2014).

All continuous covariates were standardized by subtracting the mean value and dividing by the standard deviation. Basin area, road density, and stream temperature were natural log-transformed prior to standardization. Due to large differences in the numbers of fish stocked in different basins (i.e., some basins received tens of millions more stocked rainbow trout than other basins), we standardized indices of propagule pressure using data within each basin rather

than across all basins. Thus, propagule pressure is a relative within basin measure. Given the major differences in stocking across basins, we also included an additional covariate for stocking history that described the differences in stocking intensity (total number of fish stocked) across the major river basins. We used a categorical variable to describe stocking intensity; basins were categorized as having received "low" (<2,000,000 fish), "medium" (2,000,000–8,000,000 fish), and "high" (>8,000,000 fish) rainbow trout stocking. Correlations between all continuous variables were low ($r < 0.45$; Table S1). There was no evidence that center timing of flow, slope, and basin area were related to the presence or degree of hybridization; hence, those results are not presented hereafter.

2.3 | Statistical analysis

General linear and general linear mixed models were used to test for relationships between spatiotemporal variation in admixture and variables hypothesized to influence hybridization dynamics. An information theoretic approach (AIC) was used to compare support for different model structures and to select best-supported models. Specifically, we examined how abiotic and biotic variables were related to three response variables: the presence of rainbow trout alleles in a sample ($N = 582$); pRBT in sites where rainbow trout alleles were detected ($N = 246$); and changes in pRBT over time ($N = 98$). pRBT values across space were logit-transformed prior to analyses to bound the response from $-\infty$ to ∞ rather than 0.0 to 1.0. Temporal changes in pRBT were calculated by subtracting the pRBT value in the most recent sample by the pRBT value from the oldest sample. Models focused on the presence of rainbow trout alleles used a binomial error structure (i.e., a general linear model), while models focused on pRBT across space and temporal changes in pRBT used a Gaussian error structure (i.e., normal linear model).

We used the model selection approach described in Zuur et al. (2009) to select the optimal fixed and random model structure. We first fit a series of linear and general linear models with all combinations of the predictor variables and retained all models within 10.0 AIC units of the model with the lowest AIC value. We eliminated models that contained uninformative parameters—parameters that did not reduce AIC by 2.0 relative to the more parsimonious model (Arnold, 2010). We then examined whether random effects were supported by the data (i.e., a basin or ecoregion random intercept), and as a final step, we accounted for heteroscedasticity (via covariates in the model error structure) or remaining spatial autocorrelation. We used Program R and the package lme4 to fit general linear mixed models and nlme to fit linear models with covariate structure for heteroscedasticity and spatial autocorrelation using generalized least squares estimation. All model comparisons via AIC used maximum likelihood estimation, but parameter estimates from the best-supported linear models were conducted using restricted maximum likelihood (Zuur et al., 2009).

To account for potential differences in hybridization dynamics across space (presence of admixture, pRBT, or changes in pRBT) that were not accounted for by the a priori-defined candidate predictor variables, we grouped samples according to major river basin or ecoregion. These groupings were used as random intercept terms. For the presence/absence data, we grouped population samples according to major river basins ($N = 17$; Figure 1a). Given that there were fewer locations with temporal data or locations where rainbow trout alleles were detected, we grouped populations based on major ecoregions ($N = 4$; Fig. S1). For each response variable, we also tested whether an exponential autocorrelation structure addressed any additional lack of independence among samples. There was evidence that the exponential autocorrelation error structure helped address some spatial autocorrelation for models explaining temporal changes in pRBT and pRBT across space (see Section 3).

Model fit and performance were examined using confusion matrices for general linear models (presence of RBT alleles) and root mean square predictive error (RMSE) for the linear models (pRBT across space and temporal changes in pRBT). Confusion matrices were generated using 0.5 as a prediction threshold for the presence of rainbow trout alleles in a sample and were used to calculate predictive accuracy (true prediction rate), specificity (true negative rate), and sensitivity (true positive rate). However, we note that the primary focus of this work is to identify environmental and biotic factors influencing hybridization across large spatial scales in recent decades (not predict future dynamics).

We also tested for significant changes in hybridization over time. At each location with long-term data, we used binomial t tests to test for significant ($p < .05$) changes in pRBT. The binomial t test accounts for sampling variation in the historical and contemporary samples, that is, differences in the number of diagnostic loci (alleles) and sample sizes used to calculate pRBT over time.

3 | RESULTS

3.1 | Spatial patterns of invasive hybridization

We used general linear mixed models to identify factors influencing spatial patterns in rainbow trout hybridization across 17 major river basins. Propagule pressure, stocking history, stream temperature, road density, and year were strongly related to the presence of rainbow trout alleles across the Northern Rockies (Tables 1 and 2). On average, rainbow trout genes were more likely to be present in locations with higher propagule pressure, stream temperature, and road density (Figure 2a–c). Although temperature was strongly related to the presence of rainbow trout alleles, cold stream temperatures clearly did not act as a barrier to admixture (Figure 3). The majority (58%) of hybridized sites and nearly half (45%) of sites with a considerable rainbow trout genetic contribution (>10% pRBT) had mean August stream temperatures that were less than 11°C. Additionally, 35% of sites with nonhybridized cutthroat trout had mean August stream temperatures greater than 11°C.

The likelihood of detecting rainbow trout alleles in any given sample increased over time (Figure 4). This relationship could suggest that hybridization is becoming more widespread, reflect increasing probability of detection with greater numbers of molecular

TABLE 1 Parameter estimates from best-supported models explaining patterns in rainbow trout hybridization across space and time

Response variable	Parameter	Estimate	*SE*	*p*
Presence	Intercept	0.005	.254	.983
	PPI	0.307	.094	.001
	Temp	0.254	.104	.015
	Road	0.307	.101	.002
	Year	0.403	.102	.000
	Stock—Low	−1.3226	.467	.005
	Stock—Med	−0.110	.403	.785
pRBT	Intercept	−2.178	.144	.000
	PPI	0.420	.102	.000
	Temp	0.156	.091	.088
	Year	−0.324	.085	.000
	Stock—Low	−0.687	.315	.030
	Stock—Med	0.223	.213	.295
Temporal change	Intercept	0.021	.007	.006
	PPI	0.006	.003	.064
	Precip	−0.004	.004	.373
	Precip × PPI	−0.006	.003	.036

For the response variables "presence" and proportion rainbow trout admixture (pRBT), the intercept term is the "high stocking" (stock—high) effect. "PPI", propagule pressure index; "Temp", mean August stream temperature, "Road", road density; "Precip", May precipitation; and "Year", year of genetic sample.

TABLE 2 Best-supported general linear mixed models explaining the presence or absence of rainbow trout alleles across space

Model	Random	AIC	ΔAIC
PPI + Temp + Road + Stock + Year	Basin	**734.21**	**0.00**
PPI + Road + Stock + Year	Basin	738.39	4.18
PPI + Temp + Stock + Year	Basin	741.94	7.73
PPI + Temp + Road + Year	Basin	737.35	3.14
Temp + Road + Stock + Year	Basin	743.04	8.83
PPI + Road + Year	Basin	742.38	8.17
PPI + Temp + Year	Basin	743.97	9.76
PPI + Temp + Road + Stock + Year		743.31	9.10

The table only includes models with fixed effects structure within 10 AIC units of the best-supported model. River basin was used as a random intercept for all models. The best-supported model is highlighted in bold.

markers, or both. Statistical support for a random intercept term for river basin (Table 2) provides evidence that the average probability that rainbow trout alleles are present at a site varied among major river basins, even after accounting for stocking intensity. Interestingly, there was strong evidence that river basins that received "low" levels of stocking had significantly lower occurrence of rainbow trout admixture, but there was no difference in average presence of admixture between basins that received "moderate" and "high" levels of stocking (Table 1). Overall, predictive accuracy of the best-supported model explaining variation in the presence of rainbow trout alleles was 0.71 (i.e., 71% of model predictions matched the observed data), sensitivity was 0.54, and specificity was 0.83 (Table S2). Thus, the best-supported model was better at predicting where rainbow trout genes were absent, rather than where rainbow trout genes were present. Predictive accuracy varied across river basins (0.55–0.88; Table S2).

Similar to the presence of rainbow trout alleles, the degree of rainbow trout admixture—pRBT within a sample at invaded sites—was explained by propagule pressure, stocking intensity, stream temperature, and year (Tables 1 and S3). Rainbow trout admixture was higher in locations that historically experienced greater propagule pressure and stocking intensity, but residual variation in pRBT also increased with higher levels of propagule pressure (Fig. S2). Inclusion of propagule pressure in the variance structure of the best-supported model decreased AIC by 78.3 relative to competing models (Table S3), highlighting that the consequences of rainbow trout stocking on contemporary patterns of hybridization were increasingly heterogenous with increasing stocking. Unlike the presence of admixture, pRBT at invaded sites actually decreased over time, a pattern that may reflect spatial shifts in monitoring (a shift toward sampling locations where pure westslope cutthroat trout may still be present), rather than decreases over time (hybridization is increasing at most sites with repeat sampling). After accounting for some spatial autocorrelation in the data (Table S3), the RMSE for best-supported model was 0.132.

3.2 | Temporal changes in invasive hybridization

We used binomial t tests to test for changes in pRBT in samples from those sites where there were temporal genetic data were available. The pRBT in a sample increased over time in half ($N = 49$; 50%) of the sites with temporal data and decreased in only five sites (Figure 4); 44 sites remained non-hybridized. After accounting for differences in sample size due to variation in number of molecular markers and individuals genotyped, pRBT significantly increased at 31 locations (63% of sites where pRBT increased). Importantly, the majority ($N = 29$; 74%) of the sites where pRBT increased (both significant and nonsignificant changes) were initially nonhybridized.

Linear models were used to identify which abiotic and biotic factors contributed to changes in pRBT over time. Temporal changes in hybridization were best explained by an interaction between propagule pressure and May precipitation (a surrogate for peak streamflow) (Tables 1 and S4), where the greatest increases in hybridization, on average, occurred at locations with the lowest precipitation and highest propagule pressure (Figure 2b). Residual variation strongly increased with higher propagule pressure (Fig. S3a) and lower precipitation (Fig. S3b), and including covariates that accounted for this heterogeneity substantially improved the best-supported models (decreased AIC by ~50; Table S5). Essentially, pRBT did not increase in sites with low propagule pressure and high precipitation. Conversely, pRBT often increased at sites with high propagule pressure and low precipitation, but there was considerable variation at the lowest

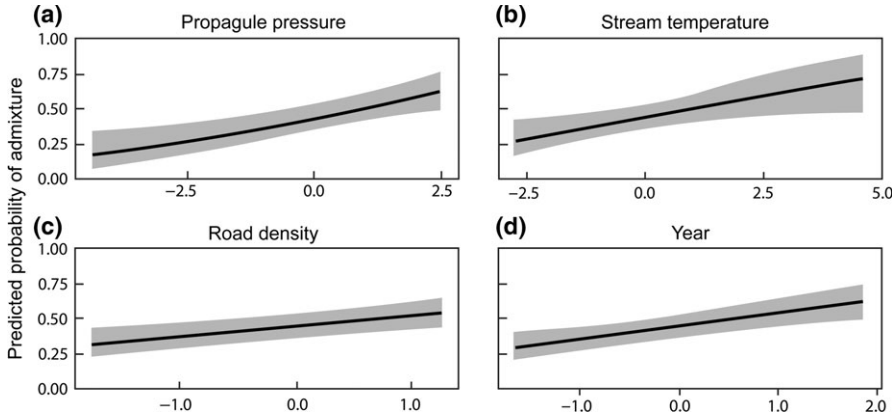

FIGURE 2 Predicted probability of detecting rainbow trout alleles in westslope cutthroat trout populations in the Northern Rocky Mountains as a function of (a) propagule pressure, (b) stream temperature, (c) road density, and (d) year of sample. All covariates are standardized. Parameter estimates are from the best-supported general linear mixed models predicting the presence of rainbow trout alleles, and the figures depict marginal effects for each variable (i.e., predicted probabilities are based on mean values for all other covariates)

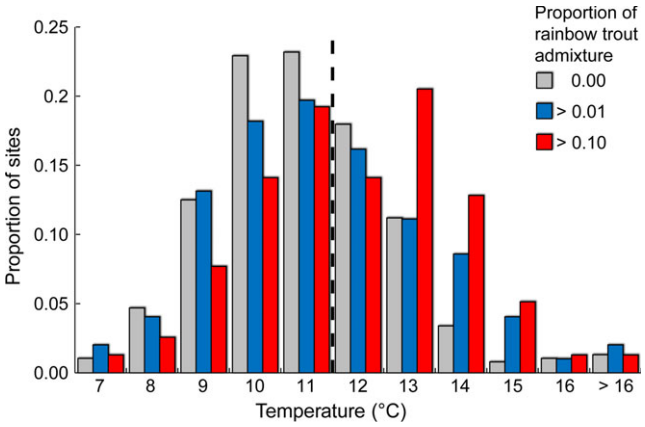

FIGURE 3 Cold stream temperatures did not prevent invasion of non-native rainbow trout and hybridization with native cutthroat trout. Proportion of sites with varying degrees of proportion rainbow trout alleles relative to summer (August) mean water temperature. The dotted line represents 11°C as a reference to the cold-water climate shield hypothesis (Isaak et al., 2015)

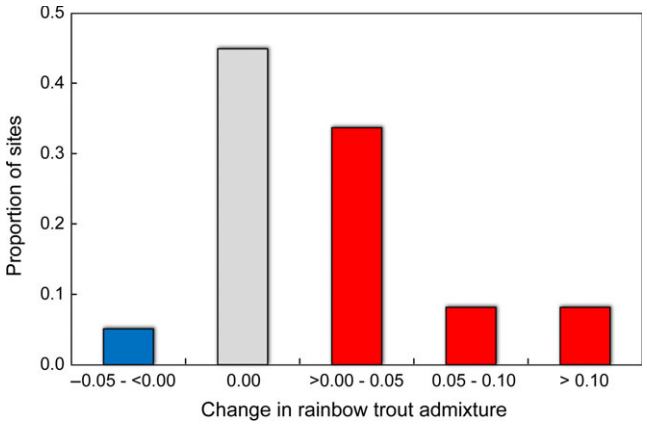

FIGURE 4 Changes in rainbow trout hybridization over time. Proportions of sample sites that experienced different levels of change in proportion rainbow trout admixture (pRBT) over time (bars). Temporal changes in pRBT were calculated by subtracting the pRBT in the most recent sample by the value from the oldest sample (mean = 18 years, *SD* = 6)

value of precipitation and highest values of propagule pressure. After accounting for some spatial autocorrelation in the data (Table S4), the RMSE for best-supported model explaining temporal changes in pRBT was 0.004. Genetic results (Tables S5 and S6) and summary statistics for spatial and temporal covariates and response variables (Tables S7 and S8) by major river basin can be found in the Supporting Information.

4 | DISCUSSION

Extensive spatiotemporal data from multiple salmonid hybrid zones demonstrate that human actions can have profound effects on biodiversity that are long-lasting and exacerbated by climatic variation and change. We found that widespread introductions of an invasive species and recent climatic variation were associated with the spread of introgressive hybridization in native cutthroat trout populations across the Northern Rocky Mountains, USA. The infiltration of non-native genes into native trout genomes continues to increase across a broad geographic region despite cessation of widespread stocking of nearly 200 million fertile rainbow trout over 40 years ago. The spread of these invasive genes into native populations across space and through time was primarily correlated with historic propagule pressure and stocking intensity and exacerbated by warmer stream temperatures, higher road densities, and lower spring precipitation (e.g., reduced spring flows). These results suggest that pervasive effects of human-mediated introductions of invasive species are entwined with climate and other human stressors, with irreversible evolutionary consequences for threatened species and biodiversity.

We show that hybridization can spread rapidly during climate-mediated range expansion of invasive species using a high-resolution spatiotemporal dataset across a broad geographic area. The actual

biological mechanisms that promote climate-driven admixture between rainbow and cutthroat trout are likely multifaceted, potentially reflecting interspecific differences in spawning and incubation times (Muhlfeld et al., 2014), physiological requirements (Rasmussen, Robinson, Hontela, & Heath, 2012), and interspecific differences in philopatry (Lowe, Muhlfeld, & Allendorf, 2015). High spring flows and flood disturbances caused by spring precipitation during spawning (i.e., reproduction) strongly reduce invasion success of rainbow trout (Fausch et al., 2001). Likewise, periods of low spring flows and reduced peak pulses in snow melt can facilitate rapid expansion of introduced rainbow trout and subsequent hybridization with native cutthroat trout (Muhlfeld et al., 2014). Rainbow trout also have a higher upper temperature tolerance and greater growth capacity at warmer temperatures than cutthroat trout (Bear et al., 2007), which may reflect a postzygotic mechanism for the observed prevalence of hybridization in warmer stream environments (Rasmussen et al., 2012). Finally, continuous immigration of dispersing (i.e., termed "straying" in the salmonid lexicon) hybrids is a primary mechanism promoting the spread of hybridization between these species, further accelerating this process (Boyer et al., 2008; Kovach et al., 2015). Dispersal rates in salmonids appear to increase with temperature (Muhlfeld et al., 2014; Westley, Dittman, Ward, & Quinn, 2015) and dispersal capacity can evolve quickly in invading populations (Phillips, Brown, & Shine, 2010), suggesting that climate and contemporary evolution may interact to accelerate the spread of rainbow trout and their hybrids. Our results and future climate change predictions—reduced late spring and summer flows, and warmer stream temperatures (Isaak et al., 2015; Jones, Muhlfeld, Marshall, Mcglynn, & Kershner, 2014; Pederson, Graumlich, Fagre, Kipfer, & Muhlfeld, 2010; Pederson et al., 2011)—suggest that climate change will further erode native cutthroat trout genomes in the coming years.

Our index of propagule pressure—an intrabasin relative measure incorporating distance from stocking sites, number of outplanted rainbow trout released at stocking sites, and barriers to upstream movement—was the most consistent driver of hybridization across both space and time. Differences in stocking intensity across river basins also explained spatial patterns in rainbow trout hybridization. Together, these findings corroborate extensive literature showing that propagule pressure and introduction history play a critical role in the establishment and spread of invasive species, a central tenant in invasion biology (Blackburn & Duncan, 2001; Lockwood, Cassey, & Blackburn, 2005), including hybridization between salmonid species (Bennett et al., 2010). Until now, however, our ability to disentangle the evolutionary impacts of co-occurring climatic variation and biological invasions has been hampered by the low spatiotemporal resolution of data on climate, biological response (e.g., quantitative estimates of non-native genetic admixture), and human releases of non-native organisms (Staudt et al., 2013; Suarez & Tsutsui, 2008).

In addition to propagule pressure, stocking history, and climate, other factors may have contributed to variation in the outcome of invasive hybridization, including standing genetic variation, adaptive introgression, the timing and location of introductions, and habitat quality (Strayer et al., 2006). For example, the positive association between the incidence of hybridization and road density, here used as an index of human disturbance and access, suggests disturbance facilitates invasion and consequently genetic introgression or that roads simply act as conduits for undocumented introductions of rainbow trout (Harbicht et al., 2014; Heath et al., 2010). Multiple lines of evidence (increasing variation in pRBT with higher stocking and lower model sensitivity relative to specificity) highlight that other deterministic (e.g., natural selection) and stochastic factors (e.g., genetic drift) strongly influence the evolutionary outcomes of human-induced contact between native and invasive species.

Although abiotic variation appears to influence invasive hybridization, there were no environmental factors that wholly prevented invasion and upstream expansion of hybrid zones. For example, we found that a majority (58%) of hybridized sites occurred in relatively cold streams (<11°C), and 35% of streams supporting pure cutthroat trout were warmer than 11°C (Figure 3). Recent studies postulated that cold stream temperatures (<11°C mean summer temperature) may preclude invasion by most non-native aquatic invasive species, including non-native rainbow trout, and suggested that these temperature thresholds should be used to delineate climate refugia for native species (Isaak et al., 2015, 2016). However, these studies did not quantify levels of non-native introgression in native cutthroat trout populations. Our empirical results quantifying spatial patterns and temporal changes in non-native genetic admixture in populations across a wide range of environmental conditions clearly show that cold temperatures do not prevent rainbow trout invasion and subsequent hybridization with native cutthroat trout; these findings are consistent with hybridization studies on these species at smaller geographical scales (Muhlfeld, McMahon, Boyer, et al., 2009; Yau & Taylor, 2013). Conservation prioritization strategies based mainly on thermal criteria may ignore a substantial proportion of existing native diversity in habitats that are likely closer to thermal optima for cutthroat trout (Bear et al., 2007) and seriously underestimate the threat of rainbow trout introgression in headwater streams.

Hybridization increased over time in many native trout populations, the vast majority of which were previously nonhybridized. The evolutionary consequences of invasive hybridization are long-lasting, irreversibly disrupting native genomes that have evolved over millennia. Because the progeny of hybrids are hybrids (i.e., will possess genes from both parental taxa), the presence of even a few hybrids in the breeding population is enough to ensure the propagation of non-native genes, even in the face of strong selective pressures against hybrid progeny (Epifanio & Philipp, 2000). Hybrid trout in this region appear to have substantially reduced fitness relative to pure cutthroat trout (Kovach et al., 2015; Muhlfeld, Kalinowski, et al., 2009), but chronic immigration of fish from historical stocking locations appears to overwhelm strong selective costs (Kovach et al., 2015), especially during periods of climatic warming (Muhlfeld et al., 2014). Genomic extinction may be inevitable for many nonisolated cutthroat trout populations if hybrid source populations are not eliminated. Isolated (above-barrier) populations are likely to remain nonhybridized, but these populations might not be viable in the long

term and might be in need of assisted gene flow (i.e., genetic rescue) (Whiteley, Fitzpatrick, Funk, & Tallmon, 2015). Unfortunately for the conservation of cutthroat trout, a key insight from invasive species as models of evolution is that reproductive isolation between species often takes millions of years to be complete (Sax et al., 2007).

Interestingly, results from the spatial and temporal data were relatively consistent—both sources of information emphasized that stocking history and climate influence hybridization between these species. However, we acknowledge that sample sites were not randomly or systematically selected; instead, sampling was primarily conducted to determine the genetic status of known westslope cutthroat trout populations. Currently, nonhybridized westslope cutthroat trout populations predominantly occur in small, high-elevation streams, although they were historically present along the entire gradient of cool-water habitats throughout their historical range (Shepard et al., 2005). Any bias in the genetic dataset is due to the fact that local and regional biologists sampled areas they knew or believed that pure or mostly pure westslope cutthroat trout were present. In other words, streams with fish that have predominant rainbow trout phenotypes were less frequently genotyped. This bias is also reflected across space, where some river basins have far more genetic data than others. Nevertheless, the data clearly show that hybridization significantly increased in many populations over time, the vast majority of which were initially nonhybridized, indicating a strong pattern of increasing hybridization that may ultimately lead to genomic extinction of extant pure populations of cutthroat trout. Thus, downplaying the threat of rainbow trout hybridization for westslope cutthroat trout conservation is ill advised, because it does not reflect the current temporal trajectory of the spread of admixture between these species or the widespread genomic extinction of westslope cutthroat trout across much of their range, particularly east of the continental divide (Muhlfeld et al., 2016; Shepard et al., 2005).

Another broad-scale study recently found that abiotic (e.g., water temperature) and biotic (e.g., source effects) factors were associated with spatial patterns of hybridization between rainbow and westslope cutthroat trout (Young et al., 2016). The paper concludes that genomic extinction is unlikely and that ecological segregation between these taxa, primarily due to cold temperatures, will prevent rainbow trout invasion and subsequent hybridization in some areas. These conclusions differ from ours for three primary reasons. First, the temporal genetic data used in our study clearly show that hybridization is increasing and that cold temperatures do not prevent rainbow trout invasion and hybridization with cutthroat trout (see discussion above). Second, our results strongly suggest that propagule pressure and proximity to historical stocking locations were primary factors influencing spatial and temporal patterns in hybridization. Temperature may play an important role in mediating hybridization, but dismissing the importance of historical, ongoing, and future propagule pressure is not supported by our data nor results from Young et al. (2016); despite their use of imprecise proxies for propagule pressure (e.g., distance to streams with high mean annual discharge and warm summer water temperatures), they also detected significant relationships between this proxy variable and spatial patterns in admixture. Finally, Young et al. (2016) predict substantial increases in hybridization, including losses of up to 45%–74% of existing nonhybridized populations, due to future climate warming. These predictions—although alarming—may actually be conservative. Young et al. (2016) assume that recent warming rates (1993–2011) are representative of warming rates expected over the next 50–100 years and use a 1.0°C increase in temperature as an "extreme" scenario. The same temperature model used in their spatial analysis predicts an average increase in stream temperatures of 1.3°C by 2040 and 2.15°C by 2080 using a "moderate" A1B scenario (Isaak et al., 2015), values that are much higher than their "extreme" scenario (i.e., greater losses of cutthroat trout would have been predicted had values used in other similar studies been applied). Overall, nonhybridized westslope cutthroat trout populations occupy only a fraction of their historical range (10%–22%; Shepard et al., 2005). Thus, the genomic extinction of additional populations or lineages—a scenario being realized currently and one that will only proceed into the future—represents a critical threat to westslope cutthroat trout conservation.

Our findings underscore the ecological and evolutionary consequences of climate change and legacy introductions for native trout threatened by human-mediated hybridization, as well as impacts on native biodiversity. Our study also highlights the importance of broad-scale and long-term studies for understanding the effects of invasive hybridization and climate change. Results show that climatic changes are interacting with legacies of species introduction and habitat degradation to threaten native cutthroat trout, as invasive rainbow trout continue to expand their range. Remaining nonhybridized populations represent only a fraction of the historic distribution and genetic diversity of cutthroat trout (Allendorf & Leary, 1988; Shepard et al., 2005), underscoring the urgency of addressing invasive hybridization through proven conservation strategies (e.g., suppression of non-natives, installation of barriers, habitat restoration and protection) that prevent genomic extinction of additional populations and eventually entire lineages (Al-Chokhachy et al., 2014; Muhlfeld et al., 2012; Pierce, Podner, & Carim, 2013). More broadly, our results suggest that mitigation of chronic human stressors—translocation of species and habitat modification—that interact with climate to promote the expansion of invasive species will be crucial for conserving biodiversity (Hauer et al., 2016). Likewise, the mitigation of these chronic stressors may represent more feasible targets for slowing the ecological and evolutionary effects of climate change than its root causes.

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REFERENCES

Al-Chokhachy, R., Muhlfeld, C. C., Boyer, M. C., Jones, L. A., Steed, A., & Kershner, J. L. (2014). Quantifying the effectiveness of conservation measures to control the spread of anthropogenic hybridization in stream salmonids: A climate adaptation case study. *North American Journal of Fisheries Management*, *34*, 642–652.

Allendorf, F. W., & Leary, R. F. (1988). Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology*, *2*, 171–184.

Almodovar, A., Nicola, G. G., Ayllon, D., & Elvira, B. (2012). Global warming threatens the persistence of Mediterranean brown trout. *Global Change Biology*, *18*, 1549–1560.

Amish, S. J., Hohenlohe, P. A., Painter, S., Leary, R. F., Muhlfeld, C., Allendorf, F. W., & Luikart, G. (2012). RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. *Molecular Ecology Resources*, *12*, 653–660.

Araguas, R. M., Sanz, N., Pla, C., & García-Marín, J. L. (2004). Breakdown of the brown trout evolutionary history due to hybridization between native and cultivated fish. *Journal of Fish Biology*, *65*, 28–37.

Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management*, *74*, 1175–1178.

Bear, E. A., McMahon, T. E., & Zale, A. V. (2007). Comparative thermal requirements of westslope cutthroat trout and rainbow trout: Implications for species interactions and development of thermal protection standards. *Transactions of the American Fisheries Society*, *136*, 1113–1121.

Behnke, R. J. (1992). *Native trout of western North America*. Bethesda, MD: American Fisheries Society.

Bennett, S. N., Olson, J. R., Kershner, J. L., & Corbett, P. (2010). Propagule pressure and stream characteristics influence introgression: Cutthroat and rainbow trout in British Columbia. *Ecological Applications*, *20*, 263–277.

Blackburn, T. M., & Duncan, R. P. (2001). Determinants of establishment success in introduced birds. *Nature*, *414*, 195–197.

Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and the past, present, and future of biotic interactions. *Science*, *341*, 499–504.

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 lewisi*). *Canadian Journal of Fisheries and Aquatic Sciences*, *65*, 658–669.

Carim, K., Eby, L., & Pierce, R. (2015). Does whirling disease mediate hybridization between a native and nonnative trout? *North American Journal of Fisheries Management*, *35*, 337–351.

Comte, L., & Grenouillet, G. (2013). Do stream fish track climate change? Assessing distribution shifts in recent decades. *Ecography*, *36*, 1236–1246.

Epifanio, J., & Philipp, D. (2000). Simulating the extinction of parental lineages from introgressive hybridization: The effects of fitness, initial proportions of parental taxa, and mate choice. *Reviews in Fish Biology and Fisheries*, *10*, 339–354.

Escalante, M. A., García de León, F. J., Dillman, C. B., de los Santos Camarillo, A., George, A., Barriga-Sosa, I., de los, A., ... Manel, S. (2014). Genetic introgression of cultured Rainbow Trout in the Mexican native trout complex. *Conservation Genetics*, *15*, 1063–1071.

ESRI (2015). *ArcGIS*. vol. *10.3*. Redlands, CA: ESRI.

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*, 1438–1455.

Gozlan, R. E., Britton, J. R., Cowx, I., & Copp, G. H. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, *76*, 751–786.

Harbicht, A. B., Alshamlih, M., Wilson, C. C., & Fraser, D. J. (2014). Anthropogenic and habitat correlates of hybridization between hatchery and wild brook trout. *Canadian Journal of Fisheries and Aquatic Sciences*, *71*, 688–697.

Hauer, F. R., Locke, H., Dreitz, V. J., Hebblewhite, M., Lowe, W. H., Muhlfeld, C. C., ... Rood, S. B. (2016). Gravel-bed river floodplains are the ecological nexus of glaciated mountain landscapes. *Science Advances*, *2*, e1600026.

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*, 77–90.

Hellmann, J. J., Byers, J. E., Bierwagen, B. G., & Dukes, J. S. (2008). Five potential consequences of climate change for invasive species. *Conservation Biology*, *22*, 534–543.

Hitt, N. P., Frissell, C. A., Muhlfeld, C. C., & Allendorf, F. W. (2003). Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*, 1440–1451.

Isaak, D. J., Young, M. K., Luce, C. H., Hostetlerc, S. W., Wengerd, S. J., Petersone, E. E., ... Nagel, D. E. (2016). Slow climate velocities of mountain streams portend their role as refugia for cold-water biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 4374–4379.

Isaak, D. J., Young, M. K., Nagel, D. E., Horan, D. L., & Groce, M. C. (2015). The cold-water climate shield: Delineating refugia for preserving salmonid fishes through the 21st century. *Global Change Biology*, *21*, 2540–2553.

Jones, L. A., Muhlfeld, C. C., Marshall, L. A., Mcglynn, B. L., & Kershner, J. L. (2014). Estimating thermal regimes of bull trout and assessing the potential effects of climate warming on critical habitats. *River Research and Applications*, *30*, 204–216.

Kelly, B., Whiteley, A., & Tallmon, D. (2010). The Arctic melting pot. *Nature*, *468*, 891.

Kovach, R. P., Hand, B. K., Hohenlohe, P. A., Cosart, T. F., Boyer, M. C., Neville, H. H., ... Luikart, G. (2016). Vive la résistance: Genome-wide selection against introduced alleles in invasive hybrid zones. *Proceedings of the Royal Society of London B: Biological Sciences*, *283*, 9.

Kovach, R. P., Muhlfeld, C. C., Al-Chokhachy, R., Dunham, J. B., Letcher, B. H., Kershner, J. L. (2016). Impacts of climatic variation on trout: a global synthesis and path forward. *Reviews in Fish Biology and Fisheries*, *26*, 135–151.

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 of London B: Biological Sciences*, *282*, 9.

Lockwood, J. L., Cassey, P., & Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, *20*, 223–228.

Lockwood, J. L., & McKinney, M. L. (2001). *Biotic homogenization*. New York, NY: Springer Science+ Business Media.

Lowe, W. H., Muhlfeld, C. C., & Allendorf, F. W. (2015). Spatial sorting promotes the spread of maladaptive hybridization. *Trends in Ecology & Evolution*, *30*, 456–462.

Marie, A. D., Bernatchez, L., & Garant, D. (2012). Environmental factors correlate with hybridization in stocked brook charr (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 884–893.

McKay, L., Bondelid, T., Dewald, T., Johnston, J., Moore, R., & Rea, A. (2012). *NHDPlus version 2: User guide*. United States Environmental Protection Agency. Retrieved from ftp://ftp.horizon-systems.com/nhdplus/NHDPlusV21/Documentation/NHDPlusV2_User_Guide.pdf

McKinney, M. L., & Lockwood, J. L. (1999). Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends in Ecology & Evolution*, *14*, 450–453.

Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 5446–5451.

Muhlfeld, C. C., Albeke, S. E., Gunckel, S. L., Writer, B. J., Shepard, B. B., & May, B. E. (2015). Status and conservation of interior redband trout in the Western United States. *North American Journal of Fisheries Management*, *35*, 31–53.

Muhlfeld, C. C., D'Angelo, V. S., Downs, C., Powell, J. D., Amish, S. J., Luikart, G., . . . Kalinowski, S. T. (2016). Genetic status and conservation of westslope cutthroat trout in Glacier National Park. *Transactions of the American Fisheries Society*, *145*, 1093–1109.

Muhlfeld, C. C., D'Angelo, V., Kalinowski, S. T., Landguth, E. L., Downs, C. C., & Tohtz, J. (2012). A fine-scale assessment of using barriers to conserve native stream salmonids: A case study in Akokala Creek, Glacier National Park, USA. *Open Fish Science Journal*, *5*, 9–20.

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*, 328–331.

Muhlfeld, C. C., Kovach, R. P., Jones, L. A., Al-Chokhachy, R., Boyer, M. C., leary, R. F., . . . Allendorf, F. W. (2014). Invasive hybridization in a threatened species is accelerated by climate change. *Nature Climate Change*, *4*, 620–624.

Muhlfeld, C. C., McMahon, T. E., Belcer, D., & Kershner, J. L. (2009). Spatial and temporal spawning dynamics of native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, introduced rainbow trout, *Oncorhynchus mykiss*, and their hybrids. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1153–1168.

Muhlfeld, C. C., McMahon, T. E., Boyer, M. C., & Gresswell, R. E. (2009). Local habitat, watershed, and biotic factors influencing the spread of hybridization between native westslope cutthroat trout and introduced rainbow trout. *Transactions of the American Fisheries Society*, *138*, 1036–1051.

Olden, J. D., Poff, N. L., Douglas, M. R., Douglas, M. E., & Fausch, K. D. (2004). Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution*, *19*, 18–24.

Ostberg, C. O., & Rodriguez, R. J. (2004). Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes*, *4*, 26–29.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669.

Pederson, G. T., Graumlich, L. J., Fagre, D. B., Kipfer, T., & Muhlfeld, C. C. (2010). A century of climate and ecosystem change in Western Montana: What do temperature trends portend? *Climatic Change*, *98*, 133–154.

Pederson, G. T., Gray, S. T., Ault, T., Marsh, W., Fagre, D. B., Bunn, A. G., . . . Graumlich, L. J. (2011). Climatic controls on the snowmelt hydrology of the northern Rocky Mountains. *Journal of Climate*, *24*, 1666–1687.

Phillips, B. L., Brown, G. P., & Shine, R. (2010). Evolutionarily accelerated invasions: The rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology*, *23*, 2595–2601.

Pierce, R., Podner, C., & Carim, K. (2013). Response of wild trout to stream restoration over two decades in the Blackfoot River basin, Montana. *Transactions of the American Fisheries Society*, *142*, 68–81.

Rahel, F. J. (2000). Homogenization of fish faunas across the United States. *Science*, *288*, 854–856.

Rahel, F. J., & Olden, J. D. (2008). Assessing the effects of climate change on aquatic invasive species. *Conservation Biology*, *22*, 521–533.

Rasmussen, J. B., Robinson, M. D., Hontela, A., & Heath, D. D. (2012). Metabolic traits of westslope cutthroat trout, introduced rainbow trout and their hybrids in an ecotonal hybrid zone along an elevation gradient. *Biological Journal of the Linnean Society*, *105*, 56–72.

Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, *27*, 83–109.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., & Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, *421*, 57–60.

Rubidge, E. M., & Taylor, E. B. (2005). An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. *Conservation Genetics*, *6*, 369–384.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., . . . Rice, W. R. (2007). Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, *22*, 465–471.

Shepard, B. B., May, B. E., & Urie, W. (2005). Status and conservation of westslope cutthroat trout within the western United States. *North American Journal of Fisheries Management*, *25*, 1426–1440.

Simberloff, D. (2014). Biological invasions: What's worth fighting and what can be won? *Ecological Engineering*, *65*, 112–121.

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., . . . Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology & Evolution*, *28*, 58–66.

Sorte, C. J. B., Ibanez, I., Blumenthal, D. M., Molinari, N. A., Miller, L. P., Grosholz, E. D., . . . Dukes, J. S. (2013). Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecology Letters*, *16*, 261–270.

Staudt, A., Leidner, A. K., Howard, J., Brauman, K. A., Dukes, J. S., Hansen, L. J., . . . Solórzano, L. A. (2013). The added complications of climate change: Understanding and managing biodiversity and ecosystems. *Frontiers in Ecology and the Environment*, *11*, 494–501.

Strayer, D. L., Eviner, V. T., Jeschke, J. M., & Pace, M. L. (2006). Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution*, *21*, 645–651.

Suarez, A. V., & Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Molecular Ecology*, *17*, 351–360.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*, 494–499.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., . . . Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*, 389–395.

Wenger, S. J., Isaak, D. J., Luce, C. H., Nevillea, H. M., Fauschc, K. D., Dunhamd, J. B., . . . Williams, J. E. (2011). Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 14175–14180.

Wenger, S. J., Luce, C. H., Hamlet, A. F., Isaak, D. J., & Neville, H. M. (2010). Macroscale hydrologic modeling of ecologically relevant flow metrics. *Water Resources Research*, *46*, W09513. doi:10.1029/2009WR008839.

Westley, PAH, Dittman, A. H., Ward, E. J., & Quinn, T. P. (2015). Signals of climate, conspecific density, and watershed features in patterns of homing and dispersal by Pacific salmon. *Ecology*, *96*, 2823–2833.

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. *Trends in Ecology & Evolution*, *30*, 42–49.

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*, 2093–2106.

Yau, M. M., & Taylor, E. B. (2013). Environmental and anthropogenic correlates of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*). *Conservation Genetics*, *14*, 885–900.

Young, M. K., Isaak, D. J., Mckelvey, K. S., Wilcox, T. M., Pilgrim, K. L., Carim, K. J., . . . Schwartz, M. K. (2016). Climate, demography, and zoogeography predict introgression thresholds in salmonid hybrid zones in rocky mountain streams. *PLoS ONE*, *11*, e0163563.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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