

MECHANISMS OF RAINBOW TROUT HYBRIDIZATION WITH NATIVE
CUTTHROAT TROUT IN THE LAMAR RIVER OF
YELLOWSTONE NATIONAL PARK

by

Kurt Conrad Heim

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DEDICATION

I dedicate this dissertation to my wife Meghan, who has stood beside me and provided unwavering support while I learned how to do science during my graduate school career; also, to Mork.

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ABSTRACT

Invasive hybridization—when invasive species interbreed with native species—is a pervasive conservation issue. Hybridization presents difficult management decisions and poses complex problems at the intersection of contemporary evolution, invasion biology, physiology, and landscape ecology. I examined the invasion scenario playing out in the Lamar River watershed of Yellowstone National Park where native Yellowstone cutthroat trout (YCT) are undergoing hybridization with introduced rainbow trout (RT). I first test the hypothesis that a breeding timing mismatch of an invasive species (i.e., RT often spawn far earlier than YCT, and eggs are scoured by snowmelt runoff) can be overcome by hybridization with a locally adapted native species, that spawn later (Chapter 2). I found support for this hypothesis. Spawning timing was strongly related to the degree of non-native admixture at the individual level, indicating that hybridization alters breeding timing in ways that could benefit hybrids. At the population level, hybrid spawning timing closely matched that of native taxa and was strongly correlated to stream flow conditions. Overall, these data suggest poorly matched spawning timing is unlikely to serve as a strong mechanism limiting invasive hybridization of YCT. Next, I mapped the spatial distribution of hybridization in the Lamar River watershed (Chapter 3). Many non-hybridized populations persist in the upper watershed, whereas a prominent source of RT in the lower watershed has likely contributed inordinately to the spread of introgression. Because hybridized populations occurred across a full range of environmental conditions, I do not predict that abiotic conditions (i.e., cold water, late-stream flow, high elevations, or small stream size) will prevent the spread of hybridization in the long-term. As such, management interventions are recommended in chapter 4 and 5. I developed a simple taxonomic key to identify hybrids and guide selective removal efforts (Chapter 4). Additionally, I used volunteer angler surveys to estimate that recreational anglers visiting the watershed (~10,000 per year) could likely harvest a substantial number of non-native trout that would contribute to genetic management goals. I conclude by providing recommendations for management and monitoring (Chapter 5).

CHAPTER ONE

GENERAL INTRODUCTION

Extinction by Hybridization, Cutthroat Trout, and Controversy

The word extinction is most often associated with a complete and irreversible loss of a population, species, or taxonomic group. A tangible entity that once existed, no longer does. Habitat modification, overharvest, trophic cascades, and the introduction of non-native species are usually involved; however, invasive species can lead to extinction by another, more cryptic route that is not as widely recognized.

Native taxa can go extinct by hybridization with invasive species. Epifanio and Philipp (2001: 349) define genomic extinction as a situation when "... taxa expressing monophyletic genotypic combinations" go extinct. This definition implies that any native taxon (i.e., a population, metapopulation, or species) with a polyphyletic evolutionary history facilitated by humans is genomically extinct, no matter the degree of admixture. This conceptualization of genomic extinction has proven to be controversial in conservation. At the core of the issue is whether the strict definition of Epifanio and Philipp (2001) is useful for conservation purposes, or, does a population need to have a certain degree of non-native admixture before it really is "extinct" (Campton and Kaeding 2005)? Should we conserve hybrid populations, which ones, and how should hybrid populations be treated under the Endangered Species Act?

The reasoning set forth in Allendorf et al. (2001; "The problems with hybrids: setting conservation guidelines") continues to be the most widely accepted approach.

This framework suggests that conservation decisions in the face of invasive hybridization are context-specific, hard to generalize, and choosing some acceptable level of admixture to apply in all scenarios is inappropriate. Consider a taxa that now only exists in hybridized form in a single population. This population would represent the remaining evolutionary legacy of that taxa left on earth, and so would be of high conservation value despite non-native admixture. The Florida Panther is an example (Johnson et al. 2010). Alternately, if many genetically unaltered populations of a taxon persist, then hybrid populations may be less valuable, or even a problem (Allendorf et al. 2001). Whereas most would agree that the Florida Panther is an entity deserving of protection, the later situation is more nuanced and potentially controversial.

Such is the case for all subspecies of native cutthroat trout (*Oncorhynchus clarkii* spp.) in western North America that are experiencing invasive hybridization with introduced rainbow trout (*O. mykiss*, RT). Rainbow trout are native to the Pacific Northwest, but are widely introduced outside of their native range to provide sport-fishing opportunities (Halverson 2010). When sympatric with cutthroat trout, RT produce fertile offspring, which can facilitate rapid introgression of non-native genes into native populations. Overall, a fair number of non-hybridized cutthroat populations remain (Shepard et al. 2005, Gresswell 2011), and it has been argued that conservation of sympatric hybrid populations is unwarranted. Protecting hybrid populations could maintain vectors to further spread introgression across native trout metapopulations.

This issue came to head when westslope cutthroat trout (*O. c. lewisi*, WCT) were declined for listing under the Endangered Species Act, in part, because slightly

hybridized populations were considered in the rangewide species assessment (Campton and Kaeding 2005). Campton and Kaeding (2005) note their concerns about a strict hybridization criteria (i.e., Epifanio and Phillips 2001) based on “any detectable introgression”, which would render all slightly hybridized populations eligible for eradication under the auspices of the ESA. They also note a lack of scientific evidence that slightly hybridized populations (i.e., < 1% RT admixture) are less likely to persist than non-hybridized populations. Allendorf et al. (2004) describe an alternative view, suggesting that introgression can only spread because all progeny of hybrids will be hybrids, any introgression will disturb local adaptations of native trout, and even a population with a single hybrid ancestor 100 years ago should be considered hybridized.

Disagreement continues among scientists on many topics related to hybridization as evidenced by a high number of response papers in scientific journals expressing alternative perspectives to published research. Interpretations of evidence differ on several topics: whether cutthroat and RT will inevitably form hybrid swarms (McKelvey et al. 2016, Kovach et al. 2017), whether human mediated hybridization is a useful conservation tool to promote resiliency to climate change (Hamilton and Miller 2016, Kovach et al. 2016b), whether hybridization increases rates of dispersal (Lowe et al. 2015, Phillips and Baird 2015), if cold water will prevent expanding introgression (Muhlfeld et al. 2017, Young et al. 2017), and how relevant increasingly powerful genomic techniques are to applied conservation (Shafer et al. 2015). Finally, stepping back even further to a more fundamental topic, Rohwer and Marris (2016) challenge the often implicit assumption by conservation biologists that preservation of “genetic

integrity”, “pure genomes”, and non-hybridized populations is a moral duty. Indeed, Rhymer and Simberloff (1996) also noted the pejorative connotations of the hybridization lexicon (i.e., “genetic assimilation”, “contamination”, “genetic deterioration”, “genetic pollution”). For multiple reasons, both scientific and social, I agree “It is an understatement to say that hybridization is a complex business!” (Stone 2000: 354).

Therefore, each hybridization scenario requires careful examination of genetic, ecological, and social information across multiple spatial scales to prioritize conservation and management decisions. However, there is no avoiding some degree of “discretion” in the interpretation of scientific data and application of conservation guidelines in the context of hybridization (Lind-Riehl et al. 2016). In my dissertation I provide a thorough investigation of the hybridization scenario playing out in an important conservation population of native trout, and contribute some new perspectives to unsettled scientific questions.

Solving the Hybrid Problem in the Lamar River Watershed

In the case of Yellowstone cutthroat trout (*O. c. bouvieri*, YCT), fisheries managers are unified in a general approach to prioritization of populations based on multiple criteria (Al-Chokhachy et al. 2018). From a genetics perspective, non-hybridized populations are most highly valued, populations with < 1% admixture are considered core conservation populations, and those with admixture < 10% are classified as conservation populations. A recent rangewide status assessment also incorporated a portfolio of biological data for all extant YCT populations (e.g., length of stream occupied, water

temperature) to rank conservation units with the highest probability of persistence into the 21st century (Al-Chokhachy et al. 2018). This effort identified conservation units of high value to prioritize, yet, the task of managing these populations is allocated to regional fisheries managers that must make decisions based on the best available scientific knowledge, interpretation of local genetic and ecological information, perceptions of hybridization risk at the local scale, and societal limitations. In my dissertation I examine in detail the ongoing invasion scenario in the Lamar River watershed of Yellowstone National Park (YNP), a stronghold for non-hybridized YCT that are increasingly threatened by invasive hybridization with RT.

The Lamar River watershed is a large interconnected fluvial system that is relatively isolated from downstream threats of non-native species, and supports a YCT metapopulation of high conservation value. The watershed (1,731 km²) occurs mostly within YNP, and flows 78 km from the headwaters to the confluence with the Yellowstone River. This large and nationally protected river system promotes life-history diversity of YCT and important metapopulation processes that promote long-term persistence (Hilderbrand and Kershner 2000, Al-Chokhachy et al. 2018). Over 600 km of riverine habitat are likely occupied by trout (Chapter 2), which is remarkable considering most other conservation populations of non-hybridized YCT occupy much shorter sections of stream habitat (Al-Chokhachy et al. 2018). A second important feature of this conservation unit is that it is isolated from downstream sources of RT and other non-native fishes common in the lower Yellowstone River. Knowles Falls on the Yellowstone River (22 km downstream of the Lamar River confluence) serves as an important natural

fish movement barrier, and the Lower Falls of the Yellowstone River (33 km upstream from the Lamar River confluence) can be considered an upstream boundary to this conservation unit.

The extensive fluvial connectivity within the watershed, however, renders the metapopulation vulnerable to expanding hybridization with RT. Although generally isolated from invasion by outside the conservation unit, a well-established RT population is present in Buffalo Fork Creek (within the Lamar River watershed), and is likely contributing to spreading introgression (Ertel et al. 2017). Hybridized trout were first reported in upper Slough Creek in the early 2000's, which suggests that the spatial distribution of hybridization may be expanding (Ertel et al. 2017). As such, a better understanding of the hybridization process is required to inform conservation decisions to protect this important conservation unit from extinction by hybridization.

The mechanisms that govern invasive trout hybridization are complex and generally well studied, yet it remains unclear how RT admixture continues to increase within local populations, and spread across space despite demonstrably lower hybrid fitness (Muhlfeld et al. 2009, Kovach et al. 2015, 2016a). It is hypothesized that RT are recruitment limited where snowmelt runoff co-occurs with the spawning period (i.e., a phenological mismatch). When introduced, RT often conserve early spawning timing (March – May) that is adaptive in their native range, but can be maladaptive and limit their invasion potential in new environments (Fausch et al. 2001). Recruitment limitation via a phenological mismatch would also prevent local increases in population admixture if hybridized fish consistently expressed maladaptive spawning timing (Muhlfeld et al.

2014). Yet, the continued spread of introgression across nearly all-possible abiotic conditions suggests such a limiting mechanism is not present. In chapter two I test the hypothesis that interspecific hybridization and introgression of native genes, which likely influence early spawning timing, can help an invader overcome a breeding timing mismatch. The implications of this study extend beyond cutthroat trout.

In the third chapter of my dissertation I summarize an extensive genetic dataset to characterize the spatial distribution and extent of hybridization in the Lamar River watershed to guide management decisions and expectations for the future risk of spreading introgression. Two principles, that are hotly debated, currently guide hybridization risk assessments for native trout. First, cold water and flow regimes at high elevation sites are predicted to mediate introgression (Young et al. 2016); that is, some scientists consider cold water (specifically $< 11^{\circ}\text{C}$) to shield native trout from upstream invasion by non-native salmonids (Isaak et al. 2015). Secondly, propagule pressure and source proximity can, in some cases, overwhelm abiotic resistance. Therefore, cold water alone may not prevent expanding invasion (Hitt et al. 2003, Muhlfeld et al. 2017). I examine which of these principles is most pertinent to the invasion scenario in the Lamar River by modeling the spread of invasion with abiotic and biological predictor variables. The results of this study suggest introgression is unlikely to be limited by abiotic gradients in the watershed, though important semi-permeable movement barriers have likely prevented more widespread invasion. The results of Chapter 2 and 3 underscore a pressing need for continued management intervention.

The final chapters (Chapter 4, 5) of my dissertation are directly related to ongoing management efforts and are geared towards reducing uncertainty in the outcomes of specific management decisions (Bal et al. 2018). There is growing recognition that selective removal of hybrid trout can decrease the degree of RT admixture within populations, and reduce the probability of expanding introgression (Kovach et al. 2018). To be effective, selective removal requires that the invasive taxa can be correctly and quickly identified in a fieldwork setting, and that a sufficient number of organisms be removed to achieve biological objectives. In Chapter 4 I develop a simple taxonomic key for standardized hybrid identification, and estimate the potential catch of recreational anglers in the watershed. Lastly, in Chapter 5, I provide an overview of the management options for the Lamar River watershed, and suggest a framework for continued genetic monitoring.

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CHAPTER TWO

INTERSPECIFIC HYBRIDIZATION SOLVES A PHENOLOGICAL BREEDING
MISMATCH FOR A NONNATIVE INVADER

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Kurt C. Heim

Contributions: Designed study and developed research questions, collected the data, analyzed the data, wrote the manuscript

Co-Author: Thomas E. McMahon

Contributions: Assisted with study design, discussed results, edited the manuscript

Co-Author: Clint C. Muhlfeld

Contributions: Helped develop outlines for manuscript, discussed results, edited the manuscript

Co-Author: Brian D. Ertel

Contributions: Assisted with study design and data collection, edited the manuscript

Co-Author: Todd M. Koel

Contributions: Helped develop study design and obtained funding, edited the manuscript

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Kurt C. Heim, Thomas E. McMahon, Clint C. Muhlfeld, Brian D. Ertel, Todd M. Koel

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Abstract

Species and populations breed synchronously with conditions that maximize fitness in home environments, however, temporal breeding adaptations may be maladaptive in novel environments (i.e., phenological mismatch) and limit invasion potential differentially across space and time. We demonstrate that hybridization and introgression with native, locally adapted taxa may overcome a breeding timing mismatch to benefit an invasive genome. Introduced rainbow trout (RT) often spawn before snowmelt runoff and eggs get washed away in Rocky Mountain streams, whereas native Yellowstone cutthroat trout (YCT) are locally adapted to spawn after runoff. We estimated spawning timing of genotyped fish (YCT, hybrids, and RT) in 15 populations in Yellowstone National Park. Native trout spawning timing (14 population means, $n = 133$ individuals) was associated with multi-annual averaged conditions (i.e., temperature, runoff timing, stream size) reflecting local adaptations. Unexpectedly, hybrid spawning timing (7 populations, $n = 112$) was more strongly associated with abiotic variation. Hybrids spawned very early in streams where early spawning is considered advantageous (early-runoff or intermittent), but as late or later than YCT where late spawning is advantageous (late and scouring runoff). Hybrids with higher proportions of YCT admixture spawned later, affirming that introgression influenced trait variation. Rainbow trout ($n = 3$) were encountered in a single location expected to strongly favor early spawning (an intermittent stream); hybrids were abundant and widespread across the full range of abiotic conditions. Thus, the invasive RT genome, carried by hybrids with highly variable and well matched spawning timing, may invade native metapopulations

even as non-hybridized RT are limited. Early spawning by RT and hybrids, however, is likely to be beneficial in future climate scenarios. Snowmelt runoff timing is increasingly earlier (1 day/decade since 1927) and more variable in our study area, and stream intermittency is increasing across the Rocky Mountains. This shifting adaptive landscape could accelerate the already ominous loss of native cutthroat trout by favoring early spawning phenotypes. Ecosystems around the globe are experiencing similar shifts towards earlier phenology; if invasive hybridization hastens the evolutionary response to new adaptive landscapes for breeding phenology, it could be increasingly common in a rapidly changing world.

Introduction

The timing of breeding is a critically important life-history trait that often differs among species and populations due to strong adaptations to seasonally available resources. For example, birds breed during times of the year that maximize forage availability and hence chick survival (Baker 1939); riverine fishes match reproduction to the local flood pulse (Junk et al. 1989); and plants flower at times that maximize pollinator attraction (Elzinga et al. 2007). Breeding date can be thought of in terms of an adaptive landscape (i.e., the relationship between fitness and trait or genotypic variation) that is shaped by the phenology of resources governing reproductive success (Figure 2.1). Breeding too early or too late relative to the local fitness optima results in a resource mismatch and is selected against (i.e., Cushing 1990). When resource phenology is variable across space, selection acts differentially to produce asynchrony in peak

breeding timing across species and populations, a phenomenon that contributes diversity and aggregative stability to metapopulations and ecosystems (Den Boer 1968). To the extent that well matched breeding timing is adaptive in native environments, poorly matched breeding timing can also limit the success of invaders.

When a species arrives in a novel environment, it may either conserve its reproductive phenology or change it to match the new environment (i.e., plasticity). In the first scenario, an invasive species may have high reproductive success in habitats that are similar to its native range, but poor breeding success when conditions are different and conserved breeding phenology is maladaptive (i.e., phenological mismatch). This mechanism explains the differential invasion success of some fish, bird, and plant species across space (Chuine and Beaubien 2001, Fausch et al. 2001, Inoue et al. 2009, Luna et al. 2017) and time (Muhlfeld et al. 2014). Alternatively, invader plasticity may overcome phenological mismatches (Davidson et al. 2011, Wolkovich and Cleland 2011), however; there are limits to breeding timing plasticity imposed by the evolutionary history of species (Whitlock 2002, Fausch 2008).

Natural selection reduces genetic and phenotypic diversity, and thus locally adapted taxa are likely to have a relatively narrow reproductive window that might not overlap with fitness optima in a new environment (Figure 2.1, H_0). Strong local adaptations may also preclude the potential for a longer-term evolutionary response because selection purges genetic variation that is not useful locally, but could be in other environments (Whitlock 2002, Verhoeven et al. 2011). While there are examples of evolutionary responses to track changing fitness optima for breeding timing (Loe et al.

2005, Kovach et al. 2012), such responses are limited by standing genetic variation and take at least several generations. A rapid evolutionary mechanism that allowed the invading taxa to extend its phenotypic range of breeding timing, and quickly match a local fitness peak, could facilitate rapid invasion.

Invasive hybridization – interbreeding between invasive and native species – is widely considered to be maladaptive for native taxa (Allendorf et al. 2001, Muhlfeld et al. 2009a), yet few studies have examined how hybridization and introgression affects the breeding timing of the invading genome. Hybrids often have intermediate phenotypes to parental taxa (including breeding timing, Henderson et al. 2010), and therefore within a single generation hybrid individuals could shift breeding timing closer to local fitness optima. In this study, we test the hypothesis that interspecific hybridization can help an invader overcome a breeding timing mismatch in stream salmonids. Salmonids are ideal organisms to test this hypothesis because hybridization and introgression are common among closely related species and widespread stocking has created sympatry between many previously allopatric species. Moreover, salmonids exhibit both species and population specific adaptations to the timing and magnitude of streamflow conditions of the natal stream habitats (Gresswell et al. 1997, Beechie et al. 2008, Gharrett et al. 2013). Therefore, quantifying how various levels of introgression influence the timing of spawning provides an ideal situation to test whether invasive hybridization may be adaptive or maladaptive in nature.

Invasive hybridization with rainbow trout (*Oncorhynchus mykiss*, RT), the world's most widely introduced fish, threatens all remaining subspecies of cutthroat trout

(*O. clarkii* spp.) in western North America. Rainbow trout and cutthroat trout produce fertile offspring and introgression often continues until monophyletic native cutthroat trout lineages are lost (i.e., genomic extinction, Epifanio and Philipp 2000, Muhlfeld et al. 2017). In their native range, RT spawn earlier in the spring during increasing flows associated with rain events, whereas cutthroat trout are adapted to spawn later as flows decline following peak runoff in snowmelt dominated systems (Gresswell et al. 1997, Quinn 2011). High spring flows and peak pulses in the snowmelt hydrograph can limit RT recruitment outside their native range because such flows scour eggs and recently hatched fry from spawning nests (Fausch et al. 2001). Despite demonstrably lower fitness of hybrids relative to native cutthroat trout (Muhlfeld et al. 2009a), hybridization is common among these species across a wide range of environmental conditions (Boyer et al. 2008). How invasive RT admixture can increase in local populations, and spread across native trout metapopulations despite lower hybrid fitness is considered paradoxical (but see Phillips and Baird 2015) and has motivated several mechanistic hypotheses including differential rates of dispersal by hybrids (Muhlfeld et al. 2009b, Kovach et al. 2015), or the “genomic ratchet” effect (i.e., that all hybrids beget hybrids; Epifanio and Philipp 2000, Lowe et al. 2015).

Here, we use an extensive spawning dataset to assess how the timing of spawning may influence the spread of invasive hybridization. Since hybrids often display intermediate spawning timing (Henderson et al. 2000, Muhlfeld et al. 2009b, DeRito et al. 2010), it is possible that introgression of native alleles or gene-complexes could facilitate invasion by “matching” breeding timing to local stream conditions and facilitate

further introgression. Can invaders steal beneficial genetic variation from locally adapted native taxa? This could occur even if other components of fitness acted against hybrids (Muhlfeld et al. 2009a), helping to explain this purportedly paradoxical scenario. We examined spawning timing of 248 individuals with varying degrees of RT admixture that spawned in wide range of streams differing markedly in runoff timing, water temperature, size, and elevation. Our objectives were to (1) characterize the variation in abiotic conditions and phenology of snowmelt runoff in spawning habitats (i.e., the selective forces in the adaptive landscape, Figure 2.1), (2) determine how native cutthroat trout spawning phenology corresponds with variation in abiotic conditions (i.e., test for evidence of local adaptations, Figure 2.1), and (3) examine how hybridization influences population and individual spawning timing in heterogeneous spawning environments. Can hybrids match local fitness optima for spawning timing in ways that would facilitate continued hybridization, or should we expect poorly matched spawning timing of hybrids to be a strong mechanism limiting continued invasion (Figure 2.1, H_0 vs. H_a)? ‘

Methods

Study Area

Our study was conducted in the Lamar River watershed (1,731 km²) within Yellowstone National Park (YNP), which flows 78 km from the headwaters (elevation = 3,015 m) to the confluence with the Yellowstone River (1,829 m) (Figure 2.2). Mean annual discharge is 25 m³/s and mean annual peak flow is 231 m³/s. A canyon bisects the lower and upper Lamar River, and barriers to upstream movement exist on Slough Creek

(~3 m waterfall), Soda Butte Creek, and Buffalo Fork Creek (Figure 2.2). Yellowstone cutthroat trout (*O. c. bouveri*, YCT) are the only native salmonid; RT were introduced into the watershed in the early 1900s. Presently, parental RT are only commonly found in Buffalo Fork Creek, a tributary to Slough Creek (Figure 2.2), but RT x YCT hybrids (CTX) are common in the lower portion of the Lamar River watershed (Chapter 3).

Spawning Location and Timing

Radio Telemetry. We radio-tagged 136 trout (YCT, RT, and CTX with varying degrees of admixture) captured in mainstem locations during 2015 and 2016 and tracked them to spawning areas in 2016 and 2017 to estimate spawning location, spawning date, and the distance and direction of the spawning migration. Fish were captured and tagged in the Lamar River from the confluence with the Yellowstone River upstream to Flint Creek, and in lower Slough and Soda Butte creeks below migration barriers (Figure 2.2). Tagging was conducted from July to September, and fish were monitored as they made spawning runs in the following year(s). Fish were sampled by angling ($n = 80$), backpack electrofishing ($n = 11$), and boat electrofishing ($n = 45$). Trout were anesthetized with AQUI-S (New Zealand Ltd., Lower Hutt, New Zealand), and a small tissue sample was collected from the upper lobe of the caudal fin for genetic analysis. A transmitter (Lotek Wireless Inc., Newmarket, ON, Canada, MCFT2-3BM [8 g] or MCFT2-3FM [11 g]) less than 3% of fish body mass was surgically implanted and fish were released near their point of capture.

We attempted to locate fish twice a week during spawning periods (April – August) in 2016 and 2017 using aerial and ground based tracking. In 2016 we conducted 15 telemetry flights and 38 ground tracking surveys between April 1 and August 1, and fish eventually used in final analysis ($n = 51$) were detected an average of 19.5 times ($sd = 7.4$). In 2017 we conducted 15 flights and 33 ground surveys, detecting individual fish an average of 19.3 ($sd = 6.6$) times. Telemetry error estimates were made for all detections based on calibrated models relating received signal strength to distance-to-transmitter (Heim et al. 2018). We assumed a fish spawned if it made a distinct upstream or downstream movement (between April and July) towards a spawning site following a prolonged period of no movement, and (usually) made a distinct post-spawning movement (Figure A.1). All other fish were considered non-spawners and excluded from further analysis. We used the sequence of detections for an individual to estimate pre-spawning location, spawning location, spawning date, and two estimates of spawning migration distance (i.e., a minimum, and a maximum) that reflected uncertainty due to radio telemetry error (Heim et al. 2018). Additional details are provided in Appendix A.

Passive Integrated Transponder Tag Monitoring. Additionally, we gathered spawning timing and location estimates by marking 1,339 fish with passive integrated transponder (PIT) tags and detecting spawning runs into and out of tributaries with remote stream-wide antennas (Figure 2.2). Fish were captured, tagged, and released in mainstem sites including the Lamar River ($n = 599$), lower Slough Creek ($n = 102$), and lower Soda Butte Creek ($n = 268$), using raft electroshocking and angling (2015, 2016). These samples included fish of mixed population origin, which were later detected

making spawning runs into streams monitored with PIT antenna (Figure 2.2). Spawning tributaries were also sampled multiple times with backpack electrofishing during each spawning season to tag additional fish (Ditch Creek, $n = 152$; Hidden Creek, $n = 61$; Rose Creek = 99, Chalcedony Creek, $n = 58$; Table A.1). All captured fish were anesthetized, measured, fin clipped, and gently squeezed to determine spawning condition (presence of milt or eggs). A 23-mm half-duplex PIT tag (Oregon RFID Inc., Portland, Oregon, USA) was inserted into the dorsal sinus of fish ≥ 250 mm, or, a twelve-mm PIT tag was inserted into the body cavity of fish between 120 and 249 mm. Half-duplex PIT tag readers (Oregon RFID Inc., Portland Oregon, USA) with paired, stream-wide antennas recorded the date, time, antenna number, and unique ID of each passing PIT tagged fish. The overall antenna detection efficiency was high across sites and years (most > 0.90 , Table A.1). Lastly, two additional sites not monitored with PIT antenna were sampled with backpack electrofishing during the spawning period (Flint Creek, $n = 20$ fish; Crystal Creek, $n = 16$).

With the aforementioned data, we used three methods to estimate spawning date. First, if a complete spawning run was detected remotely at PIT antenna (i.e., enter date and exit date), we used the median date of the spawning run as the spawning date estimate. Second, if a fish was tagged within a tributary monitored with PIT antenna during the spawning period, we used the median date between capture date and exit date (via PIT antenna) as an estimate for spawning date. If the fish was not observed exiting the tributary, we either used the date of capture as the spawning date if it was ripe when handled, or excluded it from analysis. Similarly, for Flint Creek and Crystal Creek, which

were not monitored with PIT antenna, we only considered ripe fish for analysis and estimated spawning date as the date of capture. We used a site-specific size-at-maturity cutoff (Table A.2) to exclude individuals detected remotely that were likely immature. In total, 162 spawning date and locations estimates were made with PIT tag detections, and 59 were made by directly sampling ripe fish. In combination with 55 estimates made with radio telemetry, our final dataset included 276 estimates of 248 individuals (some fish spawned in multiple years), all of which were genotyped.

Genetic Analysis

We used species-diagnostic single nucleotide polymorphism (SNP) loci to estimate RT admixture (pRT) of individual fish that provided useful spawning data. Genotyping was performed at the University of Montana Conservation Genetics Laboratory using methods and genotyping assays described in detail elsewhere (Bingham et al. 2016). Briefly, a micro-fluidic SNP-chip (Fluidigm Corp., San Francisco, CA) was used to run 95 SNP assays on all samples; 19 of the loci were diagnostic for YCT, 19 for YCT, and 17 for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT). Our final marker set used for analysis included 18 RT, 16 WCT, and 18 YCT diagnostic markers after excluding probable non-diagnostic loci (Chapter 3). We estimated individual YCT, WCT, and RT admixture within individuals (Kalinowski 2010), and classified any individual that was heterozygous at more than 90% of YCT and RT markers (i.e., interspecific heterozygosity, H_i) as a first generation (F1) hybrid. Classification of F1s using this criterion was used because hybrids with $H_i = 1.00$ (e.g., true F1's) were absent from the dataset and fish with $H_i > 0.90$ biologically represent a similar mating event as a

first generation cross. For example, mating between a slightly hybridized RT (pRT ~0.95) and a YCT would produce such a genotype.

Spawning Patch Characteristics

We identified 15 distinct spawning streams (i.e., patches), and characterized them in terms of abiotic properties that are known to influence spawning timing of stream salmonids (flow timing, flow volume, stream temperature, and elevation). We hypothesized that fish would spawn earlier in smaller, warmer, lower elevation streams with earlier runoff conditions. All fish spawning in the same tributary were grouped into the same patch, and two mainstem spawning patches in the Lamar River were delineated (one upstream of Lamar Canyon, one downstream; Figure 2.2).

We obtained modeled estimates of streamflow timing, flow volume, and stream temperature that were available for all of the 15 spawning patches. To describe long-term snowmelt runoff timing, we used modeled center timing of flow mass (Wenger et al. 2010, CFM). This is calculated as the date of the water year (starting October 1) at which 50% of flow has moved passed a given point; therefore, earlier values indicate earlier snowmelt runoff timing at a site and should drive earlier spawning timing. We also extracted mean annual flow estimates (MAF, in m^3/s) to describe stream size (Wenger et al. 2010). Thermal variation across patches was described with mean August stream temperature estimates from the NorWeST website (Isaak et al. 2017). We also collected empirical measures of streamflow and water temperature to validate modeled estimates, and to estimate the annual hydrograph at eight spawning patches.

Hydrographs were estimated at six sites during the study by deploying HOBO water level (and temperature) loggers (Pocasset, Massachusetts, USA) in PVC stilling wells to continuously record depth, and measuring stream discharge (m^3/s) five to ten times each year across a range of flows to develop site-specific rating curves. Exponential or polynomial regressions were used to relate measured discharge to water depth, and to generate hydrographs for each site. Logger data were also used to summarize mean May and June water temperatures at sites. Data were extracted from the U.S. Geological Survey gage stations on the Lamar River (#06188000, discharge and temperature), and Soda Butte Creek (#061879015, discharge only).

Data Analysis

We characterized abiotic conditions and asynchrony in spawning patches using modeled data for all 15 sites, and measured data (temperature, stream discharge, date of peak flow, and peak flow volume) where applicable. To assess whether the modeled data accurately reflected gradients in abiotic conditions, we used Spearman's rank correlation (r_s) and compared modeled estimates to measured data.

To examine spawning timing of YCT and hybrid trout, we first summarized the aggregate dataset by taxa ($n = 276$ spawning date estimates, $n = 248$ individuals) and compared spawning timing to the mainstem Lamar River hydrograph with kernel density estimates. We tested for differences in spawning timing between CTX and non-hybridized trout using Mann-Whitney U -tests for the 2016 and 2017 datasets; low sample sizes in 2015 ($n = 35$) precluded statistical analyses of these data. Next, we tested for associations between population mean spawning timing (by taxa) and abiotic variables

expected to reflect local adaptations using Pearson's correlation coefficient and linear regression ($p < 0.10$). Modeled abiotic conditions were compared to overall site-specific spawning timing means across all years and measured data were compared to mean spawning timing estimates made within years.

We used linear mixed effects models and an information theoretic approach (AIC) to examine how pRT and abiotic conditions influenced individual spawning timing (Burnham and Anderson 1998). We expected that increasing levels of pRT would lead to earlier spawning, and that abiotic patch conditions would either have an additive or interactive effect (with pRT). We used a reduced dataset that included only fish from hybridized spawning patches ($n = 7$) and only the first spawning observation for repeat spawning fish (for a total $n = 173$) to avoid repeated measures on individuals. The resulting dataset included 112 CTX, 3 RT, and 58 YCT. We classified each individual's spawning patch as small or large (based on MAF), early or late (based on CFM), and cold or warm, based on medians from the modeled datasets. However, after binary classification there was very low variation in individual pRT across cold and warm sites, and MAF and CFM coded as binary variables were perfectly co-linear. We therefore considered only CFM in subsequent models. We tested a null model, one with CFM as an additive term, and one with it as an interaction term with pRT. All models include year as a random intercept, and Akaike's information criterion was used to evaluate the relative model performance.

We compared spawning site fidelity, migration distance, and migration direction between hybridized and non-hybridized fish. We considered a fish to display site fidelity

if it spawned in the same patch in multiple years, whereas spawning in multiple patches in subsequent years was considered evidence of dispersal. We compared spawning migration distances between YCT and CTX using a Welch's two-sample *t*-test and a procedure that accounted for potential biases of telemetry error. In *t*-tests, minimum spawning migration distances (Figure A.1) were used for the group (i.e., YCT or CTX) with the greater mean, and maximums were used for the group with the lower mean. This minimized the potential for telemetry error to result in spurious statistical results.

Results

We obtained a total of 276 estimates of spawning date and location for 248 individual fish during 2015 ($n = 35$), 2016 ($n = 85$), and 2017 ($n = 156$) that ranged widely in the level of RT admixture (Table 2.1, Figure A.2). In total, 54% of fish were YCT ($n = 133$), 1% were RT ($n = 3$), and 45% were CTX ($n = 112$). Hybrids with pRT from 0.01 – 0.40 were least common ($n = 13$, 12%), the most common genotype was from 0.40 – 0.60 ($n = 61$, 54%), followed by fish from 0.60 – 0.97 ($n = 38$, 31%)(Figure A.2).

Hybrids were common downstream of Lamar River canyon (5/6 patches) and rare upstream of this feature (2/9 patches), whereas YCT spawned in streams throughout the entire watershed (14/15 patches) (Table 2.1, Figure 2.2). Hybrids and native fish were sympatric in six patches, eight patches included only native trout, and one patch contained only hybrids.

Spawning patches were characterized by substantial variation in conditions expected to influence spawning timing (Figure 2.3). Spawning patches were heterogeneous in total flow volume (modeled MAF, range = 0.01—13.89 m³/s), runoff phenology (modeled CFM, range = April 8 – May 11), water temperature (range = 9.8—12.6°C), and elevation (range = 1,812 m – 2,144 m) (Figure 2.3). On all accounts, empirical measures were strongly correlated to modeled estimates of flow timing, volume, and stream temperature indicating that they served as a good surrogate for sites where we did not collect empirical data (Appendix A).

Despite spatial differences, CTX spawned in patches with the highest and lowest flow volume (Lamar River Lower, MAF = 13.9 m³/s; Crystal Creek, MAF = 0.01 m³/s), those with the latest and earliest runoff timing (Buffalo Fork Creek, CFM = April 8; Crystal Creek, CFM = May 11), and across nearly the full spectrum of water temperatures in patches (Figure 2.3). No abiotic conditions appeared to differentiate hybridized from non-hybridized patches (Figure 2.3, Chapter 3).

Spawning Timing at the Basin Scale

When considering the entire dataset (i.e., entire Lamar River basin, including 15 populations), YCT and CTX showed distinct differences in spawning timing (Figure 2.4). Yellowstone cutthroat trout consistently spawned on the descending limb of the Lamar River hydrograph in 2016 and 2017, while most hybrid trout spawned prior to the final peak in discharge (Figure 2.4). Overall, runoff conditions were higher in 2017 (peak flow of 275 m³/s) than 2016 (129 m³/s), but the timing of runoff and the shape of the descending hydrograph limbs were similar between years. Warm weather led to multiple

sharp peaks in each year's hydrograph; yet, the peak of YCT spawning timing in both years occurred after the last of these peaks (Figure 2.4). Hybrid spawning, however, was associated with increasing discharge (Figure 2.4). In 2016 hybrids spawned an average of 16-days earlier than native trout (CTX mean = May 29, range = May 7 – June 28, $n = 43$; YCT mean = June 14, range = May 16 – July 6, $n = 42$; $U = 299$, $p < 0.001$), and in 2017 hybrids spawned an average of 26-days earlier than native trout (CTX = May 24, range = April 13 – July 9, $n = 80$; YCT mean = June 19, range = May 16 – July 18, $n = 76$; $U = 632$, $p < 0.001$).

Spawning periods for native and hybrid trout overlapped and hybrids spawned over longer periods of time in both 2016 and 2017 (2016, CTX = 52-day period, YCT = 51-day period; 2017, CTX = 87-day period, YCT = 63-day period) (Figure 2.4).

Spawning Timing at the Population Scale

When considering spawning timing at the population scale, we found that hybrid spawning timing was strongly associated with abiotic conditions in ways that closely matched local adaptations of native YCT. Hybrids spawned later in streams where sympatric YCT spawned late (i.e., cold, large, and late flow conditions), and earlier in streams where YCT spawned earlier (i.e., warm, small, early flow conditions; Figure 2.4). At one extreme, hybrid spawning occurred on June 27 (2016, 2017 mean) in the mainstem Lamar River that has late and scouring peak flows. At the other extreme, hybrids spawned a month earlier in a small intermittent stream (Hidden Creek, May 22), with early peak flow, and strong selection potential to promote early spawning. Hidden Creek dried completely on June 25 in 2016, and June 30 in 2017, and hybrids spawned

41($sd = 14$) days prior to the date of desiccation. Six YCT spawned in this stream, yet only 26 ($sd = 12$) days prior to desiccation (Figure 2.4). This stream was also the only location where RT were encountered ($n = 3$).

Yellowstone cutthroat trout and hybrid populations consistently spawned on the descending limb of the hydrograph (Figure 2.4), but mean spawning timing differed among populations depending on abiotic conditions (Figure 2.4, Table 2.2.). Pearson's correlations indicated that both YCT and hybrid trout populations spawned earlier in patches with (1) low MAF (i.e., were smaller) (2) early long-term runoff conditions (i.e., low CFM values), (3) early observed dates of peak flow, (4) warmer temperatures, and (5) in lower elevation reaches. Mean spawning timing of hybrid populations was more strongly correlated to abiotic conditions than YCT (7/10 tests, Table 2.2). Although low sample sizes precluded many significant correlations ($p < 0.10$), 3/10 tests were significant for hybrids and none were significant for YCT populations.

Native YCT and hybrid population spawning timing tracked abiotic conditions in similar way, but hybrids still spawned earlier than native trout given similar abiotic conditions (i.e., different intercepts, Figure 2.5). Slopes of MAF regressions (native $\beta_1 = 2.25$, hybrid $\beta_1 = 3.86$) suggested a stronger influence of stream size on hybrid spawning timing than native spawning timing. This would lead to increasingly similar spawning timing in large habitats between RT and YCT (i.e., a 9-day difference in the Lamar River [lower] patch, $MAF = 13.9 \text{ m}^3/\text{s}$) but stronger temporal separation between taxa in smaller habitats (i.e., a 20-day difference in Crystal Creek, $MAF = 0.01 \text{ m}^3/\text{s}$). Regression slopes were similar between YCT and RT for the CFM regressions (Figure 2.5).

Spawning Timing at the Individual Scale

Increasing amounts of RT admixture lead to earlier spawning timing at the individual level, but the effect was different in streams with different runoff timing in the direction that matched adaptive expectations (Figure 2.6, Figure A.3). The presumably maladaptive influence of RT admixture on spawning timing was weak in patches with late-runoff timing (regression slope $\beta_I = -12.54$), but strong in small streams with early runoff (regression slope $\beta_I = -27.50$, Figure 2.6). The top model (AIC = 1381.49) included an interaction term between pRT and flow timing of the spawning patch, and outperformed an additive model ($\Delta\text{AIC} = 7.07$) and a null model that included only pRT ($\Delta\text{AIC} = 28.5$). In a stream with early flow, each 10% increase in pRT is expected to lead to spawning 2.7-days earlier; a parental RT (pRT = 1.00) is expected to spawn 27-days prior to a parental YCT (Table 2.3). In a stream with late flow, a 1.2-day difference in spawning date is expected with each 10% increase in pRT (i.e., pRT slope + interaction term $[-27.50 + 14.96]$; Table 2.3); parental RT and YCT are expected to spawn only 12 days apart (Figure 2.5). This is 15 days closer in time compared to the expected temporal separation in a stream with early flow.

First generation hybrid spawning timing was well matched to local runoff conditions. In streams with late flow, mean F1 spawning timing was June 13 ($sd = 18$, range = May 12 – July 9), which differed significantly from timing of F1s spawning in streams with early runoff timing (mean = May 25, $sd = 11$, range = May 10 – June 14, $U = 16.5$, $p = 0.008$). Similarly, fish with high heterozygosity ($H_i > 0.80$) and pRT estimates

from 0.40—0.60, but not classified as F1s, spawned across a wide range of dates but generally matched local conditions well (Figure 2.6, Figure A.3).

Spawning Migration Differences by Taxa

Inter-annual site fidelity to spawning patches was high and similar between YCT and CTX. Twenty-four fish were documented spawning in two years (n YCT = 13, n CTX = 10, n RT = 1), and two spawned in all three years of the study (n YCT = 1, n CTX = 1). All of these fish displayed inter-annual site fidelity, spawning in the same patch across years. Genotypes of hybrids showing inter-annual site fidelity ranged from pRT of 1% to 77%, but none were classified as F1s.

Native trout spawning migrations were over twice as far as hybrid trout migrations (YCT mean = 13.91 km, range = 1.14—29.01; CTX mean = 6.18, range = 0.15 – 28.01; $P < 0.005$). A few hybrids migrated long distances (> 10 km, $n = 5$), but all of these were in the downstream direction by fish that began migrations in the upper watershed, and migrated to the lower watershed to spawn among other hybrids (Figure 2.6). In contrast, native trout that began migrations in the upper watershed, nearly always migrated long-distances upstream (15/17 migrated upstream, 12/15 were > 10 km), leading to complete spatial isolation of radio tagged YCT in the upper watershed during the spawning period. Although we observed (1) spawning migrations downstream through Lamar River canyon and (2) post-spawning migrations upstream through Lamar River canyon, no fish moved upstream through the canyon coincident with high discharge.

Discussion

Rainbow trout invasion potential is limited in regions, local habitats, or time periods where flood-disturbance co-occurs with reproductive phenology; a primary mechanism leading to this limitation is egg-scour and fry displacement by early-spawning RT (Fausch et al. 2001, Beechie et al. 2008; Inoue et al. 2009; Kitanishi et al. 2010; Muhlfeld et al. 2014). However, the spatial spread of RT genes via introgressive hybridization does not appear to be as strongly constrained. Our results support the hypothesis that introgression with native trout may benefit the invasive genome by alleviating a breeding timing mismatch. Hybrid populations spawned on the descending limb of the hydrograph and tracked abiotic conditions in the same way as locally adapted cutthroat trout populations. Fish with more YCT admixture spawned later, validating that spawning timing was influenced by introgression. This implies that RT invasion of streams with late and scouring streamflow may be facilitated by access to an extended gene pool provided by hybridization. Extreme early spawning demonstrated by RT and hybrids is, however, unlikely to be maladaptive in all scenarios and could lead to colonization of “empty temporal niches” (Sanz-Aguilar et al. 2015) like intermittent streams. Furthermore, early spawning is expected to be advantageous as snowmelt runoff becomes earlier and more variable as a function of climate change (Leppi et al. 2012). Fitness landscapes for temporal breeding adaptations are rapidly shifting for many taxa across the globe (Bowers et al. 2016); our study suggests that invasive hybridization may hasten the evolutionary response to match shifting breeding timing optima, exacerbating rates of genomic extinction by hybridization in nature.

Asynchrony in Selective Pressures in a Trout Metapopulation

Spawning habitats were asynchronous and variable in conditions that shape local breeding timing adaptations. Center timing of flow mass varied by 32 days, measured dates of peak flow spanned an 80-day period, and differential rates of warming during the spawning period were documented. Such asynchrony in resource phenology provides a diverse habitat template for temporal adaptations by native taxa (Southwood 1977, Armstrong et al. 2016); but also could differentially limit invasion potential of an invader with conserved breeding timing (Sanz-Aguilar et al. 2015). The fact that hybrids between RT and native trout occurred across the full spectrum of environmental variation in spawning habitats (also see Chapter 3) provided the first piece of evidence that invasion is not constrained by mismatched spawning timing. Rather, hybrid spawning timing appeared to be well matched to the highly asynchronous conditions in the study area. This finding differs from previous assessments, but the scale of the datasets used in prior studies provides an explanation for this discrepancy.

Comparison of mainstem river hydrographs to hybrid breeding timing has given an impression of maladaptive behavior in previous studies (Henderson et al. 2000, Muhlfeld et al. 2009b, DeRito et al. 2010). This is not surprising because it is the natal stream environment (not the mainstem river) that creates differential selective pressures for spawning timing (Lisi et al. 2013). We would have come to a similar conclusion had we only included mainstem river hydrology in our analysis. Hybrid spawning timing peaked coincident with sharp increases in flow in the Lamar River. However, at this time peak flow conditions and flood-disturbance probability had long-passed in streams with

smaller basin areas where hybrids were actually spawning (i.e., Crystal Creek, Hidden Creek, Rose Creek). In their native range, RT spawning migrations are cued by rain-events that provide access to small spawning tributaries (Erman and Hawthorne 1976, Quinn 2011), where local egg-scour potential may be much lower (Montgomery et al. 1999) and peak earlier than mainstem sites. This cued response to increasing flows appears to be conserved outside of their native range, yet our results suggest it is not always maladaptive.

Well Matched Spawning Timing of Native and Hybrid Trout Populations

Two prominent selective pressures act in opposing directions to produce local fitness optima for spawning timing in salmonids in western North America. Early spawning is selected against where eggs or fry are swept from nests by late snowmelt, whereas spawning too late can limit growth potential for offspring and lead to poor survival (Quinn and Peterson 1996). Juvenile fish that are larger (i.e., born earlier as a function of early-spawning parents) have competitive advantages over conspecifics (Abbot et al. 1986), alter the behavior of subordinate conspecifics or species to their advantage (Fausch and White 1986), and thus will accrue growth and survival advantages (Quinn and Peterson 1996). Consistent with a similar study in 27 hydrologically diverse tributaries to Yellowstone Lake (Gresswell et al. 1997), native YCT in the Lamar River watershed spawned later in streams with late streamflow, and those with persistent cold temperatures in the spring. Alternatively, YCT spawned earlier where the selective pressures of egg-scour peaked early and then subsided along the descending limb of the

hydrograph. These same associations are demonstrated across the entire salmonidae family (Quinn 2011, Lisi et al. 2013), and are widely considered to be a result of local adaptations to natal stream environments (Gharrett et al. 2013).

Gharrett et al. (2013) showed by review of literature and empirical data that spawning timing is (1) heritable and variable, and such variation (2) leads to fitness differentials across environments or in common garden experiments, and (3) there are multiple mechanistic links between spawning timing and fitness. Although we did not measure the fitness consequences of trait variation across habitats, the degree to which populations match established trait or phenotypic optimum is strongly informative about local adaptation (Kawecki and Ebert 2004). In our interpretation of hybrid spawning timing, we rely on native trout to inform habitat-specific optima (Kawecki and Ebert 2004) and wealth of prior literature that provides mechanistic links between this behavior, streamflow conditions, and fitness.

To our knowledge, we provide the first detailed comparison of hybrid trout spawning phenology to local abiotic conditions in a hydrologically complex drainage network, and our results show that hybrid spawning timing is closely in tune with the selective pressures of the spawning environment. Mean spawning timing of hybrid populations was strongly correlated with abiotic variation, to a degree higher than native trout. This occurred because (1) hybrid trout often spawned far earlier than native trout in small streams with early peak flow but, (2) spawned very late – often later than sympatric native trout – in large, mainstream locations characterized by late peak flow. Other studies confirm this broad temporal spawning period of hybrids that encompasses the

entire native spawning period (Henderson et al. [2001], CTX = 113 day period, YCT = 71 days; DeRito et al. [2010], CTX = 84 days, YCT = 48 days) but have lacked hydrological data for spawning tributaries to make local comparisons. Our use of modeled and measured hydrological data across 15 strongly asynchronous spawning habitats show that this widely varying spawning timing was not haphazard—but was consistent with adaptive expectations (Gresswell et al. 1997, Quinn 2011, Lisi et al. 2013). By comparing RT admixture to individual spawning timing we also validated that trait variation was influenced by introgression, but this analysis also provided some evidence of phenotypic plasticity.

Interactive Effects of Nonnative Admixture and Runoff Timing

Our examination at the individual level affirmed the genetic influence on spawning timing, but also revealed genotype x environment interaction. The more invasive DNA present in an individual, the earlier it was predicted to spawn. In RT, spawning timing is a polygenic trait (Sakamoto et al. 1999), yet there may also be single genes with a very strong influence on spawning timing (Prince et al. 2017). Therefore, with a higher contribution of RT admixture an individual would be more likely to have inherited multiple quantitative trait loci of small effect, or perhaps one of large effect. Yet, fish with similar genotypes spawned at different times depending on runoff conditions in the spawning habitat—providing evidence for a plastic behavioral response. High plasticity is well-recognized as an advantageous trait for invasive taxa (Davidson et al. 2011), and we found exceptionally high phenotypic variation in fish with similar

amounts of RT admixture (especially F1s). Such plasticity could also exacerbate the spread of introgression in native trout populations.

Limitations: Was Introgression Beneficial
for the Invading Genome?

We only encountered and measured spawning timing of three RT in our study, which prevented a more detailed assessment of the breeding timing limitations of the invader prior to introgression. In the Rocky Mountains, mean spawning timing of RT is generally in late April and May (Yellowstone River, MT = April 26 [DeRito et al. 2010]; Flathead River MT = May 7 [7 year mean from Muhlfeld et al. 2009b]; Madison River, MT = May 1 [Downing et al. 2002]; South Fork Snake River, ID = May 20 [Henderson et al. 2001]) though individual redd construction has been reported as late as June 9 (Downing et al. 2002). Consistent with these studies, the three RT in our study spawned from May 9 to May 16, and were all encountered in a location strongly expected to favor early spawning (i.e., an intermittent stream, discussed below). These observations suggest some limitations to the breeding range of RT outside of their native environment, yet RT are also well known for high genetic and phenotypic variation. In hatchery environments selective breeding can rapidly alter spawning timing, advancing it by as much as 7 days per generation, and hatchery broodstock programs generate populations that spawn in every month of the year (Siitonen and Gall 1989). Therefore, there is indeed adaptive potential in the RT genome to match nearly any spawning timing optima in nature; however, the fact that RT are limited across the globe where high flood-probability occurs from March – June shows that microevolution does not regularly overcome this

mismatch (Fausch et al. 2001, Inoue et al. 2009; Kitanishi et al. 2010). Introgression with locally adapted taxa represents access to an extended gene pool that could quickly facilitate microevolution of well matched spawning timing, relative to non-hybridized RT. Prior studies have generally focused on fitness reductions (relative to native trout) resulting from introgression with RT (Muhlfeld et al. 2009a, Kovach et al. 2015); a different perspective is to consider how hybrid fitness compares to the non-hybridized invader.

Three prior studies have demonstrated that hybrids between RT and cutthroat trout have reduced fitness (Muhlfeld et al. 2009a, Kovach et al. 2015, 2016), but these are not inconsistent with the notion that introgression can benefit the invasive genome. The mechanisms leading to reduced fitness have not yet been identified (Drinan et al. 2015), and it is considered a paradox that hybridization is proliferating given lower hybrid fitness relative to native trout (Lowe et al. 2015). Increased dispersal probability following introgression is a prominent hypothesis to explain the spatial pattern of invasion (Kovach et al. 2015). But, to spread introgression, a RT or hybrid must successfully spawn in a novel environment. Our study contributes a new perspective; following introgression and dispersal from a hybrid source, individuals with wide-ranging (including much later than RT) and perhaps plastic spawning timing carry portions of the invasive genome into new populations. Whereas a long-distance dispersal event of a RT with conserved maladaptive spawning timing would be unlikely to lead to introgression, this may not be the case for hybrids that may be more successful vectors for the invasive genome. Since introgression is a unidirectional process (Epifanio and

Philipp 2000), any fitness benefits conveyed to hybrids, relative to the invasive non-hybridized individuals, could have a lasting evolutionary consequence.

Indeed, hybrids tended to have higher fitness than parental RT in one study (Muhlfeld et al. 2009a), and although there is strong selection for most RT genes or loci during invasive hybridization, some are under strong positive selection (Hohenlohe et al. 2013, Kovach et al. 2016, Bay et al. *in press*). Our study implicates that higher fitness of hybrids relative to RT (i.e., Muhlfeld et al. 2009a) could be a function of (1) increased recruitment via a lack of egg-scour by late spawning behavior and (2) an increase in the probability of encountering reproductively active mates in a novel environment.

Moreover, because spawning timing is strongly heritable and likely controlled by several genes of high importance (Prince et al. 2017), selection could strongly favor genes associated with early spawning in some environments (i.e., intermittent streams) even as other portions of the RT genome resulting in fitness consequences occurring at other life-history stages were selected against. Additional studies using parentage analysis or genomic methods (Muhlfeld et al. 2009a, Kovach et al. 2016) but including information on spawning timing could help reveal the true adaptive benefits of introgression relative to parental RT in novel environments.

Hybrid Site Fidelity and Short Migrations

Our measures of phenotypic traits related to dispersal did not provide evidence that RT introgression increases migration distances or decreases spawning site fidelity, as has been hypothesized as a vector for hybridization spread (Boyer et al. 2008, Lowe et al. 2015). Rather, we found that hybrids migrated shorter distances, more often in the

downstream direction, and expressed inter-annual site fidelity at equal rates to native YCT. It was also noteworthy that all radio-tagged hybrids in the upper watershed migrated long-distances downstream to spawn among other hybrids—leading to spatial isolation of YCT from hybrids in the upper watershed. Overall, we interpret these results regarding limited dispersal probability with reservation; site fidelity and dispersal probability may be related to access to mates (Einum et al. 2006), temporally variable (Radinger and Wolter 2014), or increase in future climate scenarios (Muhlfeld et al. 2014). We acknowledge the limitations of studying migration traits in a limited number of individuals during a short time period. Regardless, hybrid spawning site fidelity and short migrations may be one reason that introgression has not spread more widely in the Lamar River watershed since abiotic conditions do not appear to preclude invasion (Chapter 3).

Intermittent Streams as an Empty Temporal Niche?

Our study identifies intermittent streams as potential “empty temporal niches” ripe for invasion by RT or hybrids that may have a distinct advantage in such habitats over native trout (Sanz-Aguilar et al. 2015). The empty temporal niche hypothesis posits that invading taxa that conserve breeding timing may have high invasion potential if they exploit a temporal breeding window not used by native taxa. The invasion success of several exotic bird species in the Mediterranean supports this hypothesis (Sanz-Aguilar et al. 2015). Intermittent streams may similarly represent empty temporal niches that are currently unused by native trout species, or, provide only periodic recruitment success to native trout. In Hidden Creek, RT and early spawning hybrids bred as early as April,

providing ~ 2.5 months for embryonic development, while native trout spawned in this stream with only an average of 26 days prior to desiccation. If the phenology of water availability (i.e., duration, timing) matches life history needs of aquatic taxa, and is to some extent predictable, temporary aquatic habitats can be widely exploited for spawning (Heim et al. *In revision*). Indeed, use of such habitats is a well-recognized adaptive behavior for both RT and Pacific salmonid species in the Pacific Northwest (Erman and Hawthorne 1976, Heim et al. *In revision*); but, we are aware of no examples of YCT using such locations. The fact that climate change is increasing the frequency of intermittent streams (Jaeger et al. 2014) forebodes that such locations could be inordinately prone to invasion by nonnative trout in the future. More broadly, the adaptive landscapes for breeding adaptations across both aquatic and terrestrial taxa are also shifting markedly due to climate change.

Interspecific Hybridization and Shifting Adaptive Landscapes

Populations are constantly evolving to match the selective pressures of their biological and abiotic environments, in a so-called evolutionary arms race—populations or species that can't keep up with the changing fitness landscape do not persist. Climate change has shifted the fitness landscape for many taxa with regards to breeding timing. Birds, fish, and mammals are breeding as much as a month earlier than historic times (Loe et al. 2005, Kovach et al. 2012, McQueen and Marshall 2017). This is also true for stream salmonids, as snowmelt runoff is occurring increasingly earlier across all of western North America (Leppi et al. 2012), and in the Lamar River it has shifted significantly (about 1 day earlier per decade since 1927; Appendix B). To match new

fitness optima, a population must possess sufficient genetic and phenotypic variation (i.e., standing variation), and must reach new fitness optima before another taxa fills in the unoccupied niche space (Fausch 2008). If another species gets there first then priority effects and biotic resistance (Mack et al. 2000) could exacerbate continued maladaptive breeding timing and lead to native species decline. Our study highlights a compelling mechanism by which introgression could hasten the shift to reach an earlier breeding timing optima and act to further spread hybridization.

Invasive hybridization can rapidly introduce new genetic material into a population that can immediately expand the range of phenotypic possibilities—perhaps in ways not possible for non-hybridized native taxa due to limited standing genetic variation (Hamilton and Miller 2016). It is not a new concept that invasive species may benefit from hybridization. But, these studies generally focus on the benefits of multiple donor sources of the same species of invading taxa, such that intraspecific hybridization creates high genetic diversity for natural selection to work with (Ellstrand and Schierenbeck 2000, Verhoeven et al. 2011). While some have argued that adaptive introgression (between species) could become increasingly useful in conservation to increase genetic variation in wild taxa (Hamilton and Miller 2016), the same mechanism may prove problematic from an invasive hybridization perspective. If hybridization between species leads to genetic change for earlier, more variable, or otherwise well matched breeding timing—it could hasten the shift of native taxa towards new local fitness optima. This could exacerbate the spread of hybridization. We hypothesize that this may presently be occurring in native trout as introgression of RT alleles for earlier breeding lead to

colonization of ‘empty temporal niches’. From the alternative perspective, we have also shown that an invasive genome can likely benefit from ‘stealing’ genetic material from native taxa for adaptive breeding timing allowing it to overcome a breeding timing mismatch.

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Tables and Figures

Table 2.1. Counts of Yellowstone cutthroat trout (YCT), and YCT x rainbow trout hybrids (CTX) that spawned in 15 hydrologically diverse spawning patches from 2015 to 2017. Only three rainbow trout were observed spawning in this study, all in Hidden Creek (not shown, included as CTX in count). The proportion of individuals in each patch that were CTX or rainbow trout is shown (pHyb), as is the mean spawning date by taxa and difference between taxa (days) when trout were sympatric (in days).

Patch	YCT	CTX	pHyb	YCT spawn	CTX spawn	Difference
1. Ditch Cr. †	31	0	0	19-Jun	-	
2. Hidden Cr. ‡	6	57 (11)	0.91	31-May	22-May	-9
3. Buffalo Fork Cr.	1	5 (3)	0.83	6-Jul	14-Jun	-22
4. Slough Cr.	7	11 (4)	0.61	11-Jun	31-May	-11
5. Crystal Cr.	0	17 (1)	1	-	21-May	
6. Lamar R. Lower	1	4 (2)	0.8	23-Jun	27-Jun	+4
7. Rose Cr.	39	17	0.3	13-Jun	2-Jun	-11
8. Soda Butte Cr.	1	0	0	1-Jul	-	
9. Pebble Cr.	4	1	0.2	14-Jun	25-Jun	+11
10. Chalcedony Cr.	20	0	0	24-Jun	-	
11. Cache Cr.	1	0	0	16-Jul	-	
12. Lamar R. Upper	5	0	0	26-Jun	-	
13. Flint Cr.	8	0	0	-	-	
14. Calfee Cr.	1	0	0	23-Jun	-	
15. Miller Cr.	8	0	0	22-Jun	-	

Note: † designates an unnamed tributary to the Yellowstone River at 44.92, -110.40, and ‡ designates an unnamed tributary to Slough Creek at 44.92, -110.34

Table 2.2. Pearson's product moment correlation between abiotic conditions in spawning patches, native cutthroat trout spawning timing, and hybrid trout spawning timing. Modeled conditions are compared to site-specific mean spawning timing across all years, while measured conditions are compared to site and year specific spawning timing estimates. Spawning timing units are day of year and significant correlations are in bold.

Type	Abiotic conditions	Native spawning timing	Hybrid spawning timing	<i>n</i> YCT	<i>n</i> CTX
Modeled	Center timing flow mass	0.44	0.63	13	7
	Mean annual flow	0.43	0.71	13	7
	August temperature	-0.08	-0.38	13	7
	Elevation	0.25	0.3	13	7
Measured	Date of maximum flow 2016	0.59	0.54	4	4
	Date of maximum flow 2017	0.51	0.96	6	4
	Mean May temperature 2016	-0.49	-0.33	5	5
	Mean May temperature 2017	-0.14	-0.62	5	5
	Mean June temperature 2016	-0.58	-0.55	5	5
	Mean June temperature 2017	-0.66	-0.88	5	5

Table 2.3. Parameter estimates from a model predicting individual spawning date using individual degree of nonnative ancestry (pRT), and the interactive effect streamflow timing in the spawning habitat (early or late).

Term	Estimate	<i>SE</i>	<i>T</i>	<i>p</i>
Intercept	161.83	3.06	52.80	< 0.001
RT admixture	-27.50	3.65	-7.52	< 0.001
Flow timing (late)	7.18	3.92	1.83	0.06
Interaction	14.96	8.72	1.71	0.08

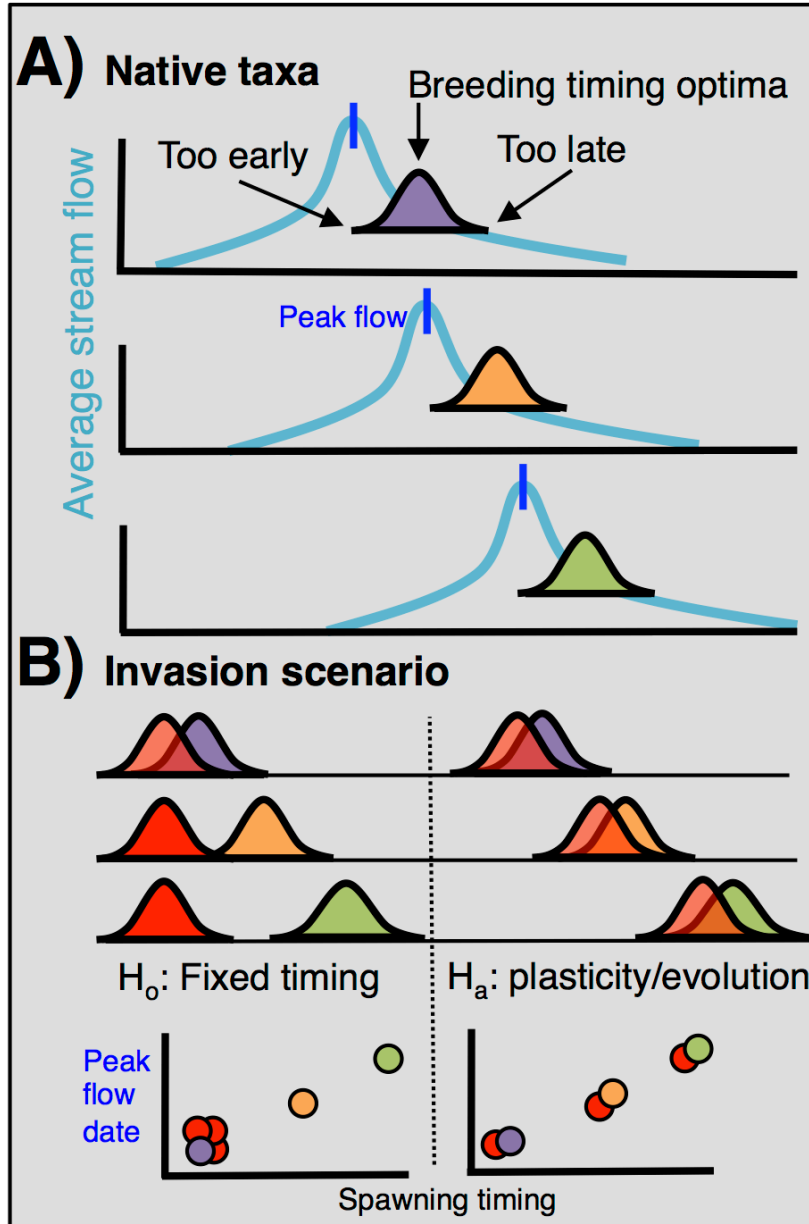


Figure 2.1. Native trout are locally adapted to spawn during the receding limb of the hydrograph; too early may lead to egg-scour, too late limits potential offspring growth during year-1 (A). Different flow regimes in streams lead to different selective pressures for spawning timing (an early, average, and late spawning population are shown). An invasive species with fixed early-breeding timing (H_0) would be most successful in patches with early streamflow, and temporal reproductive isolation with native taxa would also be weakest (purple patch) facilitating hybridization (B). Invasion and hybridization would be environmentally mediated in other patches (tan and green patches). Invader plasticity or adaptation could lead to weak abiotic resistance to invasion and hybridization (H_a).

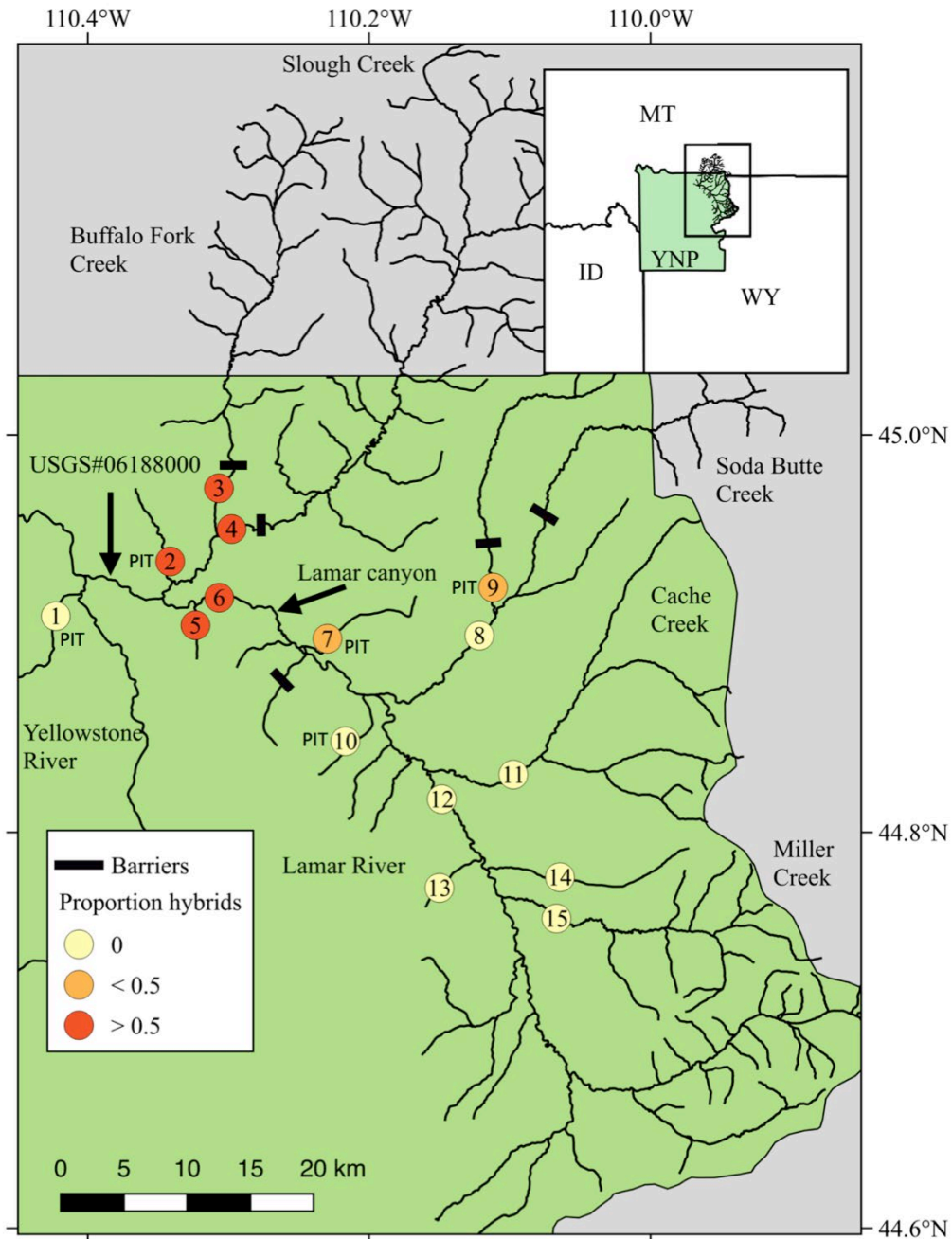


Figure 2.2 Lamar River watershed in Yellowstone National Park and National Forest land in Montana and Wyoming (grey). Spawning patches are shown and numbers correspond with table 1. Patches monitored with stream-wide passive integrated transponder antennas (PIT) are labeled.

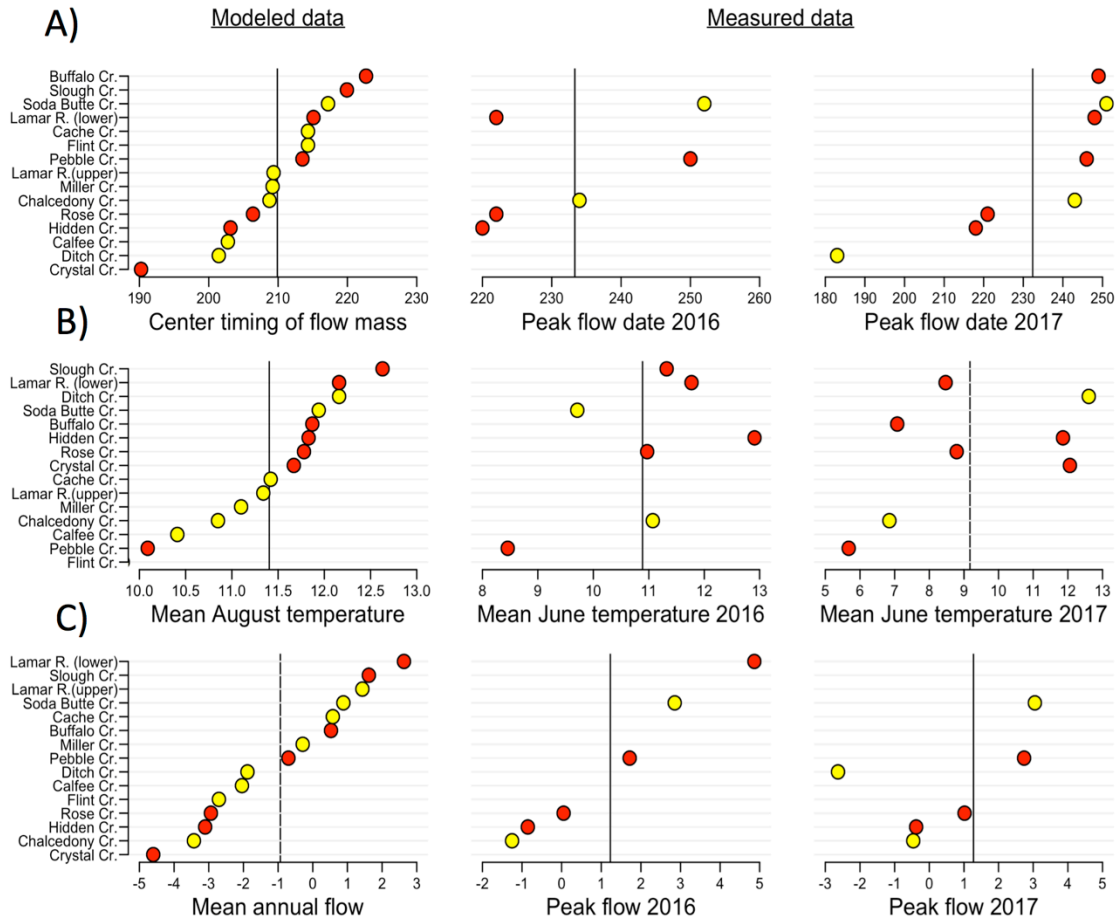


Figure 2.3. Asynchrony in runoff timing (A), variation in thermal conditions (B), and flow magnitude (C), among spawning patches in which spawning timing of native and hybrid trout was evaluated. Patches are sorted according to modeled center timing of flow mass (A), mean August stream temperature (B), or mean annual flow. Red circles are patches where hybrids spawned; only native trout spawned in patches indicated by yellow circles. The mean value is shown as a vertical bar, calculated within each individual panel.

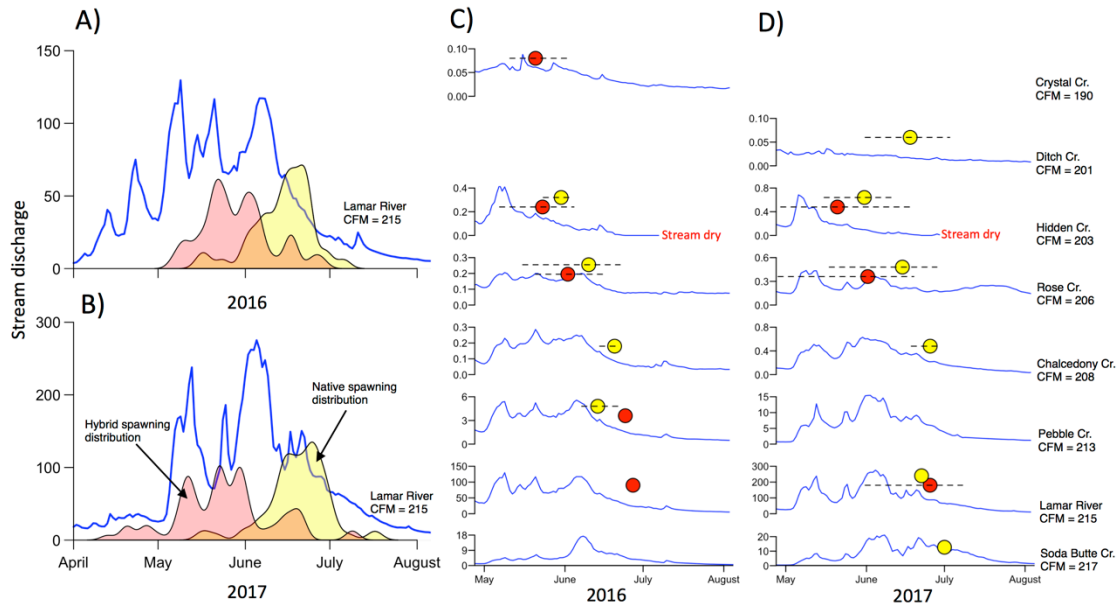


Figure 2.4. Spawning timing of native cutthroat trout (yellow) and hybrid trout (red) in relation to the hydrograph in the Lamar River in 2016 (A) and 2017 (B). Spawning timing of the entire dataset is shown with a kernel density smoother. Panel C and D show mean (and range, dashed lines) of spawning timing estimated locally within patches in 2016 and 2017, compared to locally estimated hydrographs at eight spawning sites. Sites are sorted according to long-term average runoff timing (i.e., center timing of flow mass, CFM, with earlier runoff streams at the top). Note: the Crystal Creek hydrograph was estimated in 2015 and is shown with spawning timing data from 2015 and 2016.

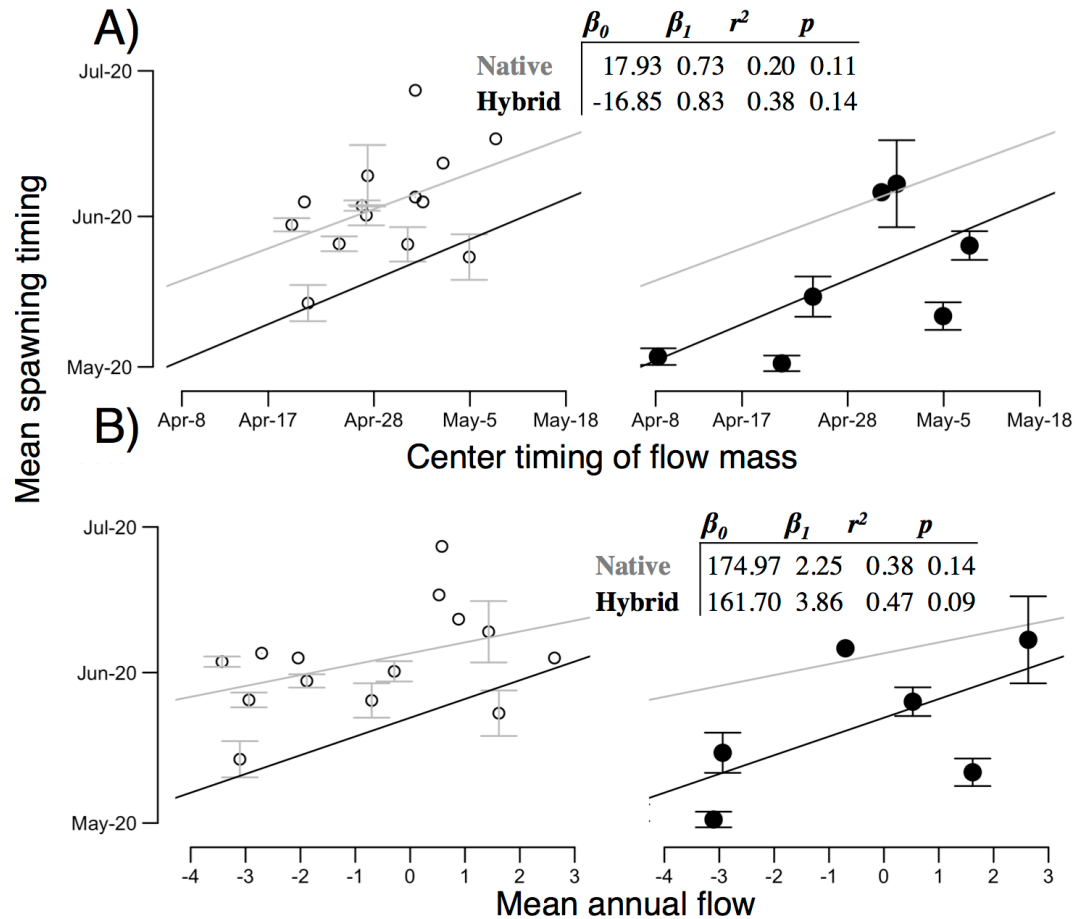


Figure 2.5. Mean spawning timing of native trout and hybrid trout populations in relation to center timing of flow mass, a metric of streamflow timing (A) and mean annual flow on the log scale (B). Spawning timing data are summarized across years (2015—2017, $\pm$ SE). Consistent slopes indicate similar responses to asynchronous flow conditions (A) and runoff volume (B) between taxa, but different intercepts show that native trout spawn, on average, later than sympatric hybrid trout.

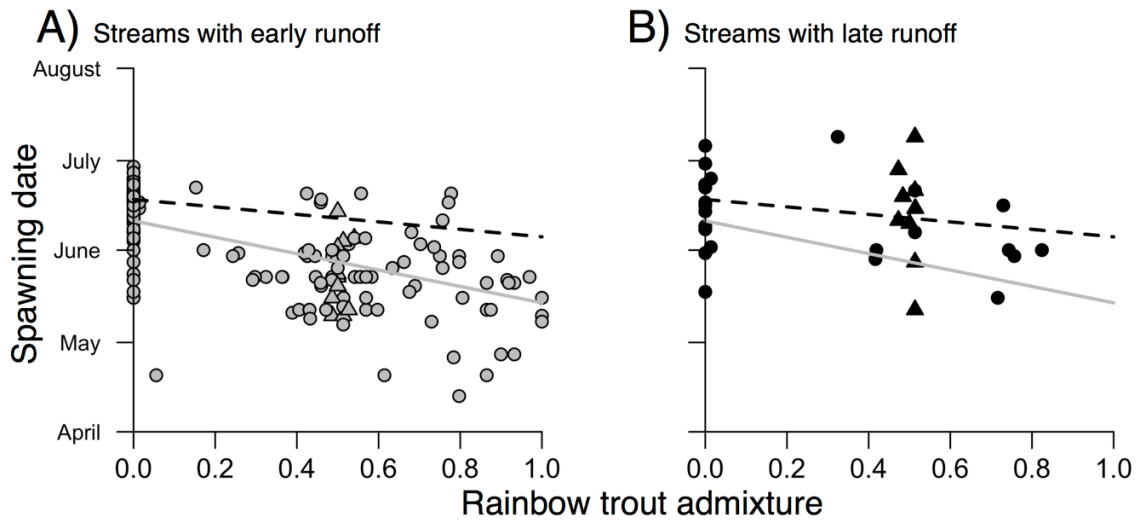


Figure 2.6. The effect of rainbow trout admixture on individual spawning date in (A) streams with early runoff and (B) streams with late runoff fitted with a linear mixed effects model (Table 3). Regression slopes (late and early) are plotted on both panels for comparisons, and triangles represent F1 hybrids

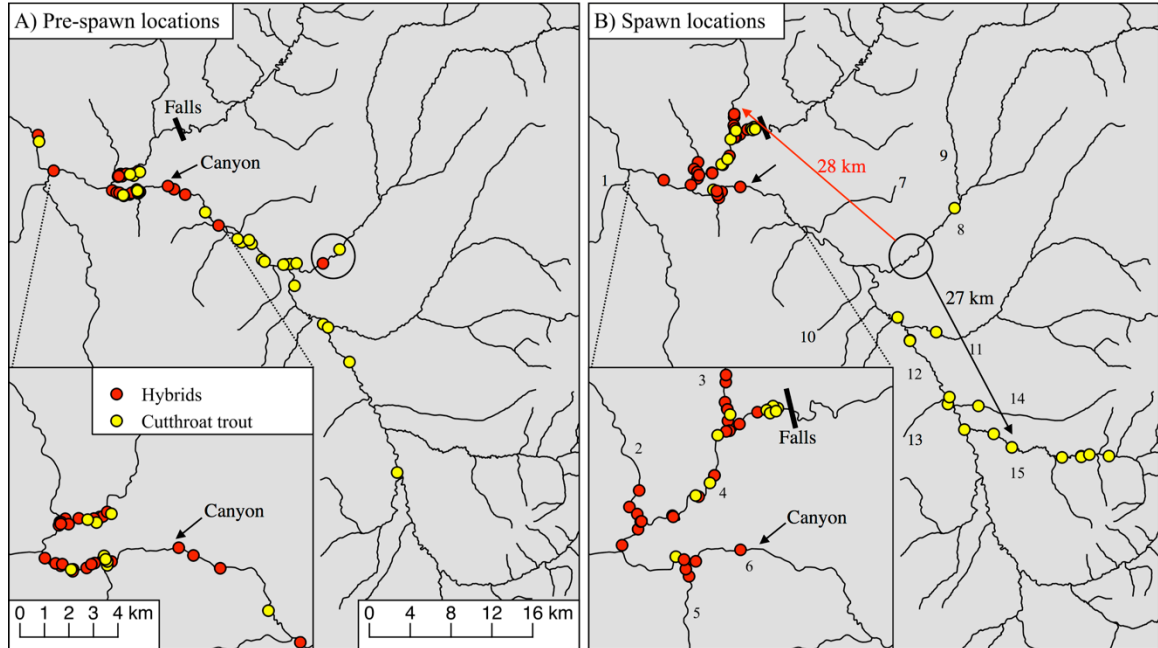


Figure 2.7. Spawning migrations of 51 radio tagged trout with known genotypes (2016 and 2017 data combined), depicting the distribution of pre-spawning locations (panel A) and spawning locations (B) (insets within each panel show lower watershed in detail). There was complete spatial reproductive isolation by taxa in the upper watershed, but not in the lower watershed (*panel B, inset*). A Yellowstone cutthroat trout and a hybrid shared the same pre-spawning location (elevation = 2020 m), but eventually spawned 55 river kilometers apart (highlighted in panel B) at an elevation difference of ~ 240 m. Numbered locations correspond with table 2.1 and figure 2.1.

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CHAPTER THREE

INVASIVE HYBRIDIZATION IN A HIGH-ELEVATION STRONGHOLD:
GENETIC STATUS OF YELLOWSTONE CUTTHROAT TROUT IN
THE LAMAR RIVER OF YELLOWSTONE NATIONAL PARK

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Kurt C. Heim

Contributions: Designed study and developed research questions, collected the data, analyzed the data, wrote the manuscript

Co-Author: Thomas E. McMahon

Contributions: Assisted with study design and development of research questions, discussed results, edited the manuscript

Co-Author: Steven T. Kalinowski

Contributions: Helped develop outlines for manuscript, discussed results, edited the manuscript

Co-Author: Brian D. Ertel

Contributions: Helped develop study design, assisted with data collection, edited the manuscript

Co-Author: Todd M. Koel

Contributions: Helped develop study design and obtained funding, edited the manuscript

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Kurt C. Heim, Thomas E. McMahon, Steven T. Kalinowski, Brian D. Ertel, Todd M. Koel

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Abstract

Invasive hybridization threatens many taxa worldwide, and protecting strongholds for non-hybridized native species is needed to conserve biodiversity. Yellowstone cutthroat trout (*O. clarkii bouveri*, YCT) in the Lamar River watershed in Yellowstone National Park represent an important stronghold for the subspecies but are threatened by hybridization with rainbow trout (*O. mykiss*). Thirty populations ($n = 1,359$ individuals) were tested at 52 species diagnostic markers to (i) assess the spatial distribution of hybridization to guide conservation efforts and (ii) determine the relative role of abiotic and biotic factors influencing the spread of hybridization. The occurrence and degree of hybridization declined rapidly with fluvial distance from an original RT stocking location in a large (~ 43 km stream length) and historically fishless tributary (Buffalo Fork Creek). Population genetic samples from Buffalo Fork Creek consisted of 99% RT admixture (pRT) at the headwaters ($\sim 2,500$ m, estimated temperature of 8°C), but decreasing amounts of pRT at lower elevations (pRT = 72%; $1,900$ m, $\sim 12^{\circ}\text{C}$). Fluvial distance to the original stocking location was the best predictor of the presence and degree of hybridization in 29 populations across the Lamar River watershed. All populations within 37 km were more than 1% pRT ($n = 10$), regardless of elevation, or estimates of stream temperature, mean annual flow, and runoff timing. Beyond this distance, all tested populations were less than 1% pRT ($n = 19$). Under some circumstances RT and hybrids can invade cold, high-elevation, and small stream habitats. Also, mainstem sites and an intermittent stream were important hybrid spawning locations, yet such habitat types are often excluded from genetic status assessments. This

work highlights how outliers to invasion patterns established at rangewide scales may be of critical importance to conservation efforts at local watershed scales.

Introduction

All subspecies of native cutthroat trout (*Oncorhynchus clarkii* spp.) of the Northern Rocky Mountains have been adversely impacted by introductions of non-native rainbow trout (*O. mykiss*, RT). Rainbow trout often replace native trout entirely, in other scenarios, RT interbreed with cutthroat trout until all remaining individuals are hybrids and the native taxa is considered extinct (Allendorf et al. 2001). For example, the Yellowstone cutthroat trout (*O. c. bouveri*, YCT) is completely extirpated from 52% of its native range, and only 28% of the historic range is now occupied by non-hybridized populations (Gresswell 2011). Dramatic declines of both YCT and westslope cutthroat trout (*O. c. lewisi*, WCT) have motivated a substantial effort to identify non-hybridized populations to prioritize for conservation and better understand mechanisms influencing RT hybridization. Predicting hybridization risk is a topic of much interest.

Two general principles are currently used to assess the risk of hybridization spreading; the first is that cold water will mediate its spread (Isaak et al. 2015; Young et al. 2017). Cutthroat trout have a lower metabolic rate and incipient lethal temperature than RT, and also spawn later in the year than RT (Bear et al. 2007; DeRito et al. 2010; Rasmussen et al. 2012). These traits are hypothesized to provide an ecological advantage for cutthroat trout at high-elevation sites characterized by cold water and late-snowmelt runoff that may scour eggs of late-spawning RT from redds (Fausch et al. 2001; Young et

al. 2016). Across the range of cutthroat trout, introgression is often rare above 1700 m in elevation (Weigel et al. 2003; Gunnell et al. 2008), and is believed to decrease rapidly where mean August temperatures are below 11°C (Isaak et al. 2015). Within watersheds, the degree of hybridization often decreases along an elevation and temperature gradient (Gunnell et al. 2008; Bingham et al. 2016). Some scientists posit that cold water (i.e., < 11°C) will prevent RT invasion and introgression (Young et al. 2016), yet, others note that introgression may occur in nearly any abiotic conditions if cutthroat trout are close to a historic or modern day source of RT (Hitt et al. 2003; Loxterman et al. 2014).

An alternative perspective is that hybridization risk is more strongly associated with distance to invasion source and propagule pressure than abiotic gradients (Hitt et al. 2003; Muhlfeld et al. 2017). Rainbow trout admixture is consistently higher near ostensible invasion sources such as historic stocking locations, and at sites that are in close proximity to modern-day sources of established, self-sustaining populations of RT and hybrids (Gunnell et al. 2008; Bennett et al. 2010; Loxterman et al. 2014). These examples suggest that invasion is constrained more so by demographic than abiotic factors (Hitt et al. 2003), and some authors consider it unlikely that cold water is sufficient to prevent expanding hybridization (Kovach et al. 2017). One of the challenges to understanding the relative role of abiotic and biotic mechanisms of invasion, however, is strong covariance between abiotic gradients (i.e., temperature, elevation), likely sources of historic invasion, and contemporary RT source connectivity (Muhlfeld et al. 2009; Loxterman et al. 2014).

Disentangling the relative influence of abiotic conditions from source connectivity is challenging because RT were most often stocked at lower-elevation sites with warm temperatures (Muhlfeld et al. 2009). For example, over 20 million RT were stocked in the Flathead River, MT, at low elevations and this has played a major role in the distribution of present-day hybridization (Muhlfeld et al. 2014). Is introgression absent or limited at cold, high-elevation sites because of ecological differences between taxa (Young et al. 2016), or, simply because these sites are farther from historic or modern day RT sources (Kovach et al. 2017)?

The answer to this question is not merely of academic interest but is integral to guide conservation efforts of remaining strongholds for non-hybridized cutthroat trout. A strong shielding effect of cold water would suggest that native populations occurring in cold water are safeguarded from genomic extinction (Isaak et al. 2015; Young et al. 2016), even if connected RT populations exist at lower elevations. Alternatively, if introgression were an expected outcome for any native population with a nearby RT source—there would be a more urgent incentive to take proactive conservation measures. In this study, we assess the relative role of abiotic and biotic factors in the spatial distribution of hybridization between RT and YCT in the Lamar River watershed of Yellowstone National Park (YNP). Our overall goals are twofold. First, this study serves as a comprehensive genetic status assessment for this watershed to prioritize conservation and management efforts. Secondly, a rather unique invasion scenario—involving low RT stocking intensity (250,000 RT, relative to 16 million YCT), in a high-elevation watershed (1,800 – 3,000 m), with a modern day RT source population occurring at 2,500

m—provides an opportunity to evaluate spatial patterns of introgression in a scenario where abiotic conditions are not strongly collinear with RT source connectivity.

The Lamar River YCT metapopulation is of high conservation value and represents a hopeful stronghold for non-hybridized YCT to persist into the 21st century (Al-Chokhachy et al. 2018). Whereas many YCT populations occur in short, isolated stream segments and are unlikely to persist in the long-term (Hilderbrand and Kershner 2000), YCT in the Lamar River watershed occur as a functioning metapopulation inhabiting a large, nationally protected watershed (Ertel et al. 2017). High fluvial connectivity, metapopulation processes, and life-history diversity promote resiliency to anthropogenic stressors (Williams et al. 2015), yet also render the entire system vulnerable to invasion from RT. Prior genetic surveys identified many non-hybridized YCT populations in the upper watershed, but also suggest that introgression may be expanding (Ertel et al. 2017). The National Park Service (NPS) recently implemented several large-scale conservation efforts in the watershed including barrier construction to prevent hybrid dispersal, chemical treatments to eliminate non-native sources, and mandatory angler harvest for RT and hybrids (Ertel et al. 2017). However, a comprehensive assessment of the current genetic status of YCT in the watershed has not been completed, nor has an examination of the relative influences of factors that may mediate the spread of invasion. Our objectives were therefore, to characterize the (*i*) spatial distribution of native YCT and hybridized populations, as well as hybrid individuals throughout the drainage and (*ii*) examine the relative role of abiotic and biotic

factors in the determining the current distribution of hybridization to guide expectations for the future spread of invasion.

Methods

Study Site

The Lamar River watershed encompasses a 1,731 km² area that is mostly within YNP and flows 78 km from the headwaters (elevation = 3,015 m) to the Yellowstone River (1,829 m) (Figure 3.1). Mean annual discharge is 25 m³/s and mean annual peak-flow is 231 m³/s (U.S. Geological Survey gaging station #06188000). A movement barrier on the Yellowstone River (Knowles Falls), 22 km downstream from the confluence of Lamar River, prevents upstream dispersal of non-native fish that are common outside of YNP (Ertel et al. 2017). Within the Lamar River watershed, canyons (on Lamar River, Buffalo Fork Creek, and Soda Butte Creek) and waterfalls (Slough Creek, Pebble Creek) are considered semi-permeable fish movement barriers and noted (Figure 3.1). 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 (Figure 3.1). An artificial barrier was constructed in 2017 on Slough Creek (just upstream of the falls, Figure 3.1) to further isolate the upper Slough Creek watershed from hybrid dispersal. Additional management actions include a chemical treatment to eradicate non-native brook trout (*Salvelinus fontinalis*) from Soda Butte Creek upstream of Ice Box canyon (2015, 2016), mandatory angler harvest of RT and hybrids (2013—present), and annual mechanical removal of RT and hybrids (2013—

present) from upper Slough Creek (YNP boundary to falls) and the Lamar River (from Soda Butte confluence to Lamar River canyon, Figure 3.1).

Stocking History and Distribution of Rainbow Trout

In the Lamar River watershed, 250,000 RT were reportedly stocked (1932—1955) in five locations, far less than the 16 million cutthroat trout stocked in 18 locations (Varley 1981, Table C.1). One prominent location was in Trout Lake, a small (0.05 km²) waterbody used from the 1920s to 1940s as a RT egg collection facility. Although a small stream connects Trout Lake to Soda Butte Creek, it does not appear this served as a prominent source of RT to the Lamar River watershed. Buffalo Fork Creek, a historically fishless watershed where RT have become widely established, is now considered the primary source from which RT disperse to interbreed with YCT (Ertel et al. 2017). Hybridization is prevalent in several populations in the lower Lamar River watershed, and in Rose Creek in the upper watershed (Figure 3.1). Introgression has not yet been detected in many tributaries in upper Slough Creek and the Lamar River near their headwaters.

Spawning Population and Mainstem Site Samples

We gathered tissue samples from 730 fish to genotype and assess the occurrence and degree of hybridization in 29 stream dwelling populations of trout and one lake dwelling population. We use the term population to describe a group of individuals that spawned or were born in the same tributary or mainstem spawning site. Several precautions were taken to assure these criteria were met. First, radio telemetry, passive

integrated transponder tags, and sampling during spawning periods were used to assign adult fish ($n = 217$) to 14 spawning populations (Figure 3.1, Table 3.1, Chapter 2). No evidence of straying in repeat spawning fish ($n = 25$) was documented, yet we recognize that some of these adults could have been assigned to non-natal populations. A tissue sample was collected from the upper lobe of the caudal fin, and fish that provided useful spawning data were genotyped (described below).

Second, we sampled small tributaries where samples containing fish of mixed natal origin would be unlikely. Inter-tributary movements during the non-spawning period are common in salmonids (Gresswell 2011), and in the Lamar River watershed fish occur in mixed stock aggregations in streams as small as Soda Butte Creek (mean annual flow, MAF = $2.42 \text{ m}^3/\text{s}$) during summer and fall (Chapter 2). Therefore, we did not target streams $> 2.0 \text{ m}^3/\text{s}$ MAF for population sampling during the non-spawning period. Sixteen sites meeting this criterion were sampled with electrofishing, angling, or gill nets from 2011 to 2017. Fish were collected from reaches of $\sim 5 \text{ km}$ to avoid sampling related individuals, and a small tissue sample was taken from each fish. Lastly, eight populations identified in Chapter 2 (i.e., via telemetry or sampling during the spawning period) that were $< 2.0 \text{ m}^3/\text{s}$ were sampled with angling and electrofishing to increase sample sizes for genetic analysis (Table 3.1). A total of 513 individual fish were thus added to the genetic population samples collected in Chapter 2.

We also sampled fish from mainstem sites ($n = 629$) to collect fish with unknown population membership including the Lamar River ($n = 379$), Slough Creek ($n = 199$), and Soda Butte Creek ($n = 51$) using angling and electrofishing (2012—2017).

Geographic positioning system coordinates (GPS) were recorded for each individual, typically to within 1 km of the sample location, and genetic results (see below) were depicted graphically with spatially explicit kernel density estimates mapped to the river network (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 ($n = 1,359$). Population genetic status was described by the occurrence of introgression, the degree of hybridization (%RT admixture, pRT) and the proportion of individuals in the population that were identified as hybrids (pHyb). Genotyping was performed at the University of Montana Conservation Genetics Laboratory using methods and genotyping assays described in detail elsewhere (Bingham et al. 2016). Loci genotyped included SNPs diagnostic for WCT, YCT, and RT. After screening and removing probable non-diagnostic loci (i.e., potential ancestral polymorphisms, Figure C.1), our final marker set included 18 RT, 16 WCT, and 18 YCT diagnostic markers. The set of SNPs (WCT, YCT, and RT) were used to estimate the genetic contribution of the three potentially hybridizing taxa to the 30 population samples and each individual fish (i.e., RT admixture, YCT admixture, and WCT admixture, Kalinowski 2010). Fish with interspecific heterozygosity (H_i) greater than 0.90 were classified as first generation hybrids (F1s), and histograms of individual pRT within populations are used to describe the genotypic pattern of hybridization.

Abiotic Conditions, Source Connectivity, and Occupied Habitat

We used modeled datasets to describe abiotic conditions expected to influence hybridization. We expected the occurrence and degree of hybridization to be influenced by elevation and temperature (negative associations), as well as stream size and snowmelt runoff timing (positive associations [increased hybridization in larger sites with early runoff]). We used the NHD-Plus Version 2 medium resolution (1:100,00-scale) stream layer available from the NorWeST website (Isaak et al. 2017) as a base map, which included stream temperature predictions (mean August temperature 2002—2011) and elevation. A database was built in Quantum GIS (Quantum GIS Development Team 2016) and further attributed with mean annual flow (MAF, m^3/s) to represent stream size and center timing of flow mass (CFM, day of water year beginning on October 1st) to represent snowmelt runoff timing (Wenger et al. 2010). The CFM predictions were validated (Chapter 2) to adequately represent variation across sites by comparing it to measured date of peak flow (Spearman's $r_s = 0.75$ in 2016, $r_s = 0.98$ in 2017), and MAF was collinear with cumulative drainage area (Pearson's $r = 0.99$) so it is a good indicator of stream size. Mean August stream temperature was measured at seven sites in the Lamar River watershed to validate the accuracy of NorWeST predictions. Six HOBO loggers (Onset Corporation, Pocasset, Massachusetts, USA) were deployed at road accessible sites (2015 = 2, 2016 = 4, 2017 = 5) and data were acquired from the U.S. Geological Survey gage on the Lamar River (#06188000, 2015—2017). Modeled NorWeST estimates adequately captured variation among sites ($r = 0.90$), but were on average 2.33°C colder than measured data (range = 0.98 — 3.40°C). Absolute error (i.e.,

the difference between measured and modeled stream temperature) was, however, strongly correlated to elevation ($r = -0.93$) indicating bias systematically decreased at higher elevation sites (Figure C.2). We proceed to use an uncorrected NorWeST dataset because it adequately describes among site variation, and so we can compare results to studies using this same dataset.

To represent the historic and continued effects of RT dispersal on hybridization (i.e., biotic effects), we considered the site where the Hidden Lake outlet stream flows into Buffalo Fork Creek (Figure 3.1, site #5) as the historical source of parental RT. This location is the only location in the Buffalo Fork Creek drainage reportedly stocked with RT and, consistent with this stocking history, has the highest pRT of any population we sampled in this study. Fluvial distance from this location to the center of each spawning population was calculated.

We used the aforementioned stream network to estimate the kilometers of stream likely used by fish for spawning (in the entire watershed) and quantify the extent of stream inhabited by populations with different genetic status. A concurrent telemetry study (Chapter 3) indicated that essentially all available stream habitats accessible to fish (i.e., size, temperature, flow timing) are used for spawning in this watershed. We therefore used a MAF cutoff of $0.009 \text{ m}^3/\text{s}$ and a stream slope cutoff of $> 11\%$ to trim the network and exclude habitat not likely to support spawning fish, and also excluded intermittent streams (except for site #1, an intermittent stream where spawning fish were documented). This resulted in 568 river km of potential spawning habitat. Genetic results were used to categorize sections of the stream network as 1) genetically tested and pRT =

0.00, 2) $pRT \leq 1\%$ (“core conservation population”), 3) $pRT > 1\%$ and $\leq 10\%$ (“conservation population”), 4) $pRT > 10\%$, and 5) not tested. These categories are those used by federal and state management agencies throughout the range of YCT (Al-Chokhachy et al. 2018).

Data Analysis

We tested for differences in abiotic conditions between hybridized sites ($pRT > 0.00$) and non-hybridized sites ($pRT = 0.00$) using a Mann-Whitney U-test. We were also interested in whether abiotic conditions were sufficient to distinguish non-hybridized from hybridized sites. Non-overlapping distributions of abiotic conditions in hybridized compared to non-hybridized sites would indicate strong abiotic resistance to invasion, whereas overlapping distributions suggest invasion is not limited by abiotic conditions. We examined these with kernel density estimates fit in the R programming environment (R Core team 2017).

We used logistic regression to evaluate the relative importance of four abiotic variables (temperature, MAF, CFM, elevation) and distance to RT source in the occurrence of hybridization at 29 sampled stream dwelling populations. As a response variable, we initially used a pRT threshold of 1% to classify populations for consistency with similar studies (Young et al. 2016). However, this lead to modeling issues because the source connectivity variable perfectly separated the dataset (i.e., all hybridized sites were between 3 and 37 km from Hidden Creek, and all non-hybridized sites were between 45 and 83 km away). Therefore, we used a threshold of 0.00 pRT to classify populations. We considered all combinations of non-correlated predictor variables ($r <$

0.5) as candidate models ($n = 13$ models) and selected the best approximating model with Akaike's Information Criteria corrected for small sample size (AIC_c). Hosmer—Lemeshow goodness-of-fit tests indicated that the logistic models provided a good fit to the presence or absence of introgression at a site. Area under the receiver operating curve was also used (AUC) as a measure of model performance, which ranges from 0.5 (i.e., the model poorly predicts dichotomous response) to 1.0 (the model perfectly predicts the response)(Zuur et al. 2009). Because sites in upper Buffalo Fork Creek (#4, #6) were historically fishless and could overly bias modeling results (i.e., native YCT were not present to resist invasion) we also ran models excluding these sites.

We used linear regression to evaluate the relative importance of independent variables to predict the proportion of individuals in the population that were hybridized with RT (pHyb). Proportions of hybrid individuals within populations were adjusted by $(pHyb(N-1) + 1/2/N)$ to remove zeros then logit transformed to meet assumptions of linearity. Initial model exploration revealed a non-linear relationship between pHyb and source distance and pHyb and elevation; therefore, we included elevation and source distance as second order polynomial terms. As with the logistic regression, we tested 13 models and ranked them according to AIC_c , and also ran models excluding sites from upper Buffalo Fork Creek.

Results

Spatial Pattern of Hybridization

Half of the sampled populations contained evidence of RT hybridization (15/30), and the degree of hybridization was highest in Hidden Lake (site #5, $pRT = 99.8\%$, elevation = 2,362 m), one of five known RT stocking sites in the watershed (Table 3.1, Figure 3.1). Most individuals in Hidden Lake were RT ($pRT = 1.00$, 18/21 fish, Figure 3.2). A small stream (~ 1.5 m width) runs from Hidden Lake to Buffalo Fork Creek. Near this confluence a population sample from Buffalo Fork (site #4; 2,303 m) consisted of predominantly RT individuals (15/20) and pRT was 99.5%; upstream from this site, at the highest elevation population sampled (site #6; 2,465 m) an equal number of hybrids and RT were encountered (10/20) and pRT was 98.8%. Overall, in the upper Buffalo Fork Creek samples (outside of YNP), RT was the most common genotype (43/61, 70%) and all fish sampled had individual pRT estimates greater than 95%. Although a legacy of YCT introgression was detected at all sites, YCT were altogether absent.

Increasing amounts of YCT admixture were detected in lower Buffalo Fork Creek, at a site 500 m lower in elevation. In the population sample inside of YNP (site #3; 1935 m), YCT admixture increased ($pRT = 72.42\%$), parental RT were absent, and a YCT was documented spawning here with radio telemetry (Table 3.1, Chapter 2). Although RT were absent, fish with pRT greater than 95% were common (Figure 3.2). The predominance of RT and fish with pRT greater than 95% in the Buffalo Fork Creek drainage (upper and lower), relative to all other population samples in the Lamar River watershed was noteworthy. Outside of the Buffalo Fork Creek drainage, only three RT

(3/642, 0.4%, all from site #1), and one hybrid with individual pRT greater than 95% (1/642, 0.2%, site #12) were encountered in the spawning population samples.

The degree of population hybridization was notably higher in the lower watershed (downstream of the Lamar River canyon and a waterfall on Slough Creek), relative to the upper watershed (Figure 3.3). All populations in the lower watershed were substantially hybridized (pRT > 10%, 5/5 populations) whereas only a single population in the upper watershed (excluding Buffalo Fork Creek) was substantially hybridized (1/22 populations, site #13). In both the upper Lamar and Slough Creek watersheds, the most hybridized populations were the ones with the shortest fluvial distance to the lower watershed (sites #13, #7, #8; Figure 3.1, 3.3).

The distribution of hybrids sampled from mainstem sites (i.e., not assigned to a population sample) was consistent with spatial patterns of population hybridization (Figure 3.3). In the lower Lamar River below the canyon, 49/87 (56%) of genotyped fish were hybrids, above the canyon only 11/292 (3%) were hybrids. Below the falls on Slough Creek, 91.5% of fish were hybrids (54/59) versus only 6.4% of fish above the falls (9/140). All 51 fish sampled in Soda Butte Creek, upstream from Lamar River Canyon, were YCT.

In contrast to the restricted distribution of hybrids, YCT were widely distributed. Yellowstone cutthroat trout were sampled in all but one population sample (site #12) outside of the upper Buffalo Fork watershed (26/27 populations). Similarly, YCT were also sampled in all mainstem locations in the lower watershed (Figure 3.3).

Genotypic patterns of individual pRT were markedly different in populations in the lower, compared to the upper watershed. Populations in the lower watershed provided evidence of multiple generations of backcrossing and continued contact between parental taxa as evidenced by many fish with pRT near 50% (Figure 3.2). In contrast, the genotypic distribution in populations of the upper watershed (Abundance Creek [site #9], Pebble Creek [#16], Soda Butte Creek [#18], and Miller Creek [#26]) indicated a lack of continued contact between parental taxa and hybridization that occurred several generations ago (Figure 3.2). Outliers to this pattern occurred in the three aforementioned hybrid populations directly upstream from semi-permeable movement barriers. Hornaday Creek (site #7) and Elk Tongue Creek (#8), in Slough Creek just upstream of the falls, provide evidence of recent contact between RT and native trout (Figure 3.2). Rose Creek (site #13) was the only population in the upper watershed with pRT greater than 10%, and although YCT were the predominant genotype, they co-occurred with a wide-ranging distribution of hybrids from pRT 2% to 80%.

Extent of Occupied Habitat

We estimated 568 km of river habitat are likely to support spawning populations in the Lamar River watershed, and tested the genetic status of 380 km (67% of the watershed). A total of 216 km was tested and exhibited no evidence of hybridization (pRT = 0.00, 56% of tested), 280 km was tested and classified as “core conservation populations” (pRT $\leq$ 1%, 74% of tested), 15 km was classified as “conservation populations” (pRT $>$ 1% $\leq$ 10%, 4% of tested), and 84 km was $>$ 10% pRT (33% of

tested). The Buffalo Fork Creek drainage contains 46 km of habitat supporting populations > 70 % pRT.

Abiotic and Biotic Correlates of Hybridization

Sites containing hybrid populations were significantly lower in elevation (mean = 2,072 m; range = 1,865—2,464 m) and significantly warmer (mean = 10.8°C; range = 8.0—12.6°C) than those containing native populations (mean elevation = 2,233 m; range = 2,023—2,427 m [Mann-Whitney $U = 164$, $p = 0.009$]; mean temperature = 9.9°C; range, 8.1—11.7°C [$U = 53$; $p = 0.023$]). Streams with hybrid populations did not differ in MAF (mean = 1.8 m³/s; range = 0.01—13.9 m³/s) or CFM (mean = 214; range = 190—231) from those supporting native populations (mean MAF = 0.84 m³/s; range = 0.03—3.82 m³/s [$U = 107$, $p = 0.86$]; mean CFM = 213; range = 203—226 [$U = 113$, $p = 1$]).

However, hybrid populations occupied a wider range of conditions than native populations (Table 3.2, Figure 3.4). Hybrids were present at the coldest, highest elevation stream with the latest streamflow timing we sampled (Buffalo Fork Creek [upper], site #6, 8.0 °C, 2,426 m, CFM = 231), and also the warmest, lowest elevation, and largest site (Lamar River [lower], 12.5°C, 1,865 m, MAF = 13.9 m³/s). The smallest site, with the earliest peak flow was also occupied by a hybrid population (Crystal Creek, site #12, MAF = 0.01 m³/s, CFM = 190). Such variation in abiotic conditions among hybridized and non-hybridized sites led to weak univariate correlations between pHyb (the proportion of individuals hybridized) and temperature ($r = 0.28$), MAF ($r = 0.22$), CFM ($r = 0.21$), and elevation ($r = -0.36$) (Figure C.3).

The occurrence of hybridization was strongly associated with fluvial distance to the ostensible RT source, Hidden Lake, and the best approximating logistic model included only distance-to-source but no abiotic covariates (Table 3.3). As discussed (see methods), distance-to-source perfectly separated hybridized from non-hybridized sites when using an introgression threshold of 1% used to classify core conservation populations (hybrid population mean distance-to-source = 22 km; range = 3–37 km; native population mean = 61 km; range = 45–83 km). All populations within 37 km of Hidden Lake were hybridized; all populations past this distance were classified as core conservation populations ($p_{RT} < 1\%$). The best approximating logistic model included only distance-to-source had a high classification success rate ($AUC = 0.85$), and outperformed a model that also included temperature ($\Delta AIC_c = 1.51$). This second ranked model suggested warmer sites were more likely to be hybridized, but only marginally improved classification success ($AUC = 0.88$, Table 3.4). Excluding sites 4 and 6 (from Buffalo Fork Creek) yielded the same top five ranked models, in the same order (Table 3.3), indicating our results were not overly biased by inclusion of these sites that were historically fishless. Odds ratios from the top model indicate a population 50 km away from Hidden Lake is 55 times more likely to be non-hybridized than one 0 km from Hidden Lake ($1.104 * 50$) (Table 3.4). Results from the linear model also strongly support the important role of source dynamics.

The proportion of hybridized individuals within populations (p_{Hyb}) was strongly associated with distance-to-source, and the best approximating model included both distance-to-source and streamflow timing (CFM). First, this relationship was clearly

strong by assessing the univariate relationship visually (Figure 3.5) and by simple correlation tests ($r = 0.86$) (Figure C.3). Our top performing linear model included distance-to-source as a second order polynomial term and CFM, and explained 87% of variation in pHyb (Table 3.3, Table 3.4). The same model was selected with or without Buffalo Fork sites 4 and 5. A negative association between CFM and pHyb suggested that earlier streamflow timing leads to higher rates of hybridization, and a positive association of temperature (in the second ranked model) suggests pHyb increases with colder temperatures (Table 3.4). However, both the CFM and temperature effect estimates overlapped zero and added little explanatory power to the model excluding abiotic variables.

Discussion

Fluvial connectivity and distance to a contemporary source of RT best explained the occurrence and degree of hybridization in the Lamar River watershed. Abiotic gradients did not appear to prevent introgression because (i) RT have colonized an entire, presumably cold, high-elevation watershed, (ii) hybrid populations occupied a wider range of abiotic conditions (temperature, flow timing, flow volume, elevation) than YCT, (iii) introgression radiates from a single high-elevation source, and lastly (iv) hybridization models suggested only secondary roles of abiotic conditions. Despite the preponderance of introgression in the lower watershed, two semi-permeable movement barriers (a waterfall and a canyon) appear to have prevented more widespread invasion. Our study provides further evidence that abiotic conditions should not be expected to

invariably prevent continued introgression in cutthroat trout metapopulations (Muhlfeld et al. 2017). This underscores the need for prompt management actions if native cutthroat trout are to be spared from genomic extinction.

Unlikely Sources of Rainbow Trout and Hybrids

Buffalo Fork Creek is a prominent contemporary source of invasive RT, but is a rather unusual RT source because abiotic conditions here are expected to strongly limit RT invasion potential (Fausch et al. 2001). This drainage was predicted to be cold ($\sim 8^{\circ}\text{C}$), had the latest peaking stream flow timing in our study, and was occupied across the entire length (2,500 m – 1,935 m) by RT and highly admixed fish. This is exceptional because cold water is expected to favor native trout (Rasmussen et al. 2012; Isaak et al. 2015; Young et al. 2016), late and potentially scouring peak flows are predicted to limit RT recruitment (Fausch et al. 2001; Muhlfeld et al. 2014), and RT and hybrids are not often reported above 1,700 m in stream habitats (Weigel et al. 2003). Because Buffalo Fork Creek was historically fishless, a lack of biotic resistance by established trout populations probably contributed to RT colonization. Indeed, the invasion biology literature provides many examples of priority-effects contributing to invader success (Shulman et al. 1983; Mack et al. 2000). However, uneven stocking ratios (3,500 RT compared to 173,000 YCT in the drainage, Table C.1), and the aforementioned poor conditions for RT still renders this a rather idiosyncratic scenario. This scenario shows that RT and hybrids are not fundamentally limited from inhabiting cold streams with late peak-flow above 2,500 m. The invasive potential of RT and hybrids is further highlighted by successful colonization of small and intermittent streams at lower elevations.

Hybrids spawned in habitats not typically included in genetics assessments or widely regarded as important. An unnamed intermittent stream supported the second most hybridized population within the portion of our study area in YNP. Spawning in small and intermittent streams is considered an adaptive behavior in the native range of RT and can contribute as much as 50% of overall production in metapopulations (Erman and Hawthorne 1976). Hybrid populations also used large mainstem spawning habitats, which has been documented in multiple telemetry studies of YCT (Henderson et al. 2000; DeRito et al. 2010). Such locations are typically excluded from genetic assessments because they are either difficult to sample, or perceived to be of marginal importance (Boyer et al. 2008; Young et al. 2016). This, however, could stifle discovery and recognition of import hybrid sources and suggests that future genetic surveys should include these types of spawning habitats to fully understand invasion dynamics. Moreover, if native populations using these sites were inordinately vulnerable (or resistant) to hybridization, not including them in studies to identify associations between introgression and abiotic conditions could bias inferences.

The Relative Role of Abiotic and Biotic Factors to Explain Hybridization

Fluvial distance to a prominent RT source (Hidden Lake, and the Buffalo Fork Creek watershed) best explained the spatial pattern of hybridization. Distance from source perfectly predicted the occurrence of introgression at the threshold used to designate core conservation populations. All tested populations within 37 km of Hidden Lake were more than 1% RT ($n = 10$), regardless of abiotic conditions, and all tested

populations beyond this distance were less than 1% RT ($n = 19$). Furthermore, logistic and linear regressions performed substantially better when including distance-to-source, but inclusion of abiotic features added little explanatory power. Our work supports many prior studies that have emphasized source connectivity as a primary factor influencing the spatial distribution of hybridization (Gunnell et al. 2008; Muhlfeld et al. 2009; Bennett et al. 2010; Loxterman et al. 2014) and points to a stepping stone pattern of invasive hybridization (Boyer et al. 2008).

The spatial distribution of hybridization radiated from the confluence of Buffalo Fork Creek and Slough Creek in the lower watershed, but appeared to be limited by the presence of two semi-permeable movement barriers. We did not include these two features (the Lamar River canyon, and falls on Slough Creek) as model covariates because both are semi-passable during certain flow conditions (Chapter 2, Ertel et al. 2017), but we strongly suspect they have played an important role in preventing more widespread hybridization. It is probable that these barriers are only passable by fish under certain flow conditions (Chapter 2), such that dispersal past these points does not occur at continuously high rates. The high degree of RT admixture in Rose Creek (the first site upstream of Lamar Canyon) and Hornaday Creek (the first site upstream of Slough Falls) relative to other sites in the upper watershed support this interpretation. Following a stepping-stone model of invasion (Boyer et al. 2008), these two sites would be expected to receive the highest number of dispersing hybrids from the lower watershed (Chapter 3).

An important limitation of our study is that we used modeled datasets to characterize stream habitats, but several points suggest these will not fundamentally change our conclusions. The NorWeST dataset accurately reflected gradients in temperature across sites in our study ($r = 0.90$), and the VIC model of Wenger et al. (2010) accurately reflected stream size and runoff timing (Chapter 3). This indicates correlations between abiotic gradients and introgression should not be strongly influenced. However, the absolute values of temperature were consistently too cold, especially at low-elevation sites (Figure C.2). This raises an important question: how cold is Buffalo Fork Creek? If it was indeed far warmer than the predicted 8.0°C (site #6, 2,465 m) then it would be less surprising that it has been invaded by RT. Realistically, we cannot know the absolute temperature without empirical measurements, yet expect it to be somewhere near 8 or 9°C given the increasing accuracy of modeled data at higher elevations in our study area (Figure C.2) and, that nearby sites in the NorWeST database (~20 km) at similar elevations averaged 9.1°C. Because NorWeST temperature data are widely used in similar studies (Young et al. 2016; Muhlfeld et al. 2017; Al-Chokhachy et al. 2018) some comparisons to prior studies are useful.

Although hybridized populations occurred at lower elevations and higher stream temperatures, on average, than native populations, introgression was present across the full thermal and elevation gradient in the watershed. In general this supports the notion that introgression is more common under certain abiotic conditions (Young et al. 2016) and occurs along an elevation gradient (Weigel et al. 2003), but also that cold water and high elevations does not invariably shield native populations from invasion (Muhlfeld et

al. 2017). For example, hybridization was present at two sites predicted to be below 9°C, including the Upper Buffalo Fork Creek sample (pRT = 98.9%) and Elk Tongue Creek sample (pRT = 3.6%). As mentioned we cannot confirm the temperature at these locations, but relative to other studies using the same NorWeST dataset, this is exceptionally cold. Young et al. (2016) examined 558 sites and an area covering 150 times the watershed area of our study (250,000 km² vs. 1,730 km² in our study) and only 4% of sites ($n = 24$) were predicted to be as cold as Buffalo Fork Creek (site #6). Interestingly, ten of these coldest sites also contained evidence of introgression (10/24, 41%, max pRT = 30%, Young et al. 2016). From a management perspective, a lack of abiotic resistance to introgression underscores the importance of locating and eliminating RT sources that threaten native trout populations of high conservation value.

Management Implications

Our findings provide a path forward to conserve native YCT in the Lamar River watershed. It does not appear environmental gradients will shield native populations from expanding introgression—especially in the long-term (i.e., the next hundred years)—suggesting a need for management actions (Ertel et al. 2017). Given that Buffalo Fork Creek is the most prominent threat to expanding introgression, the NPS has begun plans for managing RT by means of a chemical treatment. Our study also identified populations in the upper watershed that are undergoing hybridization (Rose Creek, Hornaday Creek, Elk Tongue Creek) that could potentially be the next stepping-stone to facilitate expanding invasion (Boyer et al. 2008). These populations, now identified, can be targeted for actions to eliminate or reduce hybrid dispersal potential to core conservation

populations that persist in the upper Lamar River and Slough Creek watersheds. We are hopeful that YCT in the Lamar River watershed can be preserved well into the 21st century.

Conclusions

Recent studies have focused on evaluating spatiotemporal patterns of introgression across broad geographic areas, and provide insights that are robust to nuances and variation across watersheds (Young et al. 2016; Muhlfeld et al. 2017). Our study was narrowly focused, and thus we do not know how broadly applicable some of our results are (i.e., prevalence of intermittent stream spawning, ability of RT to persist in streams above 2,500 m). Regardless, the important nuances of the invasion scenario playing out in the Lamar River watershed, even if they are outliers to more general trends, are of critical importance to local conservation efforts. We agree that regional model results linking introgression thresholds with temperature are useful to guide prioritization efforts at broad spatial scales (Isaak et al. 2015; Young et al. 2016); yet, we affirm the importance of local genetic surveys. Just as “every stream is likely to be individual and thus not really very easily classifiable” (Hynes 1975), so too are the nuanced and context-dependent invasion scenarios that play out differently in native trout metapopulations across western North America. In the Lamar River watershed, RT have colonized a high-elevation and likely very cold habitat. However, because RT have invaded a historically fishless watershed does not mean they will inevitably invade all cold habitats elsewhere. Finally, many authors (us included) have interchangeably interpreted introgression studies focusing on WCT and YCT and other cutthroat trout

subspecies, yet it may be that these invasion scenarios are governed by different mechanisms. A synthetic review on this topic would be immensely useful, for both guiding conservation decisions and continued research priorities regarding this widespread invasion scenario.

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Tables and Figures

Table 3.1. Sampling information and results of genetic testing.

#	Stream	Sample size		YCT	RT	CTX (F1)	pHyb	%RT
		Telem	Capture					
1	Unnamed (Slough) ^a	66	6	7	3	62 (7)	0.90	57.1
2	Slough (lower)	18	0	7	0	11 (4)	0.61	32.3
3	Buffalo (lower) ^a	6	21	1	0	26 (2)	0.96	72.4
4	Buffalo (middle) ^a	0	20	0	15	5 (0)	1	99.6
5	Hidden Lake ^a	0	21	0	18	3 (0)	1	99.8
6	Buffalo (upper)	0	20	0	10	10 (0)	1	98.8
7	Hornaday	0	19	16	0	3 (0)	0.16	4.8
8	Elk Tongue	0	25	18	0	7 (0)	0.28	3.6
9	Abundance ^b	0	51	50	0	1 (0)	0.02	0.03
10	Slough (upper)	0	29	29	0	0 (0)	0	0
11	Lamar (lower)	5	0	1	0	4 (2)	0.80	42.2
12	Crystal	17	4	1	0	20 (0)	0.95	45.4
13	Rose	56	9	44	0	21 (0)	0.32	11.1
14	Chalcedony ^c	20	4	20	0	4 (0)	0	0
15	Soda Butte (lower)	1	0	1	0	0 (0)	0	0
16	Pebble (lower)	5	0	4	0	1 (0)	0.20	0.3
17	Pebble (upper)	0	25	25	0	0 (0)	0	0

Table 3.1 Continued

18	Soda Butte (upper) ^a	0	23	20	0	3 (0)	0.13	0.1
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	0.2
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: Telem, samples from a concurrent telemetry study (Chapter 2); Capture, samples collected by angling or electrofishing (i.e., not from Chapter 2); YCT, number of Yellowstone cutthroat trout; RT, number of rainbow trout; CTX (F1), number of hybrids followed by number of F1 hybrids; pHyb, the proportion of individuals with detectable rainbow trout admixture in the sample; %RT the percent rainbow trout admixture in the sample.

^aalso contained < 0.5% WCT admixture.

^ba 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 #3550) also revealed low amounts of RT admixture. This is interpreted as evidence of RT introgression.

^ccontained no evidence of RT admixture but four individuals had < 2% WCT admixture.

^dtwo 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.

Table 3.2. Abiotic conditions present in trout populations of the Lamar River watershed, and fluvial distance from Hidden Lake. Streams with bold names contained evidence of rainbow trout introgression.

#	Stream	Lat.	Long.	EL	T	MAF	CFM	D
1	Unnamed (Slough)	44.941	-110.312	1891	11.8	0.04	203.1	24
2	Slough (lower)	44.929	-110.344	1891	12.6	5.00	220.2	18
3	Buffalo (lower)	44.958	-110.309	1935	11.9	1.69	222.7	15
4	Buffalo (middle)	45.045	-110.297	2303	9.5	1.17	227.5	3
5	Hidden Lake	45.064	-110.275	2362	-	-	-	-
6	Buffalo (upper)	45.14	-110.251	2465	8.0	0.35	231.3	10
7	Hornaday	44.962	-110.225	2012	10.9	0.10	216.2	27
8	Elk Tongue	44.985	-110.200	2071	8.8	0.07	219.0	31
9	Abundance	45.093	-110.048	2205	10.1	0.26	213.2	57
10	Slough (upper)	45.130	-110.134	2269	10.6	1.01	225.7	54
11	Lamar (lower)	44.916	-110.324	1865	12.5	13.89	215.1	26
12	Crystal	44.911	-110.322	1913	11.7	0.01	190.2	27
13	Rose	44.899	-110.229	2029	11.8	0.05	206.4	37
14	Chalcedony	44.849	-110.202	2024	10.9	0.03	208.8	45
15	Soda Butte (lower)	44.894	-110.127	2032	11.7	2.34	217.4	49
16	Pebble (lower)	44.915	-110.113	2078	11.4	0.50	213.5	52

Table 3.2 Continued

17	Pebble (upper)	44.931	-110.113	2171	9.3	0.50	213.5	54
18	Soda Butte (upper)	44.980	-110.067	2144	11.1	1.61	218.3	61
19	Cache (lower)	44.827	-110.129	2086	10.7	1.78	214.3	51
20	South Cache	44.832	-110.049	2170	10.5	0.50	213.2	59
21	Cache CC3	44.875	-110.044	2366	9.8	0.31	216.6	62
22	Cache (upper)	45.140	-110.251	2409	9.4	0.79	215.9	67
23	Flint	44.786	-110.123	2209	8.1	0.07	214.3	56
24	Lamar (middle)	44.787	-110.116	2093	11.3	3.83	209.4	56
25	Calfee	44.782	-110.088	2278	8.6	0.13	202.8	59
26	Miller	44.753	-109.938	2205	10.1	0.75	209.2	75
27	Willow	44.708	-110.086	2212	9.1	0.08	204.7	67
28	Mist	44.622	-110.120	2427	8.6	0.23	207.3	82
29	Little Lamar	44.642	-109.958	2411	9.7	0.41	212.2	83
30	Lamar (upper)	44.694	-109.945	2340	9.9	0.71	215.3	83

Note: EL, elevation in meters; T, modeled mean August stream temperature in °C; MAF, mean annual flow in m³/s; CFM, center timing of flow mass in day of water year beginning on Oct 1; D source, fluvial distance from Hidden Lake outlet stream in kilometers; latitude and longitude are expressed in decimal degrees.

Table 3.3. Model selection results for logistic regression using abiotic and biotic variables to predict the occurrence of hybridization (rainbow trout admixture > 0.00), and a linear model to predict the proportion of hybrid individuals within a population.

Response	With Buffalo Fork Creek			Without Buffalo Fork Creek		
	Model	AIC _c	ΔAIC _c	Model	AIC _c	ΔAIC _c
Hyb: Logistic	D	29.12	0.00	D	29.05	0.00
	D + T	30.63	1.51	D + T	30.59	1.54
	D + CFM	31.58	2.46	D + CFM	31.53	2.48
	D + MAF	31.61	2.49	D + MAF	31.59	2.54
	D + T + CFM	33.33	4.21	D + T + CFM	33.35	4.3
pHyb: Linear	D + D ² + CFM	93.37	0.00	D + D ² + CFM	88.05	0.00
	D + D ² + CFM + T	95.57	2.20	D + D ²	89.68	1.62
	D + D ²	95.60	2.23	D + D ² + CFM + T	90.88	2.83
	D + D ² + T	96.65	3.28	D + D ² + T	91.47	3.42
	D + D ² + MAF	97.75	4.38	D + D ² + MAF	92.01	3.96

Note: Hyb, a dichotomous response variable indicating if a population was hybridized or not; pHyb, the proportion of individuals with RT admixture in a sample; D, distance to rainbow trout source; T, stream temperature; CFM, center timing of flow mass; MAF, mean annual flow with natural log transformation.

Table 3.4. Model coefficients for the top performing logistic and linear regression models to predict hybridization using abiotic and biotic variables.

Model	AUC	r^2	Variable	Estimate	SE
Logistic #1	0.85	-	Intercept	-4.755	1.913
			D	0.099	0.036
Logistic #2	0.88	-	Intercept	0.558	5.327
			D	0.088	0.035
			T	-0.460	0.463
Linear #1	-	0.88	Intercept	10.547	5.653
			D	-13.634	1.104
			D ²	5.755	1.092
			CFM	-0.058	0.026
Linear #2	-	0.88	Intercept	7.742	6.433
			D	-13.187	1.209
			D ²	6.008	1.129
			CFM	-0.053	0.027
			T	0.161	0.174

Note: D, distance to rainbow trout source; CFM, center timing of flow mass; AUC, area under the receiver operating curve; T, stream temperature.

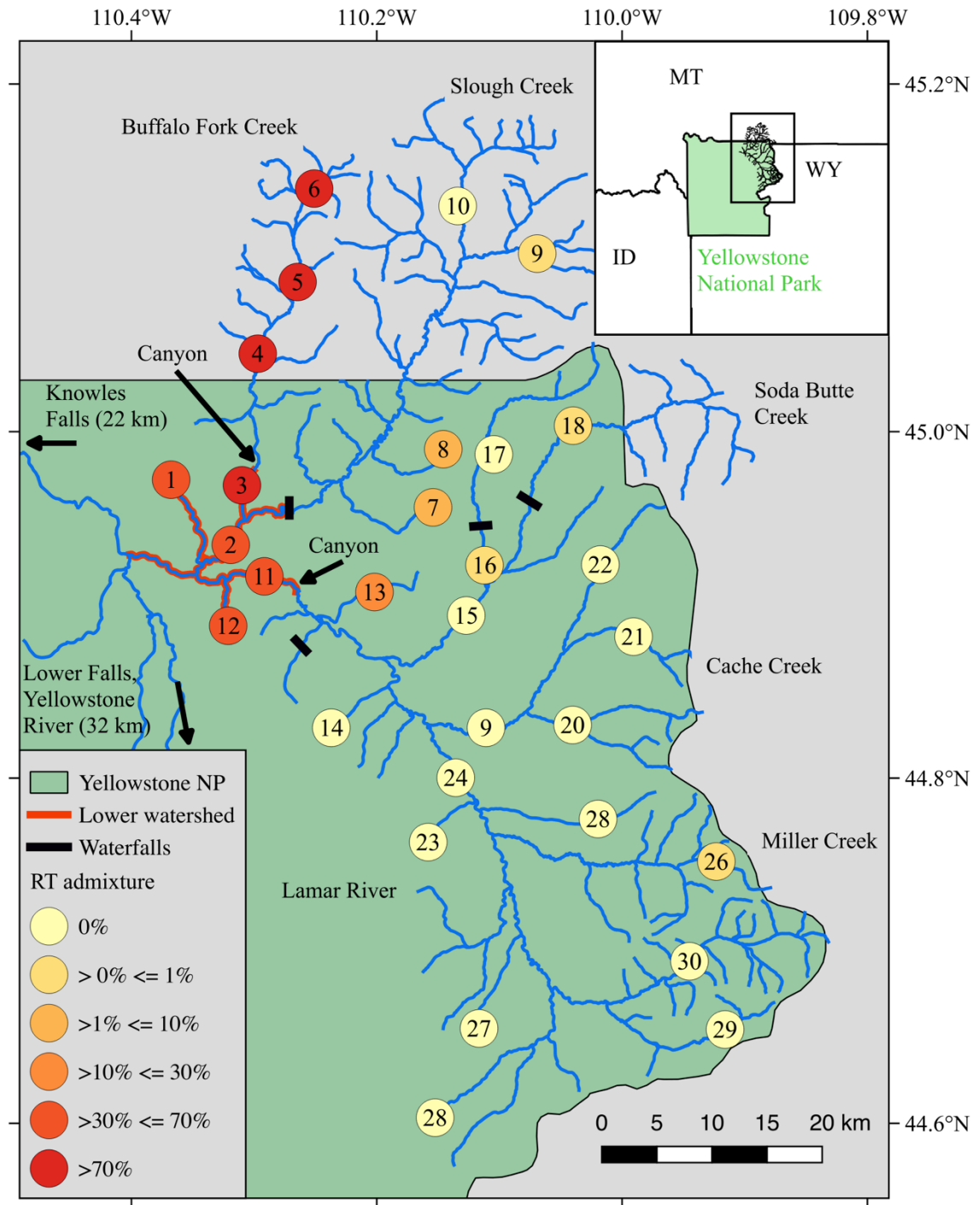


Figure 3.1. Map of the study area in Lamar River watershed in Yellowstone National Park with locations of populations genetically tested for rainbow trout admixture. The Lamar River flows into the Yellowstone River. Knowles Falls is 22 km downstream from this confluence; the Lower Falls of the Yellowstone River is 32 km upstream.

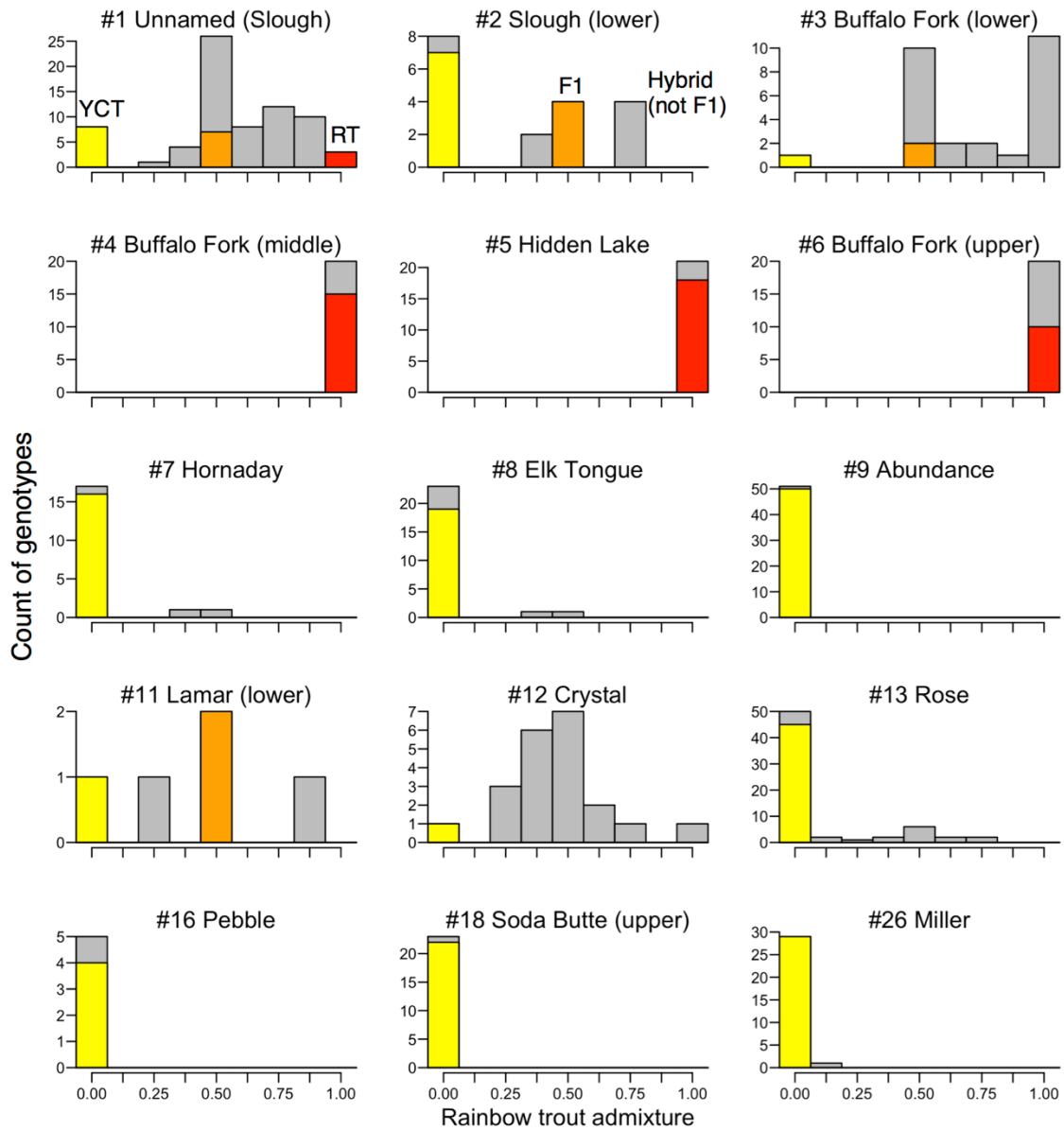


Figure 3.2. Distribution of individual rainbow trout admixture estimates within 15 populations where introgression was detected.

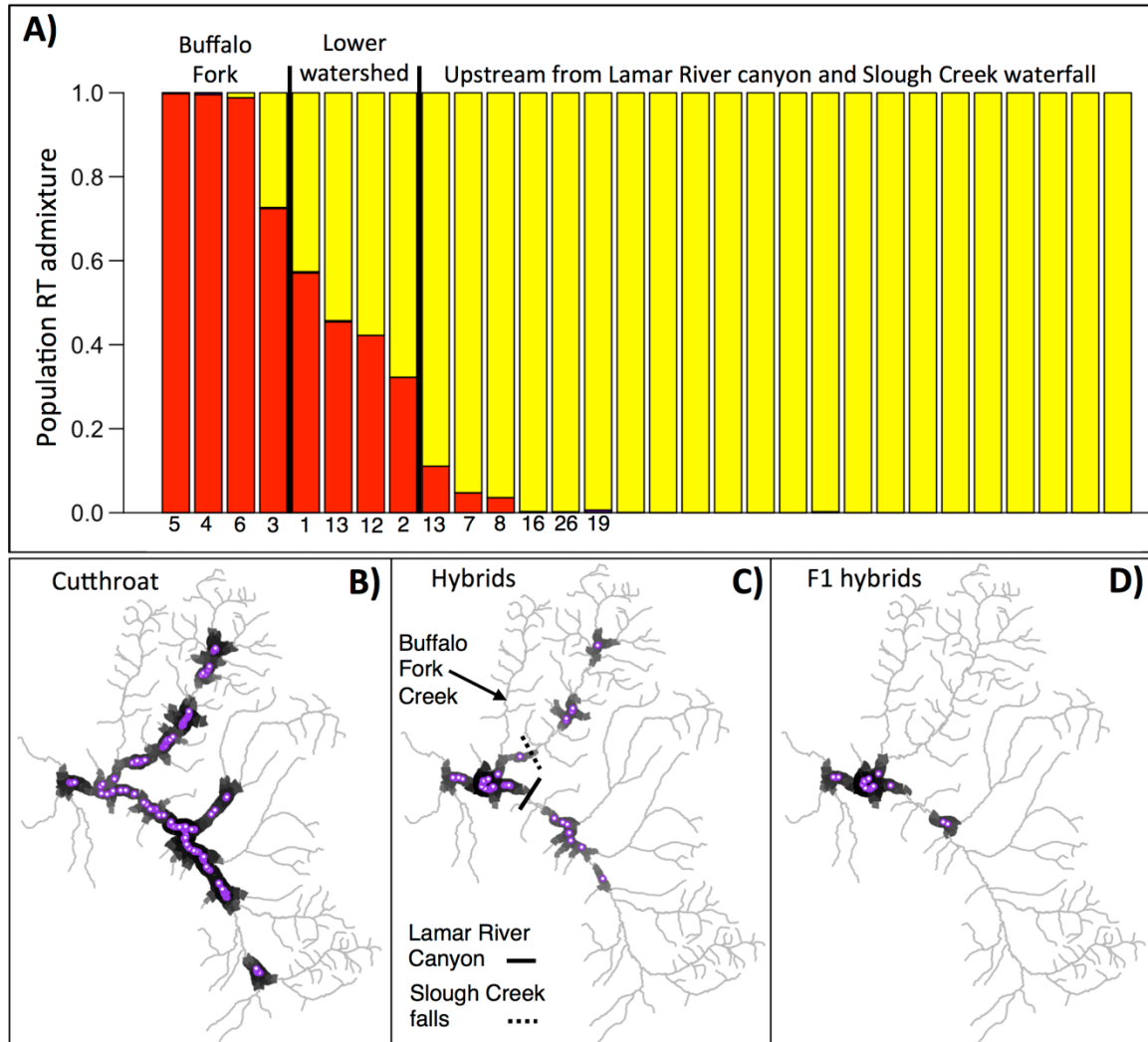


Figure 3.3. Population admixture estimates of 30 populations, and their relative location within the Lamar River watershed. Populations are sorted according to admixture estimates and labeled according to site codes (table 3.1). Three bottom panels display the distribution of Yellowstone cutthroat trout (B), hybrids (C), and F1 hybrids (D) sampled from mainstem river sites. Each panel (B—D) shows the result of a kernel density smoother (independently calculated for each panel) depicting the concentration of genotyped fish on the river (B, $n = 506$), hybrid trout (C, $n = 123$), and F1 hybrids (D, $n = 29$). High concentration areas are shown in darker greyscale and points from which kernels were estimated are shown (many are overlapping).

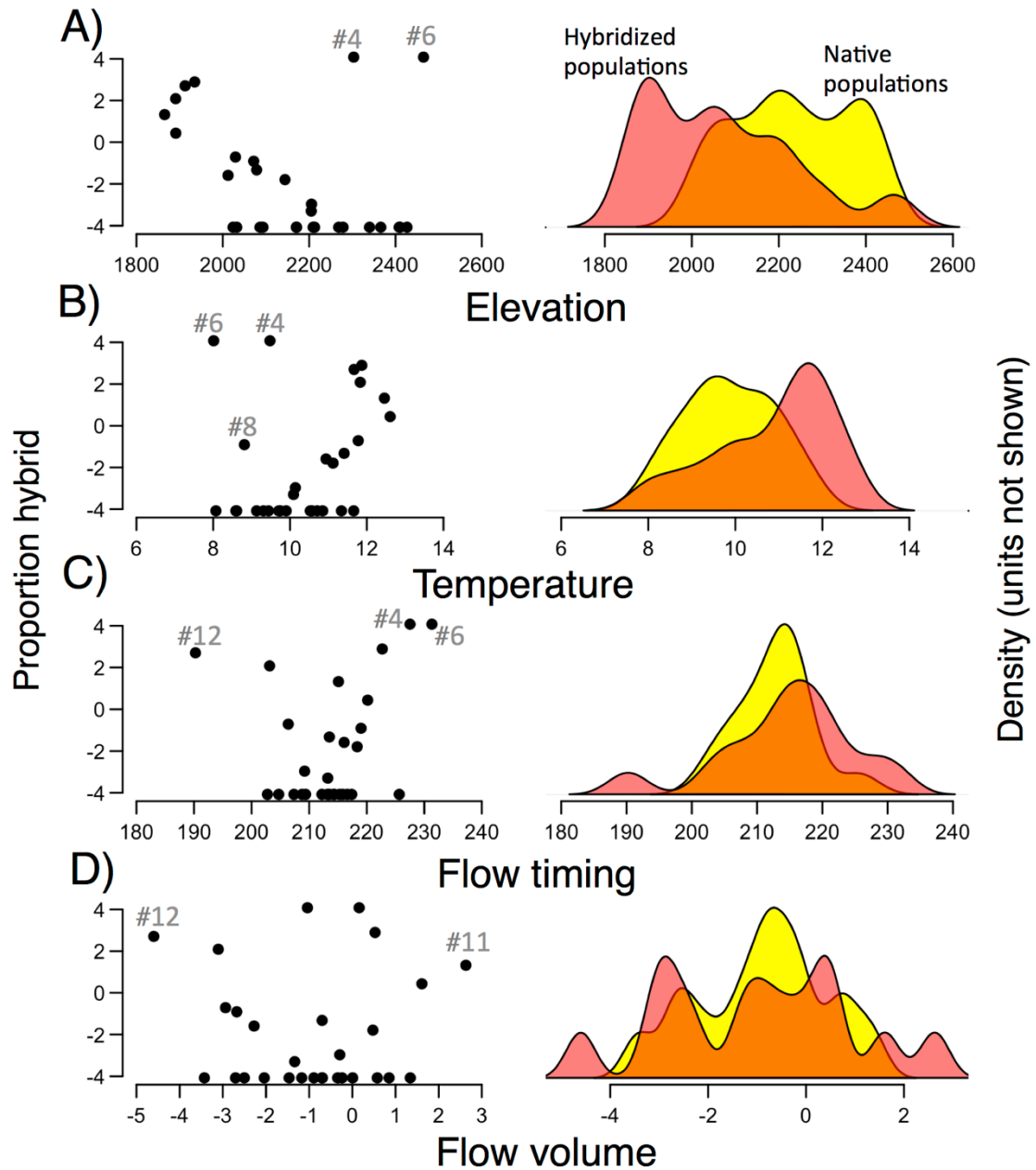


Figure 3.4. Relationship between the proportion of individuals within populations that had rainbow trout admixture (pRT) and environmental variables elevation (M), modeled stream temperature ($^{\circ}\text{C}$), modeled streamflow timing (center timing of flow mass, units are day of water year), and flow volume (mean annual flow, m^3/s). The left panel shows the relationship, the right panel shows the distribution of conditions in hybridized sites and native trout sites with density smoothers.

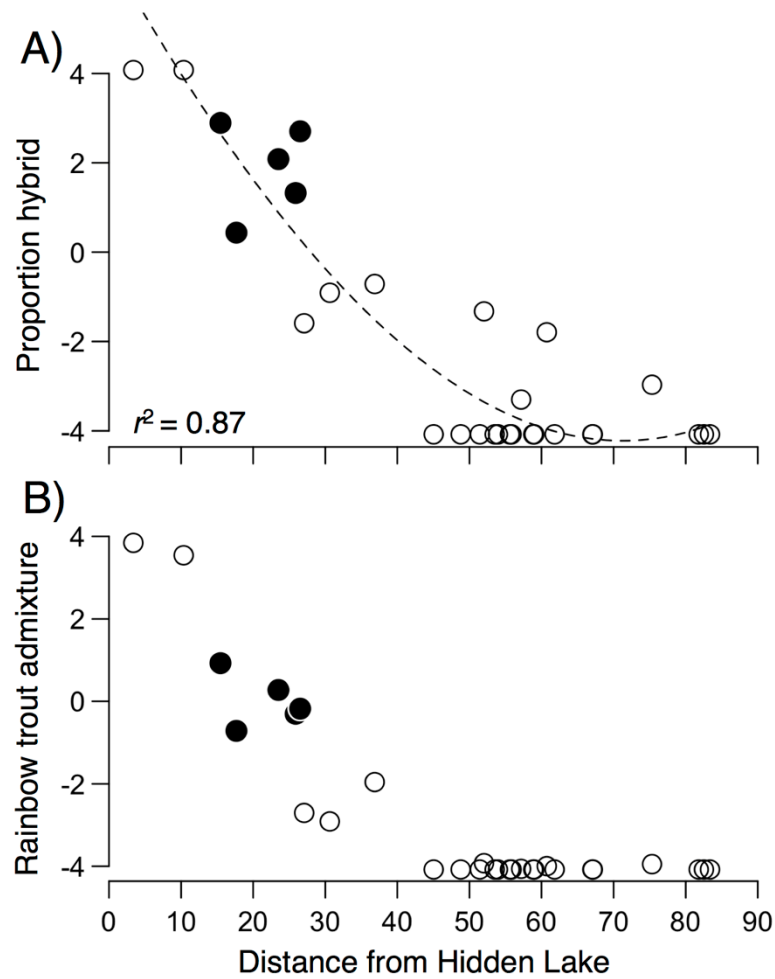


Figure 3.5. The relationship between distance from Hidden Lake and the proportion of hybrid individuals at a site (logit transformed)(A), and the proportion of rainbow trout admixture at a site (logit transformed) (B). Panel A shows the top performing linear model fit (table 3.2). Filled points are in the lower watershed, open points are in the upper watershed.

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CHAPTER FOUR

LEVERAGING PUBLIC HARVEST FOR GENETIC MANAGEMENT:
IDENTIFICATION AND EXPLOITATION OF INVASIVE HYBRID
TROUT IN YELLOWSTONE NATIONAL PARK

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

Author: Kurt C. Heim

Contributions: Designed study, developed research questions, collected the data, analyzed the data, wrote the manuscript

Co-Author: Thomas E. McMahon

Contributions: Helped with study design, discussed results, edited the manuscript

Co-Author: Brian D. Ertel

Contributions: Helped with study design, assisted with data collection, edited the manuscript

Co-Author: Todd M. Koel

Contributions: Helped with study design, obtained funding, edited the manuscript

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Kurt C. Heim, Thomas E. McMahon, Brian D. Ertel, Todd M. Koel

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Abstract

Leveraging public harvest can be a cost-effective invasive species management tool, but target taxa must be correctly identified and removed at rates that achieve biological objectives. We explored the potential role of recreational anglers to curtail expanding hybridization between invasive rainbow trout (*O. mykiss*; RT) and native Yellowstone cutthroat trout (*O. clarkii bouvieri*; YCT) in the Lamar River watershed in Yellowstone National Park. We sought to (1) develop a hybrid identification key and (2) estimate angler use, catch, and potential exploitation rates. Trout ($n = 251$) collected from 14 locations were genotyped to estimate RT admixture, assessed for seven morphological features, and an identification key was built with recursive partitioning. A single-choice dichotomous key (white pelvic fin tip present/absent) correctly classified 93% of fish as native (YCT) or non-native (RT or hybrid); success increased to 97% when a second criterion was added (head spot count ≥ 6). Using angler surveys (2013—2017), we estimated that 10,000 anglers catch 50,000 fish annually. In a popular road-accessible area near the upstream extent of hybridization, we estimated that most trout are caught and released ~ 4 times each year. The combination of high angler participation, substantial catch rates, and an accurate and easy to use identification method indicate that leveraging public harvest is a promising management strategy. Invasive hybridization is a global conservation problem, and genetic tools are increasingly used in conservation efforts. We demonstrate that collecting simple morphological data concurrently with genotypes can help determine useful criterion for hybrid identification to guide selective removal and genetic monitoring efforts.

Introduction

Early detection and eradication of new invaders is ideal, but when eradication is not an option, maintenance control is often used to control invasive species once they have become established (Mack et al. 2000). A variety of physical, chemical, and biological methods can be used to mitigate negative impacts to native taxa (Frazer et al. 2012; Simberloff 2014). To be effective, a sufficient number of invasive organisms must be removed, yet, costs of achieving such numbers can be substantial, required in perpetuity, and may deprive funding from other conservation opportunities (Pimentel et al. 2005; Simberloff 2014). Thus, careful consideration is required before implementing a maintenance removal program.

Hunting and fishing for invasive animals can be used to reduce populations at little cost to agencies while providing recreation and outreach opportunities for the public (Nuñez et al. 2012). For example, a successful campaign promoting spearfishing for invasive lionfish (*Pterois spp.*) reduced populations and predation on native fishes (Frazer et al. 2012). Lionfish are excellent table fare and can be marketed by fisherman, which has provided incentive for participation (Nuñez et al. 2012). Agency supported bounty programs are also used to bolster participation by rewarding participants monetarily for each invasive individual removed (Mack et al. 2000; Flinders et al. 2016). Such approaches, however, can have negative consequences. In Australia there is public interest and participation in invasive cane toad (*Rhinella marina*) removal, but distinguishing cane toads from native toads can be challenging and some native

individuals are inadvertently killed (Somaweera et al. 2010). Though public harvest can be a powerful tool, it will only be useful if target species can be identified accurately.

Field identification of non-native species can complicate maintenance control efforts, especially when invading species hybridize with native taxa further convoluting identification (Meyer et al. 2017a). If hybrids between introduced and native taxa are fertile, non-native genes can spread across the native species through introgressive hybridization (Allendorf et al. 2001). Such cases generate challenges for resource managers tasked with conserving the genetic integrity of native taxa. For example, hybrids may be difficult or impossible to identify in the field, necessitating costly and time-consuming molecular genetic methods to assess population status (Allendorf et al. 2001). Invasive hybridization is a conservation issue particularly common in the fishes because eggs are fertilized externally, and pre-zygotic isolating mechanisms are often weak between related taxa (Allendorf and Leary 1988).

One of the greatest threats to native cutthroat trout (*Oncorhynchus clarkii* spp.) is invasive hybridization with non-native rainbow trout (*O. mykiss*, RT). The widespread introduction of RT has contributed to the decline of all cutthroat trout subspecies in western North America (Allendorf and Leary 1988), and introgression often proceeds until native genomes are lost (Allendorf et al. 2001). For example, Yellowstone cutthroat trout (*O. c. bouvieri*, YCT) have been extirpated from 58% of its native range, and only 28% of remaining populations are non-hybridized (Gresswell 2011). These genetically unaltered populations are considered the highest conservation priority, and various approaches are being used to protect them.

Management actions include isolation of populations from hybrid sources using barriers, chemical removal of non-native source populations, and selective removal of hybrids and RT (Al-Chokhachy et al. 2014; Ertel et al. 2017). Before a population has become a hybrid swarm (i.e., all fish are hybrids), selective removal can be effectively used to curtail further loss of native genotypes (Al-Chokhachy et al. 2014; Kovach et al. 2018). This can be advantageous as compared to complete eradication with chemical treatments and re-stocking native trout, because the local adaptations of the native population can be conserved. However, a long history of failed attempts to control invasive salmonids has shown that capturing sufficient numbers of fish to effect a biological change requires substantial effort, particularly in large streams (Hansen et al. *in press*). Because YCT, RT, and their hybrids are highly regarded sport fish (Quist and Hubert 2004), leveraging public harvest could be a useful strategy to supplement removal efforts by management agencies (Flinders et al. 2016).

Selective removal will be effective only if hybrids can be easily and reliably distinguished from native taxa and removal rates are high enough to achieve biological or genetic objectives. We addressed these two uncertainties in the Lamar River watershed of Yellowstone National Park (YNP), which supports one of the few remaining metapopulations where large, migratory, and non-hybridized YCT are still present. The extensive fluvial connectivity of this system promotes important life-history diversity, yet, also makes it vulnerable to upstream invasion of RT and RT x YCT hybrids (CTX) which are now common in the lower watershed and appear to be increasing in frequency upstream (Ertel et al. 2017, Figure 4.1). This watershed is also a premier fly-fishing

destination, drawing anglers from around the world. Recognizing the potential role of recreational harvest in reducing the frequency of CTX and perhaps preventing invasion upstream, YNP implemented mandatory harvest regulations for RT (but not CTX) in 2013. Regulations specified for field-based RT identification (i.e., absence of characteristic cutthroat trout red lower jaw slash) however, likely resulted in the release of many CTX (Meyer et al. 2017a). A recent study in Idaho found that about 50% of non-hybridized RT have faint cutthroat slashes (Meyer et al. 2017a); this same study found that white pigmentation on fin tips is reliably absent from YCT (99.5%), but present in 80% and 57% of F1 and post-F1 hybrids, and 100% of RT. Therefore, in 2017 YNP updated regulations to include mandatory harvest of field identifiable CTX, suggesting the presence of a white fin tips as a useful feature to guide identification. Although the results of Meyer et al. (2017a) provide a useful guide for morphological identification in YNP, wide-ranging patterns in pigmentation are well documented in salmonids (Nicieza 1995; Seiler et al. 2009), substantiating the need for local validation of phenotypic criterion for identification. Therefore, the objectives of our study were to further test the reliability of field-based hybrid identification by (1) developing and testing a simple dichotomous key to identify hybrid trout using a paired genetic and morphological dataset collected from the Lamar River watershed; and examine the potential role of angler harvest in the Lamar River by (2) estimating angler use, catch, and exploitation rates.

Methods

Study Area

The Lamar River watershed encompasses an area of 1,731 km² and is mostly within YNP (Figure 4.1). Buffalo Fork Creek is considered the primary source from which RT disperse to interbreed with native YCT (Ertel et al. 2017, Chapter 3). Hybridization is prevalent in several spawning populations in the lower watershed and Rose Creek in the middle watershed (Figure 4.1, Chapter 3). Introgression has not been detected in many tributaries in upper Slough Creek and the Lamar River near their headwaters.

Fish Sampling and Morphological Assessment

Trout ($n = 251$) from 14 sites were assessed for morphology and genotyped to estimate individual proportion of RT admixture (Figure 4.1, Table 4.1). Fish were sampled from June to September in 2016, and from March to August in 2017 using angling ($n = 45$), backpack electroshocking ($n = 189$), and gillnets ($n = 17$). Although sampling was not conducted randomly across space, during a given sampling event fish were randomly selected for genetic and morphological assessments. Fish were anesthetized using AQUI-S (New Zealand Ltd., Lower Hutt, New Zealand), fork length was measured (mm, 72 – 480, mean = 209), a photograph was taken, and a tissue sample was obtained from the upper lobe of the caudal fin and preserved for genetic analysis. Fish were then transferred to a recovery bath where we visually assessed seven morphological features. We used the same features as Meyer et al. (2017a), but recorded

them as ordinal scale variables (Figure 4.2) rather than binary (i.e., presence/absence). These included white anal fin pigmentation (0 = absent, 1 = weak, 2 = strong); white pelvic fin pigmentation (0 – 2); pink ‘rainbow’ stripe along midline (0 – 2); orange cutthroat slash on lower jaw (0 – 3); relative density of spots below the lateral line (0 – 2); and ventral coloration (white = 0, white/orange = 1, orange = 2). We also counted the number of head spots (Figure 4.2). A laminated version of Figure 4.2 was carried in the field and used as a reference for the biologist handling the fish and ranking morphological features. Each fish was also identified as YCT, RT, or CTX in the field, based on the general criteria presented in Meyer et al. (2017a). Classification of morphological features (i.e., is this an orange cutthroat slash – 1, or slash – 2?) and fish identification in the field was generally discussed among multiple biologists present when not immediately straightforward to the biologist handling the fish. Because of this, we did not account for individual biologist handling the fish in our analysis. Photographs provided a means to validate any suspected errors in data recording.

We collected additional information on maxilla length from digital photographs. The same biologist analyzed all photographs, and assigned a value based on the maxilla length relative to the posterior end of the eye (0 = maxilla to eye, 1 = maxilla slightly past eye, 2 = maxilla well beyond eye). Maxilla length was also measured to the nearest mm using the program ImageJ (Abramoff et al. 2004), and expressed as a proportion of fork length for each fish.

Genetic Analysis

We used species-diagnostic single nucleotide polymorphism (SNP) loci to estimate the proportion of RT admixture (pRT) of individual fish. Genotyping was performed at the University of Montana Conservation Genetics Laboratory using methods and genotyping assays described elsewhere (Bingham et al. 2016). Loci used included SNPs diagnostic for westslope cutthroat trout (*O. c. lewisi*, WCT), YCT, and RT. The set of SNPs (WCT, YCT, and RT) together were used to estimate the genetic contribution of each of the three potentially hybridizing taxa to an individual's genotype (Kalinowski 2010). After screening and removing non-diagnostic loci (Appendix D), our final marker set included 17 RT, 17 WCT, and 19 YCT diagnostic markers.

Estimation of Angler Catch

We used volunteer angler report (VAR) cards and fishing permit sales (2013 – 2017) to quantify angler effort and catch. Cards are issued to all YNP fishing permit holders, and anglers are asked to report on their fishing experience from a single location only (i.e., one river, stream, or lake), and return the card to NPS. The NPS distinguishes several reaches on the Lamar River, Soda Butte Creek, and Slough Creek to organize and summarize responses (i.e., reaches shown in Figure 4.1); we use data from the five most commonly fished reaches in the Lamar River watershed. For each reach we report the average number of VAR cards returned and catch rates (i.e., number of fish reported caught per angler, by species) during the 2013—2017 fishing seasons.

To account for the population of anglers that fished, but did not return VAR cards, we used a simple extrapolation procedure to estimate (1) the total number of fisherman

that fished each reach annually and (2) estimate total annual catch per reach. The total number of fishing permits sold annually in YNP provides a good estimate of the number of anglers fishing (in all of YNP), and relative rate at which VAR cards are returned for specific locations provides a rough estimate of how angler effort is distributed across locations in YNP. A demonstration is most useful to convey our approach; in 2017 a total of 46,144 permits were issued; 81 anglers returned VAR cards reporting on fishing in Soda Butte Creek, out of an overall 1,510 anglers that returned VAR cards. We therefore estimate that in 2017, about 5.3% of fisherman visiting YNP fished Soda Butte Creek (81 VAR cards for Soda Butte Creek/1,510 VAR cards total) so about 2,307 anglers (5.3% of 46,144 permits sold) is our estimate. Then, we reduced the aforementioned catch rates (fish/angler by species for each reach) by 50% to account for angler overestimates of fish caught, and over-representation of successful anglers in survey responses (Carline 1972; Sullivan 2003), and multiplied this by the estimate of anglers fishing for each reach. This was done for all reaches from 2013 – 2017.

To reduce populations via mechanical removal, it is the exploitation rate (i.e., fraction of the population removed) that is relevant rather than absolute numbers; therefore, we calculated exploitation rate for one reach where a mark-recapture population estimate was possible. A two-pass mark recapture population estimate was conducted in July 2016 using raft electroshocking over the approximately 7-km Lamar River (middle) reach. Length-frequency distributions of fish caught by raft electroshocking were similar to the distribution vulnerable to angling (Figure D.1), therefore, this population estimate should adequately represent the number of fish

vulnerable to angling. Population estimates were made with the Chapman modification of the Peterson formula (Chapman 1951) and 95% confidence intervals constructed with a Poisson distribution in the FSA package in R (Ogle 2017; R Core Team 2017). Potential exploitation rate was calculated by dividing the VAR estimated number of fish caught per year (2013—2017 average) by the population estimate.

Statistical Analysis

We used recursive partitioning ('rpart' routine in program R; Therneau and Atkinson 2018) to determine which of the seven morphological features best distinguished native YCT ($pRT = 0$) from fish with RT admixture ($pRT > 0$, including non-hybridized RT). This method uses independent quantitative or categorical variables to split the dataset into categories and maximize classification success of a single response variable independently and repeatedly until a specified stopping point is reached. We specified for the algorithm to stop once minimum group size of 10 was reached. Anal fin coloration was almost perfectly collinear with pelvic fin coloration, so was not included in the recursive partitioning analysis. Since our focus was to develop a key specifically for use in the Lamar River watershed, we excluded fish from the Yellowstone River and Ditch Creek for a total of 227 fish used in this analysis. To avoid over fitting, the cost complexity value was set to 0.001 and the tree was pruned to minimize the 10-fold cross-validated error.

Results

We collected paired morphological and genetic information for 153 YCT, 55 CTX, and 43 RT (Table 4.1, Figure 4.3). Individual RT admixture (pRT) estimates of CTX ranged from 0.07 to 0.93, indicative of multiple generations of backcrossing, but also continued contact between parental taxa (Figure 4.3). Individuals with low pRT (i.e., slightly hybridized YCT) were relatively scarce; only two hybrids had $pRT < 0.25$ and only 6 had pRT values < 0.40 (Figure 4.3). We detected WCT admixture in only 4 of 251 individuals (2%), WCT admixture proportion was always less than 0.06, and all these individuals also had $pRT > 0.40$. We therefore assumed WCT admixture negligibly influenced observed phenotypic variation. Parental RT ($pRT = 1.00$) were found in only 3 of 14 sites (21%), and were concentrated in the Buffalo Fork Creek Drainage (Figure 4.1). Hybridized fish were widely distributed (6/14 sites, 43%) but far more common in the lower watershed ($n = 51$) relative to sites in the upper watershed ($n = 4$). In contrast, parental YCT were found in nearly all sampled sites (86%, 12/14 sites).

Field Based Classification Success

Assuming 100% genetic classification accuracy, we correctly identified fish in the field as YCT, CTX, or RT 96% of the time (241/251 correct classifications) by following the guidelines presented in Meyer et al. (2017a). Classifying CTX as RT was the most common misclassification (4/10), which all involved CTX with $pRT > 0.80$. Three CTX ($pRT = 0.07, 0.25, \text{ and } 0.46$) were misclassified as YCT, and three YCT as CTX. Under the current management policy of harvesting RT and CTX, we would have released three

of the 98 (3%) CTX and RT that were handled. Only three of the 153 YCT (2%) handled would have been mistakenly killed. These misclassified YCT were all from Ditch Creek (outside of the Lamar River watershed), where YCT had an unusually high number of head spots (see below).

Useful Morphological Features to Identify Hybrids

Based on simple summary statistics and visualization of the dataset (Figure 4.3, 4.4), it was clear that the presence of white anal and pelvic fin tips was the most consistent feature to distinguish hybridized from non-hybridized fish. One hundred percent of YCT lacked white fin tips on the pelvic fin, in contrast, 84% of fish with $pRT > 0$ had at least faint white pigmentation. The results for anal fin coloration are similar, except one YCT had a faint white fin tip (seen as lone red bar amongst yellow bars in Figure 4.4). The presence of white fin tips could not be confirmed in the photograph of this fish (fish id = 7594) suggesting this was a data recording error. Individual fish nearly always received the same classification score for anal and pelvic fins (240/251, 96%) indicating consistent pigmentation patterns across fins at the individual level.

The presence of a pink coloration along the midline and head spot counts also differed among taxa (Figure 2.2, 2.4), but were not as consistent as white-fin tips to distinguish taxa. Whereas most RT and CTX had at least a faint pink midline stripe, use of this feature alone would preclude recognition of 39% of CTX and 25% of RT. Head spots were useful since YCT had very few (Figure 2.3), but fish from Ditch Creek represented a noteworthy outlier. Here, YCT had an unusually high number of head spots (mean = 11, range = 0 – 32, $sd = 9$), whereas all other YCT included in the dataset had a

mean of 0.49 head spots (range = 0 –12, $sd = 1.5$). Since fish in Ditch Creek presented no other features indicative of hybridization except head spots, we somewhat conservatively classified them in the field as CTX only if they had an extreme head spot count. Had we used a 5 head spot rule (Meyer et al. 2017a), 16 (compared to 3, as described above) YCT would have been misclassified as CTX.

Maxilla length, ventral coloration, ventral spot density, and lower jaw pigmentation were not particularly useful at distinguishing taxa (Figure 2.4).

Classification Tree Built with Recursive Partitioning

The classification trees built with recursive partitioning included only two nodes and very accurately classified fish (Figure 4.4). The first dichotomous choice was whether a fish had any white pigmentation on the pelvic fin (or not) and yielded 93% classification success (i.e., this feature alone correctly classified 212/227 fish). In a culling scenario following this single choice key, 15 fish with non-native alleles would be released but no YCT would be killed. The second choice was whether or not the fish had 6 or more head spots, and increased classification success to 97%. In a culling scenario following this key, three YCT would be killed and four fish with non-native alleles released into the environment (Figure 2.4).

Variation in Morphology with Size and Admixture

An analysis of morphological variation compared to pRT and fork length of YCT is provided in the supplementary materials (Appendix D). In sum, as pRT increased, individual morphology became more similar to parental RT (Figure D.2). We also found

evidence for ontogenetic changes in YCT morphology; as fish size increased, characteristics of YCT typically became more pronounced (Figure D.3).

Angler Effort, Catch, and Potential Exploitation Rate

Fishing effort and angler participation in the Lamar River watershed is substantial; we estimated an average of 10,514 anglers catch 53,076 trout each year. In the five-year period between 2013 and 2017, an average of 44,008 (range = 42,259 – 46,144, $sd = 1,559$) fishing permits were issued annually in YNP. An annual average of 1,621 anglers (range = 1,397 – 1,922, $sd = 214$) returned their VAR cards; of these, an average of 384 (range = 286 – 443, $sd = 57$) reported fishing in the Lamar River watershed. The average number of reported total fish caught in the watershed from VAR cards was 3,894 (3,716 YCT, 17 CTX, and 161 RT), with an average catch rate of 10.09 fish per angler.

Angler use varied considerably among the five reaches; lower Soda Butte Creek consistently receiving the most fishing pressure (2,258 estimated anglers) and anglers reported the highest catch rates (13.55 fish/angler) of predominantly YCT (Table 4.2). Reported numbers of CTX and RT (combined in Table 4.2) caught were highest in lower Slough Creek and Lamar River (lower) (Table 4.2), yet YCT captured still outnumbered non-native fish in all reaches. Overall, in these five fishing locations combined, 130 RT and CTX are reported caught annually, and we extrapolate this to be 869 annually for the angling population.

Our estimate of potential exploitation rate was higher than 1.00 (i.e., more fish are caught, than are present) in the middle Lamar River. The mark-recapture population

estimate for the middle Lamar River was 1,882 (95% confidence interval 1,010 to 3,805). A conservative estimate of exploitation rate (if all fish were harvested) is made by comparing reported fish caught to estimated population size. In this scenario, 32% (601/1,883) of fish in the middle Lamar River are captured annually (but recall that this only reflects the catch of about 4% of visiting anglers). Comparing estimated population size ($n = 1,882$) to the extrapolated angler catch ($n = 8,006$) suggests that the number of fish caught is 4.25 times greater than the number of catchable sized trout. In other words, we expect each fish is caught about 4.25 times each fishing season. Applying this to the lower and upper confidence intervals for our population estimate yields exploitation rates of 2.10 to 7.92.

Discussion

Our study shows that most hybrid trout in Lamar River watershed can be reliably identified by morphology and that anglers could likely harvest a substantial number of them. A mandatory harvest regulation has great potential to curtail the spatial expansion of introgression, and reduce population RT admixture. Tens of thousands of individuals of various species are genotyped annually to estimate non-native admixture for conservation purposes. We demonstrate how collecting morphological data concurrently with genotypes can be used to assess the reliability of field-based hybrid identification and standardize long-term monitoring efforts. Genetic analysis remains essential to detect low levels of non-native admixture, yet morphological assessments may provide an inexpensive and user-friendly alternative for some conservation purposes.

Hybrid Identification and Benefits of a Standardized Key

A lack of individuals with low amounts of RT admixture (i.e., ≤ 0.40) benefitted classification success. We had high power to detect pRT levels below 0.05 (~95% probability of detection) so a scarcity of hybrids with RT admixture less than 0.40 suggests that either (1) they are present but we failed to sample them or (2) they are rare in the watershed. Sampling bias is unlikely since we did not selectively choose individuals to include, and this general pattern is present in a more extensive genetic dataset (Chapter 3). Irregular genotypic hybrid distributions of YCT x RT crosses are common (Henderson et al. 2000; Bingham et al. 2016) suggesting that F1s may be more likely to backcross with parental RT (compared to parental YCT), or, F1 x YCT crosses suffer differential fitness consequences. While our classification success benefited from a lack of fish with low admixture—that are harder to identify by morphology (Meyer et al. 2017a)—the fact that fish with such genotypes are rare indicates high classification success should be expected in this watershed.

Our study supports previous findings that hybrids and RT are readily distinguishable from YCT in the field, but adds to previous studies by demonstrating the utility of a standardized key for distinguishing hybrids. A simple two-choice key provided a 97% success rate—just as good as our field-based decisions that took into account a suite of features. Other studies report similarly high success rates of 94%, 94%, 87%, and 91% (Henderson et al. 2000; Campbell et al. 2002; DeRito et al. 2010; Meyer et al. 2017a), when identification is made based on trained biologist opinion. However, we are not aware of other studies that have developed classification trees to standardize

field-based hybrid identification, which we believe has several practical benefits. First, the frequency of hybrids captured in surveys is often used as a trend indicator for monitoring (Ertel et al. 2017; Kovach et al. 2018) but could be biased by differences in the criteria that biologists use to distinguish hybrids. This would be especially true over long time spans where multiple biologists and technicians are involved in data collection. Rather than recording fish as “YCT” or “Hybrid” in the field, an alternative is to record fish species category based on selected morphological features (i.e., as 1 - “Hybrid – white tips”; 2 - “Hybrid – head spots” [no white tips but ≥ 6 head spots]; or 3 - “Hybrid – suspected” [other features suggest introgression]). This adds only slight complexity to data recording in the field, but allows for more meaningful analysis of long-term trends robust to staffing differences across time. Secondly, key development provides a scientific basis for which criteria to use for angler regulations, with an expected rate of error. Using a gestalt of other features that vary along a continuum (e.g., cutthroat slash coloration, spot distribution, belly coloration) may still be useful in agency culling programs involving trained biologists. Yet, their application in a fieldwork setting will be imperfect, and such complex classification methods are unlikely to be useful for public harvest criteria that must be clear-cut.

We conclude that the most reliable and consistent feature to identify YCT hybrids is the presence of white pigmentation on the pelvic or anal fin. This supports the suggestion by Meyer et al. (2017a) that this result is applicable across the range of YCT. Head spot counts are perhaps the second most reliable feature, but we do not advocate that a strict head spot count of 5 (Meyer et al. 2017a) or 6 (our study) be used without

prior testing in populations of interest. Whereas Meyer et al. (2017) attributed high head spot counts of some YCT as genotyping errors, we had high power to detect low amounts of RT admixture and found a single population (Ditch Creek) that systematically expressed high head spot phenotypes. We interpret this as evidence of inter-population variation in YCT morphology, which highlights the usefulness of locally validating phenotype x genotype relationships.

Angler Catch in the Lamar River Watershed

Estimates of annual catch are likely influenced by unit non-response bias and should be interpreted with some caution (Fisher 1996). In extrapolating VAR responses to the population of anglers, we took several conservative steps including reducing reported catch rates by 50% to account for potential exaggerated catch rates (Carline 1972; Fisher 1996; Sullivan 2003). The greatest uncertainty, however, comes from the assumption that VAR cards are returned for specific locations at the same rate that those areas are fished. Anglers fishing for longer periods of time with more fishing experience are probably (1) more likely to target certain waterbodies than novice anglers fishing elsewhere, and (2) return surveys because of a deeper interest in the fishery (Fisher 1996). We therefore do not expect that survey response rate will perfectly predict allocation of fishing effort; however, several points suggest the general conclusions drawn from our analysis are robust to such uncertainties.

A focused study in the Yellowstone River estimated trout were captured 9.7 times annually (Schill et al. 1986), similar to our high estimated potential exploitation rate of 4.25 (i.e., fish are probably caught 4 times each) in the middle Lamar River. In high

participation recreational fisheries, capture rates such as this are not uncommon (Kozfkay and Dillon 2010). Indeed, on multiple occasions we marked fish captured by angling during a concurrent fish movement study (Chapter 2), and subsequently recaptured the same fish within hours. Unlike other public harvest programs, where incentive to participate is lacking or must be encouraged by a bounty, there is no lack of interest in recreational fishing in YNP and participation is high. Collectively, our results and relevant information strongly support the notion that most fish in road accessible portions of the Lamar River watershed are captured multiple times during each fishing season.

Leveraging Public Harvest—Will it Work?

Whether public harvest can have the desired conservation outcomes depends on 1) anglers ability to distinguish hybrids; 2) anglers willingness to follow regulations; and 3) whether the resulting exploitation rates will help to reduce hybridization. A white-fin tip criterion for harvest is most promising to use as a single criterion for angler harvest, and should be rather straightforward for anglers to use in the field. Although some hybrids lack white fin tips and would evade harvest, all YCT evaluated in our study lacked white fin tips and would be required to be released. This would minimize unintentional harvest of native species that has been problematic in other public harvest scenarios (Schmetterling and Long 1999; Somaweera et al. 2010). Additional studies would be useful to follow up on our study to more directly test the abilities of the public to distinguish the presence of white fin tips—which can in some cases, be quite faint. We expect a more realistic issue, however, is a lack of harvest by an angler population deeply committed to catch and release.

Catch and release fishing is an important tool in fisheries management and many anglers have a deep personal commitment to releasing fish unharmed (Lewin et al. 2006). This may be motivated by myriad factors including ethics, conservation, or maintaining sustainable angling opportunities. In fact, many anglers prefer fishing for non-native trout compared to cutthroat trout (Quist and Hubert 2004), and thus we do not expect full compliance with a mandatory harvest regulation. Compliance will depend on whether or not anglers understand the purpose and motivation for the regulation—and agree that preventing invasive hybridization is a cause worth fighting for (Simberloff 2014). An important part of this process is clear public communication, which can be done through seminars, social media, web-material, and stakeholder meetings. To this end, crafting clear and positive communication strategies (‘save Lamar cutthroat’) rather than negative messages (‘kill the rainbows’) will be essential (Crowley et al. 2017). Finally, we hope that the results of this study can help anglers to recognize their potential role in active fisheries management. A sentiment we encounter is “what difference can a single angler make?”, a perspective that overlooks the cumulative impact of thousands of anglers on a fishery (Lewin et al. 2006).

A final, yet essential consideration is whether or not the expected rate of public harvest will address conservation objectives of preventing the upstream spread of hybridization and reducing non-native population admixture. Hybridization spreads as hybrids from source populations disperse, often upstream, to colonize native populations (Allendorf et al. 2001). In the Lamar River watershed, the highest angling pressure and potential exploitation rates are at the upstream front of the invasion (upper Slough Creek,

middle Lamar River, and lower Soda Butte Creek, Chapter 3). Our results suggest that if hybrids disperse to this area, they are very likely to be captured by anglers (and hopefully harvested). In a sense, this spatially concentrated exploitation could serve a similar function as physical barriers do in preventing upstream invasion, yet without negative consequences to native YCT (Novinger and Rahel 2003).

Rainbow trout admixture can be reduced by focused selective removal effort of just 1—3 passes with electrofishing equipment annually in streams of similar size to the Lamar River (Al-Chokhachy et al. 2014; Meyer et al. 2017b; Kovach et al. 2018). In 2013, NPS began conducting annual two pass removal efforts on upper Slough Creek and the middle Lamar River, yet it is probable that anglers could sample far more trout (over a 160 day fishing season) than NPS biologists during several days of targeted electrofishing. For example, from 2012 – 2014, biologists removed 36 RT and CTX from upper Slough Creek (Koel et al. 2015), while we expect that during this time anglers caught about 150. Similarly, from 2013 to 2014, biologists removed 21 RT and CTX from the middle Lamar River (Ertel et al. 2017), whereas we estimated anglers caught about 400. Continued monitoring of both the spatial extent and local degree hybridization will be required to evaluate the efficacy of these efforts (Chapter 5), but in summary this study supports the continued effort to use public harvest as part of a multifaceted selective removal program.

Conclusions

Invasive hybridization is a problem expected to increase in a warming climate and managers are increasingly confronted with difficult decisions about how to best conserve

remaining species strongholds (Muhlfeld et al. 2014). Isolating populations above barriers from connected sources of RT is the most reliable way to prevent hybridization. Yet, physical isolation of conservation populations limits life-history diversity, leads to loss of genetic diversity, and subjects populations to demographic problems associated with small population size (Fagan and Holmes 2006). Balancing the threat of invasion, with the problems associated with isolation will remain a challenge for managers of native trout populations in the foreseeable future. Our study indicates that morphological features can reliably be used to distinguish hybrids from YCT with low error rates, and that selective removal may play a key role in conservation within the Lamar River watershed. In addition to directed agency efforts by the NPS, engaging the public to attain genetic management goals appears to be a worthwhile, and potentially very effective, management strategy.

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Tables and Figures

Table 4.1. Number Yellowstone cutthroat trout (YCT), rainbow trout (RT), and YCT x RT hybrids (CTX) used for genotype-phenotype comparison. Ditch Creek (our name for an unnamed Yellowstone River tributary) and the Yellowstone River are outside of the Lamar River drainage; all other locations are within the Lamar River drainage.

Collection site	YCT	CTX	RT
1. Ditch Cr.	22	1	0
2. Yellowstone R.	1	0	0
3. Hidden Cr.	2	33	2
4. Buffalo Fork Cr.	0	0	20
5. Hidden Lake	0	0	21
6. Slough Cr.	0	4	0
7. Crystal Cr.	1	13	0
8. Lamar R. (middle)	1	0	0
9. Rose Cr.	1	3	0
10. Chalcedony Cr.	2	0	0
11. Calfee Cr.	25	0	0
12. Miller Cr.	23	1	0
13. Mist Cr.	30	0	0
14. Lamar R. (upper)	15	0	0
15. Little Lamar R.	30	0	0
Totals	153	55	43

Table 4.2. Volunteer angler report (VAR) summary from locations outside of the Lamar River (between Knowles Falls and Lower Falls of the Yellowstone River), and two locations within the Lamar River watershed (Slough Creek lower, Lamar River below Canyon) for relative comparison. Data include 3,156 VAR responses collected from 2001 to 2017. The VAR stream codes are shown exactly as they appear in the database provided by NPS; abbreviations are as follows: n, number of VAR cards received from 2001 to 2017 reporting on each waterbody; n Non, the number of rainbow trout and hybrids reported caught; n YCT, the number of Yellowstone cutthroat trout reported caught; ratio, the ratio of rainbow trout and hybrids to the total reported; CPUE, catch per unit effort

Reach	Anglers _r	Anglers _{ex}	YCT/angler _r	CTX/angler _r	YCT _r	RT + CTX _r	YCT _{ex}	RT + CTX _{ex}
Lamar R. (L)	34(10)	914 (248)	6.99(1.91)	1(0.46)	243(110)	31(9)	3,287 (1,443)	213(58)
Lamar R. (M)	59(16)	1,605 (446)	9.9 (2.96)	0.6(0.7)	568(169)	33(41)	7,803 (2,342)	203(218)
Slough Cr. (L)	43(12)	1,162 (311)	5.01(1.54)	1.38(1)	202(46)	50(17)	2,732 (409)	348(144)
Slough Cr. (U)	41(12)	1,117 (285)	12.41(3.26)	0.19(0.17)	522(244)	8(7)	6,969 (2,825)	54(48)
Soda Butte Cr.	82(13)	2,258 (495)	13.55(2.07)	0.09(0.04)	1,119(314)	8(4)	1,5324 (4,648)	51(22)

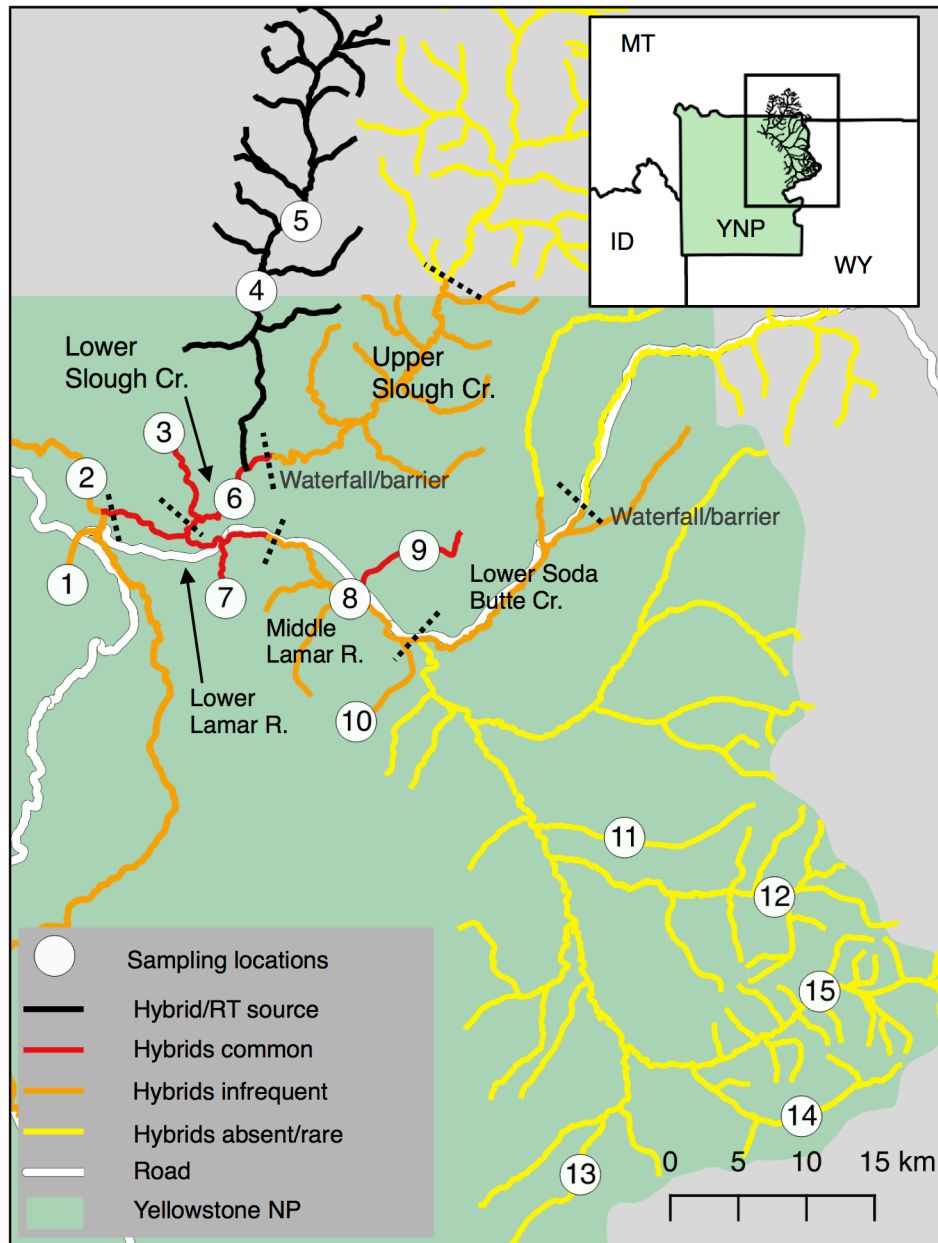


Figure 4.1 The Lamar River watershed in Yellowstone National Park, depicting the general frequency of non-native and hybrid trout, and reaches designated for estimating angler catch (named with black font, separated by dotted lines), and sampling sites where fish were collected for genotype – phenotype comparisons. Sample numbers correspond with table 4.1.

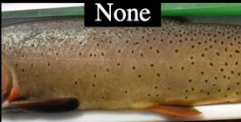
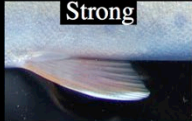
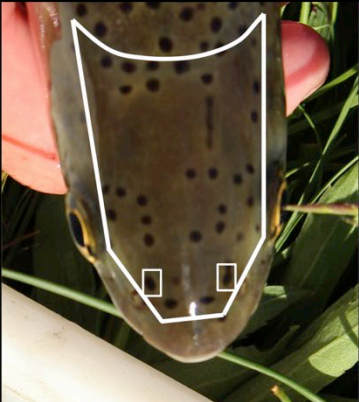
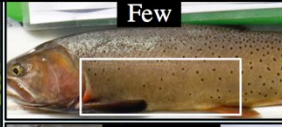
A) Tip white				B) Rainbow stripe				C) Belly color			
None	YCT	CTX	RT	None	YCT	CTX	RT	White	YCT	CTX	RT
	100	28	0		98	39	25		27	54	93
Weak				Faint				Intermediate			
	0	28	7		2	44	63		60	28	7
Strong				Strong				Orange			
	0	44	93		0	17	12		13	19	0
D) Slash				E) Number head spots*				F) Belly spots			
None	YCT	CTX	RT					Few	YCT	CTX	RT
	0	9	84						64	44	40
Faint								Moderate			
	9	48	16						35	33	46
Moderate								Many			
	41	27	0						1	23	14
Strong				*Area were head spot counts were made, spots adjacent to nares not counted							
	50	15	0								

Figure 4.2 Morphological features recorded from trout in the field. These exact photographs were included in a laminated reference guide and carried in the field to facilitate consistent attribution. Shown next to each series of photographs (A – E) is the percent of genotyped individuals of a given taxa (YCT, CTX, RT) that received each phenotypic score. Column totals indicate the percentage of fish in each taxa showing the particular trait. Panel F shows the region from which head spot counts were made, and the two spots typically associated with nares that were not included.

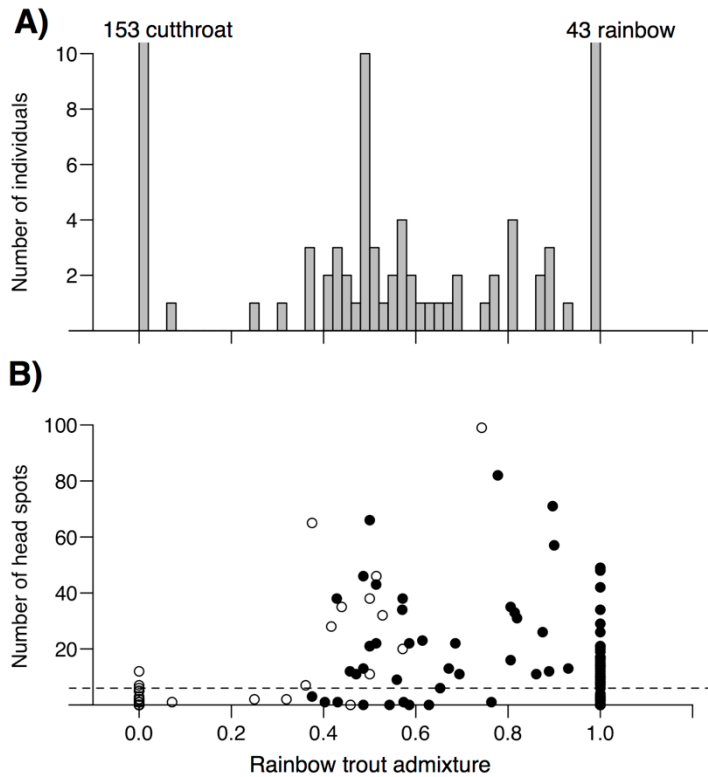


Figure 4.3. Distribution of rainbow trout admixture (pRT) of individual trout used for genotype-phenotype comparison (A), and the relationship between pRT, head spot count, and pelvic fin coloration (panel B). Fish with either white tips present are shown as closed circles, and open circles show fish with white tips absent from pelvic fins. Fish with white fin tips or ≥ 6 head spots (points above dashed line) would be classified as hybrids based on our final key. Fish from Ditch Creek are excluded in panel B.

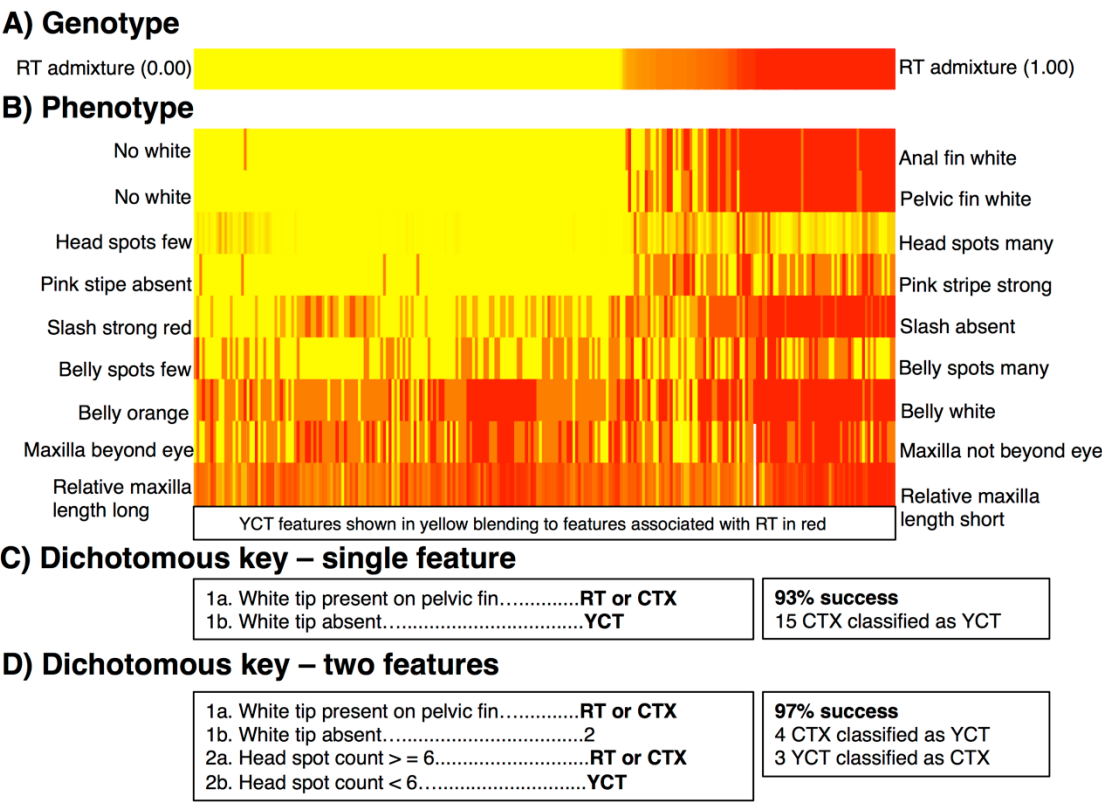


Figure 4.4. The correlation between genotype and phenotype of 251 trout including (panel A) Yellowstone cutthroat trout (YCT, yellow), a range of hybrid genotypes (CTX, orange), and Rainbow trout (RT, red). Each fish is represented by a narrow vertical bar with the proportion of RT admixture represented as a color gradient (i.e., native cutthroat trout on the left with increasingly hybridized fish to the right). Narrow vertical bars in panel B show the morphological data associated with genotyped fish represented in panel A (i.e., narrow columns in panel A and B are aligned vertically to represent the same individual fish). Within each row (panel B) ordinal scores for morphological features are represented as a color gradient. Vertical consistency in color between RT admixture (panel A) and morphology (panel B) shows strong relations between genotype and phenotype. Panels C and D depict classification trees with highest success rate to distinguish YCT from RT and hybrids using a single feature (C) or two features (D) with associated errors shown.

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CHAPTER FIVE

MANAGEMENT CONSIDERATIONS FOR NATIVE TROUT
CONSERVATION IN THE LAMAR
RIVER WATERSHEDIntroduction

In this final chapter I integrate the collective results of my studies to (1) summarize the main genetic threats to Yellowstone cutthroat trout (YCT) due to rainbow trout (RT) introgression in the Lamar River watershed (LRW), (2) outline conservation action recommendations consistent with the Native Fish Conservation Plan (NFCP) for Yellowstone National Park (YNP; Koel et al. 2010) and, (3) provide recommendations for monitoring and further research.

Desired Conditions

The NFCP for YNP describes three classes of desired conditions for native fish populations with associated quantitative measures to evaluate the success of conservation projects (Koel et al. 2010). The primary desired condition is a genetically unaltered population (RT admixture $[p_{RT}] = 0.00$, and no admixture with other cutthroat trout subspecies) that is not sympatric with non-native fish, and secure from future invasions. Populations are considered secure if either artificial or natural barriers separate all potential non-native fish populations. Secondary desired conditions arise when restoration of a genetically unaltered population is not feasible, and conservation actions are employed to prevent increasing p_{RT} , or loss of native fish by demographic processes.

Quantitative goals for conservation projects to achieve secondary desired conditions are reducing the abundance of sympatric non-native fish by at least 90% over 10 years, reducing pRT to a level below 10%, or, reducing an existing level of pRT by 50%. Lastly, conservation goals aimed at tertiary desired conditions are quantitatively characterized by reducing non-native population abundance or pRT by 50%.

Summary of Threats to Yellowstone Cutthroat Trout in the Lamar River Watershed

Spatial patterns of invasion point to Buffalo Fork Creek as the single contemporary source of RT within the watershed (Chapter 3). Fluvial distance to Hidden Lake (an original stocking site in the Buffalo Fork Creek watershed) is the single best predictor of the occurrence and degree of hybridization in quantitative models of hybridization (Chapter 3). All sampled populations within 37 km of this site ($n = 10$) had greater than 1% RT admixture, while all sites further than this ($n = 19$) had less than 1% pRT (Chapter 3, Fig 3.4). This pattern is consistent with a stepping-stone model of invasion (Boyer et al. 2008), whereby dispersal between adjacent populations facilitates expanding hybridization.

My studies do not support the notion that higher elevation habitats and thermal gradients are likely to prevent invasion of conservation populations that are connected to hybrid or RT sources (Chapter 3). This conclusion supports a continued effort to reduce or eliminate RT and hybrid populations, or isolate native populations or metapopulations to prevent continued expansion of introgression where necessary.

Recognizing that the waterfall on Slough Creek upstream from the campground was passable during certain flow conditions, the National Park Service (NPS) built a barrier in 2017 (Ertel et al. 2017). This effectively isolated the entire upper watershed from this prominent source of RT. I will consider this as a separate scenario for conservation action recommendations (i.e., Upper Slough Creek Conservation Unit) from the remainder of the watershed. Continued threats to the Upper Slough Creek Conservation Unit include populations that produce hybrids locally, potentially including Hornaday Creek ($pRT = 4.76\%$) and Elk Tongue Creek ($pRT = 3.61\%$), and other hybrid sources that may exist but I did not identify.

The Lamar River canyon is passable by fish in the upstream direction (Chapter 2), and therefore the upper Lamar River is subject to continued dispersal from Buffalo Fork Creek and all hybrid sources in the lower watershed (Figure 3.1). Although hybrid dispersal upstream via Lamar River canyon appears to be infrequent (Chapter 2), rates of dispersal in salmonids can be variable across time or increase following periods of warmer water temperature (Keefer and Caudill 2014, Bett et al. 2017), which are likely in future climate scenarios. Thus, fluvial connectivity to Buffalo Fork Creek and hybrid populations in the lower watershed is a persistent threat to the upper LRW. Upstream from the Lamar River canyon, hybridization is very limited, except in Rose Creek ($pRT = 11.08\%$). Therefore, hybrid dispersal from Rose Creek also represents a possible means by which hybridization could spread in the upper watershed.

Non-native trout sources outside of the LRW, including the Yellowstone River (YSR) and its tributaries between Knowles Falls and the Lower Falls of the YSR, are also

potential threats. My dissertation research was generally limited in geographic scope to within the LRW, yet movement data from Chapter 2 showed that movements between the YSR and the Lamar River were common. The threats of invasion from the YSR remain a topic of uncertainty. In the research needs section (below), I provide some guidance for future studies to better understand this potential threat.

Potential Conservation Actions

All Conservation Units

Angling and harvest of non-native fish as a conservation strategy is outlined in section 2.3.2.2 of the NFCP. Must-kill regulations are in place in the Lamar River watershed (Koel et al. 2010, Ertel et al. 2017). In chapter 3 I estimated that 10,000 anglers catch 50,000 fish annually, and could substantially reduce the frequency of hybrids if a must-kill regulation were adhered to. Thus, a first recommendation is to increase outreach efforts to garner compliance with harvest regulations (i.e., social media, seminars, signs at access points, or more detailed information in the fishing regulations).

Upper Slough Creek Conservation Unit

Ongoing Conservation Actions. An annual electrofishing survey is used to remove hybrid trout from Slough Creek beginning at the YNP border, downstream to the first meadow. Angler harvest of RT and CTX is also mandatory, and appears to be a potential mechanism to remove a substantial number of invasive trout (Chapter 4). Both of these actions should continue.

Hornaday Creek and Elk Tongue Creek. Conservation actions should focus on preventing local hybrid sources (i.e., upstream of the barrier) from establishing, or increasing in pRT. Hornaday Creek and Elk Tongue Creek represent locations that may serve as potential sources of expanding introgression (i.e., stepping-stones) in the Upper Slough Creek Conservation Unit, but are not yet extensively hybridized. The genotypic distribution of fish in these samples were both similar, and consisted of a portion of fish with low or no admixture, and a few individuals that were substantially hybridized (Figure 3.2). The latter individuals would be easily distinguished by morphology (Chapter 4) and selective removal could be done in these streams. Mechanical removal of non-native fish is a conservation strategy outlined in section 2.3.2.2 of the NCFP that is useful in recently invaded populations (Koel et al. 2010).

Lamar River Conservation Unit

Ongoing Conservation Actions. An annual electrofishing survey is used to remove hybrid trout from Lamar River in the Lamar Valley. Secondly, angler harvest of RT and CTX is mandatory, and appears to be a potential mechanism to remove a substantial number of invasive trout (Chapter 4). Both of these actions should continue.

Lamar River Canyon. There are multiple options for this conservation unit, several of which include large-scale projects. One option is to isolate the upper LRW by constructing a barrier in Lamar River canyon. Although Lamar River canyon appears to have prevented more widespread dispersal of hybrids into the upper Lamar River, it is not

a complete barrier to movement (Chapter 2). Even though current rates of dispersal appear to be low (Chapter 2), fluvial connectivity to hybrid sources in the lower watershed remains a persistent threat. The NFCP states, “ideally barriers would be constructed in or near road-accessible locations so that contractors with experience in similar projects can use heavy equipment and modern techniques to build more durable, predictably functioning structures, probably of concrete.” A barrier project on the Lamar River canyon would fit this description, and it would also provide protection from the aforementioned threat of upstream dispersal from multiple hybrid populations in the lower watershed. Regardless of effort extended, I consider it improbable that all hybrid sources in the lower Lamar River and the connected YSR can be realistically eliminated. Thus, a barrier on the Lamar River canyon would effectively isolate the entire upper system—creating an Upper Lamar River Conservation Unit. If this were to occur, smaller, more manageable hybrid sources that exist in the upper watershed could be targeted (i.e., Rose Creek).

A concern with barrier construction is eliminating movement corridors used by native fish, yet in Chapter 3 I did not find that YCT traverse this canyon during any time of the year. Exploratory analysis also showed strong genetic divergence between YCT above and below the canyon, suggesting historic gene flow through the canyon has been limited. A barrier in this location would be unlikely to disturb metapopulation functions in the upper watershed. Whether this project is considered or not, perhaps a more time sensitive conservation project involves Rose Creek.

Rose Creek Hybrid Removal. Rose Creek is the only substantially hybridized population upstream from Lamar River canyon that I identified, and should be considered a high priority for conservation action. Rose Creek could serve as the next stepping-stone to facilitate invasion of the upper Lamar River. The genotypic distribution in Rose Creek shows that unhybridized YCT are the dominant taxa, which co-occur with a wide range of hybrid genotypes (figure 3.2). As an already hybridized population, the goal would be to achieve *secondary desired conditions* (Koel et al. 2010) by reducing pRT by 50% from current level (11.08%) to less than 5%. This could be accomplished with a variety of methods including selective removal via electrofishing or construction of a weir to selectively pass fish. Alternatively, primary desired conditions could be attained by means of a chemical treatment and re-stocking with native YCT.

Buffalo Fork Creek Chemical Treatment. A chemical treatment of Buffalo Fork Creek could have far reaching benefits. Parental RT (or highly admixed fish with pRT > 95%) are almost exclusively found in Buffalo Fork Creek, yet most populations in the lower watershed (i.e., Hidden Creek, lower Slough Creek, lower Lamar River, Crystal Creek) had many fish similar in genetic composition to first generation hybrids (Figure 3.2). This strongly supports the notion that dispersal of highly admixed fish from Buffalo Fork Creek continues to increase pRT of nearby populations in the lower watershed. Therefore, a useful conservation action would be to eliminate this source population with a chemical treatment; the details of this are beyond the scope of this chapter.

Other Conservation Actions in the Lower Watershed. Most populations in the lower Lamar River watershed are of little conservation value because native taxa are infrequently encountered within populations that consist primarily of hybrids (Chapter 3). As mentioned, dispersal from any of these locations through Lamar River canyon could lead to expanding introgression. A challenge to successful conservation actions in these populations (i.e., Hidden Creek, Crystal Creek, lower Buffalo Fork Creek, Lamar River mainstem, Slough Creek) however, is that following conservation action to remove hybrids, there will be a high potential of re-colonization by non-native trout. Any focused efforts (i.e., on only one of the populations) to reduce pRT or the frequency of non-native taxa in these populations could be counterbalanced by re-colonization of non-native trout. Alternatively, the entire lower watershed could be targeted at once – yet this may not be a socially acceptable or realistic conservation action.

Genetic Reserves in the Upper Watershed. My study identified several genetically unaltered populations occurring in small headwater habitats unlikely to serve as spawning areas for migratory fish. Such locations include the upper portions of Willow Creek, Little Lamar River, Mist Creek, and Calfee Creek. In many watersheds, the preponderance and persistence of YCT is a function of downstream dispersal from isolated headwater sources (Bingham et al. 2016). Native fish can go down waterfalls, and hybrids can't go up them. As part of a multi-faceted approach to conserve non-hybridized populations into the foreseeable future, several populations could be intentionally isolated using small barriers to prevent upstream hybrid dispersal. As these streams do not appear to be used by large migratory YCT, their isolation would not

disturb life-history migrations that are important in the watershed (Chapter 2). These could be especially useful if a barrier was not implemented in the Lamar River canyon, and would serve as ‘genetic reserves’ for populations that would continue to produce non-hybridized YCT. Lastly, it appears that Amethyst Creek (upstream from the waterfall) would be an excellent stream to introduce a conservation population of YCT (as it is currently fishless, K. Heim, personal observation) and could serve a similar function as a ‘genetic reserve’ in the Lamar Valley.

Recommended Monitoring Actions and Research Needs

Genetic and phenotypic monitoring (i.e., taxa classification based on morphology, Chapter 4) should be aimed at collecting information that will help guide continued management decisions to address conservation goals and objectives. My first recommendation to NPS is to develop a specific set of management objectives and goals for the LRW; this will guide the development of a structured monitoring program that will help determine if these objectives are being met or if new actions are needed (Bal et al. 2018).

The NPS already collects data annually by electrofishing Slough Creek and Lamar River, and from Volunteer Angler Surveys. Both of these will be useful to detect long-term trends in the frequency of hybrids across the watershed; I make some simple recommendations on how to use these data. Secondly, I provide some of my perspectives for continued genetic sampling and research needs.

Annual NPS Sampling Events

The NPS conducts two electrofishing trips (one on Lamar River, one on Slough Creek) to remove hybrids. Data from these trips can be used to estimate population abundance of YCT with a simple mark-recapture estimate (Chapter 4) and summarize hybrid frequency (i.e., relative number of field-identifiable hybrids to total catch). I strongly recommend that a standardized approach to species identification and data recording in the field be used (Chapter 4) to avoid variation that arises from staffing differences and different perspectives on hybrid identification by morphology. I suggest recording fish as CTX-W (hybrid, white pigmentation on pelvic, anal, or dorsal fin); CTX-H (hybrid, ≥ 6 head spots but no white pigmentation); CTX-S (suspected hybrid that has other morphological features suggesting introgression). The potential benefits of this approach are outlined in chapter 4.

Volunteer Angler Dataset

The volunteer angler dataset (VAR) can be used to quantify trends in species composition and hybrid frequency, and may be especially useful to monitor locations that the NPS does not regularly sample (i.e., Lower Lamar River, Lower Slough Creek). Each year almost 400 VAR cards report fishing trips in the Lamar River watershed, and each of these cards can be used to calculate an independent estimate of catch per unit effort (CPUE) by species (Figure 5.1), or as an independent estimate of species composition (i.e., reported RT + hybrids caught/total fish caught).

I performed a simple exploratory analysis to highlight how these data could be used, with the methods described in the figure caption (Figure 5.1). Catch per unit effort

(CPUE) of non-native trout (RT and hybrids) has consistently decreased in the five most commonly fished reaches of the watershed (Figure 5.1). In contrast, YCT CPUE appears to be increasing (Figure 5.2). I explored the application of generalized additive models to this dataset (GAMs), which fit well and further revealed statistically significant trends in the total number of fish caught by anglers over time. In this analysis I used ‘reach’ as an additive term in the model given that visual inspection of the dataset (Figure 5.1) suggested similar trends across stream reaches. However, separate analyses could be performed for different areas to determine if trends are consistent across reaches. Moreover, such an approach could be used to test for the efficacy of management interventions. For example, following a chemical treatment of Buffalo Fork Creek, one would expect a significant decrease in the number of RT and hybrids caught in the lower watershed. This could be tested with such an approach using the VAR dataset.

Additional studies using already existing datasets could be conducted to validate the accuracy of the VAR dataset for monitoring trends. For example, a time series of annual hybrid proportion estimates from NPS sampling (i.e., Slough Creek upper watershed, Lamar River valley) could be compared to the same estimates from the VAR dataset (i.e., proportion hybrid caught by anglers). By validating the accuracy of VAR dataset in locations with paired sampling efforts, it could provide confidence that the VAR dataset was accurate for other areas not regularly visited by NPS.

Genetic Sampling

Chapter 3 lays a framework to establish a regular long-term genetic sampling framework (Table 3.1) to meet the continued monitoring needs of NPS. Sites could be

sampled at regular intervals (i.e., every 10 years) to determine trends in admixture within populations and identify the spatial spread of introgression into formerly non-hybridized sites. I also suggest adding new locations to assess the genetic status of populations I did not survey, but would focus efforts on collecting samples from locations that represent distinct breeding populations.

In prior years the NPS has extensively genotyped fish collected from mainstem locations including Slough Creek, Soda Butte Creek, and the Lamar River (used in Chapter 3). Fish collections from these locations all represent ‘mixed stock’ aggregations where fish from multiple sources likely interact during the non-spawning period. Since fish traverse nearly the entire watershed during migrations (Chapter 2), I do not see much practical value in genotyping fish from such collections. A trend may be detected (i.e., pRT of the collection is increasing or decreasing) but this is not particularly valuable for guiding management actions that should focus on targeting hybrid sources (i.e., where hybrids are produced). Since most hybrids can be reliably identified by morphology; I suggest a standardized morphological assessment be used to assess trends in hybrid frequency from mainstem samples, reserving more costly genetic analysis for collections representing distinct breeding groups.

Research Needs

A limitation of my dissertation research is that I did not examine potential non-native source populations in the YSR and connected tributaries. An important research need is to assess the potential role of hybrid dispersal into the Lamar River from the YSR. This could involve (1) additional genetic or morphological sampling in the YSR

and tributaries, (2) telemetry studies to identify movement patterns and spawning locations of non-native fish tagged in the YSR and, (3) evaluation of VAR data.

As a simple exploration of the VAR dataset, I queried and summarized VAR catch reports from all locations with fluvial connectivity to the LRW (i.e., the YSR and its tributaries between Knowles Falls and the Lower Falls). These results do not identify any prominent tributaries that would serve as non-native source populations, yet there is a high number of non-native fish captured in the Black Canyon section of the YSR. This could be explained by dispersal from the LRW (i.e., these fish are produced in the LRW and disperse to the Black Canyon), or possibly, production from an unidentified source or sources of non-native fish within the YSR. In Chapter 2 I observed several fish overwintering or spending the summer in the YSR and returning to LRW to spawn, so the former is a feasible explanation. A simple telemetry study would be useful in this regard.

Radio telemetry could be used to identify the spawning locations of hybrids and RT captured in the Black Canyon section of the YSR. Angling could be used to deploy radio transmitters in hybrids and RT in this area. Anglers reported capturing a ratio of 0.22 RT or hybrids to native YCT, and a catch rate of 1 RT or hybrid every 0.36 hours of fishing. Thus, it would likely take a large effort to deploy a sufficient number of transmitters, but it would be feasible with a team of several experienced anglers. Tracking these fish to spawning locations could be accomplished with weekly flights during the spawning period. An alternative or supplementary tracking method (that would be less expensive) is to deploy fixed telemetry receivers at the confluence of likely spawning tributaries (e.g., Lamar River, Hellroaring Creek, Blacktail Deer Creek) to

identify spawning movements. I had good success and very few technical issues with fixed radio telemetry receivers in my study (Figure 5.2), and can provide design specifications upon request. Of particular interest would be the proportion of tagged RT and hybrids from the YSR that return to spawn in the LRW. Chapter 2 identified high rates of site fidelity of hybridized fish; therefore, a high ratio of fish returning to the LRW out of those tagged in the YSR would suggest LRW as a primary source of non-native fish. Alternatively, a low ratio of these fish returning to LRW would strongly suggest that other locations in the YSR are producing hybrids. My recommendation would be to (1) carefully review the VAR dataset (i.e., Table 5.1), (2) conduct a preliminary radio telemetry study and then (if determined necessary) (3) use the results of the telemetry study to further guide genetic sampling if deemed necessary.

Conclusions and Parting Thoughts

There are multiple options to conserve native trout in the LRW. It may be that tolerating slight amounts of admixture in some populations (i.e., secondary desired conditions) is a better conservation strategy than attempting to identify and eradicate all traces of the non-native genome and achieve primary desired conditions across the watershed. Features that make the Lamar River metapopulation so valuable are extensive fluvial connectivity, high habitat diversity, diverse movement patterns, and high probability of persistence. Specific management objectives developed for this metapopulation should place value both on genetic purity, as well as other biological and ecological properties that promote resiliency (Al-Chokhachy et al. 2018).

Table 5.1. Volunteer angler report (VAR) summary from locations outside of the Lamar River (between Knowles Falls and Lower Falls of the Yellowstone River), and two locations within the Lamar River watershed (Slough Creek lower, Lamar River below Canyon) for relative comparison. Data include 3,156 VAR responses collected from 2001 to 2017. The VAR stream codes are shown exactly as they appear in the database provided by NPS; abbreviations are as follows: n, number of VAR cards received from 2001 to 2017 reporting on each waterbody; n Non, the number of rainbow trout and hybrids reported caught; n YCT, the number of Yellowstone cutthroat trout reported caught; ratio, the ratio of rainbow trout and hybrids to the total reported; CPUE, catch per unit effort.

VAR stream code	n	n Non	n YCT	ratio	CPUE Non	CPUE YCT
Buffalo Fork	2	20	0	1.00	3.75	0.00
Buffalo Creek	12	316	35	0.90	4.48	0.75
Tower Creek	130	417	121	0.78	1.02	0.36
Blacktail Deer Creek	155	14	11	0.56	0.02	0.04
Elk Creek	4	2	3	0.40	0.10	0.38
Slough Creek Lower	1037	1984	4881	0.29	0.36	0.82
Lamar River below Canyon	747	1436	4921	0.23	0.31	1.16
Yellowstone River Lower Black Canyon	291	686	2427	0.22	0.36	1.18
Yellowstone River to Tower Area	586	234	6336	0.04	0.08	2.24
Yellowstone River to Grand Canyon	170	57	4327	0.01	0.09	3.78
Antelope Creek	19	1	141	0.01	0.05	4.94
Agate Creek	1	0	11	0.00	0.00	2.75
Geode Creek	2	0	22	0.00	0.00	3.29

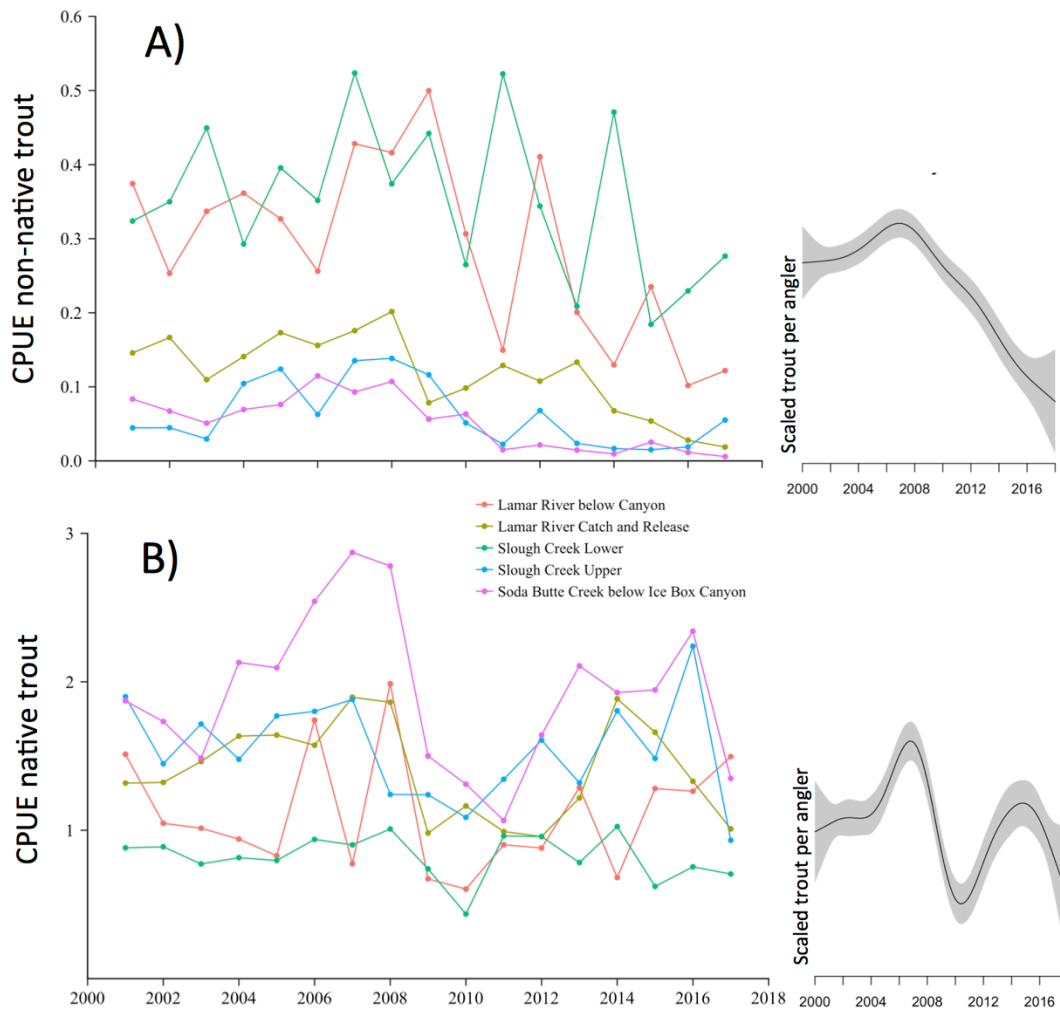


Figure 5.1. Exploratory analysis of Volunteer Angler Report dataset to examine trends in non-native trout (A) and native trout (B) catch per unit effort (CPUE, fish/hour). Data include 7,585 angler surveys reporting on fishing trips in the Lamar River watershed; the panel with multiple lines shows calculated catch per unit effort (fish/hour) for five popular fishing locations. Also shown (right) are the results of a generalized additive model (GAM) fit to the dataset with the following structure; $n \text{ fish caught}_i \sim s(\text{year}_i) + s(\text{month}_i) + \text{reach_fished}_i + \text{hours_fished}_i + \text{experience_level}_i + \text{error}_i$ where $s(\text{covariate})$ indicates an additive smoothing function and i represents an individual fisherman which is considered the sampling unit.



Figure 5.2. Fixed radio telemetry receiver station set up at the confluence of the Yellowstone River and the Lamar River.

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APPENDICES

APPENDIX A

SUPPORTING METHODS, RESULTS, TABLES, AND
FIGURES FOR CHAPTER TWO

Supplemental Methods

Radio Telemetry

We used individual sequences of telemetry locations to estimate pre-spawning location (Henderson et al. 2000, Muhlfeld et al. 2009), spawning location, spawning start and end dates (DeRito et al. 2010), and spawning date. For each individual, these were estimated based on visually reviewing a sequence of telemetry location estimates, which were represented as spatial confidence areas (i.e., polygons) as demonstrated in Figure A.1. These polygons represent the area within which we had confidence the true location of the fish was (Heim et al. 2018). Estimated error for ground tracking averaged 36 m (min = 1, max = 131 m), and aerial telemetry error was most often < 300 m (70% of detections), but as high as 1 km (1% of detections). Location estimates were represented in a geographic information system (GIS) as polygons defined by GPS points of telemetry detections and buffers equal to the estimated telemetry error (Heim et al. 2018).

This representation allowed us to distinguish movement from apparent movement while assessing sequences of detections; if subsequent detection polygons from a time series did not overlap, we considered this evidence of movement. Similarly, presence within a tributary versus at the tributary mouth was confirmed when the detection polygon overlapped only the tributary and not both the tributary and the mainstem. Because points were represented as polygons, each detection overlapped a ‘reach’ of the stream (e.g., upstream and downstream intersection of the polygon overlaid on a GIS stream layer) providing a means to incorporate error into movement calculations (Figure A.1).

Individual fish moved little during the pre-spawning period (in most cases we have 5 – 10 detections of each fish in a similar location), and therefore spawning migrations were easily distinguishable when detection polygons were first non-overlapping in the spring (Figure A.1). The last location a fish was found, before it made a spawning migration, was considered the pre-spawning location. We define spawning reach with either one or two detections, depending on whether the fish was detected within the general spawning area once or more than once. The furthest extent of the spawning migration from the pre-spawning location was always used (as either the only detection, or one of them). If a fish was detected multiple times after reaching its furthest migration extent, we defined the spawning reach using the upstream most and downstream most detection of the fish, after it reached the furthest extent of the spawning migration and before a post-spawning migration. The spawning start date was defined as the median date between when a fish first entered the spawning reach and the previous detection, and the spawning end date was defined as the median date between the last date a fish was found in the spawning reach and the first detection after it left the spawning reach. For fish that died in spawning areas, we defined the spawning end date as the date of last confirmed upstream movement (e.g., non-overlapping detection polygons) made within the spawning reach. Spawning location was defined as the center of the spawning reach, and spawning date as the median date between spawning start and end dates. Importantly, the species or genotype of individual fish was not referenced or known when reviewing and designating these spawning variables to avoid any biases related to underlying expectations.

Passive Integrated Transponder Tag Monitoring

We sampled spawning tributaries opportunistically to encounter spawning trout, attempting to visit sites multiple times during a spawning year to capture a potential range of taxa (e.g., early spawning RT, CTX, and later spawning YCT). Temporal and spatial allocation of sampling effort per month at each site for the three-year study is shown in Table A.1.

As a precautionary step to ensure that we did not include immature fish in our spawning estimate dataset, we used site-specific size-at-maturity cutoff values to exclude probably non-spawners. We used the lower 25% quartile value of observed ripe fish as the stream-specific cutoff (Table A.2). Because we did not sample fish in Pebble Creek, we used a cutoff value of 300 mm here as a conservative estimate of size at maturity (Table A.2).

When fish were sampled from a tributary that was also being monitored with a PIT tag antenna, we used the mediate date between when the fish was released, and when it was detected exiting the stream at the PIT tag station as the spawning date estimate. These fish had to be either ripe or greater than or equal to the minimum size cutoff, for that stream, to be included in further analysis. For the detection at the PIT antenna used to represent the exit-date we did not require that the fish was detected at both of the two antennas (A1 upstream, A2 downstream), though in most cases the were due to very high detection probability across sites and years (Table A.1). Because the fish was known to be released upstream of the antenna station, a detection at either of the two antennas could reliably be interpreted as a downstream movement relative to the release location.

If a ripe fish was captured, PIT tagged, and released in a tributary monitored by PIT tag antenna, but never detected exiting the stream, we used the date of capture as the spawning date.

In many cases a complete spawning run (enter date and exit date) was recorded at the PIT tag antennas, in which case we used the mediate date between the enter and exit dates as a spawning date estimate. This occurred when fish had been PIT tagged in a mainstem location, or when fish tagged in a spawning tributary exited, but then re-entered at a later date. Estimating entry date and exit date was generally straightforward, though some nuances require further discussion. Most fish exhibited a rather clear detection history where an upstream movement into a spawning tributary was observed (e.g., A2, A1) followed by at least several days of residency within the stream, and then a downstream movement was observed (A1, A2). If multiple bouts into the stream were detected, the last bout was used to represent the spawning run, as long as the length of this bout was not substantially shorter than the previous bout. For example, if a fish spent two weeks in a spawning tributary, left the stream, then came back in for a few hours—the longer of the two bouts was used. Sometimes fish made short bouts into a spawning tributary for a few days or hours, prior to making a more extended bout. In these cases the longer bout was interpreted as the spawning run. Passive integrated transponder data were analyzed manually, rather than using an automated script, so that nuanced details could be evaluated and interpreted following the exact guidelines described above. The genotype or taxa of individual fish were not known when the analyst evaluated the data to identify spawning runs.

Supplemental Results

Validation of Modeled Datasets

On all accounts, empirical measures were strongly correlated to modeled estimates of flow timing, volume, and stream temperature indicating that they served as a good surrogate for sites where we did not collect empirical data. Modeled CFM was significantly rank correlated to measured date of peak flow in 2016 ($r_s = 0.75$, $p = 0.08$, $n = 6$ sites) and in 2017 ($r_s = 0.97$, $p < 0.001$, $n = 8$ sites). Modeled temperature from the NorWeST dataset (August mean, 2002 – 2011) spanned a range of 2.81 °C (Flint Creek = 9.82 °C, Lamar River [lower] = 12.63°C) and adequately described the variation among patches in temperature during the spawning period (NorWeST compared to May 2016 mean, $r_s = 0.91$, $p = 0.004$, $n = 7$ sites; NorWeST compared to May 2017, $r_s = 0.71$, $p = 0.05$, $n = 8$ sites). Correlations were weaker for mean June temperature comparisons (NorWeST compared to June 2016, $r_s = 0.61$, $p = 0.14$, $n = 7$ sites; NorWeST compared to June 2017, $r_s = 0.65$, $p = 0.08$, $n = 8$ sites). Modeled MAF was perfectly rank correlated with our measured peak flow estimates (2016 $n = 7$ sites, 2017 $n = 8$ sites).

Tables and Figures

Table A.1. Passive integrated transponder antenna (PIT) deployment information, detection efficiency, and sampling effort (number of unique sampling events, per month) in spawning tributaries in and near the Lamar River watershed from 2015 - 2017.

Antenna specific detection efficiency is shown (first the upstream antenna, then downstream antenna) for each station. Rose Creek branches prior to entering the Lamar River, we therefore deployed PIT stations at the eastern (E) and western (W) branches; detection efficiency is shown for each. Detection efficiency was calculated as the number of unique fish detected per day at a given antenna (e.g. n at A1, n at A2) divided by the number of unique fish seen at either antenna, and averaged over the operational period. Fish were not sampled in Pebble Creek. The last five columns indicate the number of sampling events within tributaries by month and year.

Site	Year	PIT antenna	Detection efficiency	March	April	May	June	Total
Ditch Cr.	2016	N	-	0	0	0	3	3
	2017	Y	0.96, 1.00	0	1	1	6	8
Hidden Cr.	2016	Y	1, 1	0	1	2	1	4
	2017	Y	1, 0.55	1	4	5	2	12
Crystal Cr.	2015	N	-	0	1 ^a	0	1	2
	2017	N	-	0	0	2	0	2
Rose Cr.	2015	Y	E:1.00, 1.00 W:0.70, 1.00	0	2 ^b	4	4	10
	2016	Y	E:0.94, 1.00 W:1.00, 1.00	0	0	1	1	2
	2017	Y	E:0.96, 1.00 W:0.96, 0.93	0	1	1	2	4
Pebble Cr.	2016	Y	0.94, 0.91	0	0	0	0	0
Chalcedony Cr.	2015	N	-	0	0	0	2	2
	2016	Y	0.89, 1.00	0	0	0	2	2
	2017	Y	1.00, 1.00	0	0	0	1	1
Flint Cr.	2015	N	-	0	0	0	1	1

^aOne sample with a fyke net, capturing fish moving upstream

^bAll samples with a fyke net, capturing fish moving upstream

Table A.2. Number of ripe fish captured in six spawning tributaries and statistics on fork length (mm). Ripeness was determined by the presence of eggs or milt upon gentle squeezing when handled. The 25% quartile value was used as a minimum size cutoff to exclude potential non-spawners that were seen moving into these streams (Hidden, Rose, Ditch, and Chalcedony) during the spawning period at passive integrated transponder antennas.

Stream	N (ripe)	Min FL	Max FL	Mean FL	25% Quartile FL
Ditch Cr.	30	193	394	327	306
Hidden Cr.	55	152	463	311	239
Crystal Cr.	15	170	377	270	232
Rose Cr.	26	148	460	290	207
Chalcedony Cr.	22	184	411	349	348
Flint Cr.	8	146	257	183	156

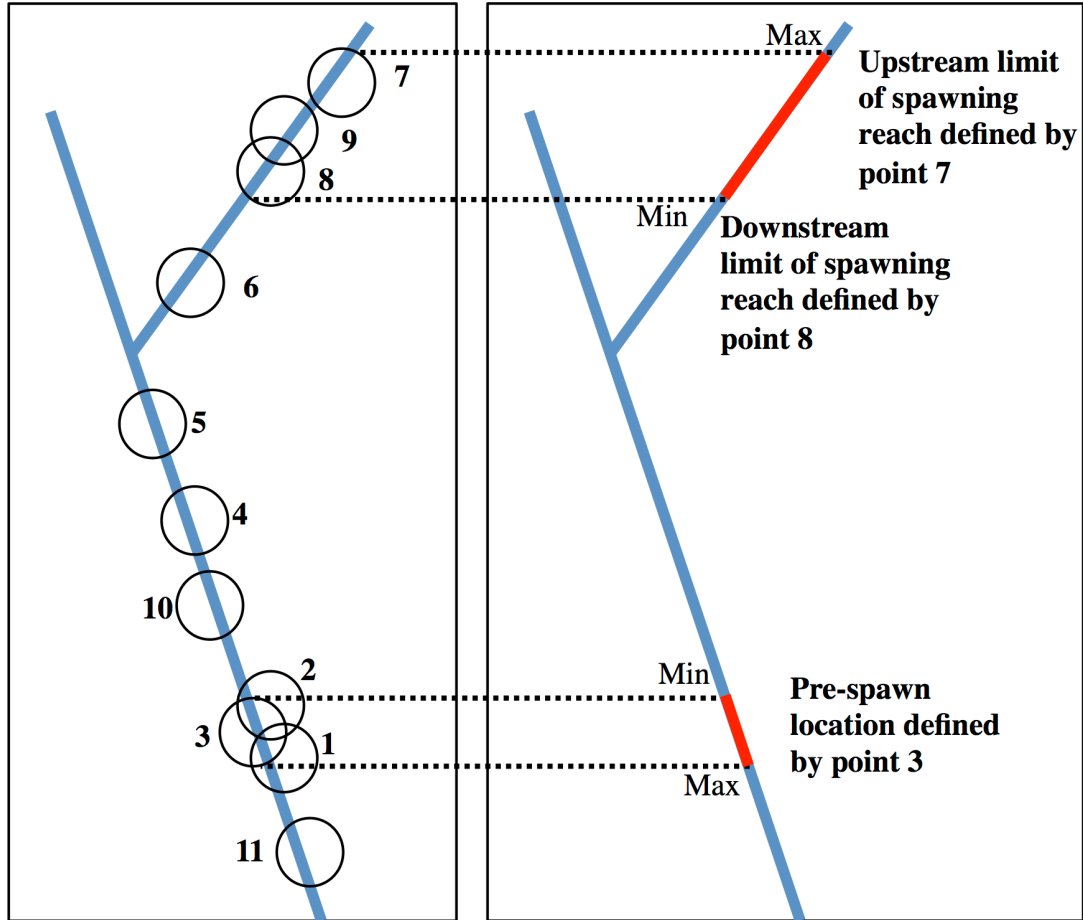


Figure A.1. Conceptual diagram of methods used to estimate spatial and temporal variables from sequence of telemetry locations, expressed as spatial confidence areas (all show with equal confidence [i.e., radius of circle] for clarity). The panel on the left shows a typical sequence of detections (1 – 11) that depicting detections of an individual fish on different dates during a spawning year. The fish was detected multiple times without moving (prior to spawning; detections 1, 2, 3) then it moved from this location to make a spawning run (point 4). The points 7, 8, and 9 represent the location that would be designated as the spawning reach, and the center of this is defined as the spawning location. Migration distance is quantified as a minimum (upper boundary of pre-spawn location to lower boundary of spawning reach) and a maximum (lower boundary of pre-spawn location to upper boundary of spawning reach) such that the minimum and maximum estimate capture the potential range of distances the fish could have traveled

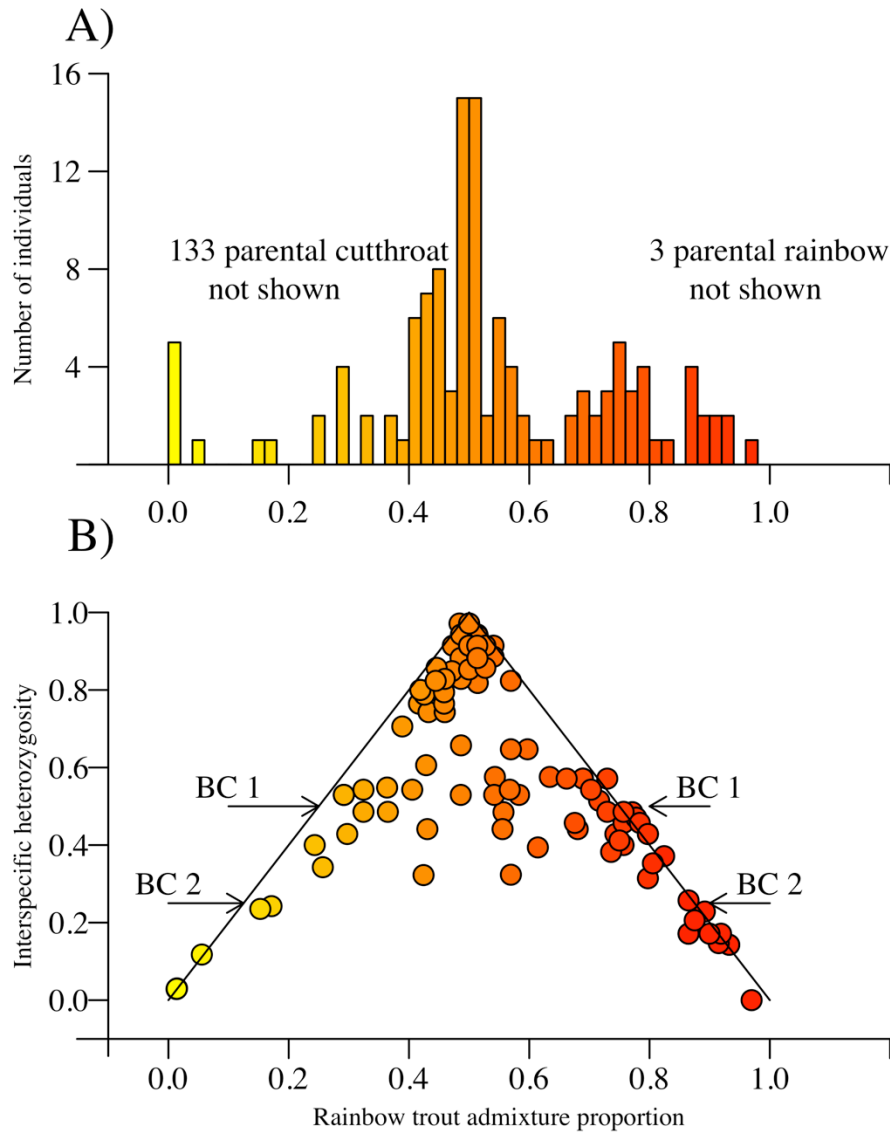


Figure A.2. Distribution of individual rainbow trout (RT) admixture estimates of 112 Yellowstone cutthroat trout x RT hybrids (panel A) and the relationship between admixture and interspecific heterozygosity (panel B) included in the final fish movement dataset. Interspecific heterozygosity is the proportion of diagnostic loci having an allele present from both taxa; first generation hybrids (F1s) will be at the apex of the triangle (interspecific heterozygosity = 1.00, RT admixture = 0.50), standard backcrosses (e.g., BC1 = F1 x RT, BC2 = F2 x RT) would be expected to fall along black lines of the triangle. An F1 x F1 cross is expected to fall in the center of the triangle (heterozygosity = 0.5, RT admixture = 0.5).

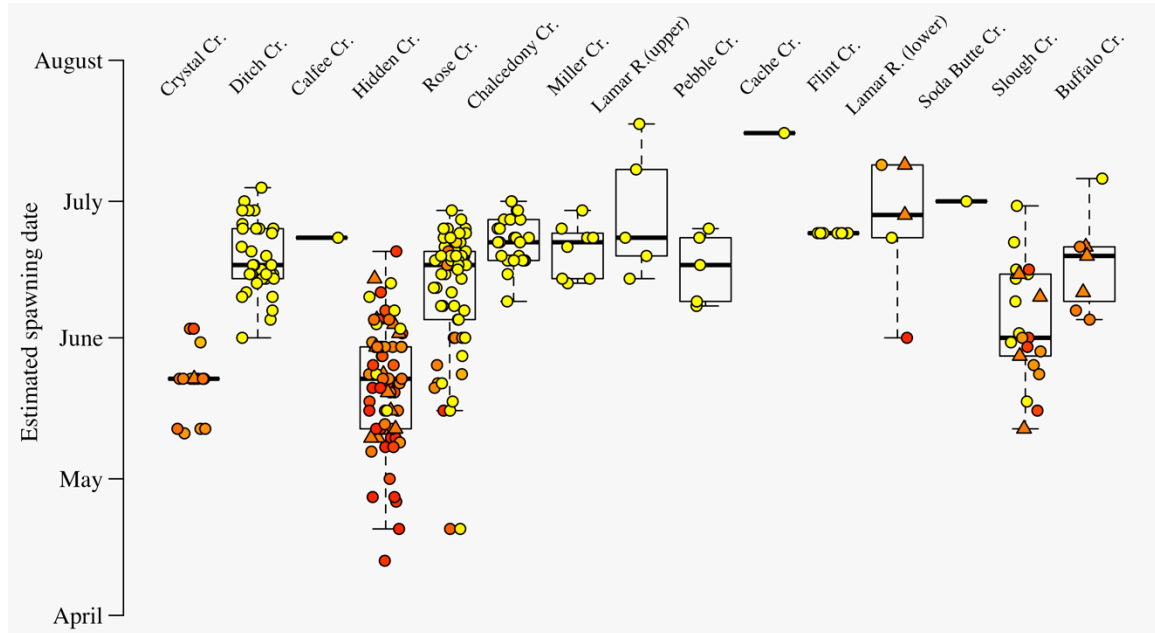


Figure A.3. Estimates of spawning date ($n = 278$) for 248 fish that spawned in 15 different patches in and near the Lamar River watershed, 2015 – 2017, ordered from left to right according center timing of flow mass of the spawning patch. Individual rainbow trout ancestry of each fish is represented by a color gradient, triangles indicate fish classified as F1 hybrids.

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APPENDIX B

ANALYSIS OF LONG TERM STREAMFLOW CONDITONS IN
THE LAMAR RIVER TO SUPPORT CHAPTER TWO

Introduction and Methods

Earlier snowmelt runoff has been observed across the Rocky Mountains, and continuing earlier trends in runoff phenology are expected to have significant impacts on the spread of rainbow trout introgression with native trout (Cayan et al. 2001, Stewart et al. 2004, Muhlfeld et al. 2014). To assess the degree that snowmelt runoff is shifting in the Lamar River, we used long-term discharge data available from the U.S. Geological survey gage site #06188000. We calculated several metrics that are described in detail elsewhere (Cayan et al. 2001, Stewart et al. 2004, Wenger et al. 2010, Leppi et al. 2012) and depicted in figure A3. Briefly, we calculated center timing of flow mass as with the equation:

$$CFM = \sum (t_i q_i) / \sum q_i$$

where t_i is time in days from the start of the water year (begins on October 1st) and q_i is the corresponding stream discharge for water year day i (Stewart et al. 2004). This value can also be described as the temporal centroid of streamflow. We also calculated the onset of spring runoff with the algorithm presented in (Cayan et al. 2001), this identifies the day of the year after which most flows are greater than average. Technically, it identifies the day on which the cumulative departure from the mean daily flow is most negative—this corresponds with the transition from winter low-flow conditions to the beginning of snowmelt. An R script included in this appendix extracts the dataset directly from USGS with a web-connection and computes the metrics. This R script can be used

to perform the same analysis for data from any other USGS gage, simply by replacing the gage ID in the R script.

With the time series dataset we compared computed metrics for the time period of 1927 – 1968 (historical data) to modern data from 1989 – 2017; a 20-year gap in the flow records provided a natural break point to split the dataset. Comparisons between historical and modern data were made with t-tests, linear regression was used to identify trends, and alpha was set a 0.05 for tests.

Results

From 1927 to 2017 the timing and magnitude of stream discharge phenology showed significant linear trends towards earlier timing. Linear regression coefficients suggest onset of spring snowmelt, center timing of flow mass, and date of maximum stream flow have changed by 0.79, 0.64, and 1.09 days per decade since 1924, and all linear regression analyses were significant (Figure B.1). Comparison of spring flow onset timing between historical and modern periods showed that the transition from winter low-flow conditions to snowmelt runoff in the spring is earlier (modern data mean 7.07 days earlier than historical data mean, 95% CI 2.3 – 11.82). Runoff timing (center timing of flow) is also earlier in modern times (5.15 days earlier, 1.07 – 9.25) as is the observed date of peak flow (9.42 days earlier, 3.94 – 14.90).

In additional to earlier stream flow timing, flow volume during the water year is trending to be higher than historical times. Mean annual flow shows a significant increase compared to historical data (historical mean = 23.40 m³/s, modern mean = 27.29, 95% CI

difference of 0.91 – 6.84), and the magnitude of peak flow is higher in current times than historically (historical mean = 213.82 m³/s, modern mean = 259.61, 95% CI difference of 12.33 – 80.37).

Modern flow patterns also showed much higher range of variability than in the past. For all tested metrics standard deviation was higher in modern times than historically (onset of spring flow historic/modern; 8.84/10.78, CFM; 8.67/8.68, MAF; 10.66/12.19). In general, these analyses show more water flowing through overall, but at an earlier time period, and with higher variation in quantity and timing.

Figure

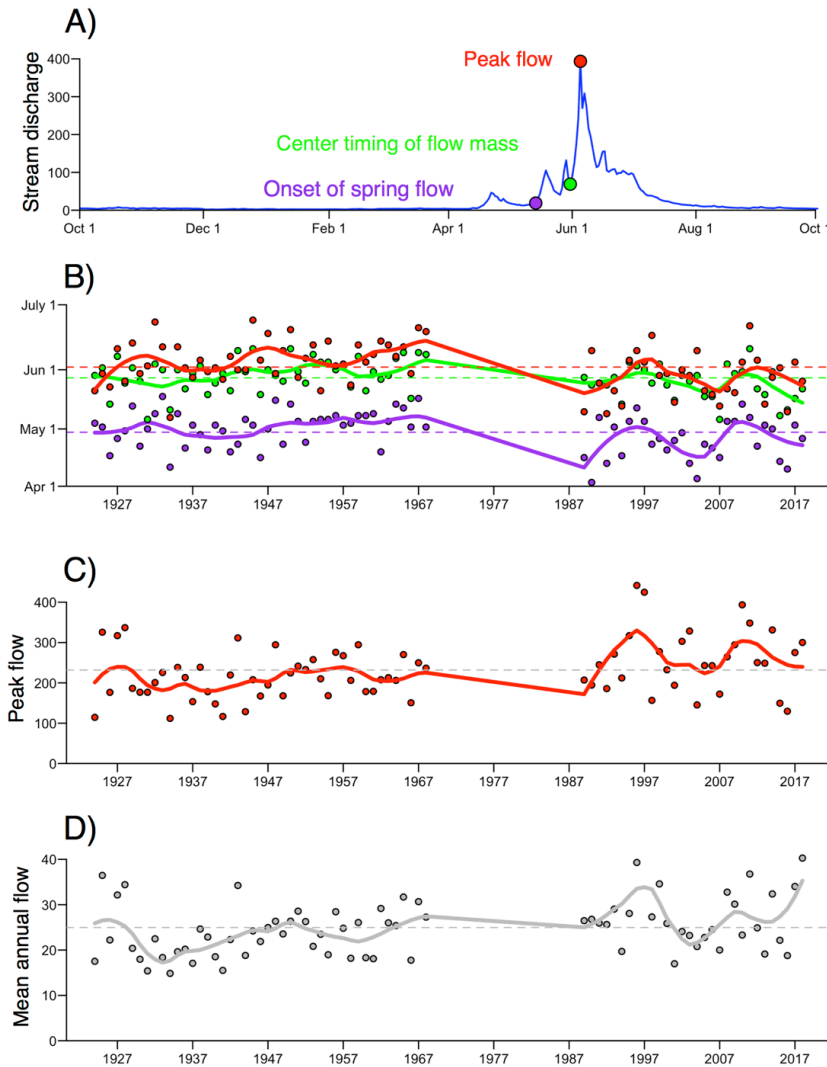


Figure B.1. Time series analysis of streamflow data for the Lamar River showing (A) a typical years hydrograph (2010 is shown) with the date of peak flow, calculated center timing of flow mass, and onset of spring flow depicted. Panel B shows these metrics (red line = peak flow date, green line = center timing of flow mass, purple line = onset of spring flow) for the 81-years of record for the Lamar River. For each metric, the dashed line represents the mean over the entire data period, and the trend lines are locally weighted scatterplot smoothers (LOESS) with a span parameter of 0.2. Panels C and D show the measured volume of peak flow and mean annual flow over the period of record.

Supporting Computer Code

```
#####GET FLOW METRICS FUNCTION#####
#this takes only a single input (the USGS gage # in quotes)
#imports all available daily stream flow data for that site
#and calculates a variety of streamflow metrics including;
#1 date of center timing of flow mass (accessed with $date_CT), units are day of water year
#2 date of observed max flow ($date_max_flow), units are day of water year
#3 maximum flow, ($max_flow, units are cubic feet per second)
#4 number of days in the data for a water year, units are number of days observed in the water year
#($n_days)
#5 mean annual flow ($mean_flow), units are cubic feet per second
#6 date of spring onset of flow ($date_spring_onset, units are day of water year)

#Author: Kurt Heim, kurtcheim@gmail.com
#Packages lfstat and waterData must be loaded to run function

GetFlowMet<-function(gage_id){
  dat<-importDVs(gage_id, code="00060", stat="00003")
  #assign water year values and reformat
  dat$dates<-as.POSIXlt(dat$dates)
  dat$wyear<-water_year(dat$dates,origin="usgs")
  #remove na vales
  dat<-subset(dat,val!="NA")
  #make a vector of years
  water.years<-unique(as.numeric(as.character(dat$wyear)))
  dat$wyear<-as.numeric(as.character(dat$wyear))
  #calculate metrics for first water year
  i=1
  w<-subset(dat,wyear==water.years[i])#get this year data
  w$wyear.day<-1:nrow(w)#assign water year day
  CT.df<-subset(w,w$wyear.day==1)# make dataframe
  CT.df<-CT.df[,c("wyear","staid")]
  CT.df$date_CT<-round(sum(w$wyear.day*w$val) /sum(w$val)) #compute CT of mass
  CT.df$date_max_flow<-subset(w,w$val==max(w$val))[1,6] #date of max flow
  CT.df$max_flow<-w[w$wyear.day == CT.df[1,"date_max_flow"],"val"]
  CT.df$n_days<-nrow(w) #how many days in water year
  CT.df$mean_flow<-mean(w$val) # mean flow
  w$dif.mean<-w$val-CT.df[1,"mean_flow"] # diff of each day from mean flow
  w$cum.dif<-cumsum(w$dif.mean) # cumulative sum of diff from mean
  CT.df$date_spring_onset<-subset(w,cum.dif==min(w$cum.dif))[6]
  CT.df# this is the data for year i

  ### loop through to do it for all other water years
  for(i in 2:length(water.years)){
    w<-subset(dat,wyear==water.years[i])#get this year data
    w$wyear.day<-1:nrow(w)#assign water year day
    CT.df.n<-subset(w,w$wyear.day==1)# make dataframe
    CT.df.n<-CT.df.n[,c("wyear","staid")]
    CT.df.n$date_CT<-round(sum(w$wyear.day*w$val) /sum(w$val)) #compute CT of mass
    CT.df.n$date_max_flow<-subset(w,w$val==max(w$val))[1,6] #date of max flow
    CT.df.n$max_flow<-w[w$wyear.day == CT.df.n[1,"date_max_flow"],"val"]
  }
}
```

```

CT.df.n$n_days<-nrow(w) #how many days in water year
CT.df.n$mean_flow<-mean(w$val, NA.rm = TRUE) # mean flow
w$dif.mean<-w$val-CT.df.n[1,"mean_flow"] # diff of each day from mean flow
w$cum.dif<-cumsum(w$dif.mean) # cumulative sum of diff from mean
CT.df.n$date_spring_onset<-subset(w,cum.dif==min(w$cum.dif))[1,6]
CT.df<-rbind(CT.df,CT.df.n)
}

CT.df
}

#####USING THE FUNCTION#####
library(lfstat)
library(waterData)

gage<-"06188000"#The Lamar River gage replace with any gage number you want
flowdat<-GetFlowMet(gage)#run the function
str(flowdat)#81 years of data for lamar

#important to get rid of data from years with incomplete data, calculations will be incorrect when data are
#missing from the full water year
flowdat<-subset(flowdat,flowdat$n_days>=365)# get rid of partial year data
cfs_to_cms<-0.0283168#conversion to metric for flow volume

#what is the mean annual flow for the 81 year dataset?
mean(flowdat$mean_flow)*cfs_to_cms # 25 cubic m per second

#what is the mean peak flow for the 81 year dataset?
mean(flowdat$max_flow)*cfs_to_cms # 231 cubic m per second

#whats the data look like?
par(mfrow=c(3,2))
plot(flowdat$year, flowdat$date_CT, type = "b")
plot(flowdat$year, flowdat$date_max_flow, type = "b")
plot(flowdat$year, flowdat$max_flow, type = "b")
plot(flowdat$year, flowdat$mean_flow, type = "b")
plot(flowdat$year, flowdat$date_spring_onset, type = "b")
plot(flowdat$year, flowdat$n_days, type = "b")

```

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APPENDIX C

SUPPORTING TABLE AND FIGURES FOR CHAPTER THREE

Table C.1. Stocking history in the Lamar River watershed. Fish stocked included Yellowstone cutthroat trout (YCT) and Rainbow trout (RT). Also shown is the ratio of RT relative to the total number stocked at each location. Data are from Varley 1981 and Montana Fish Wildlife and Parks.

Drainage	Stream	Years	YCT	RT	Ratio
Slough	Buffalo Fork Creek ^a	1916—1942	173,000	0	0.00
Slough	McBride Lake	1936—1941	52,600	9,180	0.15
Slough	Slough Creek	1920—1943	7,289,721	0	0.00
Slough	Abundance Lake	1990—2017	10,000	0	0.00
Buffalo Fork	Hidden Lake ^a	1920—1932	5,000	3,500	0.41
Lamar	Amethyst Creek ^a	1938—1940	249,850	0	0.00
Lamar	Cache Creek	1920—1942	670,000	0	0.00
Lamar	Calfee Creek	1940—1942	370,176	0	0.00
Lamar	Flint Creek	1922	40,000	0	0.00
Lamar	Lamar River	1920—1942	3,922,059	70,600	0.02
Lamar	Lamar Valley ^b	1923—1924	482,000	0	0.00
Lamar	Miller Creek	1929—1942	570,550	0	0.00
Lamar	Opal Creek	1922	40,000	0	0.00
Soda Butte	Amphitheater Creek	1922	99,500	0	0.00
Soda Butte	Shrimp Lake	1925—1928	57,000	0	0.00
Soda Butte	Soda Butte Creek	1920—1943	4,701,225	105,530	0.02
Soda Butte	Trout Lake ^d	1881—1955	2,022,010	67,720	0.03
Unknown	Foster Lake ^{a, c}	1931	0	2,500	1.00
Unknown	Twin Creek	1922	40000	0	0.00
Totals			13,269,370	249,850	0.02

^a Historically fishless.

^b Small streams in the Lamar Valley.

^c Location of this lake is not known, suspected to not actually be in Lamar Watershed.

^d A rainbow trout egg collection facility was operated here in the 1930s to 1950s.

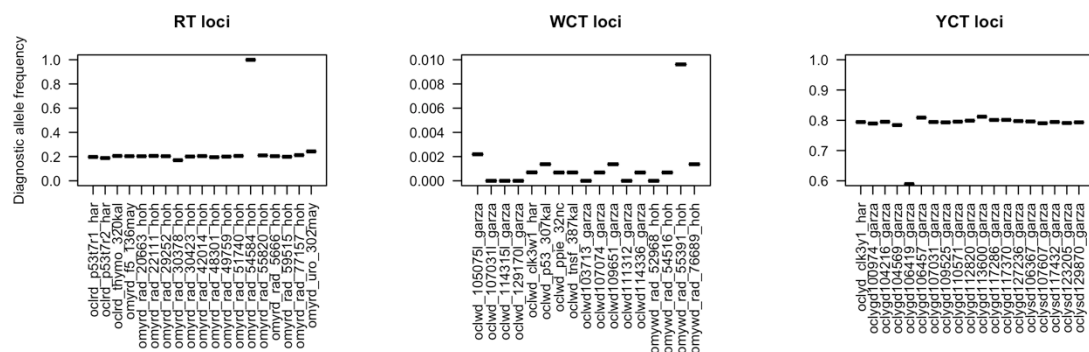


Figure C.1. Allele frequencies of diagnostic single nucleotide polymorphism (SNP) loci for rainbow trout (RT), westslope cutthroat trout (WCT), and Yellowstone cutthroat trout (YCT). A single locus was determined to be an outlier, within each marker set, and removed prior to analysis.

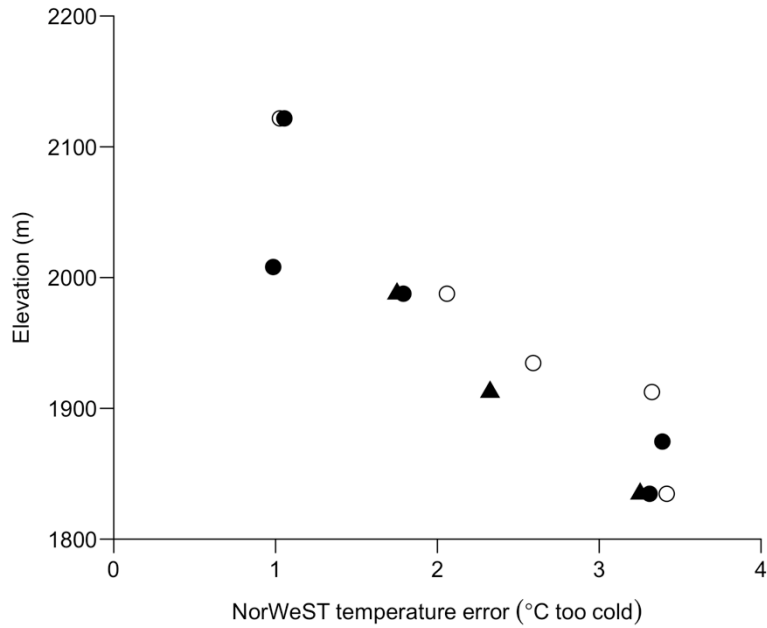


Figure C.2. Error between NorWeST model estimates of mean August temperature and measured mean August stream temperature at six sites compared to elevation. Measurements were taken in 2015 (triangles), 2016 (filled circles), and 2017 (open circles) and compared to model estimates (2002—2011 average), which were consistently too cold but were more accurate at higher elevations.

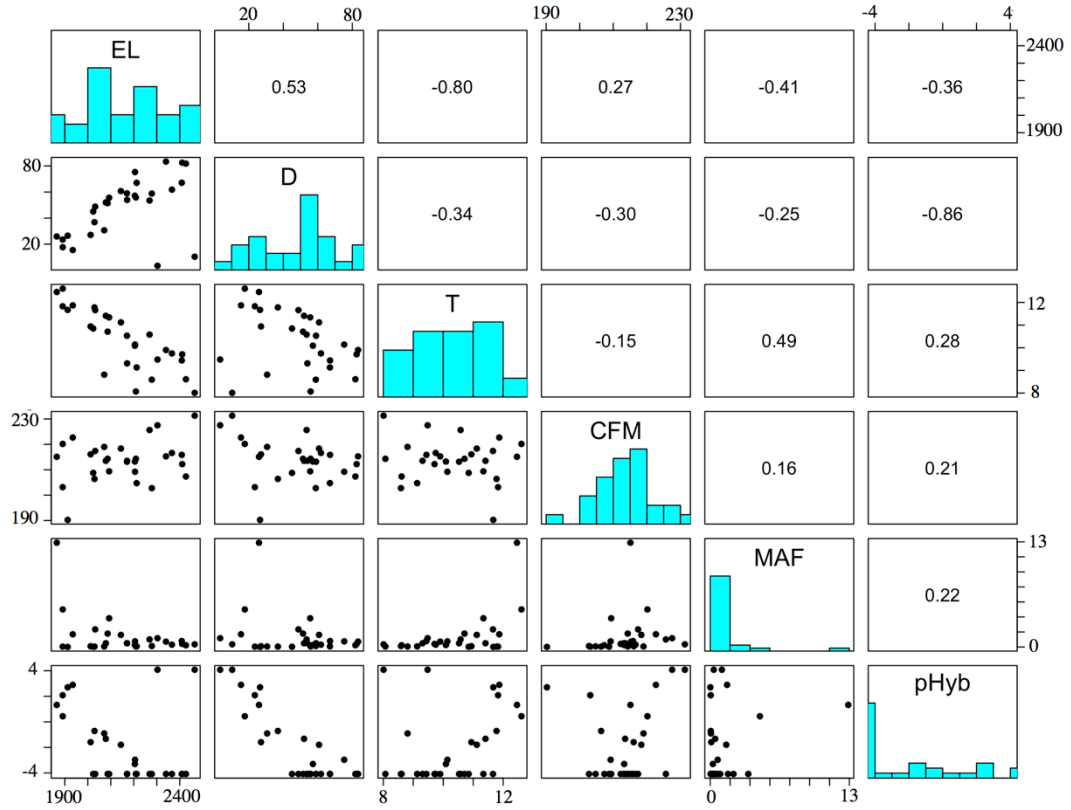


Figure C.3. Correlations between environmental variables and population hybridization for 29 sites in the Lamar River watershed. EL, elevation in meters; D, fluvial distance from Hidden Lake outlet stream in kilometers; T, modeled mean August stream temperature in °C; CFM, center timing of flow mass in day of water year beginning on Oct 1; MAF, mean annual flow in m³/s; pHyb, proportion of individuals with RT admixture in a population, logit transformed.

APPENDIX D

SUPPORTING MATERIAL FOR CHAPTER FOUR

Methods

Genetic Markers

Prior to genetic analysis we removed two markers because they appeared to be non-diagnostic. The marker *OmyRD_rad_54584_Hoh* was fixed for the non-diagnostic allele in all fish, and was therefore removed and considered non-diagnostic. The marker *OmyRD_30378_Hoh* was removed because it was the only marker suggesting eight fish from Hidden Lake and Buffalo Fork Creek were not RT, and the diagnostic allele occurred at a lower frequency (0.24) at this locus than all others (mean = 0.33, sd = 0.16, range = 0.28 – 0.35) in the dataset.

Rainbow Trout Admixture and Morphological Variation

To further investigate if morphological variation was related to the proportion of rainbow trout admixture (pRT), we used one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc test. For comparisons of pRT to quantitative variables (head spot count and relative maxilla length) we used spearman's rank correlation because of non-linear relationships, and used an alpha level of $p = 0.05$.

Ontogenetic Changes in Morphology of YCT

We visualized morphological variation of YCT in relation to fork length with boxplots.

Results

Rainbow Trout Admixture and Morphological Variation

Rainbow trout admixture varied significantly as a function of morphological classification scores for several of the tested categorical variables (Figure D.2). Significant results include cutthroat slash coloration (ANOVA, $F_{3, 93} = 55, p < 0.001$), pelvic fin tip pigmentation (ANOVA, $F_{2, 94} = 47, p < 0.001$), ventral coloration (ANOVA, $F_{2, 94} = 16, p < 0.001$), and maxilla relationship to eye (ANOVA, $F_{2, 90} = 12, p < 0.001$; note there were 4 individuals for which this variable was not recorded). Hybrids with longer relative maxilla lengths had significantly lower pRT, whereas there was no relationship between pRT and head spot counts. In all these comparisons however, there was substantial variation in pRT for a given phenotypic category, and therefore we did not attempt to fit a more complex model predicting pRT from phenotype.

Ontogenetic Changes in Morphology of YCT

When considering only YCT, cutthroat slash coloration, ventral coloration, maxilla length in relationship to eye, and relative maxilla length appeared to vary according to fish size (Figure D.3). In general, features characteristic of YCT became more pronounced as fork lengths increased. For example, cutthroat slash was strongest among large fish and faint on juveniles. This provides evidence for ontogenetic changes in morphology.

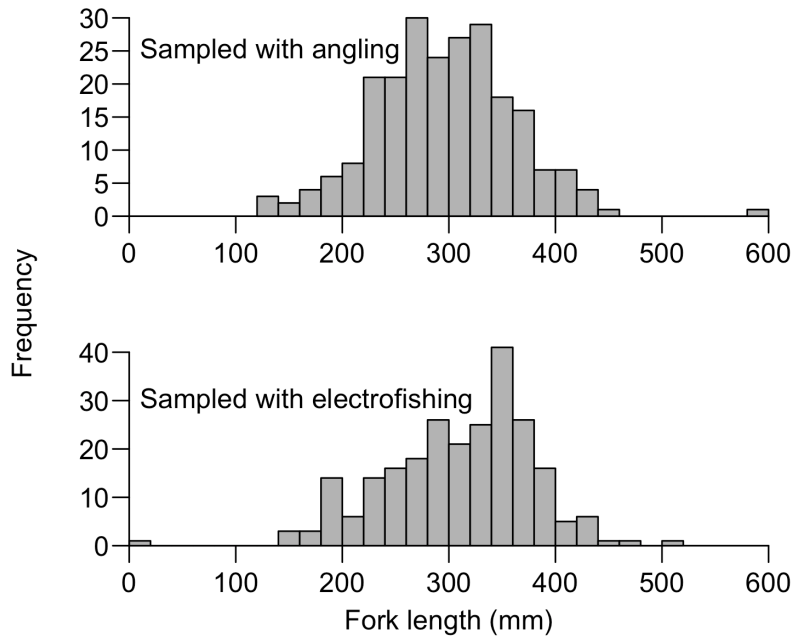
Figures

Figure D.1. Length frequency distribution of fish captured in Lamar River with angling ($n = 229$, 2015—2016) during a concurrent study (Chapters 2, 3), and length frequency distribution of fish captured with a raft electroshocker in 2016 for use in a mark-recapture population estimate ($n = 244$) in this study (Chapter 4). The similarity of the distributions suggests that our population estimate reflects a similar size distribution to fish vulnerable to angling.

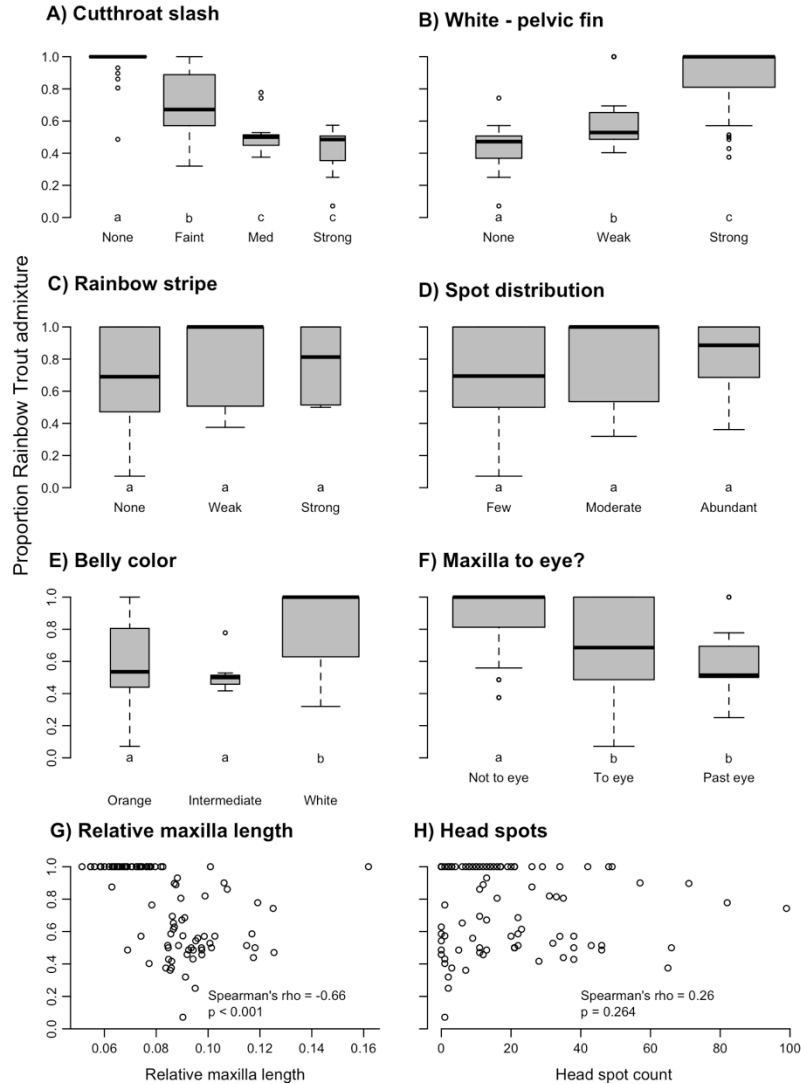


Figure D.2. Variation in rainbow trout admixture of hybrids in relationship to morphological variation. In boxplots, the horizontal line represents the median; the middle 50% of the data (25th to 75th percentiles) are within the box; whiskers indicate 1.5 times the interquartile range, values above this are shown as points. Widths of boxes are drawn according to the number of observations in that category, and different letters indicate significant differences based on ANOVAs and Tukey's HSD test ($\alpha = 0.05$).

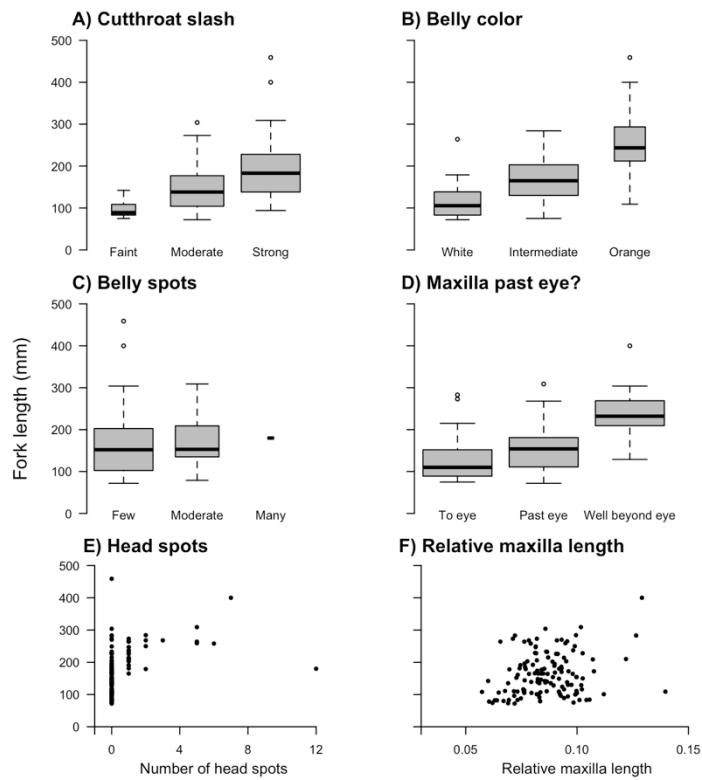


Figure D.3. Fork length distributions of Yellowstone cutthroat trout grouped by morphological categories. Features are listed in panel labels.