

Invasive hybridization in a threatened species is accelerated by climate change

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Climate change will decrease worldwide biodiversity through a number of potential pathways¹, including invasive hybridization² (cross-breeding between invasive and native species). How climate warming influences the spread of hybridization and loss of native genomes poses difficult ecological and evolutionary questions with little empirical information to guide conservation management decisions³. Here we combine long-term genetic monitoring data with high-resolution climate and stream temperature predictions to evaluate how recent climate warming has influenced the spatio-temporal spread of human-mediated hybridization between threatened native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and non-native rainbow trout (*Oncorhynchus mykiss*), the world's most widely introduced invasive fish⁴. Despite widespread release of millions of rainbow trout over the past century within the Flathead River system⁵, a large relatively pristine watershed in western North America, historical samples revealed that hybridization was prevalent only in one (source) population. During a subsequent 30-year period of accelerated warming, hybridization spread rapidly and was strongly linked to interactions between climatic drivers—precipitation and temperature—and distance to the source population. Specifically, decreases in spring precipitation and increases in summer stream temperature probably promoted upstream expansion of hybridization throughout the system. This study shows that rapid climate warming can exacerbate interactions between native and non-native species through invasive hybridization, which could spell genomic extinction for many species.

Changes in species ecology associated with climate change have been documented for a broad range of organisms^{1,6}, yet empirical understanding of how climate change influences evolutionary processes and resulting patterns of biodiversity is limited. One consequence of climate-induced range shifts is increased sympatry between previously isolated species, potentially resulting in introgressive hybridization (genes from an invasive species spread into a native species)^{7,8}. Climate-induced expansions of introgression have been predicted for many terrestrial and aquatic species, especially species that are sensitive to temperature and streamflow conditions^{2,6,9}. Although hybridization can increase the adaptive potential of closely related species through periods of climate change⁷, hybridization driven by human activities, such as translocation of species, tends to occur quickly and reduce fitness¹⁰, genomic integrity¹¹, and ultimately native species diversity¹².

Despite predictions that interspecific hybridization may increase as a result of species range shifts and human impacts, empirical evidence linking such evolutionary changes to recent climatic change is extremely scarce^{3,13}.

Salmonids—a group of fishes of enormous ecological and socio-economic value—are ideal organisms for examining how climate change facilitates hybridization between native and non-native species. Hybridization and introgression are particularly common between salmonids and other fish because there are limited pre- or post-zygotic barriers to introgression¹², and widespread introductions have created sympatry between many previously allopatric species¹¹. Moreover, the distribution, abundance and phenology of salmonid fishes are strongly influenced by climatic conditions through species-specific adaptations to water temperature and the timing and magnitude of streamflow¹⁴. Thus, ongoing climate change is expected to differentially affect salmonid species, possibly expanding zones of introgressive hybridization as some species expand their distribution and increase in abundance during periods of warming and shifting hydrologic regimes^{14,15}.

Quantifying spatial and temporal genetic changes in wild populations, including introgression, provides strong support for climate-induced evolutionary change^{13,16}. Such data, however, are limited among vertebrates, especially for rare and endangered species. Here we use long-term genetic monitoring data (1978–2008) to test the prediction that climatic variation has affected the spread of introgressive hybridization between threatened native westslope cutthroat trout and non-native rainbow trout. Cutthroat trout (*Oncorhynchus clarkii*) and introduced rainbow trout can overlap in time and space during spring-spawning and produce fertile offspring when they interbreed¹⁷. Introgression often continues until a hybrid swarm (a randomly mating population containing only hybrid individuals) is formed and all the native genomes are lost¹⁸. Introgression poses a serious threat to all subspecies of inland cutthroat trout in western North America as a result of widespread stocking of rainbow trout into historical cutthroat trout habitats; two subspecies are now extinct and five are listed as threatened under the US Endangered Species Act. The westslope cutthroat trout is the most widely distributed subspecies, and hybridization is the leading threat to the persistence of genetically pure populations; known non-hybridized populations occupy less than 10% of their historic range¹⁹.

Relative to cutthroat trout, rainbow trout prefer warmer temperatures, lower spring flows, earlier spring runoff, and tolerate

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greater environmental disturbance^{15,17,20,21}. Therefore, we tested the prediction that increased summer stream temperatures, decreased spring precipitation, and wildfire disturbance have influenced the spatiotemporal spread of hybridization throughout the Flathead River system (USA and Canada; Supplementary Fig. 1 and Supplementary Table 1). The drainage presents an ideal location to examine this prediction because it is one of America's most pristine river systems²² and does not contain other introduced species that may influence interactions between these species. However, the basin has warmed considerably over the past century, with annual average temperatures warming at two times the global average²³. From 1948 to 2008, mean annual air temperatures increased by 0.82 °C, a rate of 0.14 °C/decade, yet from 1978 to 2008 warming nearly tripled to 0.36 °C/decade²⁴. Increasing air temperatures are contributing to a decrease in spring snowpack and a shift towards an earlier spring season, resulting in peak spring runoff two to three weeks earlier than the historic average and lower spring and summer flows^{23–25}, which are directly contributing to increased summer stream temperatures^{26,27}. Changes in spring temperature and precipitation correspond strongly to these observed changes and significantly influence the timing and magnitude of streamflow in the basin^{25,28}. Furthermore, wildfire has burned approximately 16% of the basin from 1984 to 2008, potentially increasing the rate of stream warming by removing canopy cover²⁴. Such changes are hypothesized to benefit rainbow trout, as spawning and recruitment are limited by high spring flows and cold water temperatures¹⁵.

Over 20 million rainbow trout were stocked throughout the Flathead River system, primarily in low elevation areas, beginning in the late 1800s and ending in 1969⁵. Despite massive stocking efforts, genetic samples collected from the late 1970s and early 1980s detected low levels (<2%) of hybridization in just 2 of 20 sites, and a hybrid swarm (representing multiple generations of hybridization) with a predominant (92%) genetic contribution from rainbow trout was discovered in the lower valley in 1994 (Fig. 1a and Supplementary Table 1)^{5,29}. Conversely, samples from the 2000s showed introgression in 9 of the 18 previously non-hybridized sites, demonstrating that introgression increased rapidly over a 30-year period (from 10% to 52% of all sites; Fig. 1b). In the most extreme example, introgression in one population increased tenfold from 3% to 33%.

Climatic factors, including May precipitation (representing the timing and magnitude of spring streamflow^{25,28}) and summer stream temperature, were strongly related to the extent of hybridization occurring over geographic space (Fig. 1 and Table 1 and Supplementary Tables 2 and 3); however, recent wildfire disturbance was not related to hybridization levels. An interaction effect between May precipitation and proximity to the historic source of rainbow trout best explained hybridization levels across the stream network (Table 1; $R^2 = 0.81$). Introgression was substantially greater in populations where May precipitation was low (<2 cm), mean stream temperatures were >10 °C, and the source population was within 90 km (Fig. 1 and Fig. 2a). The best-supported model explaining temporal change in introgression also included an interaction between changes in precipitation and distance to source (Table 1 and Supplementary Table 4), where the greatest increases in hybridization occurred at locations near the source and with the greatest decreases in precipitation (>0.53 cm; Fig. 2b). The only population with a decrease in introgression occurred at a location where precipitation increased. Finally, there was strong evidence that an interaction between stream temperature and precipitation best explained introgression after correcting for distance ($P < 0.001$; reduction in AIC_C relative to intercept-only model was 40.7).

Invasion success of non-native species is often facilitated through complex interactions between biotic and abiotic factors operating at multiple spatial and temporal scales. In this study, covariation among spatial and environmental variables (Supplementary Table 5)

Table 1 | Best-supported models explaining spatial variation and temporal changes in rainbow trout admixture.

Model	N	AIC _C	ΔAIC _C	R ²
Spatial models				
$D \times P$	3	120.3	0.0	0.81
$D \times T$	3	136.6	16.3	0.73
$T \times P$	3	143.3	23.0	0.69
$D + P$	2	166.3	45.9	0.49
$P + T$	2	167.0	46.7	0.48
Temporal models				
$D \times P$	3	126.54	0.00	0.61
D	1	130.46	3.92	0.41
$D + T$	2	132.59	6.05	0.41
$D + P$	2	132.60	6.06	0.41
$D \times T$	3	132.66	6.12	0.47

Model covariates are represented by D = distance to downstream source of rainbow trout, P = May precipitation, T = average summer temperature. For temporal models, P and T represent change in precipitation and temperature, respectively. N = number of predictor variables.

complicate our ability to determine the relative influence of factors that promoted the rapid spread of hybridization, a situation that is common when non-native species invade from lower elevation areas. The observed temporal and spatial patterns in hybridization, however, were probably not the result of source effect alone for several reasons. First, historic genetic data, pre-dating the recent period of accelerated warming, revealed high levels of introgression in only one source population despite millions of rainbow trout released over the past century. Second, all the best-supported models explaining hybridization across space and over time included strong and consistent hydroclimatic effects (Table 1). Last, after correcting for distance to source, climatic factors still explained a significant amount of variation in hybridization levels.

We found that climatic drivers and human-mediated introductions of an invasive species have interacted to increase introgressive hybridization in nature. The rapid spread of rainbow trout hybridization was probably driven by an earlier peak and decrease in magnitude of spring streamflow. In their native ranges, rainbow trout spawn early in the spring, whereas cutthroat trout are adapted to spawn later, following high spring-flow events in snow-melt dominated systems¹⁷. High spring flows and peak pulses in the snow-melt hydrograph caused by spring precipitation can limit rainbow trout recruitment outside their native range because such flows can scour eggs from spawning nests or wash away newly emerged juveniles¹⁵. Thus, periods of reduced spring flooding and flows have probably allowed rainbow trout to proliferate and hybridize with native cutthroat trout populations. Summer water temperature was also related to the extent of hybridization, but to a lesser degree, probably owing to its direct relationship with spring precipitation and summer baseflow²⁵. Nevertheless, rainbow trout have higher thermal tolerances than cutthroat trout²¹, so the direct effects of temperature may be more important as water temperatures continue to rise.

Aquatic ecosystems in western North America are predicted to experience increasingly earlier snowmelt in the spring, reduced late spring and summer flows, warmer and drier summers, and increased water temperatures^{23,25}. Although regional spring precipitation is projected to increase, which may buffer these trends, periods of reduced and earlier spring precipitation (for example, drought or decreases in orographic precipitation enhancement²⁸) will significantly exacerbate these conditions²⁵. These climatic changes threaten to erode native trout genomes as rainbow trout continue to expand their range through climate-induced 'windows

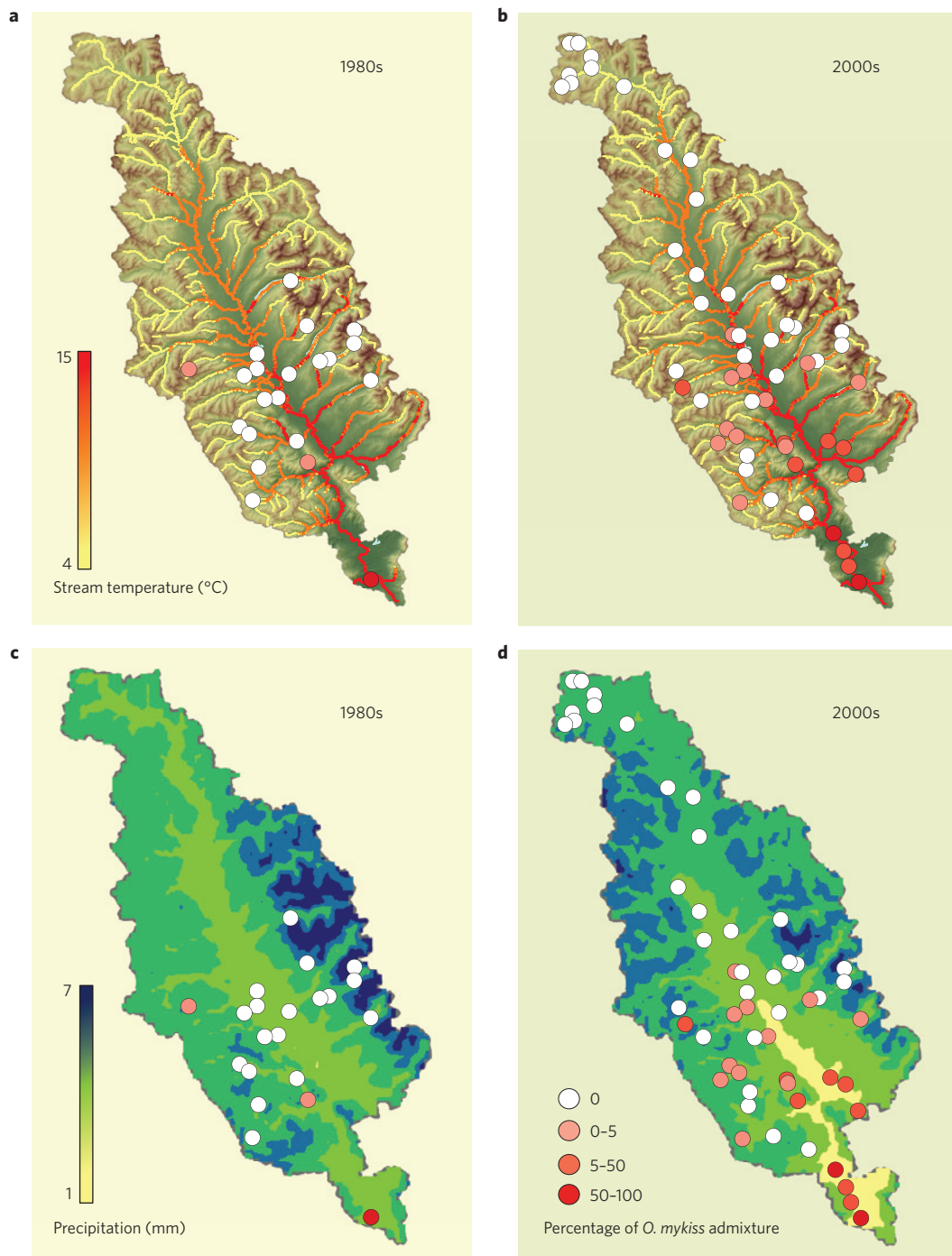


Figure 1 | Spatiotemporal spread of hybridization relative to climatic changes. **a-d**, Maps showing the spread of rainbow trout hybridization in relation to average decadal summer stream temperature (**a,b**) and May precipitation (**c,d**); 1980s (**a,c**) and 2000s (**b,d**). Sample data are included in Supplementary Table 1.

of opportunity⁷, putting many extant populations at greater risk than previously thought. Many native salmonids are already at risk of climate-induced extirpation due to physiological requirements for cold temperatures, combined with historic population declines from a century of intensive habitat alterations and species introductions²⁴. Our findings underscore the negative consequences of climate change for native trout and other species threatened by human-mediated hybridization, as well as the potential for both ecological and evolutionary impacts on biodiversity.

A major consequence of hybridization between native and non-native species is reduced fitness due to the break-up of co-adapted

gene complexes and disruption of local adaptations that have evolved together over thousands of generations¹⁰. Indeed, hybrid trout in this region have substantially reduced fitness relative to pure cutthroat trout¹⁰. For threatened populations already facing other stressors, decreases in fitness as a result of outbreeding depression could cause further decline and extirpation. Although hybridization may increase genetic diversity for some species⁷, and thus adaptive potential, genomic extinction seems imminent for cutthroat trout if hybrid source populations are not eliminated.

As predicted, hybridization can rapidly spread as invasive species expand their range as a result of perturbations in climatic

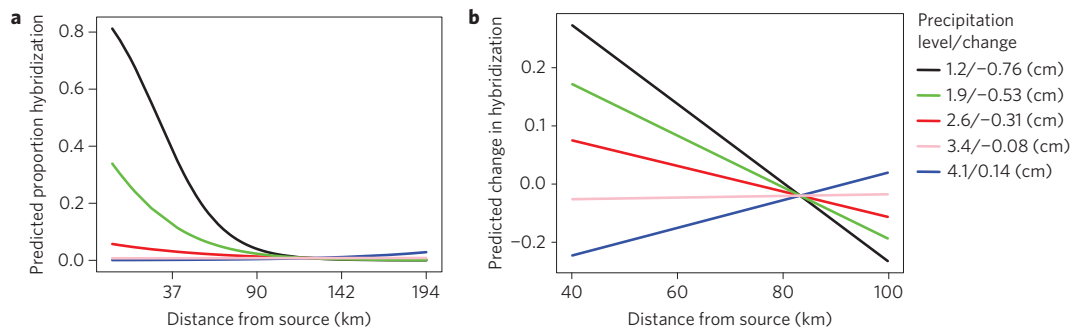


Figure 2 | Interactive effects of precipitation and source dynamics on hybridization across space and time. **a,b**, Relationships between predicted proportions of **(a)** and predicted changes in **(b)** rainbow trout admixture and distance to the downstream source of rainbow trout. The lines represent different magnitudes of May precipitation or changes in May precipitation (see legend).

conditions, with irreversible evolutionary consequences for threatened species. Protecting genetic integrity and diversity—critical for long-term resiliency in the face of environmental change—will be incredibly challenging when species are threatened with climate-induced invasive hybridization. Conservationists will be increasingly confronted by a double-edged sword: protect native genomic integrity via isolation management at the cost of losing genetic and life-history diversity, or allow introgression to proceed, causing extinction of native genomes that have evolved over millennia. Climate change will, thus, pose complex and, at present, relatively underappreciated dilemmas for biodiversity protection in the future.

Methods

Historical (1978–1984) samples were genotyped at six species diagnostic allozyme loci^{5,29}. Recent (2000–2008) samples from the 21 historically sampled populations and a further 29 populations throughout the basin were genotyped at seven diagnostic microsatellite loci^{10,30} (Supplementary Table 1). A species diagnostic locus has non-overlapping allele sizes in the two parental taxa. For our purposes, individual trout could have zero, one or two rainbow trout alleles at each locus. These molecular markers have identical statistical properties for estimating introgression at the population level (that is, detection probability does not vary by marker type). However, by using more diagnostic loci in recent samples the sampling error around our estimates is slightly reduced and there is a small increase in power to detect low levels of hybridization. With a sample size of 25 individuals (average sample size in this dataset) the power to detect 1% rainbow trout admixture is 0.95 with six diagnostic loci, 0.97 with seven diagnostic loci, and 0.99 with ten diagnostic loci. The proportion of rainbow trout admixture in each population was calculated as the number of rainbow trout alleles divided by total number of alleles genotyped.

Linear models and Akaike's Information Criterion (AIC) were used to test for relationships between variables hypothesized to influence introgression and to select the best-supported models. Specifically, we tested whether biotic and climatic variation were related to the amount of rainbow trout hybridization across sites ($N = 50$) and over time on the basis of repeat samples from the late 1970s and early 1980s and early 2000s ($N = 20$ sites; the source population was not included in the model). Before spatial model-selection analyses, proportions of rainbow trout introgression (p) were adjusted by $((p(N-1)+1/2)/N)$ to avoid zeros, then logit transformed to linearize relationships. The response variable for temporal change in hybridization was the observed proportion of introgression in recent samples minus the proportion in historical samples. To determine if climatic effects were still evident after removing the effects of distance to source, we tested for relationships between temperature and/or precipitation and residuals from the relationship between distance to source and proportion of introgression.

Predictor variables for each population included flow-connected stream distances to the source of rainbow trout^{5,20,30}, spring precipitation (April, May, June, and three month average), summer stream temperature, and the presence or absence of recent wildfire (since 1984) within each stream drainage. We tested for additive and interactive effects between covariates. Covariates were obtained for each location using ArcGIS version 10.1 (Environmental Systems Research Institute, Redlands). Average summer stream temperature conditions were predicted using a spatially explicit stream temperature model for the Flathead River basin²⁶ driven by high-resolution air temperature surfaces (800 m). Average precipitation conditions were calculated from daily precipitation surfaces (1 km) processed from National Aeronautics and Space Administration (NASA) Daymet data. Because genetic samples were collected from multiple age classes

(age-1-age-3), climatic covariates were averaged over the three years prior to genetic sampling. Average precipitation for the month of May was found to have the strongest statistical relationship with introgression of all spring months and average conditions tested. Therefore, the temporal analyses used the change in average stream temperature and May precipitation from 1980–1984 to 2000–2005 as predictor variables.

All model results (Supplementary Tables 2 and 4), parameter estimates from best-supported models (Supplementary Table 3), and correlations between predictor variables (Supplementary Table 5) can be found in the Supplementary Methods.

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Author contributions

C.C.M. had the original idea for the study. C.C.M., R.P.K., L.A.J. and R.A-C. designed the study; C.C.M., M.C.B., R.F.L. and F.W.A. collected the data; C.C.M., R.P.K., L.A.J., R.A-C., M.C.B., R.F.L., W.H.L., G.L. and F.W.A. analysed and interpreted the data; C.C.M., R.P.K., L.A.J., R.A-C., M.C.B., R.F.L., W.H.L., G.L. and F.W.A. wrote the paper. All authors discussed the results and commented on the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.