ARTICLE

Abiotic conditions are unlikely to mediate hybridization between invasive rainbow trout and native Yellowstone cutthroat trout in a high-elevation metapopulation

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Abstract: Understanding factors mediating hybridization between native and invasive species is crucial for conservation. We assessed the spatial distribution of hybridization between invasive rainbow trout (*Oncorhynchus mykiss*) and native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouveri*) in the Lamar River of Yellowstone National Park using a paired telemetry and genetic dataset. Spawning populations containing hybrids (15/30) occupied the full spectrum of abiotic conditions in the watershed (stream temperature, stream size, runoff timing), including an intermittent stream that dried completely in late June, and mainstem spawning locations. Hybrids and rainbow trout occupied an entire high-elevation (~2500–1900 m) tributary where rainbow trout ancestry was highest in headwaters and decreased downstream. Fluvial distance to this ostensible source population was the only covariate included in top hybridization models; effects of abiotic covariates and stocking intensity were relatively weak. In this watershed, abiotic conditions are unlikely to mediate continued hybridization. We conclude that management intervention is important for the persistence of nonhybridized Yellowstone cutthroat trout and highlight the value of pairing telemetry with genetic analysis to identify and characterize populations for hybridization assessments.

Résumé : Il est d'importance cruciale pour la conservation de comprendre les facteurs qui modulent l'hybridation d'espèces indigènes et envahissantes. Nous avons évalué la répartition dans l'espace de l'hybridation entre des truites arc-en-ciel (*Oncorhynchus mykiss*), une espèce envahissante, et des truites fardées de Yellowstone (*Oncorhynchus clarkii bouveri*), une espèce indigène, dans la rivière Lamar du Parc national de Yellowstone, en utilisant un ensemble jumelé de données génétiques et de télémétrie. Les populations reproductrices contenant des hybrides (15/30) occupent toute la fourchette des conditions abiotiques (température dans le cours d'eau, taille du cours d'eau, moment de l'écoulement) observée dans le bassin versant, incluant un cours d'eau intermittent qui était complètement asséché à la fin de juin et des lieux de frai dans le cours principal. Des hybrides et des truites arc-en-ciel occupaient l'entièreté d'un affluent de haute altitude (~2500–1900 m) dans lequel une ascendance de truites arc-en-ciel était maximum dans les eaux d'amont, diminuant dans les eaux plus en aval. La distance fluviale par rapport à cette population source apparente est la seule covariable incluse dans les meilleurs modèles d'hybridation; les effets des covariables abiotiques et de l'intensité de l'ensemencement sont relativement faibles. Dans ce bassin versant, il est peu probable que les conditions abiotiques modulent l'hybridation à l'avenir. Nous concluons que des interventions de gestion sont importantes pour assurer la persistance de truites fardées de Yellowstone non hybrides et insistons sur l'utilité de jumeler la télémétrie et l'analyse génétique pour cerner et caractériser les populations pour des évaluations de l'hybridation. [Traduit par la Rédaction]

Introduction

Extinction by hybridization is a prominent conservation threat, especially as introductions of non-native species increase globally (Rhymer and Simberloff 1996; Todesco et al. 2016). This is a common issue for many fishes because of weak barriers to interspecific hybridization and widespread translocation of non-native species (Mayr 1963; Rhymer and Simberloff 1996). For example, all subspecies of cutthroat trout (*Oncorhynchus clarkii* ssp.) in western North America have been adversely impacted by hybridization with non-native rainbow trout (*Oncorhynchus mykiss*). After introductions, hybridization with native trout can proceed until hybrid swarms develop, wherein all remaining individuals in a population are hybrids and the parental taxa become extinct (i.e., genomic extinction; Epifanio and Philipp 2000; Allendorf et al. 2001). Rainbow trout may also replace cutthroat trout entirely by

demographic processes. Combined with additional stressors like habitat alterations, stocking of other exotic species, and warming temperatures, many native cutthroat trout subspecies have declined to occupy only a fraction of their historical ranges (Shepard et al. 2005; Gresswell et al. 2011). Such declines have motivated a substantial effort to identify and protect remaining nonhybridized populations and to better understand mechanisms influencing hybridization outcomes.

Two general hypotheses have been forwarded to predict hybridization risk for cutthroat trout populations. The first is that abiotic conditions, namely cold water temperatures, will mediate the spread of hybridization because of ecological differences between taxa (Isaak et al. 2015; Young et al. 2016, 2017). Some laboratory studies demonstrate that cutthroat trout have a lower temperature tolerance and metabolic rate than rainbow trout (Bear et al. 2007; Rasmussen et al. 2012; Yau and Taylor 2014), and rainbow

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1434

Can. J. Fish. Aquat. Sci. Vol. 77, 2020

trout generally spawn later in the year (Henderson et al. 2000; DeRito et al. 2010). These traits are hypothesized to provide an advantage for some cutthroat populations at high-elevation sites characterized by cold water and late snowmelt runoff (that would scour eggs of earlier-spawning rainbow trout), thereby reducing the likelihood of rainbow trout colonization and subsequent hybridization (Fausch et al. 2001; Young et al. 2016). Distributional patterns of hybridization support this hypothesis. Hybridization is rare above 1700 m elevation (Weigel et al. 2003; Gunnell et al. 2008; Yau and Taylor 2013) and often decreases along an elevation and temperature gradient (Gunnell et al. 2008; Bingham et al. 2016). Therefore, some scientists posit that cold-water habitats (<11 °C) will serve as refugia for nonhybridized cutthroat trout (Isaak et al. 2015; Young et al. 2016). However, hybridization is often more strongly related to historical stocking patterns and rainbow trout source connectivity than abiotic gradients (Hitt et al. 2003; Loxterman et al. 2014). Thus, the second general hypothesis is that historical propagule pressure, fluvial connectivity to modern sources, and dispersal can overwhelm ecological resistance to hybridization.

The role of propagule pressure, a measure of the intensity of non-native introductions, is widely recognized as a strong predictor of invasion success across many taxa. This holds true for rainbow × cutthroat trout hybridization scenarios; the more rainbow trout stocked at or near a site, the more likely it is that invasive genes will introgress into the native population (Bennett et al. 2010; Muhlfeld et al. 2017). Studies have also demonstrated strong correlations between hybridization and fluvial distance from naturalized rainbow trout populations from which fish can disperse (Boyer et al. 2008; Kovach et al. 2011; Loxterman et al. 2014). These examples suggest that high numbers of rainbow trout arriving in a native cutthroat population (by direct stocking or dispersal from elsewhere) may overwhelm aforementioned patterns of ecological resistance. Therefore, cold water alone may be insufficient to prevent expanding hybridization and eventual genomic extinction (Muhlfeld et al. 2017; Kovach et al. 2017). One of the challenges to understanding the relative role of abiotic and biotic mechanisms of invasion, however, is strong covariance among abiotic gradients, historical stocking locations, and contemporary rainbow trout source connectivity (Muhlfeld et al. 2009; Loxterman et al. 2014).

Most large-scale rainbow trout stocking occurred at warmer, lower-elevation, mainstem sites, whereas stocking at cold, high-elevation, headwater streams was historically rare; this distinction is important to consider (Kovach et al. 2017). For example, over 20 million rainbow trout were stocked in the Flathead River watershed, Montana (mostly at lower elevations), and this has played a major role in the distribution of present-day hybridization, which has been spreading rapidly upstream over the past 20 years from naturalized populations (Boyer et al. 2008; Muhlfeld et al. 2014). Throughout western North America, this pattern of low-elevation-biased propagule pressure is common leading to naturalized rainbow trout sources in lower portions of watersheds (Bennett et al. 2010; Mandeville et al. 2019). An important question is raised: Is hybridization absent or limited at cold, high-elevation sites because of ecological differences between taxa and ecological resistance, or is it because these sites have had less exposure to rainbow trout?

A better understanding of hybridization risk is integral to guide conservation efforts. A strong shielding effect of cold water would suggest that many native populations are safeguarded from genomic extinction, even if fluvially connected to rainbow trout populations. Alternatively, if hybridization were likely for any native population with a connected rainbow trout source nearby, there would be a more urgent incentive to take proactive conservation measures. Here, we assess the relative role of abiotic and biotic factors in the spatial distribution of hybridization between rainbow trout and Yellowstone cutthroat trout (*Oncorhynchus clarkii bouveri*) in the Lamar River watershed of Yellowstone National Park. A unique invasion scenario — involving relatively low rainbow trout stocking intensity and a naturalized rainbow trout source occupying an entire high-elevation watershed — makes this case study distinct. We are able to evaluate spatial patterns of hybridization in a scenario where abiotic conditions are not strongly collinear with rainbow trout source connectivity and propagule pressure was, overall, very low.

The Lamar River metapopulation is also of high conservation value and represents a hopeful stronghold for nonhybridized Yellowstone cutthroat trout to persist into the 21st century (Al-Chokhachy et al. 2018). This metapopulation encompasses a large and pristine watershed, whereas most nonhybridized populations occur in isolated stream segments and face inherent demographic and genetic risks to persistence (Hilderbrand and Kershner 2000). Our objectives were to (*i*) characterize the spatial distribution of hybridization in spawning populations and individuals during the nonspawning season and (*ii*) examine the relative role of abiotic and biotic factors in the determining the current distribution of population hybridization. Ultimately, our study goals were to understand the risk of hybridization spreading across the metapopulation and formulate a management plan to prevent this from happening.

Methods

Study site

The Lamar River watershed (1731 km²) is mostly within Yellowstone National Park and flows 78 km from the headwaters (elevation = 3015 m) to the confluence with the Yellowstone River (1829 m) (Fig. 1). Mean annual discharge is 25 m³·s⁻¹ (US Geological Survey gaging station No. 06188000). Yellowstone cutthroat trout are the only native salmonid, which colonized the watershed ~10 000 years ago after Pleistocene glacial retreat (Campbell et al. 2011). About 250 000 rainbow trout were reportedly stocked (1932–1955) in five locations in the watershed across a range of elevations (~1900 to 2300 m), whereas 16 million cutthroat trout were stocked in 18 locations (Varley 1981; Fig. 1; also see online Supplementary material, Table S1[1]). Stocking records do not specify the subspecies of cutthroat stocked, though most are assumed to be Yellowstone cutthroat trout from the Yellowstone Lake Hatchery (Varley 1981). Today, rainbow trout are common only in Buffalo Fork Creek, a tributary that was historically fishless upstream of a falls near its confluence with Slough Creek (Fig. 1). A small lake near the upper end of this tributary (Hidden Lake) was stocked with 3500 rainbow trout in 1932; this is the only recorded stocking event in the entire Buffalo Fork Creek watershed (Table S1[1]).

Several prominent geomorphic features likely play an important role in hybridization dynamics. A waterfall on the Yellowstone River (Knowles Falls), 22 km downstream from the confluence of the Lamar River, prevents upstream dispersal of non-native fish that are common outside of Yellowstone National Park. Within the Lamar River watershed, several canyons and waterfalls are considered complete or partial barriers to upstream movement (Fig. 1). We designate the area downstream of the Slough Creek waterfall and Lamar River canyon as the lower watershed and the upper watershed as the area upstream from these features (Fig. 1).

Rainbow and hybrid trout have been reported in the Lamar River (upstream to Soda Butte Creek) and lower Slough Creek (below the falls) for many years. Yet, their distribution is apparently spreading. In 2003 hybrids were first reported in upper

Fig. 1. Map of the study area in the Lamar River watershed in Yellowstone National Park with locations of populations genetically tested for rainbow trout (RT) hybridization. Map produced with Quantum GIS and map data sources from USGS, Isaak et al. (2017), and Esri. [Colour online.]
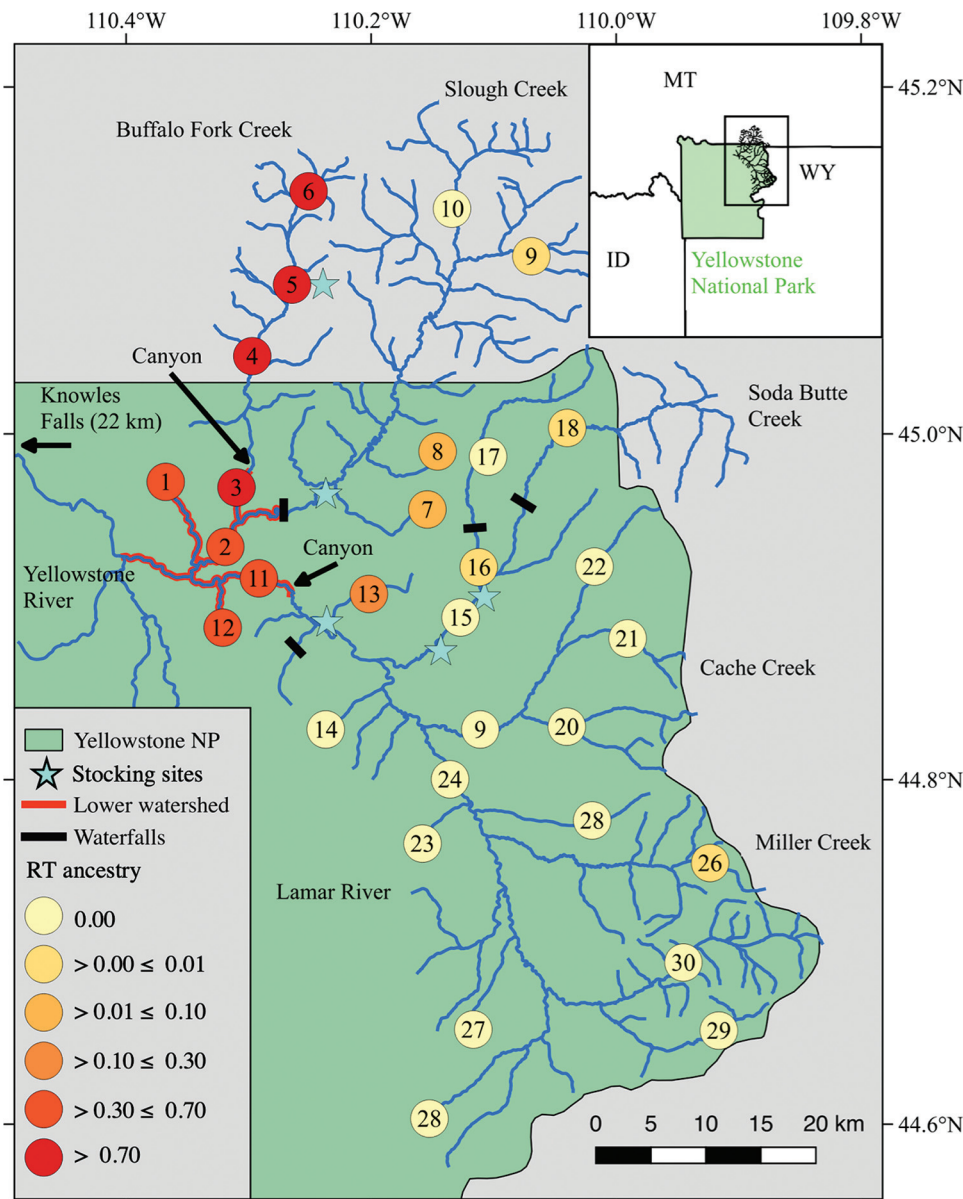
Slough Creek by anglers, and these fish were confirmed to be hybrids with genetic analysis (Ertel et al. 2017). In 2014 genetic sampling by the National Park Service detected rainbow trout alleles as far upstream in the Lamar River as Flint Creek (site 23, Fig. 1). These presumed increases in the extent of hybridization were part of the motivation for this study.

Spawning population designation and sampling

To identify and genetically characterize spawning populations throughout the watershed, we used a two-part approach. This involved (*i*) a telemetry study to track tagged and genotyped fish to spawning locations (Heim 2019), combined with (*ii*) sampling fish from small streams and assuming these reflect distinct populations for genetic analysis. Sampling of small streams designated a priori is a more standard approach but has limitations. Because of the migratory behavior of salmonids, fish captured in large mainstem sites usually constitute fish of mixed natal origin. These samples, then, do not reflect distinct breeding populations, so

mainstem sites are often excluded from studies describing the spatial distribution of hybridization. Second, populations that are important, but not designated a priori for sampling, are also excluded from analysis following this approach. The use of telemetry helped to overcome these two limitations. In total, 730 genotypes were used to describe the occurrence and degree of hybridization in 29 distinct stream-dwelling populations and one lake-dwelling population.

Radio telemetry, passive integrated transponder (PIT) tags, and sampling during spawning periods was used to assign adult fish (*n* = 217) to 14 spawning populations based on observed spawning runs or maturity status in a concurrent study (Heim 2019). Fish were observed making spawning runs into tributaries monitored with streamwide PIT antenna or assigned to spawning locations based on radio telemetry detections. This telemetry study also revealed that fish from multiple spawning populations co-occurred in tributaries as small as Soda Butte Creek (mean annual

1436

Can. J. Fish. Aquat. Sci. Vol. 77, 2020

Table 1. Sampling information and results of genetic testing.

No.	Stream	Sample size		YCT	RT	CTX (F_1)	pHyb	pRT
		Telem.	Capture					
1	Unnamed (slough)[a]	66	6	7	3	62 (7)	0.86	0.571
2	Slough (lower)	18	0	7	0	11 (4)	0.61	0.322
3	Buffalo (lower)[a]	6	21	1	0	26 (2)	0.96	0.724
4	Buffalo (middle)[a]	0	20	0	15	5 (0)	0.25	0.996
5	Hidden Lake[a]	0	21	0	18	3 (0)	0.14	0.998
6	Buffalo (upper)	0	20	0	10	10 (0)	0.50	0.988
7	Hornaday	0	19	16	0	3 (0)	0.16	0.048
8	Elk Tongue	0	25	18	0	7 (0)	0.28	0.036
9	Abundance[b]	0	51	50	0	1 (0)	0.02	<0.000
10	Slough (upper)	0	29	29	0	0 (0)	0	0
11	Lamar (lower)	5	0	1	0	4 (2)	0.80	0.422
12	Crystal	17	4	1	0	20 (0)	0.95	0.454
13	Rose	56	9	44	0	21 (0)	0.32	0.111
14	Chalcedony[c]	20	4	20	0	4 (0)	0.20	0
15	Soda Butte (lower)	1	0	1	0	0 (0)	0	0
16	Pebble (lower)	5	0	4	0	1 (0)	0.20	0.003
17	Pebble (upper)	0	25	25	0	0 (0)	0	0
18	Soda Butte (upper)[a]	0	23	20	0	3 (0)	0.13	0.001
19	Cache (lower)	1	0	1	0	0 (0)	0	0
20	South Cache	0	23	23	0	0 (0)	0	0
21	Cache CC3[d]	0	30	28	0	0 (0)	0	0
22	Cache (upper)	0	28	28	0	0 (0)	0	0
23	Flint	8	12	20	0	0 (0)	0	0
24	Lamar (middle)	5	0	5	0	0 (0)	0	0
25	Calfee	1	25	26	0	0 (0)	0	0
26	Miller	8	22	29	0	1 (0)	0.03	0.002
27	Willow	0	21	21	0	0 (0)	0	0
28	Mist	0	30	30	0	0 (0)	0	0
29	Little Lamar	0	30	30	0	0 (0)	0	0
30	Lamar (upper)	0	15	15	0	0 (0)	0	0

Note: Column data indicates the following: Telem, samples from a concurrent telemetry study (Heim 2019); Capture, samples collected by angling or electrofishing; YCT, number of Yellowstone cutthroat trout; RT, number of rainbow trout; CTX (F_1), number of hybrids followed by number of F_1 hybrids; pHyb, the proportion of hybrids in the sample; pRT the proportion rainbow trout ancestry.

[a]Also contained <0.005 westslope cutthroat trout ancestry proportion.

[b]A single allele diagnostic for RT at *omyrd_rad_2211_hoh* was detected; typically this would be considered an ancestral polymorphism, but prior testing of this population (Montana Fish Wildlife and Parks sample No. 3550) also revealed low amounts of RT ancestry. This is interpreted as evidence of RT introgression.

[c]Contained no evidence of RT ancestry but four individuals had <0.02 westslope cutthroat trout ancestry proportion.

[d]Two fish each had a single allele diagnostic for RT at the marker *omyrd_rad_5666_hoh*; this is interpreted to be an ancestral polymorphism until further genetic testing is conducted.

flow (MAF) = 2.42 m³·s⁻¹) during summer and fall (i.e., intertributary movements) but not in streams smaller than this. Based on this result, we made the assumption that on-site collections, in summer and fall, from streams with <2.00 m³·s⁻¹ MAF would be likely to include only fish natal to that tributary. To increase sample sizes for genetic characterization, we collected additional samples from some of the sites (<2.0 m³·s⁻¹ MAF) identified with telemetry. We also collected samples in other sites, not identified with telemetry (2011 to 2017; Table 1) where MAF was <2.0 m³·s⁻¹, in summer and fall and treated these as population samples. In all on-site sampling, we targeted reaches of at least 5 km to avoid sampling related individuals, and a small tissue sample was taken from each fish.

Genetic sampling of individuals in mixed aggregations

We additionally sampled fish from mainstem locations (*n* = 629), during the nonspawning season, to collect fish with unknown population membership and describe the nonspawning distribution of hybrids and Yellowstone cutthroat trout. These mainstem locations (e.g., Lamar River or Soda Butte Creek) are fished by anglers extensively (~10 000 per year; Heim et al. 2020) and are regularly sampled by the National Park Service. In contrast, remote and often very small tributaries (where many spawn-

ing populations occur) are far more difficult to sample. Thus, even though collections from these sites do not reflect distinct breeding populations, knowing where hybrids are during the summer, when fishing and sampling is possible, is valuable to managers (e.g., for potential removal efforts or monitoring). Sampled mainstem sites included the Lamar River (*n* = 379), Slough Creek (*n* = 199), and Soda Butte Creek (*n* = 51). Sampling was done using angling and electrofishing (2012–2017), and geographic positioning system coordinates (GPS) were recorded for each individual, typically to within 1 km of the sample location. Genetic results for these individuals were depicted graphically with spatially explicit kernel density estimates (Tyers 2017).

Genetic analysis

We used species-diagnostic single nucleotide polymorphism (SNP) loci to describe the hybridization status of populations (*n* = 30) and individual fish (*n* = 1359). Population genetic status was described by the occurrence (presence–absence) of hybridization, the proportion of rainbow trout ancestry (pRT) in a population, and the proportion of individuals in the population that were hybrids (pHyb). Loci genotyped included SNPs diagnostic for Yellowstone cutthroat trout, rainbow trout, and westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and were developed by Amish

et al. (2012), Campbell et al. (2012), Harwood and Phillips (2011), Kalinowski et al. (2011), and Pritchard et al. (2012). The SNPs were determined to be diagnostic using broadly distributed reference populations (12 Yellowstone, 19 westslope, and coastal rainbow trout hatchery strains from across the US). Reference populations also included three redband rainbow trout populations (*Oncorhynchus mykiss gairdneri*). Genotyping was performed at the University of Montana Conservation Genetics Laboratory using laboratory methods described in Bingham et al. (2016). After screening and removing probable nondiagnostic loci (i.e., potential ancestral polymorphisms; Figs. S1–S4[1]), our final marker set included 18 Yellowstone, 18 rainbow, and 16 westslope diagnostic markers. The set of SNPs were used to estimate pRT for each individual and each population. In the absence of westslope cutthroat trout, this estimate is simply the proportion of the total alleles genotyped that indicate non-native ancestry. If westslope cutthroat trout alleles were detected, the maximum likelihood method of Kalinowski (2010) was used to estimate the ancestry proportion of each taxon (i.e., an estimate for all three potentially hybridizing species). With an average sample size of 24 individuals, and in the absence of westslope cutthroat trout admixture, we had an 82% chance of detecting pRT as low as 0.001. With a single individual we had a 50% chance of detecting pRT of 0.01 and a 97.5% chance of detecting pRT of 0.05 (Supplementary materials[1]).

Fish were classified as first-generation hybrids (F_1s) if they had an allele from both rainbow trout and Yellowstone cutthroat trout (i.e., were heterozygous at that loci) for more than 90% of the SNPs. Although a true F_1 will be heterozygous across all such loci, many fish were heterozygous across nearly all but a few loci. This was likely because pure rainbow trout were quite rare and restricted to only two locations, yet fish with pRT of ~0.95 were frequently encountered. A Yellowstone cutthroat trout (pRT = 0.00) crossed with one such hybrid (pRT ~ 0.95) will produce individuals that are quite similar to true F_1s, but possibly homozygous at a very small proportion of diagnostic loci. Using other heterozygosity cutoffs (i.e., 0.85, 0.95) did not fundamentally change results.

Abiotic conditions, propagule pressure, and distance to hybrid source

We used stream temperature, a metric of stream flow timing (center timing of flow mass, CFM), and a proxy for stream size (mean annual flow, MAF) to describe the abiotic conditions at all defined genetic populations. A database was built in Quantum GIS (Quantum GIS Development Team 2016) using the NHD-Plus Version 1 medium resolution (1:100 000 scale) stream layer and attributed with August mean stream temperature predictions (2002–2011) from the NorWeST website (Isaak et al. 2017). We also included predictions of MAF ($m^3 \cdot s^{-1}$) and CFM (day of water year beginning on 1 October; lower values indicate earlier flow timing at a site) from Wenger et al. (2010). Predictions were joined by location to the population genetic samples. These estimates (temperature, CFM, MAF) were compared with in situ measurements and found to adequately represent variation in our study area (Supplementary materials[1]). We did note that the NorWeST predictions were consistently colder, and this bias was lowest (~1 °C) at higher elevations (Fig. S5[1]).

To represent the cumulative intensity of stocking at each genetic population, we calculated an index of propagule pressure (PPI) using the same methods as Bennett et al. (2010) and Muhlfeld et al. (2017) (Supplementary materials[1]). Briefly, PPI for a genetic population represents the cumulative number of rainbow trout that might disperse from all fluvially connected stocking locations. We also developed another covariate that represented fluvial distance to the most prominent rainbow trout population, Buffalo Fork Creek. We considered the site where the Hidden Lake outlet stream flows into Buffalo Fork Creek (Fig. 1, site 5) as an original source of rainbow trout. This location is the only location

in the Buffalo Fork Creek watershed reportedly stocked with rainbow trout and, consistent with this stocking history, has the highest pRT of any population we sampled. Fluvial distance from the Hidden Lake outlet stream to the center of each spawning population was calculated using GIS and the covariate is referred to as D-source. We also classified each population according to whether it was upstream of a presumed impassable barrier (1) or fluvially connected (0) to this site and used this as a model covariate (BAR). All falls or canyons in Fig. 1 were considered barriers (except for the Lamar River canyon). This classification was supported by on-site evaluations and the telemetry study (Heim 2019). Although infrequent and seasonal, movements (upstream and downstream) past Lamar River canyon were observed with telemetry (Heim 2019), but not past any of the other locations (Fig. 1).

Extent of occupied habitat

We estimated the kilometres of stream likely used by fish for spawning in the entire watershed and quantified the extent of stream inhabited by populations with different genetic status. We first removed streams from the GIS network using a MAF cutoff of 0.009 $m^3 \cdot s^{-1}$ and a stream slope cutoff of >11% to exclude habitats not likely to support spawning fish. Intermittent streams where no spawning fish were detected were also removed (an exception was site 1, as many fish spawned here). This resulted in 568 river km of potential spawning habitat. Genetic results from spawning populations were used to categorize sections of the stream network according to current conservation criteria (Al-Chokhachy et al. 2018) as a "pure population" (pRT = 0.00), "core conservation population" (pRT ≤ 0.01), "conservation population" (pRT > 0.01 and pRT ≤ 0.10), "highly hybridized population" (pRT > 0.10) or "unknown status population".

Data analysis

We tested for differences in conditions (abiotic = temperature, CFM, MAF, elevation; biotic = PPI, D-source, BAR) between hybridized sites (pRT > 0.00) and nonhybridized sites (pRT = 0.00) using a Mann–Whitney U test or a χ^2 test (for BAR). We also compared kernel density estimates of these covariates in hybrid versus nonhybridized sites, fit in the R programming environment (R Core team 2017). Nonoverlapping distributions suggest a that a variable strongly limits hybridization, whereas overlapping distributions suggest it does not.

We used multiple regression to examine the association between covariates (abiotic and biotic) and two response variables: (*i*) the occurrence of hybridization (present–absent, logistic regression) and (*ii*) pRT using linear regression. All covariates described above, excluding elevation, were included in modelling. For the logistic regression, populations were considered hybridized if any rainbow trout ancestry was detected (pRT > 0.00; Table 1). In the linear regression, pRT was arcsine-square-root-transformed, and only hybridized populations were included. All covariates were centered and scaled by subtracting the mean and dividing by the standard deviation, to allow for comparison of effect sizes between covariates (Schielzeth 2010). We constructed candidate models sets for all combinations of noncorrelated covariates ($r > 0.50$; Fig. S6[1]), but limited the number of terms in the model to three for logistic regression and two for linear regression because of low sample sizes ($n = 29$; $n = 14$). When scatterplots suggested nonlinear relationships, D-source and temperature were modeled as second-order orthogonal polynomial terms. Akaike's information criteria corrected for small sample size (AIC_c) and Akaike weights (w_i) were used to identify the most plausible models (Burnham and Anderson 2002). For each regression (logistic and linear), a composite model was built with models where $w_i > 0.05$ and model-averaged coefficients were calculated to address uncertainty in parameter estimates with the zero method (Grueber et al. 2011). Hosmer–Lemeshow goodness-of-fit tests suggested a good fit for the top logistic regression models, and residuals for

1438

Can. J. Fish. Aquat. Sci. Vol. 77, 2020

Fig. 2. Rainbow trout (RT) ancestry proportion estimates of 30 populations and their relative location within the watershed (Buffalo Fork Creek, lower watershed, upper watershed; panel A). Populations are sorted according to RT ancestry estimates and labeled according to site codes (Table 1; only hybridized populations are labeled). Three bottom panels depict the spatial distribution of genotyped fish, collected from mainstem sites (i.e., not assigned to a population). Kernel density smoothers are applied to the data and show Yellowstone cutthroat trout (B: $n = 506$), hybrid trout (C: $n = 123$), and F_1 hybrids (D: $n = 29$). High concentration areas are shown in darker greyscale and points from which kernels were estimated are shown (many are overlapping). [Colour online.]
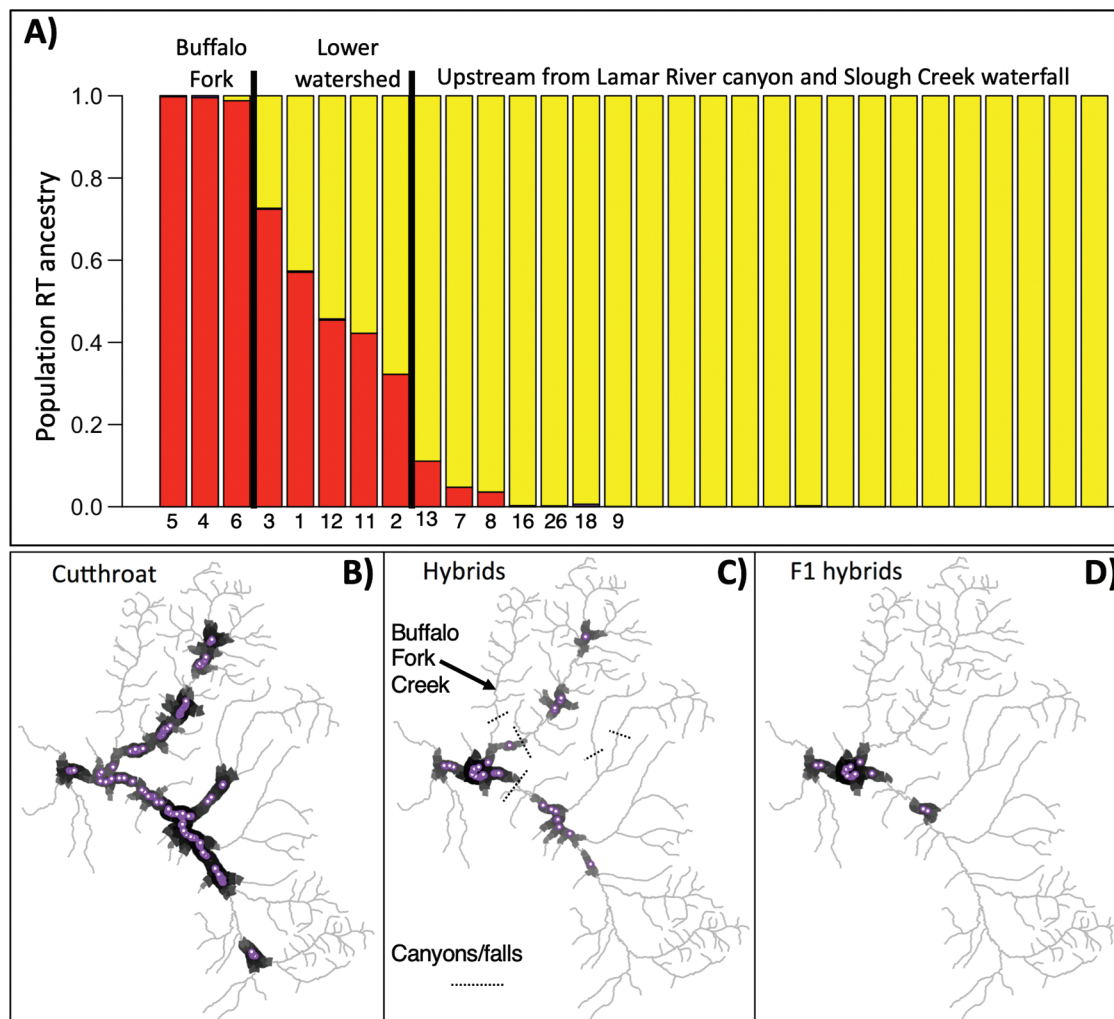
top linear models did not suggest any violations of assumptions. Because sites in upper Buffalo Fork Creek were historically fishless, and perhaps different ecological processes influenced invasions success, we also ran models without these sites. Results were similar and are presented in the online Supplementary materials (Tables S2 and S3[1]).

Results

Spatial patterns of hybridization

Half of the population samples contained evidence of hybridization; half contained only Yellowstone cutthroat trout. Hybridization was notably concentrated in the lower watershed (Slough Creek below the falls and in Lamar River downstream of the canyon; Fig. 1) and the entire Buffalo Fork Creek watershed (Fig. 1). It is visually apparent that pRT decreased with distance from this epicenter of hybridization. For example, in the upper Slough Creek and Lamar River (Figs. 1, 2), populations with the highest pRT were the ones closest to the lower watershed (Fig. 1). Figure 2A clearly illustrates this point. Though at much lower levels, hybridization was also present in distant headwater sites (e.g., site 26, Miller Creek; site 9, Abundance Creek).

Populations of upper Buffalo Fork Creek (above a canyon, Fig. 1) were mostly pure rainbow trout (population pRT range: 0.998–0.996) but also contained some hybrids with very low amounts of Yellowstone cutthroat trout ancestry. Among these sites is Hidden Lake (site 5, with a fluvial connection to Buffalo Fork Creek), the only known stocking location of rainbow trout in the Buffalo Fork Creek watershed. Here we recorded the highest degree of rainbow trout ancestry in the study (pRT = 0.998, elevation = 2362 m; Table 1). Other noteworthy points are that the highest elevation sampled in this study (Buffalo Fork Creek, site 6; 2465 m) was almost entirely rainbow trout and hybrids with individual pRT > 0.95, and pRT within Buffalo Fork Creek decreased at lower elevation (0.724 at 1935 m, site 3), a pattern opposite of what is usually reported.

We also genotyped 629 fish that were collected from mainstem sites during the nonspawning period. We made no assumption about population membership and show their sample locations and genotype category in Figs. 2B–2D. These results mirror that of spawning population samples. Yellowstone cutthroat trout were widespread, hybrids were densely concentrated in the lower watershed (but present in far upstream reaches), and F_1 hybrids were

Fig. 3. Distribution of hybrid genotypes in 15 populations where hybrids were detected, oriented to the riverscape. To distinguish genotype categories within a histogram bin, pure Yellowstone cutthroat trout are shown in yellow, hybrids in grey, and rainbow trout in red. For example, the leftmost bin includes pure Yellowstone cutthroat trout (yellow) and hybrids with individual rainbow trout ancestry proportion < 0.0625 (grey). The rightmost bin includes pure rainbow trout and hybrids with rainbow trout ancestry > 0.937. First-generation hybrids are shown in orange. [Colour online.]
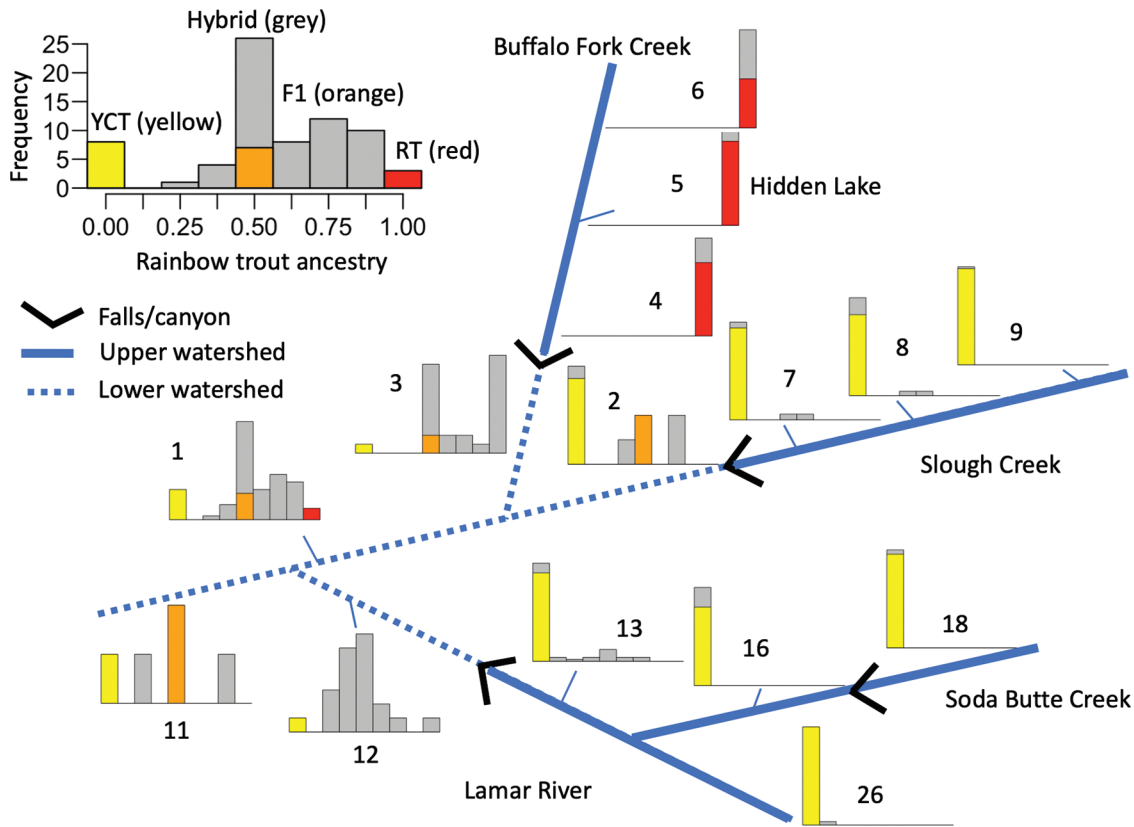
even more limited (but present upstream as far upstream as Soda Butte Creek). A single rainbow trout was sampled in Slough Creek. Differences above and below presumed movement barriers separating the upper and lower watershed were also apparent. Downstream of the Lamar River canyon, 49/87 (56%) of fish sampled from the Lamar River mainstem were hybrids, whereas above the canyon in the Lamar River main stem, only 11/292 (3%) were hybrids. Below the falls on Slough Creek, 90% of mainstem fish were hybrids (53/59) versus only 6% of fish above the falls (9/140).

Genotypic patterns in spawning populations

Hybrid populations upstream of falls or canyons were notably skewed toward either a major contribution of rainbow trout ancestry (sites 4, 5, 6 in Buffalo Fork Creek) or Yellowstone cutthroat trout ancestry (all other populations). Downstream from falls and canyons, genotype distributions become increasingly mixed, and hybrids outnumbered parental taxa (most sites in lower watershed; see Table 1 for genotype counts). This is visually striking when comparing genotype distributions in populations above and below canyons or falls on Buffalo Fork Creek (site 4 versus 3), Slough Creek (7 versus 2), and the Lamar River (13 versus 12; Fig. 3).

We found evidence of first-generation (F_1) genotypes, which are most likely offspring of fish from Buffalo Fork Creek. An F_1 individual (as we have defined it; see Methods) must have a parent with pRT > 0.95, yet fish with this genotype were almost exclusively found in Buffalo Fork Creek (thus these are likely the parents of most F_1s we encountered). In total, 82% of all fish sampled in Buffalo Fork Creek had pRT > 0.95, but only 4/624 (1%) of fish in population samples elsewhere had pRT > 95%.

In contrast with the restricted distribution of rainbow trout, Yellowstone cutthroat trout were broadly distributed, occurring in 90% (27/30) of populations. Even in the lower watershed where hybrids predominated, Yellowstone cutthroat trout were present, albeit outnumbered by hybrids. Nearly all fish in lower Buffalo Fork Creek were hybrids (26/27), barring a single Yellowstone cutthroat trout detected spawning here with radio telemetry. Similarly, passive integrated transponder (PIT) tag detections revealed seven Yellowstone cutthroat trout spawned in a small intermittent stream (site 1), where the population was mostly hybrids (pHyb = 0.86). In Crystal Creek (site 12), a single Yellowstone cutthroat trout was sampled during the spawning period. Thus, complete genetic mixing in our population samples was not evident (Fig. 3).

Extent of occupied habitat

We estimated 568 km of river habitat are likely to support spawning populations in the Lamar River watershed and used the results of spawning population sampling to attribute 380 km (67% of the watershed) with genetic status information. A total of 216 km was tested and exhibited no evidence of hybridization ("pure population" pRT = 0.00, 56% of tested), 280 km was tested and classified as "core conservation populations" (pRT ≤ 0.01, 74% of tested), 15 km was classified as "conservation populations" (pRT > 0.01 ≤ 0.10, 4% of tested), and 84 km were "highly hybridized populations" (pRT > 0.10, 33% of tested). The Buffalo Fork Creek watershed contained 46 km of habitat supporting populations with pRT > 0.70.

1440

Can. J. Fish. Aquat. Sci. Vol. 77, 2020

Table 2. Abiotic conditions present in trout populations of the Lamar River watershed and fluvial distance from Hidden Lake.

No.	Stream	Lat.	Long.	EL	T	MAF	CFM	D	PPI
1	**Unnamed (slough)**	44.941	−110.312	1891	11.8	0.04	203.1	24	60.76
2	**Slough (lower)**	44.929	−110.344	1891	12.6	5.00	220.2	18	52.80
3	**Buffalo (lower)**	44.958	−110.309	1935	11.9	1.69	222.7	15	48.24
4	**Buffalo (middle)**	45.045	−110.297	2303	9.5	1.17	227.5	3	2.91
5	**Hidden Lake**	45.064	−110.275	2362	—	—	—	—	—
6	**Buffalo (upper)**	45.140	−110.251	2465	8.0	0.35	231.3	10	2.12
7	**Hornaday**	44.962	−110.225	2012	10.9	0.10	216.2	27	8.03
8	**Elk Tongue**	44.985	−110.200	2071	8.8	0.07	219.0	31	6.72
9	**Abundance**	45.093	−110.048	2205	10.1	0.26	213.2	57	1.78
10	Slough (upper)	45.130	−110.134	2269	10.6	1.01	225.7	54	2.14
11	**Lamar (lower)**	44.916	−110.324	1865	12.5	13.89	215.1	26	73.50
12	**Crystal**	44.911	−110.322	1913	11.7	0.01	190.2	27	72.71
13	**Rose**	44.899	−110.229	2029	11.8	0.05	206.4	37	98.11
14	Chalcedony	44.849	−110.202	2024	10.9	0.03	208.8	45	86.41
15	Soda Butte (lower)	44.894	−110.127	2032	11.7	2.34	217.4	49	111.86
16	**Pebble (lower)**	44.915	−110.113	2078	11.4	0.50	213.5	52	98.25
17	Pebble (upper)	44.931	−110.113	2171	9.3	0.50	213.5	54	0.00
18	**Soda Butte (upper)**	44.980	−110.067	2144	11.1	1.61	218.3	61	0.00
19	Cache (lower)	44.827	−110.129	2086	10.7	1.78	214.3	51	62.72
20	South Cache	44.832	−110.049	2170	10.5	0.50	213.2	59	43.01
21	Cache CC3	44.875	−110.044	2366	9.8	0.31	216.6	62	37.34
22	Cache (upper)	45.140	−110.251	2409	9.4	0.79	215.9	67	28.65
23	Flint	44.786	−110.123	2209	8.1	0.07	214.3	56	50.35
24	Lamar (middle)	44.787	−110.116	2093	11.3	3.83	209.4	56	50.93
25	Calfee	44.782	−110.088	2278	8.6	0.13	202.8	59	43.32
26	**Miller**	44.753	−109.938	2205	10.1	0.75	209.2	75	19.00
27	Willow	44.708	−110.086	2212	9.1	0.08	204.7	67	28.73
28	Mist	44.622	−110.120	2427	8.6	0.23	207.3	82	13.75
29	Little Lamar	44.642	−109.958	2411	9.7	0.41	212.2	83	13.25
30	Lamar (upper)	44.694	−109.945	2340	9.9	0.71	215.3	83	12.71

Note: Column data indicates the following: Lat., latitude; Long., longitude (latitude and longitude are expressed in decimal degrees); EL, elevation in metres; T, modeled mean August stream temperature in °C; MAF, mean annual flow in $m^3 \cdot s^{-1}$; CFM, center timing of flow mass in day of water year beginning on 1 October; D, fluvial distance from Hidden Lake outlet stream in kilometres; PPI, propagule pressure index in thousands. Streams with bold names contained evidence of rainbow trout ancestry.

Abiotic and biotic correlates of hybridization

Hybrid populations occupied a wider range of abiotic conditions than Yellowstone cutthroat trout populations (Table 2; Fig. 4). Figure 4 shows that no single abiotic covariate provided clear separation between hybrid and nonhybridized sites. Hybrids were present at the highest elevation site, the coldest site, and the site with the latest streamflow timing we sampled (upper Buffalo Fork Creek, site 6; pRT = 0.988). Hybrids were also present at the warmest and largest site (lower Lamar River, site 11; pRT = 0.422) and the site with the earliest stream flow timing (Crystal Creek, site 12; pRT = 0.454).

Despite overlapping distributions (Fig. 4), sites with only Yellowstone cutthroat trout were significantly colder (mean temperature 9.9 °C; range: 8.1–11.7 °C) than those with hybrids (10.9 °C; 8.0–12.6 °C; Mann–Whitney U = 53; p = 0.023). Similarly, sites with just Yellowstone cutthroat trout were significantly higher in elevation (mean elevation = 2233 m; range = 2023–2427 m) than those with hybrids (2072 m; 1865–2464 m; U = 164; p = 0.009). There was no significant difference between MAF (U = 108; p = 0.89) and CFM (U = 80; p = 0.29) in hybridized compared to nonhybridized sites (Fig. 4).

Of the three covariates representing stocking or dispersal, only D-source varied significantly between hybridized and nonhybridized sites (Fig. 4). Hybridized sites were closer to Hidden Lake (mean = 33 km; 3–75 km) than those containing only Yellowstone cutthroat trout (61 km; 45–83 km; U = 31; p < 0.001). Moreover, if the genetic threshold used to classify "core conservation populations" (pRT < 0.01) was considered, D-source perfectly distinguished core conservation populations from other sites (Fig. 5). The occurrence of hybridization was not related to historical

stocking intensity as represented by PPI (U = 108; p = 0.89) or presumed fluvial connectivity to Hidden Lake as represented by BAR (χ^2 = 0.30; p = 0.58).

Distance to Hidden Lake was the only covariate included in the top model predicting the occurrence of hybridization and the model predicting the degree of hybridization; no abiotic covariates were included in either top model as ranked by AIC_c (Table 3). Including additional covariates added little explanatory power to models, and every top model in the confidence set (w_i > 0.05) included D-source. Despite strong support for inclusion of D-source, there was some support for models including additional covariates (Table 3). However, after model averaging, all effect sizes were small relative to D-source and had confidence intervals that overlapped zero (Table 4). Because variables were centered and scaled, covariate estimates in Table 4 can roughly be interpreted as effect sizes and are comparable to one another (Schielzeth 2010). In sum, model results all indicated that distance to presumed source of Hidden Lake was the single best predictor of hybridization. These results were generally similar with or without Buffalo Fork Creek samples (Tables S3 and S4[1]).

Discussion

We found that a cold, high-elevation tributary with late snowmelt runoff has been colonized in its entirety by rainbow trout and hybrids and is probably the main source facilitating hybridization in the Lamar River watershed. By pairing a concurrent telemetry study with genetic sampling, we also found that hybrids spawned in a wider range of abiotic conditions (temperature, stream size, flow timing) than native Yellowstone cutthroat

Fig. 4. Relationship between rainbow trout ancestry proportion (pRT, arcsine-square-root-transformed) and environmental variables elevation (metres), stream temperature (°C), streamflow timing (center timing of flow mass, units are day of water year), and flow volume (mean annual flow, m³·s⁻¹). Relationships with propagule pressure and distance to Hidden Lake, the presumed rainbow trout source, are also shown. Panels on the right show the distribution of conditions in hybridized sites (pRT > 0) and native trout sites with kernel density smoothers. [Colour online.]
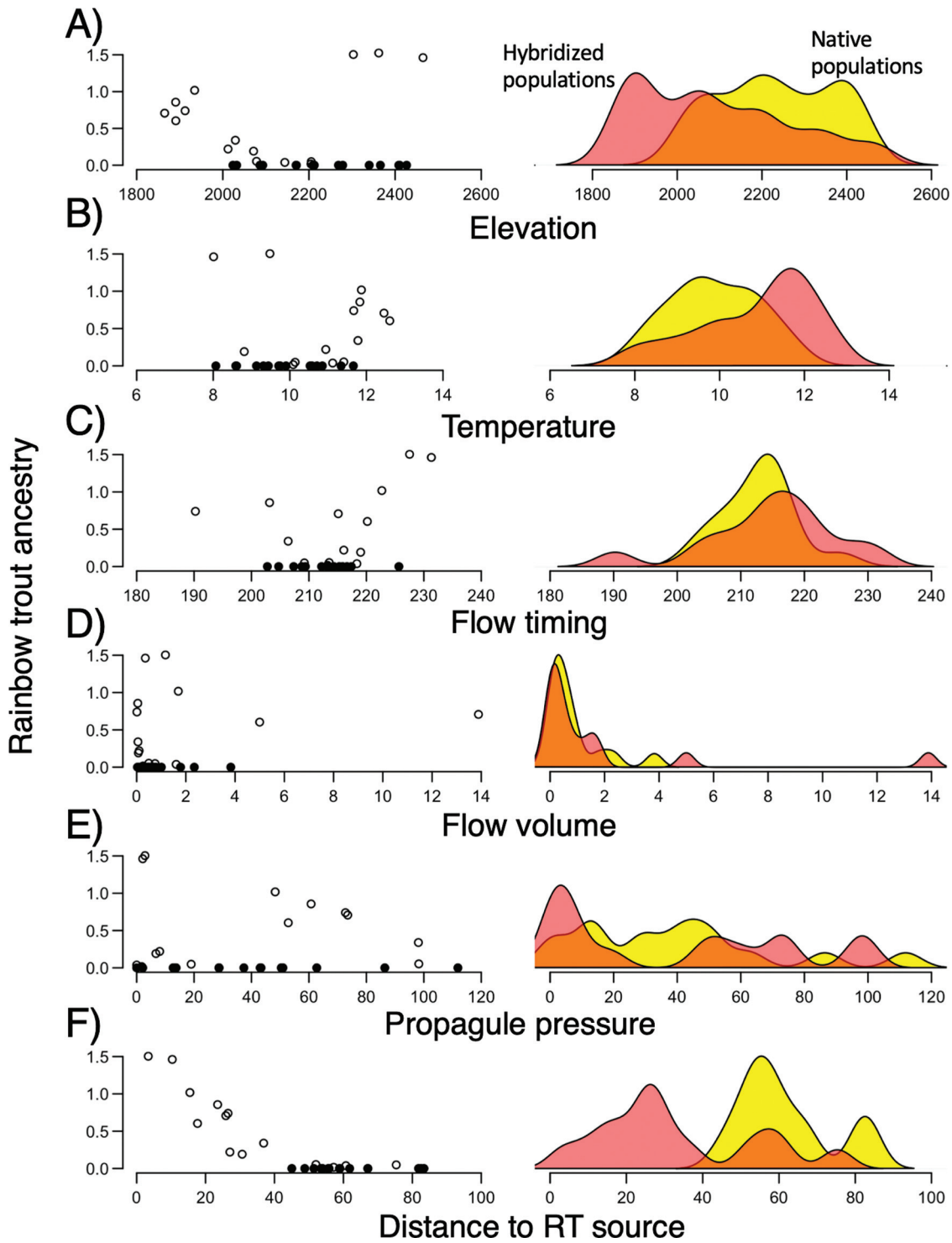
Multiple lines of evidence clearly showed that fluvial distance to a prominent rainbow trout source best explained the spatial pattern of hybridization in the Lamar River watershed. Intuitively, a population that is close to a source receives more invaders because of a higher dispersal probability. Therefore, native popu-

trout — from a small intermittent stream to large mainstem rivers. These results highlight the invasive potential of rainbow trout and their hybrids across a variety of habitat types and that hybridization outcomes in nature are nuanced, complex, and difficult to predict with certainty.

Fig. 5. Distance to rainbow trout source perfectly separated sites into those considered "core conservation populations" as determined by a 0.01 rainbow trout ancestry proportion (pRT) threshold.
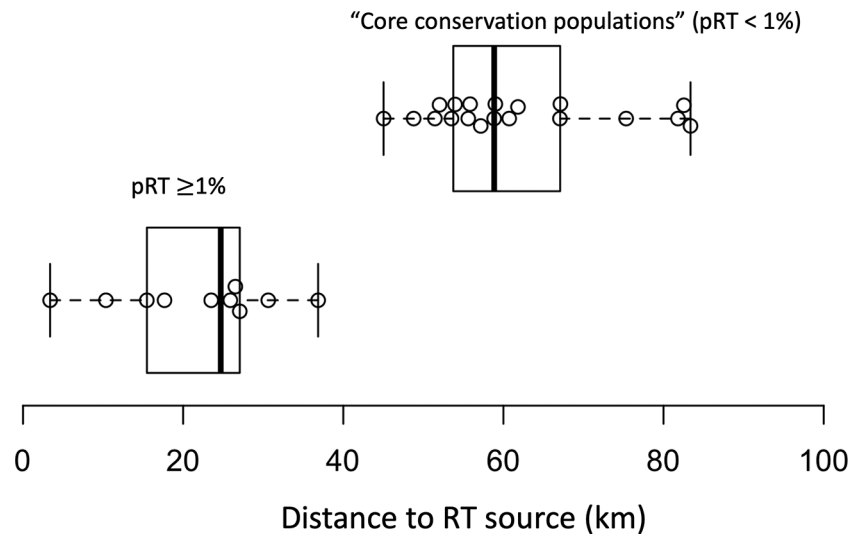
Table 3. Model selection results for top (Akaike weight (w_i) > 0.05) models predicting the occurrence of hybridization (logistic regression, $n = 29$) and the proportion of rainbow trout ancestry detected in hybrid populations (linear regression, $n = 14$) in the Lamar River watershed.

Response	Model	AIC$_c$	ΔAIC$_c$	w_i
Logistic	D	29.12	0.00	0.22
	D + BAR	30.30	1.18	0.12
	D + PPI	30.43	1.31	0.11
	D + T	30.63	1.51	0.10
	D + BAR + T	31.48	2.36	0.07
	D + CFM	31.58	2.46	0.06
	D + MAF	31.62	2.50	0.06
	D + BAR + CFM	32.41	3.29	0.04
Linear	D + D²	2.54	0.00	0.46
	D + D² + BAR	3.52	0.98	0.28
	D + D² + PPI	5.59	3.05	0.10
	D + D² + CFM	5.90	3.35	0.09
	D + D² + MAF	7.55	5.01	0.04
	D + D² + T	7.59	5.04	0.04
	BAR + PPI	20.53	17.99	0.00

Note: ΔAIC$_c$, the difference in the corrected Akaike information criteria (AIC$_c$) value compared with the top model. D, distance to rainbow trout source; T, stream temperature; CFM, center timing of flow mass; MAF, mean annual flow; PPI, propagule pressure index; BAR, movement barrier relation to Hidden Lake (connected or not connected).

Table 4. Model-averaged parameter estimates and confidence limits for logistic models predicting the occurrence of rainbow trout hybridization and linear models predicting the proportion of rainbow trout ancestry.

Response	Term	Estimate	SE	Lower 95%	Upper 95%
Logistic	Intercept	0.53	0.87	−1.24	2.31
	D	−2.39	0.93	−4.30	−0.47
	BAR	−0.44	0.99	−2.43	1.54
	PPI	−0.12	0.37	−0.87	0.62
	T	0.12	0.38	−0.63	0.88
	CFM	−0.05	0.31	−0.69	0.59
	MAF	0.01	0.24	−0.49	0.50
Linear	Intercept	0.35	0.10	0.15	0.55
	D	−0.95	0.18	−1.37	−0.53
	D²	0.37	0.20	−0.07	0.81
	BAR	0.07	0.12	−0.02	0.49
	CFM	−0.02	0.04	−0.20	0.02

Note: D, distance to rainbow trout source; T, stream temperature; CFM, center timing of flow mass; MAF, mean annual flow; PPI, propagule pressure index; BAR, movement barrier relation to Hidden Lake (connected or not connected). Estimates are derived from confidence set of models with Akaike weight (w_i) > 0.05.

lations near an established invasive source more commonly hybridize for simple numerical reasons (Gunnell et al. 2008; Boyer et al. 2008; Muhlfeld et al. 2009; Yau and Taylor 2013). This sort of "mass effect" (Urban et al. 2008) may occur regardless of which taxon or hybrid genotype has higher fitness if dispersal rates are high (Kovach et al. 2017). As new populations are invaded and hybrids produced locally, they can also become sources to further spread invasive genes in a stepping-stone pattern (Kimura and Weiss 1964; Boyer et al. 2008). Therefore, we do not expect that Hidden Lake is the sole contemporary source, but was likely the initial site from which rainbow trout invaded Buffalo Fork Creek and, thereafter, other parts of the watershed.

The importance of Buffalo Fork Creek (a cold, high-elevation site with late snowmelt runoff) as the primary hybridization source is unexpected. This is because cold water often limits rainbow trout invasion (Rasmussen et al. 2012), late and scouring peak flows can prevent rainbow trout recruitment (Fausch et al. 2001),

and hybridization is not often reported above 1700 m (Weigel et al. 2003; Yau and Taylor 2013). Because upper Buffalo Fork Creek was historically fishless, a lack of biotic resistance and competition from established native trout probably contributed to invasion success (Miller 1958; Moyle and Light 1996). Indeed, "priority effects" (i.e., the presence of one species in a location decreases the probability of invasion by another) are important in many plant and animal invasions (Shulman et al. 1983; Mack et al. 2000). Interestingly, cutthroat trout were also stocked in the Buffalo Fork Creek watershed, but were earlier (i.e., had "priority") and in higher numbers than rainbow trout (~170 000 versus 3500), but failed to colonize. In sum, we show that rainbow trout and hybrids are not fundamentally limited from inhabiting cold, high-elevation streams with late peak-flow timing in this watershed.

Potentially contributing to the successful invasion of Buffalo Fork Creek, and perhaps cold-water habitats elsewhere (Muhlfeld et al. 2017), is the high physiological and behavioral variability in rainbow trout. Expanding on prior physiological studies (e.g., Bear et al. 2007; Rasmussen et al. 2012), Yau and Taylor (2014) found that westslope cutthroat trout, in general, could tolerate colder water than rainbow trout in a laboratory setting. Yet some rain-

bow trout individuals demonstrated cold tolerance comparable to westslope cutthroat trout, highlighting the importance of considering this variability when explaining patterns of rainbow trout hybridization (Yau and Taylor 2014). In addition to physiological variability among individuals, rainbow trout also exhibit high behavioral plasticity that probably plays a role in their invasion success (Arismendi et al. 2014). Therefore, a rainbow trout hatchery strain pre-adapted by chance (i.e., late-spawning, cold-tolerant) could explain the invasion success in Buffalo Fork Creek (Fausch 2008). Finally, having persisted here for probably 90 years, these now-naturalized rainbow trout populations have had ample time for local adaptations that could increase invasiveness (Kinnison et al. 2008) and perhaps decrease pre- and postzygotic reproductive isolation with native trout when they disperse.

The lack of a strong effect of stocking intensity on hybridization is probably related to overall low stocking intensity and the role of chance in colonization success and is consistent with the mixed results reported in other studies. Rainbow trout in this watershed were stocked relatively few times, so perhaps the magnitude of propagule pressure was simply not high enough to strongly influence patterns of modern-day hybridization. Even with high propagule pressure, stocked fish often fail to establish self-sustaining populations (Miller 1958; Fausch et al. 2001), and similarly, stocking intensity has highly variable outcomes on hybridization in nature. For example, Marie et al. (2010) report a strong effect of brook trout (*Salvelinus fontinalis*) stocking on hatchery introgression in lakes of Quebec, Canada, whereas Lehnert et al. (2020) found limited hatchery introgression despite decades of stocking in streams of Nova Scotia, Canada. Cutthroat trout studies have generally shown that the extent and degree of hybridization is correlated with stocking intensity (Bennett et al. 2010; Yau and Taylor 2013), yet Muhlfeld et al. (2017) noted that as propagule pressure increased, so did the residual variation in hybridization outcomes when considering 582 sites. This means that some sites with intense stocking pressure showed low amounts of hybridization. Lastly, we also cannot rule out the possibility of unofficial plantings, perhaps in Buffalo Fork Creek, that could have influenced our results. Overall, our results emphasize that where invasive fish establish and develop self-sustaining populations — and therefore produce a lasting and continuous source of invasive individuals — can be more important than one-off and limited stocking events.

Another important result of this study is that hybrids were observed spawning in a wide range of stream conditions, some of which we would have overlooked had we not used radiotelemetry to identify spawning populations. Radiotelemetry revealed a previously unknown and highly hybridized population using an intermittent stream. Spawning in small and intermittent streams — that dry completely during part of the year — is an adaptive behavior in many fish species (Heim et al. 2019) and can contribute 50% of overall production in native rainbow trout metapopulations (Erman and Hawthorne 1976). Interestingly, the timing of flow in this stream (site 1, April–June, dries completely afterwards) was well-matched to hybrid spawning timing, which is earlier than Yellowstone cutthroat trout (Henderson et al. 2000; DeRito et al. 2010; Heim 2019). In contrast, the stream dries during the peak of cutthroat trout spawning and would not provide an adequate amount of time for incubation of eggs and offspring emigration to perennial water (Heim 2019). Increasingly, early runoff and more widespread intermittency may thus favor earlier spawning invasive fish (Muhlfeld et al. 2014), but also serve as ecological traps to taxa adapted to historical variability in hydrological conditions.

To date, the majority of large-scale genetic assessments to assess hybridization in salmonids have been conducted during nonspawning seasons and generally omit sampling of mainstem rivers and intermittent tributaries. Had we omitted sampling these sites, we would have missed some of the key hybrid populations in

the watershed. Therefore, we suggest future genetic assessments should attempt to include these types of spawning habitats to fully understand invasion dynamics. Importantly, if these types of spawning populations are common, but not included in genetic assessments, studies to understand the spatial distribution of hybridization in relation to abiotic and biotic covariates could provide an incomplete perspective.

We strongly suspect several waterfalls and canyons play an important role in spatial patterns of hybridization by facilitating downstream-biased dispersal. While upstream movements past the Slough Creek waterfall and Lamar River canyons probably occur infrequently (Heim 2019), downstream movements are likely far more common. This perspective is consistent with model results; we expect that the covariate BAR was not strongly supported because some features considered barriers are passable, in the upstream direction, under certain flow conditions. We can infer that downstream movements are common by examining the genotype distributions above and below barriers. The lower watershed (below barriers) is a region of intense hybridization where fish displaced from upper watersheds likely come into contact; rainbow trout and hybrids are provided by Buffalo Fork Creek, and nonhybridized Yellowstone cutthroat trout are provided by both Slough Creek and the upper Lamar River watershed. Fish displaced downstream inevitably spawn in a new location, with fish from different populations, leading to hybridization and a more even distribution of hybrid genotypes. This perspective is consistent with many other studies that show upstream migration barriers can structure genetic divergence in salmonids and isolate native populations from unwanted hybridization (e.g., brown trout (*Salmo trutta*), Van Houdt et al. 2005; brook trout, Torterotot et al. 2014), but also allow downstream dispersal (Bingham et al. 2016).

Our goal in conducting this study was to evaluate whether hybridization is likely to expand, or should abiotic conditions be considered adequate to limit continued invasion. Although we found hybridization was associated with warmer water and lower elevations in univariate tests, the available evidence taken collectively does not support the expectation that populations will be shielded from invasion by gradients in abiotic conditions. While pure Yellowstone cutthroat trout do persist in the lower watershed among hybrids, this is most likely a result of downstream dispersal from headwater sources (e.g., Bingham et al. 2016; Kovach et al. 2017). The limited degree of hybridization in the upper watershed is, according to model results, best explained by the long fluvial distance to a rainbow trout source, rather than the presence of a cold-water refugia. Given our results, and that most long-term genetic sampling shows increasing hybridization over the last 30 years (Muhlfeld et al. 2014, 2017, both westslope cutthroat trout studies), we are hesitant to rely on a presumed protective role of cold water to prevent further hybridization (e.g., Kovach et al. 2017) in the Lamar River watershed. Although it is appealing to draw general conclusions about what mediates hybridization in nature, the variability in hybridization outcomes between different salmonid taxon in different locations suggests caution is warranted (Yau and Taylor 2014; Lehnert et al. 2020). Detailed, local examinations of invasion scenarios, at the same spatial scale that management occurs (i.e., a population or a metapopulation) remain critical to inform management decisions.

We conclude that management intervention is important for the persistence of nonhybridized Yellowstone cutthroat trout in this watershed. An intuitive solution is to target Buffalo Fork Creek for eradication of rainbow trout and introduction of Yellowstone cutthroat trout. What if Buffalo Fork Creek contributed Yellowstone cutthroat trout, instead of rainbow trout, to the lower Lamar River watershed? We expect this would have substantial positive impacts to reduce the degree and spread of rainbow trout admixture. Additionally, targeted selective removal by the National Park Service (Ertel et al. 2017) and leveraging the

nearly 10 000 anglers visiting the watershed to remove hybrids and rainbow trout are promising strategies (Heim et al. 2020).

Acknowledgements

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