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MANAGEMENT AND CONSERVATION OF WESTSLOPE CUTTHROAT TROUT IN AN IMPACTED, CONNECTED RIVER SYSTEM

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MANAGEMENT AND CONSERVATION OF WESTSLOPE CUTTHROAT TROUT IN AN IMPACTED, CONNECTED RIVER SYSTEM

By

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Management and conservation of westslope cutthroat trout in an impacted, connected river system.

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Westslope cutthroat trout *Oncorhynchus clarkii lewisi* (WCT) is a native species of high conservation value that historically exhibited partially migratory behavior throughout its range. Long-term persistence of WCT is threatened by human habitat modification, fragmentation, introduction of non-native species, and hybridization. As a result of these changes, remnant populations in Montana have shifted toward resident populations in headwater systems and away from migratory populations in larger connected river networks. This is compounded by the historic introduction of rainbow trout *O. mykiss* (RBT) that hybridize with WCT, especially in larger river habitats. Rock Creek in western Montana, USA was historically managed as a world class RBT fishery. Despite intensive pressure from non-native species, non-hybridized migratory WCT still exist within Rock Creek. Understanding the drivers and mechanisms that have maintained this non-hybridized population of partially migratory WCT is of high importance to managers trying to manage populations in the face of multiple threats to their persistence.

First, we investigated the migratory life history of WCT in Rock Creek, including the diversity of behaviors within the population, potential drivers promoting it, and threats to this population. Second, we investigated dynamics of hybridization between RBT and WCT; specifically, what factors are shaping the pattern of hybridization across the landscape and what mechanisms are providing resistance to hybridization? Our results revealed more than a 20-fold variation in spawning migrations distances among individuals, with migratory fish returning to thirteen different tributaries. Migratory behavior was associated with larger spawning tributaries with higher adult biomass. Longer migrating individuals spawned lower in tributaries where there was greater overlap with RBTxWCT hybrids. Survival was low and not related to spawning or migration distance but was strongly related to summer habitat. Propagule pressure was the main mechanism driving the pattern of hybridization. Sites that were resistant to hybridization were further from the highest source of RBT, had more spatial and temporal assortative mating, and larger resident individuals. When propagule pressure is high it likely overwhelms localized resistance. Management and conservation of WCT, and similar partially migratory species, in these connected stream networks requires a basin scale approach that prioritizes connectivity among habitats, promotes natural flow regimes, and works to reduce abundances of non-native species.

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TABLE OF CONTENTS

APPENDIX B

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

APPENDIX A

APPENDIX B

CHAPTER 1

INTRODUCTION

Anthropogenic changes have driven a loss of biodiversity around the globe (Butchart et al. 2010). Particularly acute is the loss of freshwater fish species within their native ranges (Jelks et al. 2008; Reid et al. 2019). Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT) are an inland trout species whose distributions have been greatly reduced throughout much of their historic range (Shepard et al. 2005). This has resulted in a shift towards residency and reduction in migratory life history expression (McIntyre and Rieman 1995; Downs et al. 1997; Schmetterling 2001). Efforts to conserve these native trout have focused on habitat conservation, restoration, and population connectivity (Williams et al. 2011). However, conservation of a variety of life histories is a crucial aspect to ensure long term persistence in the face of multiple threats on the landscape (Rieman and Dunham 2000; Schindler et al. 2010; Moore et al. 2014).

WCT populations exhibit partial migration (McIntyre and Rieman 1995). Partial migration is a phenomenon where both resident and migratory phenotypes arise out of the same population and is controlled by a suite of factors and conditions (both environmental and genetic) that drive the switch between resident and migratory behavior (Chapman et al. 2011, 2012). Broadly, conditions that reduce growth rates (cold water, productivity, and competition) (Jonsson and Jonsson 1993; Olsson et al. 2006; Brodersen et al. 2008a; Wysujack et al. 2009) as well as increased predation risk (Brönmark et al. 2008; Skov et al. 2011) will generally result in higher rates of migratory phenotypes. Understanding what natal streams characteristics are associated with increased abundances of migratory phenotypes may help managers identify habitats or processes that maintain migratory life histories. Beyond understanding what characteristics promote migratory life histories, it is equally important to understand what threats migratory populations might face. Habitat fragmentation (Young 1995; Schmetterling 2003), non-native competition (Peterson et al. 2004; McHugh and Budy 2006; Al-Chokhachy and Sepulveda 2019), and hybridization with rainbow trout (*O. mykiss*, RBT) all threaten WCT populations persistence. The most pervasive of these threats is hybridization between RBT and WCT (Allendorf and Leary 1988; Shepard et al. 2005).

RBT are one of the most widely introduced fish species in the world (Halverson 2010) and have hybridized extensively with native cutthroat trout in the intermountain West. However, the variability in the extent and intensity of hybridization has led to a debate as to the major mechanisms driving the broad pattern of hybridization between these two species and the ultimate fate of non-hybridized WCT (McKelvey et al. 2016; Young et al. 2016b, 2017; Muhlfeld et al. 2017; Kovach et al. 2017). This debate centers on the tension between environmental factors limiting the spread of hybridization (Isaak et al. 2015; Young et al. 2016a, 2017; McKelvey et al. 2016) and dispersal of non-native alleles continuing to spread into non-hybridized populations (Boyer et al. 2008b; Kovach et al. 2015; Lowe et al. 2015; Muhlfeld et al. 2017). This debate highlights that we still lack a complete understanding of the mechanism that shape the landscape pattern of hybridization. Illuminating the mechanism that shape hybridization at a broader landscape will not only further our understanding of hybridization dynamics, but help prioritize and manage populations and habitats, as well as potential mechanisms that promote native species persistence.

The goals of Chapter 2 were to describe the diversity of migratory behaviors in WCT, identify tributary characteristics that promote migratory behavior, and evaluate the survival of and threats to this population of migratory individuals. The goals of Chapter 3 were to assess whether hybridization has increased or decreased over the last four decades, investigate if environmental factors were driving the pattern of hybridization beyond propagule pressure, and assess how microevolutionary forces (selection, dispersal, assortative mating, and genetic drift) may shape hybridization between these species. We focused on the Rock Creek watershed in western Montana, and collected a variety of data (genetic, habitat, demographic, and movement) to address these study goals. Rock Creek is an excellent study site to address these questions because it still retains a migratory population of non-hybridized WCT despite decades of exploitation and introductions of non-natives, there has been a dramatic change in RBT abundances over the last four decades, and the factors that have been debated as driving hybridization (temperature and propagule pressure) are largely decoupled in this landscape.

In Chapter 2 we investigated variation in spawning migratory behaviors among non-hybridized WCT, as well as the survival and potential threats to this life history. Radio tagged WCT spawned in 13 tributaries distributed throughout Rock Creek, but nearly 50% of tagged WCT returned to the West Fork of Rock Creek. Larger tributaries with higher resident adult biomass more commonly had migratory fish returning to spawn. Migration distances of WCT varied from as little as 4 river kilometers (RKM) to 94 RKM. Average annual survival for this population of WCT was low 24%, but still higher than reported for WCT in the nearby Clark Fork River (Mayfield et al. 2019). The majority of fish that survived past one year only spawned once indicating that skipped spawning is likely the predominate spawning behavior. Neither migration distance nor spawning were associated with survival, rather, summer habitat was the biggest influence on survival among migratory WCT. Fish that summered in the Clark Fork River had nearly a ten-fold reduction in survival compared to those that remained in Rock Creek. We did not find evidence of migration impediments or temperature related degradation of spawn/rearing habitats. Spawning overlap (both in time and space) with hybridized individuals was the most widespread threat to migratory WCT, followed by competition from non-natives (brown trout *Salmo trutta* and brook trout *Salvelinus fontinalis*) and low survival associated with summer habitat.

In Chapter 3 we explore changes in hybridization over the last four decades within Rock Creek, what environmental conditions might be related to the pattern of hybridization in the drainage, and whether there is evidence of localized resistance to hybridization. In general, there was little change in hybridization over the last four decades. Environmental factors (e.g., thermal regime, habitat characteristics such as fine sediment that are related to whirling disease), and historic stocking did not improve our model describing hybridization beyond propagule pressure alone. We saw strong evidence for assortative mating between genotypes (WCT, hybrids, and RBT) that was associated with site level resistance. Sites that were resistant to hybridization were further from the source of RBT, had larger bodied resident WCT and had spatial and temporal separation of the genotypes during spawning. While migratory life history was not directly associated with site level resistance to hybridization, resistant streams with migratory individuals also had larger residents.

This thesis highlights the importance of conserving migratory life histories as a mechanism of resiliency in these impacted but otherwise connected stream networks. As we work to balance life history diversity and protection of non-hybridized populations, it is often difficult to conserve both simultaneously. Rock Creek’s remnant population of migratory WCT may provide insights for maintaining both in a connected system. This thesis helps shed light on what factors might be important for maintaining both life history diversity and non-hybridized populations in Rock Creek and potentially other systems.

Half of our telemetered fish returned to a single tributary, while the remaining returned to 12 different tributaries. This pattern highlights not only the importance of identifying key specific tributaries for conservation, but also the importance of ensuring access to a wide variety of spawning streams for migratory fish. Hybridization at spawning sites was a threat to persistence of migratory WCT, but perhaps more overlooked is the importance of summer foraging habitat. While much progress has been made conserving cold-water refuges and spawning streams, warmer downstream habitats have been undervalued (Armstrong et al. 2021). These downstream habitats are crucial for migratory individuals as feeding, migrating, and overwintering habitat. Conservation of these downstream habitats is an indispensable component to maintain these migratory life histories.

We found no evidence of environmental factors limiting the spread of hybridization, but we also did not see hybridization spread over the last four decades. Rather a balance between propagule pressure, reduced hybrid fitness, and assortative mating appears to be maintaining the current pattern of hybridization. Even though the pattern of hybridization has not changed, it may be altered with disturbances or changes to the system such as climatic changes that alter flow regimes (Muhlfeld et al. 2017) or increasing RBT resistance to whirling disease (Miller and Vincent 2008; Granath and Vincent 2010). However, managers can actively manage to reduce propagule pressure and conserve habitats that promote assortative mating to limit hybridization. Managers are often faced with the dichotomy of isolating populations to prevent hybridization and lose life history variation, or connect populations to maintain life histories but risk hybridization (Fausch et al. 2009). Our study of WCT in Rock Creek suggests that conserving habitats that promote assortative mating could be another tool to maintain connected populations while promoting the persistence of WCT. Even though we saw evidence of localized resistance, the strength of that resistance is overwhelmed when propagule pressure is high. Ultimately, we continued to add to the body of evidence that suppression of non-natives is the single most effective management tool to limit hybridization (Al-Chokhachy et al. 2014; Kovach et al. 2017). Conservation and management of native trout in these larger connected networks must be conducted beyond the reach and tributary scale (Fausch et al. 2002) in order to promote migratory life histories that are critical to long-term persistence, as well as the ecological function and evolutionary legacy of native trout populations.

CHAPTER 2

VARIATION IN MIGRATORY LIFE HISTORY OF FLUVIAL WESTSLOPE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKII LEWISI)*: DIFFERENCES IN SPAWNING, SURVIVAL, THREATS, AND IMPLICATIONS FOR CONSERVATION.

Abstract

Human mediated reduction of freshwater fish distributions and abundances is driving a loss of inter and intraspecific diversity. Particularly acute is the loss of migratory life histories in riverine fishes due to habitat degradation. Not only is it important to identify causes of decline in the migratory life history, but also what conditions are maintaining life history diversity despite mounting threats. We used radio telemetry of 161 individuals to characterize phenotypic variation within the migratory life history of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT) in Rock Creek, Montana, USA from 2018-2020. We investigated variation in spawning migration distance and its relationship to spawning location, what characteristics of spawning tributaries were associated with migratory WCT, survival variation within the migratory WCT population, and assessed potential threats to migratory WCT. Individual spawning migrations varied from 4 km to 94 km; those that migrated longer distances tended to spawn lower in tributaries. Tributaries with higher discharge at base flow and higher adult (>150 mm total length) biomass were associated with an increased migratory component. Survival was not correlated with distance migrated or spawning but varied ten-fold depending on summer habitat use. Barriers and fragmentation did not impede WCT migrations in Rock Creek, nor were summer stream temperatures in natal streams predicted to exceed lethal levels within 50-year projections. Rather, hybridization with rainbow trout and WCT hybrids (*O. mykiss x O. clarkii lewisi*) is likely the greatest threat to persistence of WCT migratory life history due to high

overlap between migratory and resident individuals at spawning sites. Understanding the remaining diversity within migratory life histories and characteristics that maintain that diversity in highly impacted systems is critical for informing and prioritizing management actions in a changing world.

Introduction

Anthropogenic-driven changes to freshwater systems are the number one threat to freshwater biodiversity (Reid et al. 2019). Since 1970, populations of freshwater vertebrates have declined at more than twice the rate of marine or terrestrial populations (McRae et al. 2017). Globally, populations of large freshwater fish have declined 94% over the same time period (He et al. 2019). In North America, nearly 30% of Pacific salmon populations (Gustafson et al. 2007) and 90% of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) (Shepard et al. 2005) have been lost from their historic range. Fragmented habitats and the loss of high quality habitats reduce native fish species abundances and distribution (Jelks et al. 2008). Concurrently, there has been a loss of life history diversity that can influence a species' resilience to exploitation, disturbance, and their persistence in a changing landscape (Gamfeldt and Källström 2007; Haak and Williams 2012).

An individual fish's life history is multi-dimensional and includes factors such as age of maturity, reproductive strategies, spawning habitat types, and migration patterns. A full suite of life histories and access to diverse habitats improves a population's long-term stability (Neville et al. 2006; Schindler et al. 2010; Hellmair and Kinziger 2014; Waldman et al. 2016). The portfolio effect highlighted in the Bristol Bay salmon fishery (Schindler et al. 2010) demonstrates that populations that contained multiple age cohorts and variation in timing and location of spawning led to lower variation in abundances and resilience to disturbance. Similarly, bull trout (*Salvelinus confluentus*) meta-populations were buffered by the portfolio effect despite declining individual populations (Kovach et al. 2018b). Understanding the diversity of life history, its drivers, and potential threats are critical for conservation efforts in a changing landscape.

Salmonids display a diversity of life histories within and among species (Jonsson et al. 2019). One important aspect of life history for inland trout is migration patterns. Several salmonid species exhibit partial migration which categorizes individuals into residents and migrants (Jonsson and Jonsson 1993; Chapman et al. 2011, 2012). While both life history types spawn in tributaries, migratory individuals emigrate from their natal streams and move into larger habitats to forage, grow, and overwinter. The loss of migratory individuals in fish populations is well documented in fragmented (Ruhlé 1996; Morita et al. 2009) or over-exploited systems (Huckins et al. 2008; Scribner et al. 2012). Furthermore, the prevalence of migratory life histories in inland trout appear to be declining in unfragmented systems as well (Nelson et al. 2002; Nyce et al. 2013).

Understanding variation in the migratory life history of inland trout and what natal stream conditions promote variation in migratory life histories could not only help expand current knowledge regarding partial migration but would also help identify conservation areas in our efforts to maintain life history diversity. Partial migration is driven by both genetic and environmental factors and maintained by frequency dependent selection (i.e., smaller resident fish have lower reproductive potential, but higher survival than larger migratory fish) (Ohms et al. 2014; Gillanders et al. 2015). Migratory life histories have been shown to have moderate to high heritability in several salmonids such as rainbow trout (Hecht et al. 2015; Pearse et al. 2019; Arostegui et al. 2019) and brown trout (Lemopoulos et al. 2018; Ferguson et al. 2019). Additionally, environmental characteristics of spawning and rearing tributaries have been associated with the production of migratory fish. For example, Olsson & Greenberg (2004) found that brown trout (*Salmo trutta*) in high density, low growth stream sections were more likely to migrate than individuals in low density, high growth sections. Slower growth rates due to low food availability or colder temperatures tend to result in more migratory individuals (Olsson et al. 2006; Brodersen et al. 2008b; Wysujack et al. 2009; Crossin et al. 2016). Therefore, tributaries with environmental conditions that are expected to have lower growth rates (colder, less productive, and/or higher densities) are expected to produce more migratory individuals.

Westslope cutthroat trout is a partially migratory inland trout species; many of populations have lost much of their variation in migratory life history (McIntyre and Rieman 1995; Downs et al. 1997; Schmetterling 2001). It is estimated that non-hybridized westslope cutthroat trout (WCT) only exist in roughly 10% of their historic range, much of which is comprised of resident populations in headwater systems (Shepard et al. 2005). As a species of concern across ID, MT, WA (USA) and a listed species in AB (CAN), conserving the full range of life histories is a key conservation goal (Montana Fish, Wildlife and Parks 2007). While isolation through barriers has helped protect some WCT populations from negative interactions with non-native species (Budy and Gaeta 2018; Al-Chokhachy and Sepulveda 2019) including hybridization with non-native rainbow trout (*O. mykiss*; RBT) (Allendorf and Leary 1988; Boyer et al. 2008a; Muhlfeld et al. 2009b, 2017), habitat fragmentation associated with dams, irrigation ditches, and other barriers (Young 1995; Schmetterling 2003; Ardren and Bernall 2017; Mayfield et al. 2019) is a threat to migratory life expression. In order to conserve migratory WCT, we need to consider how these threats overlap with WCT habitat use and survival to effectively mitigate threats to existing migratory life history variation. For example, if long migrating WCT spawn lower in tributaries than resident or short migrating WCT (similar to patterns seen in steelhead studies (McMillan et al. 2007; Buehrens et al. 2013)), this would place them at higher risk of spatial spawning overlap with RBT or hybrid trout (*O. mykiss x O. clarkii* *lewisi*, RBTxWCT) that typically spawn in mainstems and lower sections of tributaries (Muhlfeld et al. 2009b).

We used radio telemetry to investigate migratory behavior of WCT in Rock Creek, MT, USA, and identify potential drivers and threats to persistence of this life history. Specifically, the first objective of our study was to (1) describe variation in migration distances among individuals and how this variation may influence timing and location of spawning, and (2) determine what stream characteristics were associated with migratory WCT. We tested the hypotheses that tributaries with higher trout densities, colder temperatures, and less overwinter habitat would be related to more migratory fish. The third objective (3) was to examine the relative influence of migration distance, whether a fish spawned, and summer habitat use on survival. Finally, the last objective (4) was to identify threats to maintaining the diversity of migratory behavior currently exhibited. We currently lack an understanding of migratory life histories in inland trout species nor have the theoretical drivers of factors driving life history variation been evaluated in these systems. This study highlights the variation in migratory phenotypes, evaluates potential environmental drivers for migratory behavior, and assesses threats to migratory behavior in a heavily utilized landscape to help managers work to conserve the full range of life histories for westslope cutthroat trout.

Materials and Methods

Study Site

Rock Creek is a 5th order river system in the headwaters of the Columbia River drainage near Missoula, Montana, USA (Fig. 2.1). The river flows 83 km from the confluence of the West Fork and Middle Fork of Rock Creek to the mouth where it empties into the Clark Fork River. The watershed encompasses 1,425 km^2 and is characterized by confined valley channels. Discharge is largely unregulated with only one dam on the East Fork of Rock Creek. There is a mixture of private, state, and federal ownership along with a variety of land use practices. Rock Creek is a blue-ribbon trout fishery and one of the most heavily fished waters in the state (MFWP 2019). Historically, the fishery was comprised of native WCT and bull trout. Like most major streams in Montana, Rock Creek was stocked with RBT until 1974 when stocking of rivers was halted. Until the early 1990's, RBT were the primary game species in the system averaging over 800 fish per mile (Liermann 2017). The arrival of whirling disease (*Myxobolus cerebralis*) in 1990's decimated the RBT population (MFWP 2019) and the fish community has shifted to mostly brown trout in the lower river and WCT in the upper river and tributaries; RBTxWCT are present throughout the system (MFWP 2021).

Capture and tagging

Montana Fish, Wildlife and Parks (MFWP) captured 80 WCT from 2018-2020 during the month of April, 81 RBTxWCT in 2019-2021 (50 in the fall and 31 in the spring), and 29 RBT (20 in the fall and 9 in the spring) (Table A.1). Fish were captured with a boat mounted electrofishing unit throughout the Rock Creek watershed and its confluence with the Clark Fork River (Fig. 2.1). We selected WCT greater than 330 mm total length (TL) for radio tagging to ensure that the WCT tagged were likely to be mature. WCT selected for tagging were anesthetized with MS-222 (tricane methanesulfonate) prior to surgery and measured for total length (mm) and weighed (g). A small fin clip from the anal fin was collected for genetic analysis. MFWP surgically implanted radio transmitters (Lotek Wireless Inc.; model MCFT2-3BM, St. John's Newfoundland, Canada) using a shielded needle technique (Ross and Kleiner 1982). Staples were used to close the surgical opening. Fish were allowed to recover from the anesthetic and then were released near where they were captured. By 2020, we had 5 frequencies and each transmitter had a unique factory programmed code to identify individuals. Expected battery life was approximately 550 days. Radio tags were equipped with a mortality indicator which would activate after 24 hours of the tag being motionless. Genetic samples collected from radio tagged individuals were analyzed for proportion of RBT admixture (pRBT) at the Montana Conservation Genetics Lab Missoula, MT. We used 39 diagnostic single nucleotide polymorphic loci that differentiate rainbow trout from westslope cutthroat trout (as described in Muhlfeld et al. 2016). We also used an additional 20 diagnostic loci that differentiate Yellowstone cutthroat trout *O. clarkii bouvieri* from WCT to quantify potential Yellowstone cutthroat trout ancestry.

Telemetry

Tagged fish were relocated either by vehicle mounted radial antenna or a handheld Yagi directional antenna and receiver (model SRX-400, Lotek Inc.) over the course of the spring, summer, and fall. We attempted to relocate fish every other day from May 1st through July 15th to identify spawning timing and location and relocated fish one or more times weekly from March to April, and from July through November, to gain information on habitat use and survival throughout the year. We estimated the spawning season as the time period two standard deviations from the mean spawning date of this study. We recorded GPS coordinates for every relocation event and coordinates were matched to nearest 150 m (0.1 mile) river mile using MFWP GIS data to calculate distance moved between each relocation. Individuals that died within three weeks of tagging may have died due to handing or tagging procedures and were not included this analysis.

To investigate how migratory distance was associated with spawn timing and location, we needed to determine spawning events. It is difficult to visually confirm spawning in spring spawning fishes due to high discharge and turbidity. We identified spawning events by either fish making a movement into a tributary or if they moved more than 15 km upstream during the spawning season within Rock Creek. As with other studies (Henderson et al. 2000; Downing et al. 2002; Muhlfeld et al. 2009b; Homel et al. 2015), we identified spawning location as the most upstream point following upstream movement and we estimated spawning date as the median day between the first and last relocation at the most upstream point observed.

Tributary characteristics

To evaluate whether tributary characteristics were associated with WCT migratory life history, we collected fish population and habitat data at 52 sites across 37 streams within the Rock Creek drainage and one site in the nearby Clark Fork River in 2019 and 2020 (Fig. 2.1). We used single pass backpack electrofishing (Smith-Root LR24) to gather information on fish population, community composition, and collected genetic samples from *Oncorhynchus spp.* at each site during this sampling. Habitat data were collected in September and October of 2020 at every electrofishing site. We tested the hypotheses that tributaries with higher trout densities, colder temperatures, and less overwinter habitat would be related to a more persistent migratory component. To test the hypothesis that colder stream temperatures would increase the number of migratory fish, we measured mean August stream temperatures as well as estimated the number of growing degree days at each site. To test whether low productivity streams were associated with increased number of migratory fish, we measured distance from the site to the confluence of Rock Creek and the elevation of the site. We expect that sites that are higher elevation and more upstream (i.e., headwater systems) are generally less productive. To test the hypothesis that less overwinter habitat would increase the number of migratory fish, we measured discharge at base-flow (m^3s^{-1}) (Hatch FH950), stream bed substrate composition, stream gradient, large woody debris, and number of pools (> 0.5 m). We expected higher discharge at base flow, a greater number of deep pools, more interstitial space, and lower stream gradient to be associated with more overwintering habitat. Additionally, we estimated both juvenile (< 150 mm TL) and adult (> 150 mm TL) biomass per m^2 and density for brook trout at every site to test the hypothesis of higher trout densities leading to more migratory fish. We placed temperature loggers (Onset Computer Corp.) near the confluences of streams to record water temperatures every 30 minutes throughout the study (2019-2021) at 48 sites across the same 37 streams where we collected habitat and fish data. We used temperature data to estimate growing degree days as a measure of stream productivity and daily mean temperature at spawning.

Threats

Migratory WCT face many human-induced threats including habitat fragmentation, climate change, non-native species, and hybridization with rainbow trout. We compiled various data sources to describe these threats within Rock Creek. Migration impediments were assessed with telemetry data and identified based on whether migrations paused at known irrigation diversions, fish passage was not possible (i.e., East Fork Reservoir and dam), or if fish were entrained and died in irrigation diversions. We assessed the threat of warming stream temperatures based on whether streams with migratory fish were predicted to stay below 20°C (ultimate upper incipient lethal temperature for WCT, (Bear et al. 2007)) based future scenarios of mean August temperature predicted by the NorWest model (Isaak et al. 2017). Finally, we examined the threat of the presence of non-native species in tributaries used by migratory fish. We used our fish sampling data to compare relative abundances of brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* among migratory and non-migratory streams. We also examined pRBT of the resident population near migratory WCT spawning sites to understand potential hybridization risk.

Data Analysis

To investigate phenotypic variation within the migratory life history of WCT (Objective 1), we used geo-referenced radio telemetry relocations to estimate movement rates, spawning locations, and spawn timing. To understand spatial and temporal differences in spawning between trout coming from the Clark Fork River and near the confluence of Rock Creek versus higher in the mainstem of Rock Creek, we fitted a generalized linear regression (Gamma distribution with a log link function) of mainstem migration distance versus mean daily temperature and relative tributary distance spawning site as predictor variables. Because migration and spawn timing in salmonids is strongly cued by water temperature (Northcote 1984; Jonsson 1991; Schmetterling 2001; Jonsson and Jonsson 2009) and we wanted to assess spawning time overlap across different tributaries and years, we used mean daily tributary temperature at the estimated time of spawning rather than calendar date. For spawning location, we standardized how far up a tributary fish spawned (given tributaries are different lengths) by calculating the percentile of how far a fish traveled up a tributary to spawn relative to the overall length of the tributary (e.g., lower 25th percentile). Because we did not know where WCT would spawn during the first study year, we did not have temperature probes deployed in 2018. To estimate mean daily water temperatures during the spawning season for tributaries in 2018, we regressed 2019, 2020 and 2021 water temperatures in the tributaries against the water temperatures at the mouth of Rock Creek (USGS station 12334510). We had high agreement for all streams that were used to estimate 2018 spawning temperatures ($R^2 = 0.70 – 0.95$). All statistical analyses were performed in R 3.6.0 (R Core Team 2019).

To investigate what tributary characteristics were associated with migratory life history (Objective 2), we used a linear discriminant analysis (MASS package; Venables and Ripley 2002) to explore whether differences in our migratory categories could be explained by habitat variables (Table 2.2). To describe the migratory component of each tributary, we categorized each stream as either: persistent, where we observed telemetered fish return in multiple years; periodic, where we observed telemetered fish return in only one year; or rare, where we did not observe any telemetered fish return. We checked for correlation among predictor variables and removed any predictors with correlation greater than 0.70. We transformed predictor variables when they violated normality assumptions of error distribution (Table 2.2). We split our data set (n=52) into training (70%) and testing (30%) datasets and used leave-one-out cross validation to assess the predictive accuracy of the model. We also used analysis of variance and Tukey's Honest Significant Difference to test for significant habitat differences between each migratory category.

We used a Cox proportional hazards model (Eq. 1) (Cox 1972; Pollock et al. 1989) with the survival package in R (Therneau and Grambsch 2000) to assess the relative influence of migration distance, whether a fish spawned, post-spawning habitat use on survival (Objective 3). We then derived estimated annual survival curves from the Cox model using the rms package (Harrell Jr. 2021).

$$\textbf{Eq 1}.\ h(t|z) = h_0(t)\exp(\beta_0)$$

Where $h(t|z)$ is the hazard for an individual at time t, $h_0(t)$ is the baseline hazard, and β_0 is the coefficient of the variables.

Mortality was assumed to have occurred during the week where we received a mortality signal from an individual's transmitter and was confirmed either by tag recovery or expert judgement (e.g., tag relocated in an eagle nest). In this model fish either died, were censored, or survived until tag failure. Fish that were never relocated again were censored out of the model at their last confirmed relocation. We used a Cox proportional hazards model to estimate the effect of year (Apr. 1 – Mar. 31), mainstem migration distance (km), spawning, and summer habitat. Summer habitat was estimated as the mean river kilometer location during Jun. – Aug. We then split the study area into three summer habitat sections: Clark Fork River, lower Rock Creek, and upper Rock Creek. We created candidate models using the dredge function in the MuMIn package (Barton 2020) and selected the best supported model based off an AIC_C and Evidence Ratio (AIC weight of the best model/AIC model).

Threat Matrix

We created a threat matrix to compare and evaluate potential threats (fragmentation, climate change, non-native species, hybridization, habitat degradation) to migratory WCT (Objective 4). To consider how these threats would influence the diversity of migration behaviors, we compared these threats categorized by migration distances (short < 50 km and long > 50 km). To assess habitat fragmentation, we used radio telemetry data to find the percentage of tagged WCT that were entrained in irrigation ditches and the percentage of tagged trout whose spawning migration was impeded based on decrease in weekly movement rates. Water temperatures beyond the thermal optimum can limit growth, reduce competitiveness, and promote non-native species expansion. We did not observe excessively warm stream temperatures or stream dewatering. Optimal temperatures for WCT are around 12-15°C (Bear et al. 2007) but some evidence suggests that they may be tolerant of even warmer temperatures (Macnaughton et al. 2021). We assessed the threat of warming summer temperatures associated with climate change by evaluating the number of streams with migratory components with current mean August temperatures greater than 20°C and those predicted to exceed 20°C mean

August temperature by 2080 based on the NorWest model (Isaak et al. 2017). We assessed the threat of non-native presence as the percentage of streams where brook trout and brown trout were detected. We assessed the threat of hybridization with rainbow trout as (1) the percentage of streams with greater than 1% site pRBT, and (2) the proportion of streams where we observed spatial and temporal overlap between hybrids and WCT spawning. More broadly, we assessed the threat of spatial and temporal overlap at the basin level using generalized linear models to explore the relationship of pRBT on spawning location and temperature at spawning.

We modeled tributary percentile (spatial overlap) and mean daily temperature at spawning (temporal overlap) as functions of migration distance and individual pRBT. For our model of tributary percentile, we fitted a Gamma distribution with a logit link, and a Gaussian distribution with an identity link for the mean daily tributary temperature model. To assess impact of habitat on survival estimated survival for summer habitat using the methods for objective three and the percentage of individuals (spawning and non-spawning) exposed to lower survival due to summer habitat conditions. We then summarized all the major threats by categorizing them as either: low, medium, or high risk. A low risk level was if the threat influenced less than 10% of the tagged fish and fewer than 2 tributaries, medium if less than 50% tagged fish and/or less than 9 tributaries were influenced, and high if more than 50% of tagged fish and/or 9 or more tributaries were influenced by the threat.

Results

Variation in migratory behavior

Telemetered WCT ranged from 334 to 450 mm TL (mean = 382 mm, SD = 25.7 mm) and weights ranged from 360 to 1025 g (mean = 571 g, SD = 140 g). We obtained 2,957 unique telemetry fixes of 80 individual WCT from April 5, 2018 – April 1, 2021. Individual fish were relocated an average of 34 times. We removed 2 individuals from the data set because they expired within three weeks of tagging. Of the 78 individuals we tracked, 52 made upstream movements during the spawning season. Forty-six WCT moved into tributaries of Rock Creek (one moved into a nearby tributary of the Clark Fork River). Annual upstream movement distance for all WCT (n= 78) ranged from 0.0 km to 93.9 km (mean 21.9 km, SD = 27.8) (Fig. 2A). Spawning migration distances (n = 52) ranged from 4.4 km to 93.9 (mean = 36.0 km ± 30.2) (Fig. 2.2B). Average weekly upstream movement rates during the spawning period averaged 5.3 km/week ± 5.0. Migration distance was significantly and positively correlated with weekly movement rates (p-value < 0.001, $R^2 = 0.49$).

We observed 46 spawning movements into tributaries (Table 2.1) during the spawning season. Three WCT moved into tributaries post-spawning season and were considering non-spawning movements. Six individuals made upstream movements greater than 15 km within the mainstem of Rock Creek during the spawning season. The remaining (26) WCT either never made spawning movements or expired prior to the spawning season. Spawning tributaries were distributed throughout the drainage (Fig. A.1). We also observed a high rate of skipped spawning by WCT. Of the 25 WCT that were alive for two spawning seasons, only a single fish spawned in consecutive years. WCT spawning peaked around the first week of June (mean = June 6th, SD =16 days). Mortality peaked about four weeks after the peak of spawning (mean = July 6th, SD = 54 days).

Typically, fish that migrated different distances (short and long) returned to the same tributaries (Table 2.1). For example, the West Fork tributary had a mean migration distance of 40 km with a standard deviation of 32 km. However, we observed only longer distance migrators (>

60 km) returning to Cowan Gulch (mean = 75.7 km ± 3.2) and mostly shorter migrators return to Stony Creek (mean = 7.9 km ± 5.5). Fish spawned at different temperatures but mean daily stream temperature of the stream at spawning was not significantly related to migration distance ($p = 0.20$). Migration distance was negatively correlated to tributary percentile ($p = 0.03$, $\beta = -2.18$, SE = 0.99). Individuals that migrated over 60 km spawned in the lower 30th percentile of the tributary (lower, closer to the tributary confluence with Rock Creek), whereas individuals that migrated less than 20 km spawned across the length of the tributary up to the 60th percentile.

Habitat characteristics of migratory spawning tributaries

We identified 13 spawning tributaries for migratory WCT in Rock Creek (Table 2.1). We classified six of the 13 tributaries as being tributaries with a persistent migratory behavior where telemetered WCT returned at least two of the three years. We observed telemetered WCT return to the other six tributaries in one of the three years. Approximately half of spawning telemetered WCT returned to the West Fork of Rock Creek.

We measured 11 variables (Table 2.2) at the tributary level to evaluate our hypotheses about what biotic or abiotic characteristics might relate to a greater WCT migratory component of the population. Of all the variables we measured, only discharge at base flow and adult biomass were significantly associated with the migratory categories (Table 2.2). Based on a Tukey's test, base flow discharge was significantly different between tributaries in the persistent and periodic categories ($p = 0.03$) and tributaries in the persistent and rare categories ($p = 0.001$), but not tributaries in the periodic and rare categories ($p = 0.88$). While the global ANOVA for adult biomass was not significant ($p = 0.59$), Tukey's test showed significant difference between persistent and rare tributaries ($p = 0.04$).

While there was no significant difference between landscape position and migratory category ($p = 0.51$), tributaries in the rare category were distributed throughout the watershed (15.6 – 112.0 km, mean = 64.1± 32.5 km from mouth), whereas tributaries in the persistent category tended to occur more upstream in the watershed (50.1 – 83.9 km, mean = 74.0 ± 14.2 km) and tributaries in the periodic category occurred more toward the lower end of the drainage (6.8 – 72.5 km, mean = 34.6 ± 25.2 km).

We used linear discriminant function to further explore if our migratory categories could be separated based on our predictor variables in a multivariate fashion (Fig. 2.3). Our training data set (70% of the total data) had a prediction accuracy of 92% (95% CI = 75%, 99%). Meaning that 92% of the time the model accurately categorized tributaries as either rare, periodic, or persistent. For the test data set to validate the model, the model had an overall predictive accuracy of 55 % (95% CI = 23%, 83%). Using leave-one-out cross-validation, the model accuracy for the entire data set was 51%.

Survival of different migratory distances

We observed 65 mortalities of WCT during the study. The remaining 13 individuals were censored from the analysis due to tag expiration or they disappeared. We fitted a Cox proportional hazards model to examine what variables may influence WCT survival in Rock Creek and to estimate annual survival. There was minimal difference between the top four models based on AIC_C, ($\Delta AIC_C < 2$) and Evidence Ratio ($ER < 2$). Only model year and summer habitat were included in all candidate models. Schoenfeld’s residuals test showed that the proportional hazards assumption of the model was met ($p = 0.64$, $\chi^2_4 = 2.53$,). Annual estimated survival for WCT was 0.24 (95% CI 0.08, 0.39). Probability of survival declined the most during the spawn and post-spawn seasons (Fig. 2.4A). We observed significant differences in survival among summer habitats. No WCT that spent the summer (Jun. – Aug.) in the Clark Fork River survived past September. There was a no significant difference in survival for WCT that summered in upper Rock Creek (0.25, 95% CI = 0.16, 0.41) and those that summered in lower Rock Creek (0.43, 95% CI = 0.27, 0.67). Survival was significantly lower for WCT that summered in the Clark Fork River (0.03, 95% CI = 0.02, 0.05) compared to those that remained in Rock Creek.

Hazard was higher (as indicated by a hazard ratio greater than one) in 2019 (p = 0.03, HR = 2.13, 95% CI = 1.06, 4.27) and 2020 (p = 0.006, HR = 3.78, 95% CI = 1.77, 8.04) compared to 2018. WCT that remained in lower Rock Creek (p = 0.004, HR = 0.30, 95% CI = 0.13, 0.67) or upper Rock Creek (p = 0.007, HR = 0.35, 95% CI = 0.16, 0.75) during the summer had reduced hazard compared to fish that summered in the Clark Fork River. There was no significant association between migration distance and survival (p = 0.09, HR = 0.99, 95% = 0.98, 1.00) nor spawning and survival (p = 0.14, HR = 1.60, 95% CI = 0.86, 2.98).

Threats to migratory life history

We did not observe substantial entrainment of migratory WCT in irrigation ditches or fish encountering barriers during their upstream migration, nor delays in migrations as measured by reduced weekly movement rates near known irrigation diversions (Table 2.3). No WCT were observed in East Fork of Rock Creek, so East Fork Reservoir dam does not appear to be a barrier to WCT migration. Two WCT were detected in irrigation ditches post-spawning but returned to the mainstem of Rock Creek before the end of the irrigation season; both fish spawned in the West Fork of Rock Creek.

There was no evidence that climate change poses an immediate or long-term risk to migratory WCT in Rock Creek. Mean August stream temperature for WCT migratory tributaries was 10.8°C (8.3 -14.4°C, SD = 1.7). The highest observed August mean daily temperature was 17.4°C. The 2080 predictions of mean August stream temperatures from the NorWest model for streams with migratory WCT in Rock Creek did not exceed 20°C.

Brook trout and brown trout were present in 26 of the 37 streams that were sampled. We detected brook trout in 19 streams and brown trout in 20. Relative densities of brook trout were lower for persistent migratory tributaries (mean = 0.02 fish/m^2, 95% CI = 0.00, 0.05) and periodic migratory tributaries (mean = 0.01 fish/m^2, 95% CI = 0.00, 0.02) than rare migratory streams (mean = 0.10 fish/m^2, 95% CI = 0.01, 0.20). However, there was no statistically significant difference between migratory categories. Brown trout were detected in 75% of migratory tributaries. There was no significant difference nor any directional trend in brown trout densities across migratory components.

We observed migratory WCT spawning at or near sites where we detected RBT alleles in the resident population and/or observed telemetered RBTxWCT spawning. The proportion of RBT admixture (pRBT) across all 52 sites ranged from 0.0 to 0.73 (mean = 0.16, 95% CI = 0.09, 0.23). For migratory WCT tributaries (n=12), average pRBT was nearly half as much as all sites combined (mean = 0.08, 95% CI = 0.01, 0.15). While there was no significant difference in site pRBT or proportion of individuals with RBT alleles associated with migratory category, persistent migratory tributaries had lower site pRBT (0.05, 95% CI = 0.00, 0.10) and proportion of individuals with RBT alleles (0.23 ± 0.19 SD) than sites in periodic (0.11, 95% CI = 0.00, 0.25) (0.36 ± 0.33 SD) and rare tributaries (0.19, 95% CI = 0.10, 0.29) (0.42 ± 0.39 SD).

We relocated 19 RBTxWCT individuals spawning in seven of the twelve WCT migratory streams during the spawning season. To assess potential temporal and/or spatial overlap between migratory WCT and RBTxWCT, indicating a higher risk of hybridization, we fitted the same generalized linear model used before but included pRBT as a variable and found that migration distance ($p = 0.007$, $\beta = -0.01$, SE = 0.004) was still significantly, negatively associated with spawning higher in the tributary. pRBT was also significantly associated with spawning lower in a tributary ($p = 0.009$, $\beta = -1.53$, SE = 0.57), indicating that individuals with higher pRBT were spatially and temporally separated during spawning from non-hybridized WCT. A generalized linear model found no significant relationship between temperature at spawning and migration distance ($p = 0.369$), but a significant, negative effect of stream temperature during spawning and individual pRBT ($p = 0.004$, $\beta = -3.98$, SE = 1.34). Meaning that fish spawned at the same temperature regardless of migration distance, but individuals with more RBT ancestry spawned at cooler water temperatures.

As described above, individuals that summered in the Clark Fork River had increased mortality. Only 9% of the population of short migration distances summered in the Clark Fork River. Of the eight WCT that summered in the Clark Fork River, three spawned in tributaries which were located in the lower and middle sections of Rock Creek. All long-distance migrators spent their summer in Rock Creek. Ultimately, the greatest threats to non-hybridized WCT in Rock Creek is the continued presence of hybridized individuals and the expansion of brown trout throughout the watershed.

Discussion

Our study is one of the first to combine radio telemetry data, habitat data, and fish population data within a single river basin to examine the diversity of behavior within migratory WCT, natal tributary characteristics, and threats to migratory WCT. Migratory WCT demonstrated a range of migration distances across a variety of spawning locations. Migratory life history was associated with larger natal tributaries with higher biomass of resident fish. Average annual survival was generally low; 60% of the mortality occurred in summer and was not driven by spawning or migration distance. The greatest threats to persistence of migratory WCT are risk of hybridization, summer habitat quality, and non-native exotics. Conserving the variation within migratory WCT life history expression will require focusing conservation on tributaries that are important natal sites for these longer migrating fish and improving habitat at multiple scales beyond the natal tributaries.

Spawning differences associated with migration distances

Several studies have described migratory life histories of *Oncorhynchus spp.* populations with access to lacustrine habitat (Meka et al. 2003; Muhlfeld et al. 2009b; Homel et al. 2015; Ertel et al. 2017). However, few studies (Henderson et al. 2000; Schmetterling 2001, 2003) have described phenotypic variation in migration distances for fluvial fish within a population. Shorter distance migrations were more common than long distance migrations, which has been shown in anadromous salmonid populations (Kristoffersen et al. 1994). These short migrators may have an advantage in terms of migratory trade-offs. Generally there is a higher energetic cost to increased migration distance resulting in size differences (Jonsson and Jonsson 2006), and reproductive trade-offs (Kinnison et al. 2001; Crossin et al. 2004). Contrary to Schmetterling (2001) who observed migration distance increase with body size, there was no correlation between fish total length and migration distance in our study. This suggests that short migrating fish have similar reproductive advantages of longer migrating individuals. Nor were we able to detect any relationship between migration distance and survival for WCT, but the smaller spatial habitat requirements of short migrators may be beneficial for WCT that spawned in the upper Rock Creek tributaries. However, the few short distance migrators that spawned in the lower tributaries of Rock Creek fish and summered in the Clark Fork River had very low survival. Overall, short distance migrators tracked in the upper and mid Rock Creek had roughly similar fecundity as long-distance migrators, but some had increased survival due to less exposure to degraded summer habitats.

There was not a significant relationship between migration distance and temperature at spawning, however, longer migrating WCT tended to spawn lower in tributaries, while WCT that migrated shorter distances spawned throughout tributaries. Studies of WCT spawning migrations in other fluvial systems show that WCT migrate on the ascending limb of the hydrograph and have a diverse range of spawning locations (Schmetterling 2001; Muhlfeld et al. 2009b; Corsi et al. 2013b). Muhlfeld et al. (2009) showed that water temperature at the start of migration was the same for WCT, RBTxWCT, and RBT, despite WCT migrating much further to reach their spawning sites. This suggests that for the migratory population of WCT cues to initiate spawning migrations and water temperatures at the time of spawning are consistent across individuals. This then would suggest that longer migrators have higher movement rates during the spawning season which is what we observed in our study.

Only 4% of WCT that survived through two spawning seasons spawned in both years similar to results observed by Schmetterling (2001). Skipped spawning in fishes is often attributed to poor condition (Johnston and Post 2009), low survival, or seasonal stressors (Rideout et al. 2005). We were not able to recapture fish each year to measure changes in condition from initial tagging, but high condition is not always associated with spawning (Burton 1994). Given the high mortality observed in the summer season for WCT in Rock Creek, stressful conditions experienced during the post-spawn/summer may be a factor in the high rate of skipped spawning. Food availability during the post-spawn season for female winter flounder

(*Pleuronectes americanus*) was associated with egg development more than any other time of the year (Burton 1994). Given the low rate of consecutive spawning and low annual survival, migratory WCT in this system function in a more semelparous manner along the iteroparous-semelparous gradient. For example, from our data the probability of an individual surviving and spawning in consecutive years is 4%. The probability that an individual spawned in three consecutive years is 0.2%. Using our range of average annual survival (8 – 39%) and assuming WCT need three years to spawn twice, the probability of surviving three years to spawn twice is between 0.05% and 5.9%.

Tributary Characteristics Associated with Migratory Fish

Migratory WCT returned to tributaries that spanned several habitat characteristics. Discharge at base flow was the only tributary characteristic that was associated with persistent migratory WCT. Resident adult biomass was higher in tributaries in the persistent than rare category. Because we observed adult resident (> 130 mm TL) WCT in all tributaries sampled, we assumed all tributaries had a resident component and no populations were entirely migratory. We did not detect an effect of colder (potentially less productive) streams associated with increased migratory fish. While cold water has been shown to promote migratory phenotypes, WCT are successful and persist as residents in cold, headwater systems (Shepard et al. 2005), slow growth associated with the cooler water temperatures in this study may not be enough to trigger a switch to migratory life history for WCT. Other studies have found that abiotic factors predicted different life histories in WCT. Heckle IV et al. (2020) found that landscape position (higher in the watershed), lower stream gradient and higher stream order tributaries were associated with fluvial versus resident WCT life history in the St. Maries River drainage in Idaho, USA. While we did not detect an effect of stream gradient or landscape position on migratory category, our observation of larger watersheds being associated with persistent migratory tributaries is similar to Heckle IV et al.'s (2020) observation of larger stream order associated with an increase in odds of being a migratory individual.

Our discriminant function analysis and training data were able to distinguish persistent migratory tributaries from periodic and rare tributaries but struggled to differentiate between streams with periodic and rare migratory categories. However, the model lacked predictive ability based on both the testing data and cross-validation. One reason for this may be that straying individuals are driving streams with periodic migratory components. Because WCT are expected to have substantial site fidelity resulting in genetic population structure (Allendorf and Leary 1988; Drinan et al. 2011), we expected that most of the fish are returning to their natal tributary. While F_{st} among non-hybridized WCT populations in Rock Creek is moderately high (0.11), F_{st} among many tributaries is much lower, often less than 0.05 (Kovach et al. *in revision*), which strongly suggests that gene flow is regularly occurring. Migratory individuals dispersing from persistent tributaries to non-natal tributaries could be confounding some of the effects of natal tributary characteristics. This could explain why we were unable to distinguish between rare and periodic tributaries based on natal tributary characteristics alone. However, one major source of uncertainty is the potential genetic contribution underpinning the migratory life history. Migratory life history has been shown to be highly heritable in steelhead (Hecht et al. 2015; Pearse et al. 2019; Arostegui et al. 2019), and brown trout as well (Lemopoulos et al. 2018; Ferguson et al. 2019) but to date, no studies have examined this in WCT. However, Strait et al. (2021) demonstrated that increasing non-native admixture with rainbow trout increased the probability of adopting a migratory life history in juvenile *Oncorhynchus spp*.

Evolutionarily, partial migration is the product of different advantages between life histories (Stearns 1976). Neither life history becomes a dominate strategy when the advantages between the two are similar or vary over time. Persistent migratory tributaries occurred at a lower frequency than tributaries where migrants are rare, suggesting some fitness advantage toward residency across the Rock Creek basin. The larger range wide shift of WCT populations towards headwater resident populations (Shepard et al. 2005) supports that migratory life histories are disadvantaged, possibly due to degraded habitats or non-native competition in large river habitats. Persistent migratory tributaries were associated with higher discharge at base flow and a higher resident adult biomass suggesting that there may be some body size advantage in these larger tributaries. Body size is important for mate selection in salmonids (Holtby and Healey 1990; Kitano 1996; Petersson et al. 1999) and often confers a reproductive advantage. In these tributaries with larger-bodied residents, the increase in body size associated with becoming migratory may be a critical advantage during spawning when stream residents are larger.

Survival differences within the migratory fish population

Although we do not know the mechanism driving the strong impact of summer habitat on reduced survival, it does highlight a need to better understand the cause of this high mortality. The relatively low annual survival of migratory WCT was not unexpected given that we captured the larger adult fish for tagging. Our estimates were within the range of observed annual survival for similar sized WCT (Mayfield et al. 2019). Similar to Mayfield et al. (2019) we observed higher fall and winter WCT survival compared to spring and summer. Interestingly, whether an individual spawned did not have a significant impact on survival. Spawning is considered stressful for salmonids (Berg et al. 1998). However, we observed mortality of non-spawning/non-migrating individuals at nearly the same rate as spawning individuals. Given that spawning was not significantly associated with decreased survival in our study and survival probability during the winter neither increased nor decreased, conditions during the summer are likely driving survival of migratory WCT in Rock Creek.

WCT that used the Clark Fork River in the summer were comprised of short distance migrators. Even though some of the longest migrators began their migrations from the Clark Fork River, these individuals summered in Rock Creek. Individuals that summered in the Clark Fork River were at a much higher risk of dying than those that remain in upper and middle Rock Creek for the summer. The upper Clark Fork River basin suffers from substantial impairment due to historic mining, dewatering, and high summer temperatures that negatively effects fish populations (Cook et al. 2015). However, the section of the Clark Fork River that was part of this study is the least impacted portion of the upper river (Mayfield et al. 2019). Low flows and warm temperatures are stressful for cold-water salmonids. Summer water temperatures were highest in the Clark Fork River section. The Clark Fork River exceeded 20°C an average of 33 times during July and August for all years of this study. Whereas maximum water temperatures in Rock Creek only exceed 20°C on average 5 times during the summer. Survival probability in WCT declines at temperatures at and above 20°C (Bear et al. 2007). Our data showed that survival is lowest in the summer and not related to spawning, suggesting that summer habitat conditions such as water temperatures may be playing a substantial role in WCT mortality.

Threats associated with migration life history

Migratory life histories generally are at higher risk of extirpation due to their need for larger, connected, complex habitat. It is not surprising that as habitat needs increase, the diversity of threats increases as well. Our analysis of threats suggests that migratory fish face the following primary threats: increasing overlap with hybrids during spawning, and poor summer habitat conditions in the Clark Fork River. Rock Creek currently does not suffer from widespread habitat fragmentation. Mayfield et al. (2019) suggested that lower survival observed in tributaries of the Clark Fork River may be attributable to irrigation canal entrainment, migration barriers, or dewatering. However, we did not find any evidence that migratory WCT were impacted by barriers or entrainment, nor were current or predicted summer stream temperatures expected to negatively affect migratory WCT.

Warming stream temperatures are a major concern for the long-term persistence of cold-water fisheries. Based on current temperatures and NorWest predictions for mean August temperatures, natal tributaries should remain in the range of WCT thermal requirements for the next 60 years. There was no difference in mean August temperature for tributaries categorized as persistent contributors to migratory behavior versus those categorized as periodic or rare. For Rock Creek, warming stream temperatures are likely not a major threat to spawning and rearing streams for WCT. None of our migratory tributaries were predicted to exceed 15.0°C mean August temperature by 2080 based on the NorWest model (Isaak et al. 2017); well below the 20°C threshold for lethal temperatures. We recognize that populations would likely be extirpated before a stream reaches consistently lethal temperatures. Heinle et al. (2020) used 18°C mean August stream temperature as an upper thermal threshold for WCT based on radio-telemetry data in the North Fork of the Flathead River near Glacier National Park. Even at this threshold, no streams in Rock Creek would be impacted for the next 50 years. Heinle et al. (2020) also predicted that WCT distribution was likely to increase under warming stream temperatures. Warming stream temperatures may open up habitats or increase abundances of WCT where they are currently limited by cold temperatures. Yet, warming stream temperatures are also associated with the spread of non-native trout, especially brown trout which have a strong negative effect on WCT populations (Bell et al. 2021).

While non-native trout species were detected in migratory tributaries, they tended to occur at lower densities in migratory tributaries than non-migratory tributaries. Brook trout negatively affect cutthroat trout where they co-occur (Dunham et al. 2002; Peterson et al. 2004) and their distributions in the Western USA appear to be associated with smaller, low gradient streams in or near unconfined valley bottoms (Wenger et al. 2011). Thus, the lower densities of brook trout in migratory streams could simply be a result of migratory WCT streams being poor habitat for brook trout. WCT migratory streams were strongly associated with larger streams whereas brook trout tend to be associated with smaller systems. Another possibility is that migratory life history is driving differences in abundances between brook trout and WCT. Competition between the two species is greatest at the juvenile stage (Griffith Jr. 1972; Peterson et al. 2004). By becoming migratory, WCT might escape the increased competition from brook trout during the juvenile stage and return much larger and with a reproductive and demographic advantage. Migratory brook trout appear to comprise a very small portion of these populations based on their limited distribution in the mainstem of Rock Creek (B. Liermann pers. comm).

Brown trout were ubiquitous but at low densities across streams with migratory WCT. Brown trout are expanding into headwater streams in the West, including Rock Creek (Al-Chokhachy et al. 2016). Studies have shown negative effect of brown trout sympatry with cutthroat subspecies (McHugh and Budy 2006; Al-Chokhachy and Sepulveda 2019). Interestingly, McHugh and Budy (2006) found decreased movement rates of Bonneville cutthroat trout *O. clarkii utah* when sympatric with brown trout. They suggest this increased sedentary behavior is due to a decrease of suitable foraging habitats absent of brown trout.

However, partial migration theory would suggest that increased predation risk (Skov et al. 2011) and lower growth rates due to increased competition should lead to more individuals becoming migratory (Brönmark et al. 2008). We saw that increased biomass of adult resident trout was positively associated with migratory behavior. If this relationship is due to competition, then the presence of larger brown trout (at their current densities) would likely not alter this mechanism. The expansion of brown trout into WCT habitats will negatively affect the long-term persistence of WCT populations. It's unclear how this will affect the expression of migratory WCT phenotypes, but coupled with other threats such as hybridization, it's likely to increase the overall threat to migratory life histories.

Hybridization is considered the most pervasive threat to WCT persistence (Allendorf and Leary 1988; Shepard et al. 2005). However, migratory WCT tributaries largely had lower resident population pRBT than non-migratory tributaries. In systems where RBT and cutthroat species occur sympatrically, spatial segregation seems to be the dominant mechanism to maintain these species (Ostberg et al. 2004; Buehrens et al. 2013). We observed spatial separation in spawning locations between WCT and RBTxWCT similar to Muhlfeld et al. (2009) who observed WCT spawning higher in tributaries, while RBTxWCT and RBT spawned lower in tributaries. However, WCT with different migratory distances experience differing levels of overlap. For shorter migrating WCT, there is a decreased risk of spatial overlap as WCT spawn throughout the length of a tributary, whereas RBTxWCT largely spawned near the mouths of tributaries. Given the typical pattern of pRBT observed in stream systems where pRBT is highest closer to the mouth and decreases moving upstream (Weigel et al. 2003; Muhlfeld et al. 2009c, 2017; Rassmusen et al. 2012), longer distance migratory WCT are likely at a higher risk for hybridization with RBT or RBTxWCT, given that they largely spawn lower in tributaries where resident population pRBT is expected to be the highest. But this spatial overlap is mitigated by temporal separation between WCT and RBxWCT. Similar to Muhlfed et al. (2009), trout in Rock Creek with higher individual pRBT spawned at cooler stream temperatures correlating to earlier spawning by RBTxWCT.

Finally, while survival related to summer habitat was severely reduced for fish that spent the summer in the Clark Fork River this only impacted a small portion of short migrators. However, because of the severity of the survival reduction, this is still an important threat to at least a specific portion of the population. Interestingly, only short distance migrators ended up in the Clark Fork River during the summer. Long distance migrators that began their migration in the Clark Fork River, summered in Rock Creek. This suggests that summer habitat may be a function of post-spawning downstream movement and that spawning populations in lower in Rock Creek may be at higher risk.

This study reveals the need to manage fisheries and populations at the intermediate scale which is difficult and often overlooked (Fausch et al. 2002). These mid-sized river habitats are increasingly threatened and crucial for supporting life history diversity. Addressing the spatial and temporal heterogeneity of habitat patch quality is vital to maintain migratory life histories (Schlosser 1991) and conservation actions for migratory life histories must be made with the entire complement of habitats in mind (Runge et al. 2014). Much focus has been placed on conserving unimpacted cold water refuge for native salmonids to offset the impacts of climate change. However, Armstrong et al. (2021) highlights how conservation planning for cold-water species under climate change tends to devalue downstream warm-water habitats. They highlight that these “seasonally warm downstream habitats” are crucial for growth during the shoulder season and that focusing on conserving cold-water summer refugee habitats would promote resident life histories over migratory life histories. Clearly, seasonally warm habitats are being extensively used by non-hybridized WCT in Rock Creek. By writing off these mid-size river in future conservation efforts we are effectively ignoring our most abundant fluvial habitat (Downing et al. 2012).

Management Implications

This study highlights the capacity for WCT to maintain a robust migratory life history in a heavily impacted system despite decades of high abundances of RBT and expansion of other exotic salmonids. Managing to promote migratory life histories is critical for broader conservation of WCT and is a stated goal of the 2007 memorandum of understanding for cutthroat trout conservation (MFWP 2007). Managing for the full range of WCT migratory life history requires: (1) Limiting the potential for hybridization between WCT and RBT and hybrids. Hybridization between these two species leads to an overall reduction in variety and expression of migratory life history among WCT populations. While brook trout and brown trout were a potential threat, their current impact on migratory WCT life history in Rock Creek is likely not as severe as hybridization. However, it is not well understood how they may impact migratory WCT specifically and managers should closely monitor spread and abundances of these exotic species. (2) Prioritizing conservation of larger tributaries, particularly impacted streams. Cold, secure, non-hybridized headwater streams were not major contributors to the migratory population. Management that ensures quality habitat, and connectivity among habitats and within the population is likely to be more successful than active management at the natal tributary trying to promote migratory life history. (3) Maintaining connectivity within the drainage. While nearly half of all our tagged WCT returned to a single tributary the remaining half were spread out among 12 other tributaries. It’s unknown what historic contribution to the migratory population has been lost in streams where connectivity has been severed (i.e., above East Fork Reservoir). (4) Working to identify and mitigate conditions limiting survival in downstream summer habitats. These downstream habitats are often more impaired than upstream habitats such that differences in survival can be severe enough to function as sink habitats or ecological traps, reducing migratory abundances and distributions.

Long-term, sustainable conservation of inland trout will require managing populations to maintain the full suite of life histories. Migratory life histories require a more varied set of management and conservation actions due to their expansive habitat needs. This means conserving not only non-hybridized headwater populations, but also protecting and restoring larger river foraging, migrating, and overwintering habitats that are vital to migratory individuals.

Table 2.1. Spawning tributaries of migratory WCT in Rock Creek 2018-2020. For migratory categories, tributaries that telemetered fish return in multiple years we identified as “Persistent”. Tributaries where fish returned only in one year we identified as “Periodic”. Mean migration distance is the average distance traveled by WCT to reach the mouth of that tributary

Tributary	**No. of Returning WCT**	**Migratory Category**	**Mean Migration Distance (km)**	**Std Dev.**
West Fork Rock Creek	23	Persistent	40.0	32.0
Stony Creek	3	Persistent	7.9	5.5
Cowan Gulch	3	Persistent	75.7	3.2
Hogback Creek	3	Persistent	18.5	27.3
Middle Fork Rock Creek	2	Persistent	42.6	5.3
Alder Creek	2	Periodic	7.1	2.5
Gilbert Creek	2	Periodic	5.7	4.5
Little Stony Creek	2	Periodic	11.7	3.9
Ross Fork	2	Persistent	24.6	5.9
Bateman Creek	1	Periodic	19.8	–
Ranch Creek	1	Periodic	20.4	–
Wahlquist Creek	1	Periodic	36.5	–
Williams Gulch	1	Periodic	5.2	–
Total	46			

Table 2.2. Habitat variables for 52 reaches across 38 tributaries within the Rock Creek drainage 2019-2020. Variables were used in univariate analysis and as covariates in the linear discriminant function analysis to investigate the relationship between natal streams characteristics and migratory categories. Bonferroni corrected p-values for each ANOVA test. * indicates significant (p-value < 0.05) difference between migratory categories. Variables were transformed where needed to meet normality assumptions of ANOVA test. Significant variables did not change with untransformed data; see supplemental (Table A.2.)

Variable	**Description**	**Mean**	**SD**	**Min**	**Max**	**Trans**	**p-val**
Mean Aug Temp	Mean stream temperature (°C) for the month of August	10.4	1.6	7.1	14.6	Identity	0.896
Growing Degree Days	Number of days where stream temperature was greater than 4°C	658.8	342.3	293.3	2033.2	Log	1.000
Elevation	Elevation (m) at the upstream end of reach	1558.1	245.3	1133.0	2140.0	Identity	1.000
Landscape Position	Distance (km) of tributary confluence from mouth of Rock Creek	54.2	30.1	6.8	92.9	Identity	0.509
Base Flow	Discharge (m^3/s) at base flow	0.26	0.35	0.02	1.57	Cube Root	0.018**
Large Woody Debris	Number of large woody debris observed within the reach	6.2	4.5	0.0	16.0	Identity	1.000
Overwinter Pools	Number of pools deeper than 0.5 m per m^2	0.005	0.005	0.0	0.019	Log	1.000
Fines	Estimated % of fine (< 2.5 mm) substrate within the reach	22.1	20.8	1.0	97.5	Log	1.000
Adult Biomass	Total biomass (g) of fish greater than 150 mm TL divided by reach area (m^2)	28.4	46.0	0.0	236.0	Cube Root	0.588*
Juvenile Biomass	Total biomass (g) of fish less than 150 mm TL divided by reach area (m^2)	6.1	5.7	0.8	26.8	Cube Root	1.000
Brook Trout Density	Number of Brook Trout per m^2	0.08	0.20	0.0	0.98	Log	1.000

Table 2.3. Threat scorecard for various threats to migratory WCT in Rock Creek, MT. Data used to assess threats included radiotelemetry, fish community assemblages, and habitat measurements across 13 migratory WCT spawning tributaries. The risk level was assessed low if it influenced less than 10% of the tagged fish and < 2 tributaries, medium if less than 50% tagged fish and/or less than 9 tributaries were influenced, and high if more than 50% of tagged fish and/or 9 or more tributaries were influenced by the threat.

Threat	**Criteria for Assessment**	**Migrations > 50 km (n=10)**	**Migrations < 50 km (n=36)**	**No. of Migratory tributaries impacted**	**Risk level**	**Notes**
Fragmentation	Percent of tagged WCT entrained	0%	6%	1	Low	Two WCT were detected in irrigation ditches that had spawned in West Fork.
	Percent of tagged WCT with reductions in weekly movement rates in spawning migration	0%	0%	NA		
Spawning tributary impairment	Mean August Temp > 20°C	NA	NA	0	Low	
	Predicted Mean August temp > 20°C	NA	NA	0		
Brown trout	Percent of tagged WCT spawning sites with brown trout present	100%	93%	9	High	Brown trout tended to occur at low densities
Brook trout	Percent of tagged WCT spawning sites with brook trout present	0%	27%	5	Med	
Hybridization	Percent of tagged WCT spawning at sites with pRBT > 1%	60%	53%	6	High	
	Percent of tagged WCT spawning site overlap with RBTxWCT	30%	14%	3		
	Percent of tagged WCT spawning temp overlap with RBTxWCT	10%	17%	4		
River habitat degradation	Percent of tagged spawning WCT summering in CFR	0%	8%	3	Med	At the population level, not much of a threat, but large threat to individuals that use that habitat

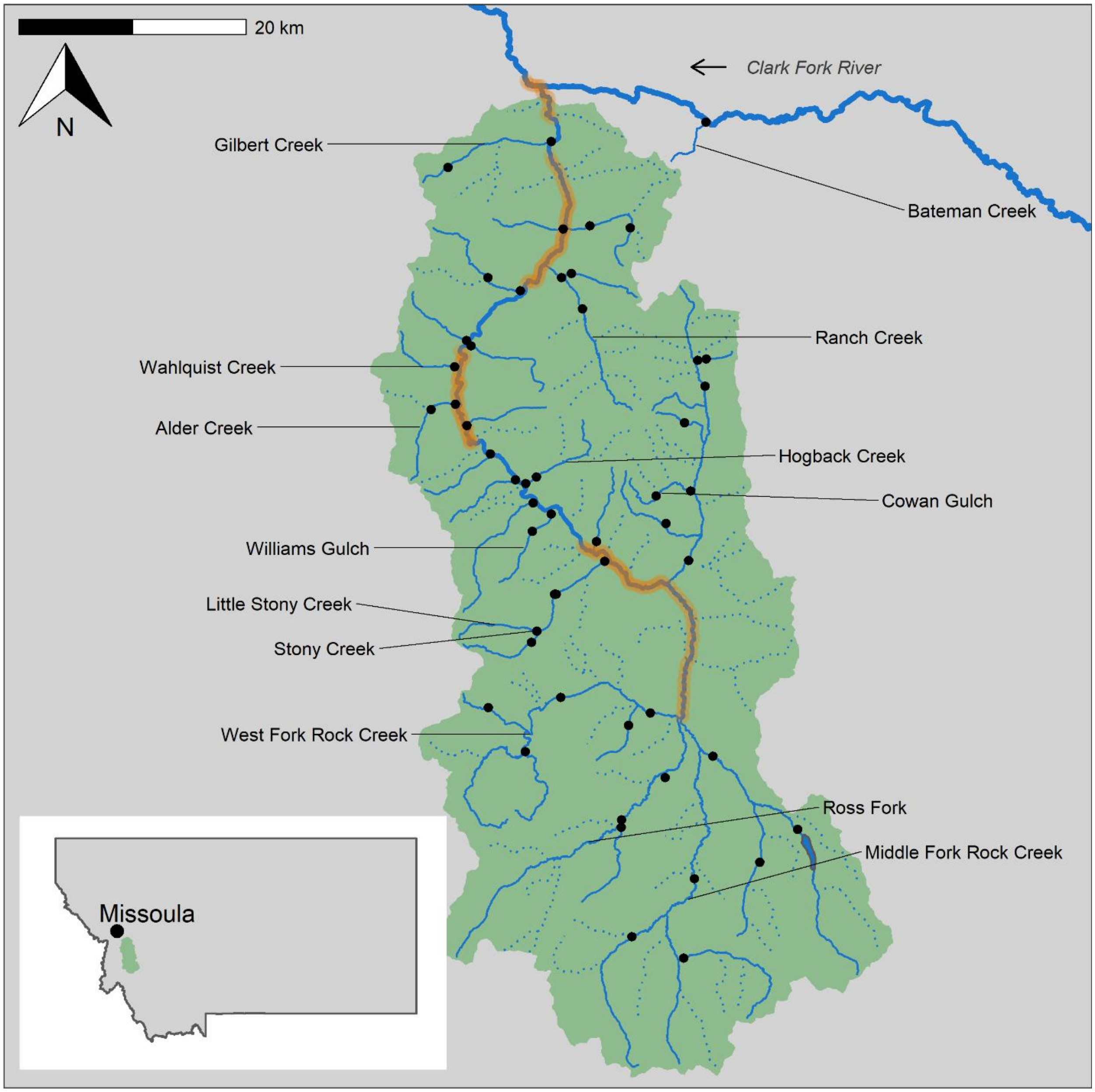


Figure 2.1. Map of the Rock Creek drainage and study area. Named tributaries are streams where WCT spawned. Orange shaded areas are where WCT and RBTxWCT were captured for telemetry. Points are tributary sampling locations. Dotted streams are small tributaries that were not sampled.

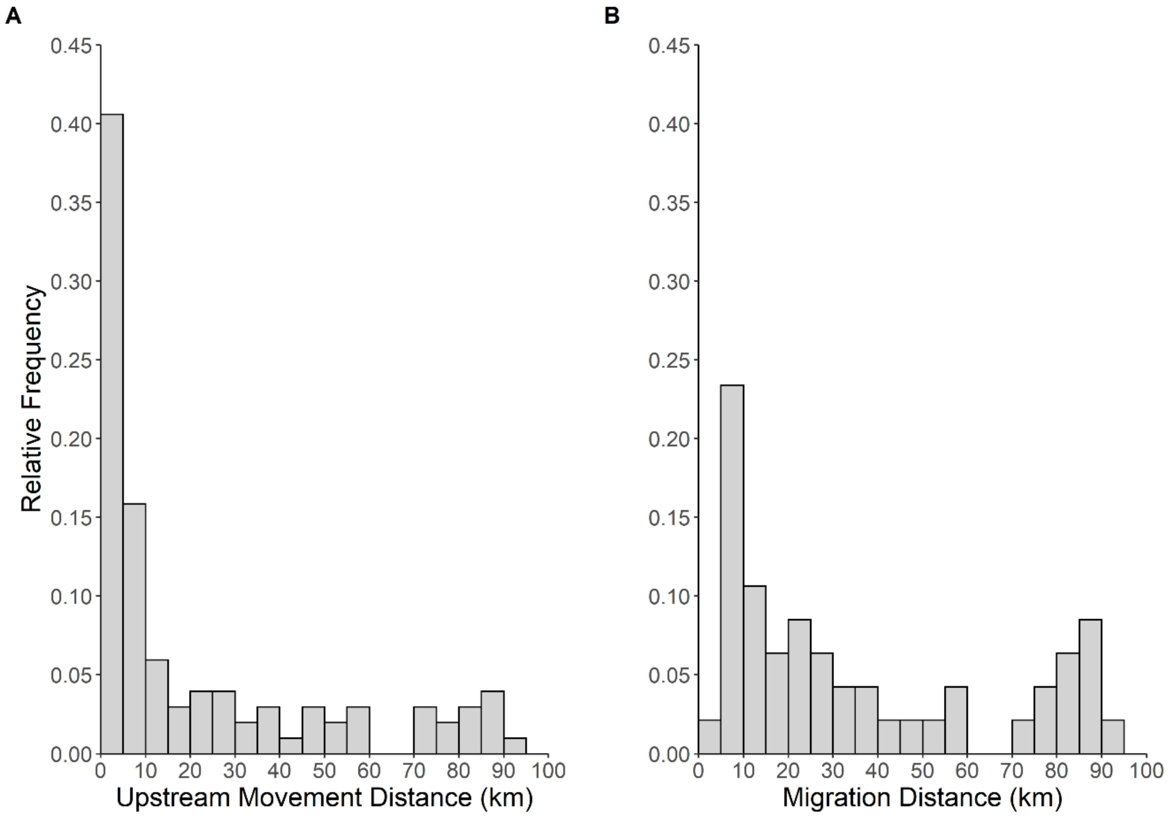


Figure 2.2. Relative frequency histograms of (**A**) annual upstream movement distances of 78 spawning and non-spawning WCT and (**B**) spawning migration distances of 52 WCT in Rock Creek, MT 2018-2020.

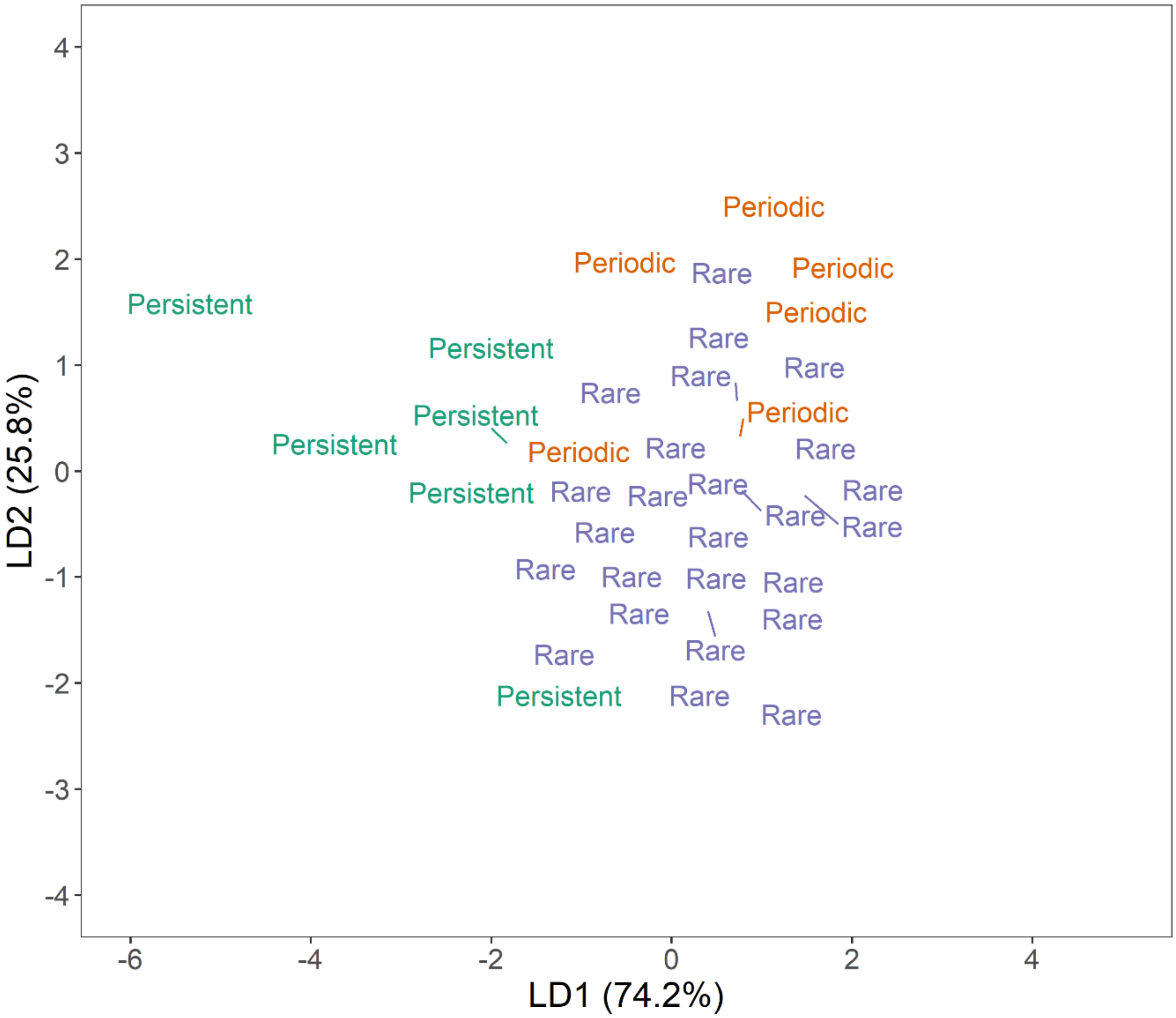


Figure 2.3. Plot of linear discriminant analysis of the entire dataset. The axes describe how much separation between the migratory categories is achieved by each axis for 37 tributaries in Rock Creek, MT. Categories indicate whether there were persistent migratory fish detected spawning in the tributary, periodic (or occasional = 1 fish in study) migratory WCT detected spawning, or rare or no WCT detected spawning in the tributary.

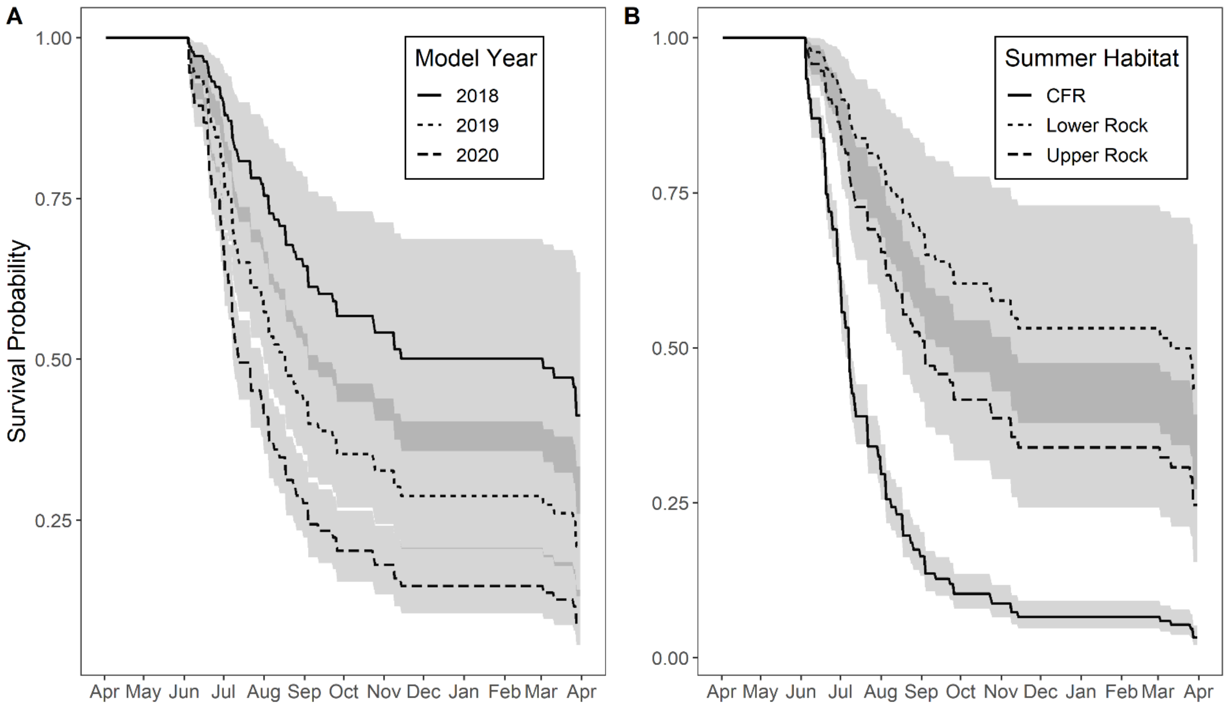


Figure 2.4. Estimated annual patterns of survival probability from April 1st through March 31st derived from the Cox Proportional Hazards model for each study year (**A**) and summer habitat (**B**) of telemetered WCT in Rock Creek, MT 2018-2021. Each line is the average estimated survival for the average individual for each value of that covariate.

CHAPTER 3

MICROEVOLUTIONARY FORCES DRIVE PATTERNS OF HYBRIDIZATION BETWEEN NATIVE WESTSLOPE CUTTHROAT TROUT *ONCORHYNCHUS CLARKII LEWISI* AND NON-NATIVE RAINBOW TROUT *O. MYKISS.*

Abstract

Human-mediated hybridization is a serious threat to biodiversity, native species persistence, and conservation. However, we often lack a complete understanding of the mechanisms that shape the pattern of hybridization across landscapes, particularly in fish. We used a framework that considered all relevant microevolutionary forces to explore potential resistance between westslope cutthroat trout *Oncorhynchus clarkii lewisi* (WCT), a species of conservation concern, and non-native rainbow trout *O. mykiss* (RBT) in Rock Creek, Montana, USA. Here we combined genetic, habitat, demographic, and movement data to examine different processes and mechanisms that drive hybridization in a watershed with historically high non-native abundances and examine potential mechanisms of localized resistance to hybridization. We did not detect any broad directional change in proportion of non-native admixture at 13 sites over the last four decades. Distance from high RBT abundances (propagule pressure from dispersal), rather than environmental resistance (e.g., water temperature and disease), or historic stocking, was associated with the current pattern of hybridization across the watershed. There was evidence of that mechanisms of ecological resistance, such as assortative mating, may be shaping the patterns of hybridization at the site level, but high propagule pressure appears to be able to overwhelm localized resistance. Sites that were farther from the highest abundances of mainstem RBT, with larger resident fish body sizes, and the increase available habitat to provide for spatial and temporal assortative mating appear to be more resistant to hybridization. By applying a

microevolutionary framework we provide a more synthetic understanding patterns of hybridization between these two species that will help inform conservation of native fishes.

Introduction

Naturally occurring hybridization has often been thought of as either a beneficial mechanism of speciation (Abbott 1992) and adaptive radiation (Seehausen 2004) or as evolutionary dead end (Arnold 1997). However, hybridization resulting from anthropogenic driven habitat modification, climate change, and translocation (intentional and unintentional) is thought to be a negative force due to the unpredictability of outcomes, threat of genomic extinction, and loss of evolutionary lineages (Rhymer and Simberloff 1996; Huxel 1999; Allendorf et al. 2001; Todesco et al. 2016; Ottenburghs 2021). Human-mediated hybridization is a major mechanism threatening the persistence of native species (Rhymer and Simberloff 1996; Allendorf et al. 2001; Grabenstein and Taylor 2018). Ottenburghs (2021) highlights 59 studies of human-mediated hybridization from 2016 to 2020, 24 of which involved fish. Hybridization between closely related fish species is particularly common relative to other vertebrates due to external fertilization, promiscuous spawning behaviors, weak mating behavioral incompatibilities, and extensive translocation by humans (Hubbs 1955; Scribner et al. 2000).

Human-mediated hybridization is a widespread phenomenon across taxa, but different conditions, mechanisms, and outcomes of initial contact can lead to widely differing patterns of hybridization within and among species (Arnold 1997). Unfortunately, our understanding of the underlying mechanisms influencing hybridization dynamics is often poor, which in turn, limits conservation opportunity and prioritization. Ultimately, the microevolutionary forces including, natural selection, assortative mating, gene flow, and genetic drift combine, to varying degrees and influence the outcome of anthropogenic hybridization events. We argue that the primary microevolutionary forces themselves provide an ideal framework for understanding hybridization dynamics. Of particular interest to conservationists tasked with addressing or mitigating human-induced hybridization is identifying the underlying mechanisms that may provide resistance to or expand hybridization in a focal species (Allendorf et al. 2001). Site scale resistance is ultimately a function of selection (intrinsic/extrinsic) (s), assortative mating (A), migration rate (m), and population size (drift) (N), which are primary microevolutionary forces (Equation 1).

Eq. 1 Site level resistance $\sim f\,(s + A + m + N)$

The latter term accounts for stochastic dynamics due to evolution in small population (i.e., all else being equal, hybridization dynamics should be more variable in small populations). We define dispersal (m) in the genetic tradition as movement from one population to another resulting in successful reproduction (gene flow). We propose using this resistance framework and mechanisms to further the understanding of hybrid dynamics among two widespread salmonid species.

Rainbow trout *Oncorhynchus mykiss* (RBT) is one of the most introduced fish species in the world (Halverson 2010) and have been cited as the leading threat to cutthroat trout persistence in the intermountain West (Allendorf and Leary 1988; Shepard et al. 2005). Hybridization between RBT and westslope cutthroat trout *O. clarkii lewisi* (WCT) is of chief management and conservation concern due to the substantial reduction in non-hybridized WCT distribution (Shepard et al. 2005) and the threat of widespread introgressive hybridization between these two species (Epifanio and Philipp 2000). In systems such as the North Fork of the Flathead River near Glacier National Park, USA, hybridization between these two species has continued to expand (Hitt et al. 2003; Boyer et al. 2008a; Muhlfeld et al. 2017) despite the cessation of stocking. However, the pattern of hybridization between these two species has been variable at local and broad scales (Muhlfeld et al. 2017) and suggests that different mechanisms might facilitate or resist hybridization between these two species. This has led to a debate over the ultimate consequences of hybridization between these two well studies species (McKelvey et al. 2016; Young et al. 2016b, 2017; Muhlfeld et al. 2017; Kovach et al. 2017).

There are two primary hypotheses in the literature regarding site level resistance of hybridization between RBT and WCT. One hypothesis posits that parental forms are segregated by ecological and physiological constraints largely associated with water temperature (implicitly this is environmentally mediated selection (s)) and that while hybridization will continue, cold water refuge (Isaak et al. 2015) will largely limit the expansion of hybridization. A competing hypothesis states that hybridization is driven by propagule pressure (dispersal rate), resulting from historic RBT stocking (Loxterman et al. 2014; Muhlfeld et al. 2017) and that as hybridization increases, propagule pressure from dispersing hybrids move non-native alleles closer to the edge of and into non-hybridized zones (Lowe et al. 2015). In essence, dispersal (m) overwhelms intrinsic and extrinsic selection (s) (Kovach et al. 2015); the consequence of this hypothesis is that it is only a matter of time before all populations become hybridized. The general landscape pattern of non-hybridized WCT occurring in headwater streams - cold, small, high elevation sites - which are often farthest from potential sources of RBT and hybridized populations which typically occur in warmer, larger low elevation locations makes it difficult to distinguish between these hypotheses.

Rock Creek, Montana, USA is an excellent study site for unraveling this landscape correlation because warm and cold tributaries are well distributed throughout the watershed (Figure 3.1), and RBT abundances have undergone dramatic changes over the last 40 years altering the dispersal distance from source populations. As we consider the known underpinnings of site level resistance (Eq. 1), we suggest that it may be useful to broaden our hypotheses around the mechanisms for site resistance to include multiple sources of extrinsic or environmentally mediated selection, intrinsic selection, dispersal, assortative mating, and genetic drift. By taking this unique approach and combining genetic, habitat, demographic, and movement data, we hope to provide a synthetic investigation of potential forces driving hybridization in Rock Creek and beyond (Table 3.1). Not only is this a useful approach to addressing hybridization dynamics of WCT, but the broader discussion of human-mediated hybridization which tends to focus only on one or two of these microevolutionary forces. We hope to further the discussion of drivers of hybridization using this multi-faceted approach.

Extrinsic or environmentally mediated selection

Many landscape level correlates have been proposed as to what environmental conditions resist hybridization such as low productivity (Rasmussen et al. 2010; Rassmusen et al. 2012), cooler thermal (Young et al. 2016a), high variation in hydrologic regimes (Fausch et al. 2001; Muhlfeld et al. 2014), higher elevation, steeper slope, and smaller stream width (Weigel et al. 2003; Muhlfeld et al. 2009c; Yau and Taylor 2013). These studies imply that environmentally mediated selection is the major force driving the landscape distribution of admixture. While different conditions have been associated with patterns of hybridization between these two species, we still lack considerable knowledge particularly regarding when, where, and how these factors play a role across the landscape.

Whirling disease (*Myxobolus cerebralis*) has been introduced throughout the Western US and is particularly virulent to RBT (Bartholomew and Reno 2002). In systems with high disease loads, RBT abundances were severely reduced (Vincent 1996). Because RBT are more susceptible to whirling disease than WCT (Vincent 2002), we would expect hybrids to be intermediate in their susceptibility and their abundances should be reduced where whirling disease prevalence is high. Carim et al. (2015) did not find any evidence that whirling disease had any impact on the size of hybrid zones in the Blackfoot River, MT. However, whirling disease prevalence was exceptionally high and widespread throughout Rock Creek and resulted in substantial declines of RBT abundances (Granath and Vincent 2010). The need for the intermediate oligochaete worm host *Tubifex tubifex*, means that habitats conducive to oligochaetes: higher order streams, with warmer temperature, and a higher proportion of fine sediments resulted in higher infection rates of salmonids (Sauter and Güde 1996; de la Hoz Franco and Budy 2004; Hallet and Bartholomew 2008). Whirling disease may be one part of a larger suite of biotic factors driving localized resistance and shaping the landscape of hybridization.

Propagule pressure or dispersal

Propagule pressure is a major mechanism that shapes the pattern of hybridization between species. Distance from source of RBT or hybrids has been shown to be a reliable predictor of the pattern of hybridization between WCT and RBT in numerous studies (Rubidge and Taylor 2005; Boyer et al. 2008a; Gunnell et al. 2008; Muhlfeld et al. 2009b, 2009c, 2017; Bennett et al. 2010; Marie et al. 2012; Yau and Taylor 2013; Kakareko et al. 2016; Heim et al. 2020). While propagule pressure can broadly describe patterns of hybridization between these two species, aberrations from the pattern of distance from source of exist, suggesting that other mechanisms (selection, assortative mating, or genetic drift) influence hybridization outcomes.

Assortative mating

One mechanism that may promote resistance to hybridization is assortative mating. Assortative mating (mate choice based on phenotype) can be a strong mechanism in maintaining reproductive isolation between heterospecifics across taxa (Culumber et al. 2014; Schumer et al.

2017; Hinton et al. 2018). Spatial and temporal differences in spawning, encounter rates, and amount of available spawning habitat affect the strength of assortative mating. Parental WCT and RBT tend to spatially and temporally segregated during spawning, with hybrids bridging the separation (Muhlfeld et al. 2009b). Additionally, assortative mating has been shown to be frequency dependent. As encounters with heterospecifics increase, “choosiness” decreases (Willis et al. 2011). Frequency of encounters on the spawning grounds could be driven by higher densities of resident conspecifics, proximity to sources of heterospecifics, or smaller habitat sizes. Other traits that influence mating success in salmonids include body size and female choice. Additionally, female choice, can influence direction of hybridization when abundances of the two species are unequal (Wirtz 1999). Studies of hybridization between migratory individuals and residents shows that males often employ sneaker strategies or are more successful when abundances of migratory forms are low (Baxter et al. 1997; Kanda et al. 2002; Ostberg et al. 2004). Lower hybrid fitness is thought to reinforce assortative mating (Kirkpatrick 2000; Baskett and Gomulkiewicz 2011), which we see with decreased fitness and selection against RBTxWCT individuals (Kovach et al. 2015, 2016).

Intrinsic or selection independent of environment

In hybrids, fitness can vary depending on genetic composition and environmental conditions (Arnold and Hodges 1995). Intrinsic outbreeding depression results in reduced hybrid fitness independent of external factors. This typically results from a breakdown of co-adapted gene complexes, (Lynch 1991) or genomic incompatibilities (Maheshwari and Barbash 2011; Ostberg et al. 2013). Studies have shown that hybrid RBTxWCT are less fit (Muhlfeld et al. 2009a) and that RBT alleles are selected against across environmental gradients (Kovach et al. 2015, 2016). Strait et al. (2020) showed increasing RBT ancestry had differential outcomes depending on environmental and demographic conditions. These studies highlight that RBT ancestry often results negative fitness consequences that can shape the distribution of hybridization.

Genetic drift associated with small population size

When populations reach small sizes, forces that determine allele frequencies dramatically shift towards stochastic chance and can drive alleles towards fixation (Wright 1931). Genetic drift has been largely absent from the discussion of hybridization. This may be because hybridization is driven by immigration of a non-native species which assumes that the population is connected. However, drift may occur through stochastic evolutionary dynamics (Carlsson et al. 1999) or if populations can become functionally isolated over time either through physical barriers or biotic conditions that limit gene flow (limiting dispersal and immigration) resulting in small effective populations sizes (Carim et al. 2016). Over time, propagule pressure and local abundances can decline and/or habitats become fragmented, resulting in smaller populations that have limited geneflow. Under these conditions the effects of genetic drift can become more pronounced, driving allele frequencies towards either end of the hybrid distribution.

For this study we had three objectives: first, we resampled sites with historic genetic data across the watershed and examined how site level hybridization has changed over the last 10 – 40 years in Rock Creek. Second, we examined whether or not environmentally mediated selection (temperature and/or *Tubifex* habitat) or historic stocking improved predictions of the presence of hybrid individuals and individual admixture over propagule pressure (distance from source) alone. Finally, we used a variety of data types, including radio telemetry, population densities, genetic, and habitat data to describe how microevolutionary forces may be influencing localized resistance to hybridization in this system. Specifically, do we see evidence of intrinsic and/or extrinsic selection (s), dispersal (m), assortative mating (A), or population size (N) influencing resistance to hybridization in Rock Creek? Ultimately, these comparisons help illuminate factors that influence resistance to hybridization, thereby helping inform management decisions on conservation actions to protect WCT, and other similar species threatened with human-induced hybridization.

Methods and Materials

Study Area

Rock Creek is a 5th order river system in the headwaters of the Columbia River drainage near Missoula, Montana, USA (Figure 3.1). The river flows 83 km and the watershed encompass 1,425 km^2 and is characterized by confined valley channels. Discharge is largely unregulated with only one dam on the East Fork of Rock Creek. Rock Creek is one of the most heavily fished waters in the state (Montana Fish, Wildlife, and Parks (MFWP) 2019). Historically, the fishery was comprised of native trout such as WCT and bull trout *Salvelinus confluentus*. Like most major streams in Montana, Rock Creek was stocked with RBT until 1974 when stocking of rivers was halted. Until the early 1990's, RBT were the primary sport fish in the system averaging nearly 800 fish per mile (

Figure 3.) making Rock Creek a renowned rainbow trout fishery. The arrival of whirling disease in the early 1990's severely reduced the RBT population. Infection intensities have continued to increase since 1998 but may have peaked in 2006. The disease was still present throughout the mainstem and major tributaries in 2008 (Granath et al. 207; Granath and Vincent 2010). The fish

community of Rock Creek has now shifted to mostly brown trout *Salmo trutta* in the lower river and WCT in the upper river and tributaries; RBTxWCT are present throughout the system (MFWP 2021).

Objective 1: Changes in admixture over time

To test for changes in the proportion of RBT admixture (pRBT) over time, we revisited 18 historic genetic sampling sites that were sampled between 1982 and 2010. Because of the strong trend of decreasing pRBT as distance from source or mainstem habitat increases, we removed four sites that were greater 4 river kilometers (RKM) apart. We used Fisher's exact test (fisher.test in R) to test the null hypothesis that the proportion of RBT alleles between historic and current sampling are not different. All analyses were performed in R (R Core Team 2019). Fisher's exact test accounts for difference in the number of molecular markers used to describe hybridization markers through time (Allozymes (n = 5), microsatellites (n=14), and SNP panel (n= 39)).

Objective 2: Environmentally mediated selection given propagule pressure

To assess the current distribution of hybridization across the landscape we sampled 51 sites across 37 tributaries (Figure 3.1) using single pass backpack electrofishing to collect genetic samples from a minimum of 20 *Oncorhynchus spp.* at each site. We sampled individuals across the length range up to 330 mm TL. Any fish greater than 330 mm TL were removed from analysis as they may have been dispersing adults from the main river (i.e., we focused our analyses on juvenile fish that likely represent the progeny of spawning events occurring in the stream of interest). Genetic samples were analyzed for proportion of RBT admixture (pRBT) at the Montana Conservation Genetics Lab Missoula, MT. We used 39 species-diagnostic single nucleotide polymorphic loci that differentiate RBT from WCT (as described in Muhlfeld et al. 2016).

Environmentally mediated selection

To assess whether colder thermal regimes limited hybridization, we measured stream temperatures using temperature loggers (Onset Computer Corp.) at 44 sites across 37 streams every 30 mins from 2019-2021. For sites within the same tributary where the NorWest model (Isaak et al. 2017) estimated mean August temperature (1993-2011) was within 0.5°C of our measured temperature, we used the temperature from the recorded site. For sites where the difference was greater than 0.5°C we placed temperature probes during the month of August 2021 and estimated the difference over that time period. We collected temperature readings across 42 sites across 37 tributaries during August from 2019-2020. Average mean August temperature was 10.8°C (±1.9°C) and ranged from 6.9°C to 16.3°C.

To assess the influence of *Tubifex spp.* habitat on patterns of hybridization, we collected discharge measurements and substrate composition measurements at every site where we collected genetic samples (n= 51) in September and October of 2020. We measured discharge at base flow (m^3s^{-1}) (Hatch FH950) at each site and visually estimated the percentage of fine sediments (< 2.5 mm) in two randomly selected 5 m sections within the electrofishing reach. We averaged the estimates of percentage of fines between the two 5 m sections for an estimate of the site as a whole. Mean discharge at base flow for 51 sites sampled was 0.31 m^3sec^{-1} (95% CI = 0.20, 0.41) and ranged from 0.01 m^3sec^{-1} to 1.57 m^3sec^{-1}. Average percentage of fines was 22% (95% CI = 16%, 28%) and ranged from 0% to 98%.

Propagule Pressure

In Rock Creek there is a trend of decreasing RBT abundances (MFWP 2021) and pRBT longitudinally along the mainstem. By using distance from the mouth of Rock Creek as a predictor variable we are tracking current abundances rather than historic stocking. In several systems historic stocking of non-natives influenced current patterns of hybridization (Loxterman et al. 2014; Muhlfeld et al. 2017; Mandeville et al. 2019). For historic stocking pressure, we searched publicly available stocking records (myfwp.mt.gov/fishMT/plants/plantreport) to determine how many RBT were stocked and where in Rock Creek.

We used distance from source as the river kilometers (RKM) from the confluence of Rock Creek based on genetic analysis of 249 *Oncorhynchus spp.* captured at three long-term monitoring sites in the mainstem of Rock Creek (Fig B.1). Within the mainstem of Rock Creek, pRBT was the highest (0.79) at the site nearest the mouth (n = 29). Parental RBT comprised the largest portion of the population (55%) at this site. Parental forms were relatively similar in abundance in the middle section (n=119) (RBT = 24%, WCT = 18%). Finally, the upper section was predominately non-hybridized WCT (64%) and only 3% were RBT. F1 hybrids were most prevalent in the middle section (12%) followed by the lower (10%) and finally the upper section (3%). Historic stocking records indicate approximately 895,000 RBT were stocked into the Rock Creek and its tributaries from 170 different stocking events 1934 to 1986; over half a million RBT were stocked into the mainstem of Rock Creek (MFWP 2021). We found records of stocking of 21 tributaries throughout Rock Creek.

In many systems, distance from source is a consistent predictor variable, therefore we included it in every model predicting hybridization and treated distance from source as a null hypothesis. We then examined whether the other hypotheses improved the explanation of pattern of hybridization. We fit a set of generalized mixed effect model with a binomial family and logistic link function with individual pRBT as the response variable and five predictor variables: Distance from mouth (current RBT distribution), historic RBT stocking, mean August temperature, discharge at base flow, and % fine substrate. We fitted another set of models with the same predictor variables and binomial response of whether an individual was a hybrid or not. Site was included as a random effect in both models. Because of the large variation in values among variables, we centered and scaled our predictors. We checked for multicollinearity between variables and ensured none of the variables used in our model exceed 0.60. Distance from source and site elevation were highly correlated (0.94) as was mean August temperature and number of growing degree days (0.63). We completed analyses using the lme4 package in R (Bates et al. 2015).

Objective 3: Microevolutionary forces of localized resistance

Propagule Pressure or dispersal

Dispersal is the key mechanism that determines connectivity between populations, and thus, is fundamental to hybrid zone dynamics. To test whether distance from source was correlated with site level resistance, we estimated propagule pressure as distance from source as described above. To assess the dispersal potential of fish with different RBT ancestry, we used radio telemetry relocations of migratory fish of each genotype to estimated home range as the RKM between the most downstream relocation and the most upstream relocation during the time that the tag was active (non-mortality). We used home range as a correlate for migration propensity assuming that genotypes with larger, more variable home ranges would represent a more mobile genotype (Radinger and Wolter 2014). We qualitatively assessed non-successful dispersal by identifying sites where migratory hybrids putatively spawned at site where we did not detect hybrid offspring (e.g., a hybrid spawning in a non-hybridized site).

Assortative Mating

To test if hybrid populations showed signs of random mating (i.e., a breakdown in assortative mating), we checked each site to see if the distribution of hybrid individuals conformed to the expectations of a hybrid swarm (random mating over time). We created a binomial distribution of expected individual pRBT from the average pRBT of the site level sample. We then compared the expected distribution of hybrid alleles with the observed distribution of hybrid alleles using a Chi-squared test. A non-significant p-value would indicate that the sample likely came from a randomly mating hybrid swarm. However, significant deviations from an expected distribution can also result from recent immigration. We assessed whether the observed distribution of hybridization could have changed due to immigration over time using a mixing parameter developed by Kalinowski and Powell (2015) to estimate how well native and non-native alleles were mixed (Table B.1).

To investigate spatial and temporal spawning separation between WCT, RBT and RBTxWCT, MFWP captured 190 *Oncorhynchus spp.* from 2018-2021 with a boat mounted electrofishing unit throughout the Rock Creek watershed and its confluence with the Clark Fork River (Table A.1). We selected *Oncorhynchus spp.* greater than 330 mm total length (TL) for radio tagging to ensure that they were likely to be mature. Fish selected for tagging were anesthetized with MS-222 (tricane methanesulfonate) prior to surgery and measured for total length (mm). A small fin clip from the anal fin was collected for genetic analysis. MFWP surgically implanted radio transmitters (Lotek Wireless Inc.; model MCFT2-3BM, St. John’s Newfoundland, Canada) using a shielded needle technique (Ross and Kleiner 1982). Fish were allowed to recover from the anesthetic and then they were released near where they were captured. We attempted to relocate fish every other day during the spawning season to identify spawning timing and areas.

We used radio-telemetry relocations to evaluate the potential for assortative mating associated with estimated spawning location, timing of spawning, and to investigate whether higher abundances of migratory WCT were associated with higher site level resistance and conversely more RBTxWCT migrants associated with lower resistance. Individuals were assumed to have spawned at the most upstream location following a rapid upstream movement during the spawning season (Apr. – Jul.) and then a downstream descent. Spawning location was calculated as the river distance in kilometers (RKM) of a fishes most upstream location in a tributary from the mouth of the tributary. We estimated spawning date as the median day between the first and last relocation at the spawning site.

To test our prediction that larger bodied residents would be associated with higher site level resistance, we first tested whether there was any difference in total length across pRBT of all genetically analyzed individuals sampled in tributaries (n = 1,179). We then assessed whether there was any association with total length and site level resistance. Because the presence of migratory life history may increase the strength of assortative mating for body size, we also investigated differences in resident body size in streams with and without migratory populations determined by our radio telemetry data.

Genetic drift associated with small population size

All else being equal, allele frequencies in small populations are more stochastic over time than in large populations. Therefore, if genetic drift is sufficiently strong to influence the outcome of hybridization events, we would expect more variation in site pRBT in smaller populations and less variation in larger populations. We assessed the potential for genetic drift to be acting on landscape patterns of hybridization by investigating variability in site level pRBT across varying densities of resident *Oncorhynchus spp*.

Results

Change in admixture over time

We tested change in admixture over time for 14 sites that met our criteria (Table 3.2). Only four sites had significant p-values indicating that the proportions likely came from different distributions. We detected an increase in pRBT in two sites that occurred lower in the drainage (RKM 23 and 39) and a decrease in pRBT in three sites that were higher (RKM 60, 62, and 85). Only two sites (Alder Creek and Welcome Creek) showed increases in site level admixture that likely came from immigration. Our current samples at those sites included previously undetected parental RBT and hybrid individuals that had higher admixture than any individuals sampled in the historic sampling.

Environmentally mediated selection given propagule pressure

We collected a total of 1,179 genetic samples from individuals across 51 sites. We detected RBT alleles at 86% of the sites we sampled. However, only 61% of sites had greater than 1% pRBT and 31% of sites had greater than 10% pRBT (Table B.1). We detected 57 (of 1,663 fish genotyped) first generation hybrids (F1) at 16 sites. We classified F1s as individuals that were heterozygous at every RBT and WCT diagnostic marker that amplified. We had a minimum of at least 36 of the 39 markers amplified for all F1 calls. Based on a mitochondrial DNA marker, 70% of F1 hybrids had maternal RBT ancestry and remaining 30% had WCT maternal ancestry. The ratio was essentially the same regardless of whether individuals were captured in the mainstem or tributaries. We tested whether environmental conditions better described the pattern of admixture than propagule pressure (distance from source) alone.

The best supported model for both response variables contained only distance from source as a predictor variable (Table 3.3) including the other variables associated with environmental mediated selection did not improve any models (Δ AICc > 2). Additional variables were not significant within the models as the standard errors around the beta coefficients all encompassed zero. Therefore, the most parsimonious model explaining spatial patterns in RBT admixture was simply distance from source.

Forces of localized resistance

To examine whether hybrid sites show evidence of random mating (no assortative mating), we tested the distribution of hybrid individuals against the expected distribution given the overall site pRBT. Only a single site conformed to Hardy-Weinberg equilibrium (Upper Willow Creek) for expected distribution of RBT alleles in a random mating population. Three sites were comprised of only hybrid individuals and two sites consisted only of hybrids and parental RBT. Because the majority of our sites do not conform to an expected distribution of hybrids, we broadly describe some patterns of hybrid distribution that we observed at the site level (Table B.1 and Figure B.4-B.5) in order to identify potential localized resistance. We observed three general patterns of site level hybridization in Rock Creek (Fig. 3.3A) that we categorized as “high”, “porous”, and “low” resistance. High resistance sites (n = 11) were comprised only of non-hybridized individuals and made up 22% of all sites. Porous sites (n=26) (Fig. 3.3B) were sites where non-hybrid fish still made up the majority of the population (> 50%), but higher admixed individuals (> 0.50 pRBT) exist at low frequencies (> 15%), and no parental RBT were detected. Porous sites made up 51% of sites. Low resistances sites (n=14)

(Fig. 3.3C) are more typical of long-term hybridization where parental forms occur at low frequencies and there is a wide distribution of individual pRBT. Non-hybrid individuals were rare (< 50%) and hybrids made up the majority of the population. Low resistance sites made up approximately 27% of all sites.

Propagule pressure or dispersal

If dispersal was acting on site level resistance to hybridization, then we expected high resistance sites to be furthest from the source. High resistance site-level pRBT patterns were associated with increasing distance from source (

Figure 3.5. Comparison of mean values and 95% confidence intervals of distance from source across different levels of site level resistance in Rock Creek, MT *2019-2020.*High resistance sites occurred further upstream than low resistance sites with porous sites generally occupying the middle sections. Even though there was a strong distance from source relationship for site-level pRBT and current RBT populations we did not detect any effect of migratory individuals of either WCT or RBTxWCT on site level resistance. Migratory individuals were observed in all three types of sites (Fig 3.7A). However, migratory RBTxWCT occurred at a lower frequency than migratory WCT at high resistance (hybrid = 6: WCT = 18) and porous (10:25) sites. Migratory forms were overall lower but more evenly represented at less (3:3) resistant sites.

Not only is distance from source a good predictor of resistance, but the home range of RBT was smaller than hybrids or WCT (including during the spawning season) suggesting a lack of broad scale movement and dispersal of RBT. Home range (measured as distance between the most downstream and most upstream locations observed) decreased with increasing pRBT (Fig. 3.4). A linear regression showed that individual pRBT was significantly ($p < 0.001$, SE = 0.21), negatively associated with home range (log transformed for normality). RBT had the smallest, least variable home ranges (mean = 6.4 km, 6.1 SD), compared to RBTxWCT (mean = 14.5 km, 18.5 SD) and WCT (mean = 32.1, 28.7 SD). Additionally, there was some indication of hybrids straying, as approximately 42% of migratory hybrids spawned at sites where the highest individual pRBT at the site was less than the migratory individual pRBT. Four hybrids spawned in non-hybridized sites.

Assortative Mating – spatial overlap, temporal overlap, and habitat size

We tagged and tracked 190 *Oncorhynchus spp.* from 2018-2021. We observed telemetered fish spawning in 17 different tributaries in the Rock Creek drainage. We predicted that spatial and temporal overlap between migratory WCT and RBTxWCT would be lowest at high resistance sites. Of the telemetered fish that spawned there was strong assortment between RBT and WCT where 90% of WCT spawned in tributaries, no RBT spawned in tributaries. Most (76%) RBTxWCT entered tributaries for spawning and tended to spawn lower in tributaries. WCT spawned throughout the length of tributaries, overlapping with RBTxWCT lower down, but also further upstream than hybrids. There was more overlap between WCT and RBTxWCT at porous and low resistance sites as spawning distance from tributary mouth for WCT decreased as resistance decreased (Fig 3.6A). WCT spawning in high resistance sites spawned significantly higher in the tributary than WCT in porous and low resistance sites. There was strong spatial assortative mating between WCT and RBT, as RBT were not observed spawning in tributaries. RBT spawned earliest, but only in the mainstem, followed by RBTxWCT, and then WCT. Only at porous resistant sites did we see a difference between spawning dates of WCT and RBTxWCT (Fig. 3.6B). High resistance and porous sites had significantly later WCT spawning dates than

WCT in low resistance sites. There was no difference in discharge at base flow (habitat size) among site types (Fig. B.2).

Assortative mating – body size, migratory life history, and female choice

We predicted that more resistant sites would have stronger indicators of assortative mating (greater body size differences among genotypes, a higher abundance of migratory individuals, and increased selectivity in female choice). Total length of migratory individuals was significantly different between species ($p < 0.001$, $f = 10.7$, $df = 2$) (Fig. B.1). Tukey's test showed both RBT and RBTxWCT had mean TL greater than WCT ($p < 0.001$); there was no difference between RBT and RBTxWCT ($p = 0.55$). However, we saw no relationship between use by our tagged migratory fish and site level resistance (Fig. 3.6A). Resident populations (TL > 75mm & < 330mm) within the tributaries showed the opposite trend with WCT (146 mm, 95% CI = 141, 150) larger on average than RBTxWCT (121 mm, 95% CI =116, 126) and again larger than RBT (106 mm, 95% CI = 90, 123).

We predicted that resident larger body sizes would be associated with higher site level resistance. Mean total lengths of all resident *Oncorhynchus spp.* were significantly greater at high resistance sites (146 mm, 95% CI = 138, 154) than low resistance sites (116 mm, 95% CI = 111, 121). Mean total lengths at porous resistance sites (137 mm, 95% CI = 133, 142) were not significantly different than high resistance sites but were significantly greater than low resistance sites. The presence of migratory life histories was associated with differences in total lengths of residents (

Figure 3.9B). Mean total length of WCT at high resistance sites with migratory component (157 mm, 95% CI = 146, 168) were significantly larger on average than high resistance sites without migratory component (133 mm, 95% CI = 123, 142). There was no difference of resident total length between migratory and non-migratory streams at porous and low resistance sites.

Genetic drift associated with small population size

We expected that if genetic drift was influencing hybridization, then we would see increased site level pRBT variation at smaller population sizes. We observed a trend of decreasing variation in site pRBT with increasing CPUE (

Figure 3.). Sites with CPUE less than 0.03 fish/shock second (n =37) saw higher mean site level pRBT (0.16) and higher variance (0.25 SD) than sites with CPUE greater than 0.03 (n = 14, mean = 0.09, 0.14 SD). Migratory fish (both WCT and RBTxWCT) were associated with lower pRBT sites at lower population sizes.

Discussion

There are likely multiple mechanism and processes occurring at different scales that shape patterns of admixture across a watershed. We show that hybridization between WCT and RBT in Rock Creek has been relatively static over the last four decades. Studies have hypothesized different environmental characteristics such as gradient, productivity, and water temperature would limit the spread of hybridization. Our study demonstrates that these characteristics did not have a measurable influence on where we observed hybrid individuals or site level admixture. Rather, proximity to the highest current abundance of RBT alone was the best predictor of the distribution of admixture at a site. Finally, in addition to dispersal (or propagule pressure), we found evidence of assortative mating at sites that showed resistance to hybridization. Our examination of potential mechanism of resistance to hybridization showed that while there is evidence of localized biological resistance, the effect of propagule pressure was likely strong enough to overwhelm localized resistance.

Changes in admixture over time

We did not detect any directional change in pRBT over the last four decades in Rock Creek. This lack of directional change in Rock Creek suggests that hybridization is not broadly increasing across the landscape, and is different from Muhlfeld et al. (2017) who found more sites that increased in pRBT as those that didn't. This points to conclusion that that the outcome of hybridization between these two species is variable. Natural hybrid zones occur where hybridizing species exist in sympatry and these zones are maintained by selection and dispersal (Barton and Hewitt 1985). In order to explore the potential mechanisms that lead to hybrid zones (versus genomic extinction), examining this system in terms of evolutionary forces may provide some insight into what mechanisms might be maintaining this current pattern of hybridization

Environmentally mediated selection given propagule pressure

Current RBT abundance (distance from source) was the variable that consistently had the most support in predicting the landscape pattern of hybridization in Rock Creek. We did not find any evidence to support the hypothesis that cold water refuge would mitigate hybridization between these two species. Similar to Muhlfeld et al. (2017), we detected hybridization at sites well below 11°C mean August temperature (Isaak et al. 2015) and some streams warmer than 11°C were non-hybridized. While we did observe the broad gradient of admixture decreasing along a longitudinal gradient, we did not find that this correlated with environmental gradients suggested in other studies (Rassmusen et al. 2012; Yau and Taylor 2013; McKelvey et al. 2016; Young et al. 2016b). We suspect that this difference is due to the lack of correlation between stream temperature and landscape position within this drainage; some of the warmest tributaries occur in the upper river. Beyond environmental factors, we did not find evidence that habitats that would support whirling disease or historic tributary stocking better describe the landscape pattern of hybridization.

Sites with habitat characteristics (e.g., more fine sediment) that we expected to support *Tubifex spp.* and therefore have experienced more severe whirling disease did not help explain the pattern of hybridization beyond distance from source. While it is still unknown how *M. cerebralis* affects survival or fitness of RBTxWCT compared to either parental form. Brook Trout x Bull Trout *Salvelinus fontinalis x S. confluentus* hybrids show intermediary susceptibility to *M. cerebralis* compared to their parental forms (Wagner et al. 2002). Our analysis of this effect may be limited because we did not directly measure *Tubifex spp.* densities, infection rates, or disease load. Studies by Granath et al. (2007) and Granath and Vincent (2010) showed that measures of whirling disease in Rock Creek are highly variable from year to year and so we chose to use measurements of habitats that would likely support *T. tubifex* consistently over time. While whirling disease does not seem to directly affect the distribution of hybrids, it may have indirectly by reducing RBT abundances across the landscape. This suggests that if whirling disease had any impact on hybridization it likely was the historic reduction of RBT abundances in the mainstem rather than currently affecting resident hybrids or RBT in the tributaries. In fact, studies have shown RBT developing resistance to whirling disease in wild populations (Miller and Vincent 2008; Granath and Vincent 2010) and if RBT populations in Rock Creek develop resistance to the disease they may eventually increase in abundance.

Unlike previous studies that showed historic stocking associated with current patterns of admixture (Loxterman et al. 2014; Muhlfeld et al. 2017; Mandeville et al. 2019), we did not find support for historic stocking in our models as a predictor of current hybridization. While historical stocking of tributaries in Rock Creek is well documented, exact locations of mainstem stocking is less well documented. Yet, Rock Creek Road follows the creek its entire length from the mouth to the confluence of the forks and we assumed that RBT were stocked along the entire length of the road. Additionally, tributaries throughout the length of the watershed were stocked. One reason why we might not have found evidence of stocking in the current pattern of admixture is that RBT abundances in the mainstem were far greater than what was stocked in tributaries. Some of these tributary stockings were one-time events and may not have ever taken hold. Additionally, there may have been undocumented stocking events. However, this lack of an effect suggests that legacy effects of stocking, at least in the tributaries, is not driving the current hybrid landscape.

Microevolutionary forces of localized resistance

By taking a broader approach to evaluating resistance of hybridization on the landscape through consideration of evolutionary forces we see evidence for multiple mechanisms shaping site level distribution of hybrids including dispersal, assortative mating, and genetic drift at small population sizes. In the sites where we see reduced resistance, we see a decline across several mechanisms that we expect to maintain resistance: distance from source, spawning location and timing, migratory life history, and body size. This highlights that a range of mechanisms are needed to provide resistance. Additionally, intrinsic selection against hybrids may be a vital component to explaining the pattern of hybridization in the absence of environmentally mediated selection.

Propagule pressure and dispersal

Biological invasion theory indicates that successful freshwater invasions hinge on high abundances of invaders (Moyle and Light 1996). Holle and Simberloff (2005) demonstrated that the intensity of non-native plant propagule pressure can overwhelm biotic and abiotic resistance of native plant communities. Resistance to hybridization decreased for sites closer to the source of RBT in the mainstem. This provides further evidence that propagule pressure is the main driver of hybridization in Rock Creek. We suggest that distance from source acts as a lottery effect, the idea being that as a site is closer to a source, more individuals with RBT alleles have more chances to be successful at reproducing despite resistance mechanisms and selection working against individuals with RBT alleles. Our results suggest that the effect of distance from source may be enough to overcome selection and resistance to hybridization and as distance from source increases, other mechanisms of resistance are enough to limit the less frequent individuals with RBT alleles.

Several studies have shown changes in life history across the continuum of hybridization for WCT and RBT (Muhlfeld et al. 2009b; Corsi et al. 2013b; Kovach et al. 2015; Strait et al. 2021). While we observed shifts in life history across pRBT, we also detected changes in unexpected directions. We observed that individuals with higher pRBT not only had smaller home ranges but were less likely to spawn in tributaries compared to WCT. This suggests alternative conclusions to other studies that have shown dispersal and migratory life history increasing with higher individual pRBT. Strait et al. (2021) and Kovach et al. (2015) showed that rates of out-migration from natal tributaries in the North Fork of the Flathead was higher as pRBT increased, but it is unknown if these individuals then dispersed long distances, remained near their tributaries, or even returned to spawn in those tributaries. Boyer et al. (2008b) highlighted the importance of long dispersing, highly admixed individuals as sources for expanding hybridization. While we did observe some hybrids, both lowly and highly admixed individuals, moving greater than 1.5 times the inter quartile range, the majority of hybrid individuals had home ranges less than 20 km. Studies have demonstrated the presence of genetic drivers of migration in RBT (Arostegui et al. 2019). It's possible that through hatchery level selection or brood stock source that RBT used to stock Rock Creek may have lacked some of the genetic variation for migratory life history. This reduced dispersal/migratory life history corresponds with distance from source being the variable with the most predictive power. If RBT and RBTxWCT were more mobile, dispersed at higher rates, and successfully spawned in non-natal streams, then distance from source would not be as strongly associated with hybridization.

Assortative mating – Spatial overlap, Temporal overlap, and Habitat size

Assortative mating is an important mechanism in maintaining separation between species, but is highly plastic and environmentally dependent (Gauthey et al. 2016). At high resistance sites there was increased spatial separation between migratory WCT and RBTxWCT, but decreased temporal separation. Porous sites saw the opposite trend where there was high spatial overlap but low temporal overlap. There was high spatial and temporal overlap at low resistance sites. WCT spawned lower in tributaries at porous and low resistance sites where we would expect more overlap with RBTxWCT. This downstream shift in spawning sites could indicate that available spawning habitat is limited (given the narrow confidence intervals compared to high resistance sites) and have less capacity for spatial separation.

In terms of timing of spawning for migratory individuals, we do not see a clear trend across different levels of resistance. Rather we see overlapping spawn times between hybrids and WCT at both high and low resistance sites, but separation at porous resistance sites. This does not necessarily indicate a breakdown of assortative mating, but highlights that it is likely a weak mechanism to resist to hybridization. Two factors that may be at work here is that hybrids show intermediate life history traits (Muhlfeld et al. 2009b; Corsi et al. 2013a) during spawning and that they are more likely to overlap in spawn timing which is highly heritable in salmonids. Secondly, males tend to arrive earlier and later than females at spawning grounds (Quinn 2018). It's unclear which phenotype (hybrids or WCT) show stronger assortative mating. Early arrived WCT males are likely to overlap with RBTxWCT females, and late RBTxWCT males are more likely to overlap with WCT females. Understanding which phenotype is more selective, whether males of either phenotype are driving the overlap in spawn timing will illuminate where potential hybridization occurs and whether it is driven by male or female choice or both.

Assortative mating – body size, migratory life history, and female choice

Assortative mating beyond spatial and temporal differences in spawning is driven by body size and mate choice (Fukui et al. 2018; Auld et al. 2019). Across the watershed we saw a decrease in total length as individual pRBT increased. We also saw that mean total length of residents decrease at low resistance sites. This means that WCT were generally larger but at low resistance sites individuals were smaller and more equal in size. This meant increased overlap in body size, particularly for resident RBT and hybrids which tended to be smaller on average. Smaller bodied individuals generally have a lower competitive ability and less reproductive success (Fleming and Gross 1994; Blanchfield et al. 2003; Jacob et al. 2007; Labonne et al. 2009). WCT at high resistance sites were generally larger so we would expect WCT males to be more competitive for larger WCT females (more fecund, greater demographic impact). As the size difference between WCT and hybrids decline, then WCT males lose the competitive advantage associated with body size and WCT females lose the reproductive capacity advantage.

We saw the opposite trend in body sizes for migratory individuals than we saw in resident populations. Migratory WCT were smaller on average than their RBT and RBTxWCT counterparts. Yet high resistance sites with migratory populations had significantly larger residents than non-migratory streams. This effect was not apparent at porous and low resistance sites. While we were not able to effectively determine sex for migratory individuals, generally migratory fish are expected to skew toward a higher proportion of females, given that the advantages of size tend to benefit females. One hypothesis may be that size-based assortative mating would favor larger resident WCT spawning with migratory WCT over smaller hybrids or RBT. High resistance sites with higher relative abundances of migratory WCT and larger resident sizes should lead to stronger assortative mating and selection for non-hybrids. Migratory individuals were more prevalent at high resistance sites, but WCT outnumbered RBTxWCT migrants three to one. At low resistance sites migratory individuals were less abundant and the ratios were equal. This conforms to the expectation that larger, more fecund migratory individuals will skew hybridization toward that genotype, particularly where one genotype is more abundant.

Female mate selection can drive asymmetric hybridization and may explain the lack of bimodal distribution of hybrid alleles that we observe at sites that show resistance. Lower encounter rates between swordtails decreased “choosiness” of females between hybrids and conspecific non-hybrids (Willis et al. 2011) and Peters et al. (2017) found asymmetrical hybridization between two species of Darwin’s finches was driven by female choice for conspecifics and hybrids that were morphologically indistinguishable. The skew toward maternal RBT ancestry in F1 hybrids suggests differential mate choice among the parental genotypes. This could occur as small WCT sneaking between larger RBT spawning in the mainstem, large RBT females choosing WCT males when RBT abundances are low, or it may indicate spawning between resident parentals in tributaries. Parental RBT were rarely observed in tributaries (either as residents or migratory spawners) so it is likely that this initial hybridization is occurring in the mainstem. Given that at the landscape scale RBT are the rarer parental form, this could indicate a breakdown in mate choice based on encounter frequency, or it could indicate that RBT are less discriminatory to begin with. If female choice were driving assortative mating then hybrids should be produced by females of the less abundant species and males of the more abundant species (Wirtz 1999). It is unknown if there is differential mate choice between WCT or RBT females.

One aspect of spawning behavior that we did not investigate is sneaking by small males. Sneaking strategies among resident males can effectively breakdown assortative mating based on body size and drive unidirectional hybridization (McGowan and Davidson 1992; Baxter et al. 1997). Sneaking strategies have not been documented in WCT but are common in other salmonids. Generally, sneaking strategies are advantageous when difference in body sizes is greater among males. Differences in total length between migratory and resident WCT was not substantial, suggesting that sneaking strategies may not be advantageous among that population. However, size differences were greater between resident WCT and migratory RBT. Choosing heterospecific can be beneficial if hybrids have increased fitness (Pfennig 2007), but given that RBTxWCT offspring generally are less fit, this should reinforce conspecific mate choice.

Intrinsic selection

Given a lack of evidence for environmentally mediated selection in our study, intrinsic outbreeding depression may be an important source of resistance. Studies have shown a pattern of selection against non-native RBT alleles (Allendorf et al. 2004; Kovach et al. 2015, 2016) and reduced fitness of hybrids (Muhlfeld et al. 2009a). Yet despite this negative selection, RBTxWCT hybrids persist and even continue to expand. Kovach et al. (2015) and Lowe et al. (2015) suggest that higher dispersal rates of hybrids helped to counterbalance selection against hybrids. It’s unclear whether there are genetic incompatibilities between WCT and RBT hybrids, particularly backcrossed individuals. However strong evidence for intrinsic outbreeding depression has been observed between distinct runs of Pacific salmon in Auke Creek, Alaska (Gharrett et al. 1999; Gilk et al. 2004) and different life histories of brook trout (Mavarez et al. 2009). Kovach et al. (2016) showed that selection against non-native RBT alleles occurred across a range of environmental conditions. Bierne et al. (2011) provides evidence that hybrid tension zones are largely shaped by intrinsic outbreeding and their location on the landscape is limited by barriers to dispersal. The result is that hybrid zones can become “trapped” along environmental gradients. At the landscape scale this gives the appearance of strong environmentally mediated selection. They state that, “[l]ocal adaptation explains the position of the genetic break but not necessarily its maintenance.” The lack of strong, consistent environmental selection against RBTxWCT hybrids in our study and others, coupled with reduced hybrid fitness and the importance of dispersal (and barriers to RBT dispersal such as limited home range and mainstem spawning habitat preference) suggests that Bierne et al.’s hypothesis might help explain how the landscape pattern of hybridization is maintained in systems such as Rock Creek.

Genetic drift associated with small population size

To our knowledge, this is the first study to investigate the potential of genetic drift to influence hybridization at a site. Habitat modification, climate change, and loss of migratory life histories can genetically isolate populations. Once gene flow is restricted, genetic diversity is reduced even in relatively large habitat sizes (Carim et al. 2017). If sites become functionally isolated after initial hybridization and/or breeding populations decline, then populations may increase or decrease site level admixture regardless of other forces, potentially creating new sources of hybrids if mechanisms that isolated these systems are “leaky.” Kovach et al. (2021) showed that while isolated populations of WCT were strongly influence by drift there was evidence that small populations without barriers were influence by drift as well, indicating that drift can be a measurable evolutionary force in both fragmented and connected systems.

Management implications and conclusions

Patterns of hybridization are complex and vary across different systems. The pattern of hybridization across the Rock Creek watershed was most related to current RBT abundances in the mainstem. Yet, assortative mating between genotypes provided resistance in this connected watershed. Localized resistance is likely not enough to maintain the current distribution of hybridization if conditions change. However, if RBT abundances were reduced in the main river, then localized resistance may help to limit hybridization. Applying Fausch et al.'s (2009) framework for managing native salmonids, this population of WCT in Rock Creek is of high conservation value (evolutionary, ecological, and socioeconomic). For intact networks with varying degrees of invasion they highlight preventing habitat degradation, maintaining natural processes, and control of non-natives.

Our results highlight the importance of continued parental RBT presence in driving patterns of hybridization within a watershed. While Rock Creek has managed to maintain a WCT population with a diverse life history, changes or disturbances to the system could alter the hybrid distribution across the landscape. The decline in RBT abundances has given mangers an opportunity to effectively limit propagule pressure. The effectiveness of targeted RBT and hybrid removals in large, connected river systems is still unclear, but promising (Al-Chokhachy et al. 2014; Kovach et al. 2018a). Targeted removal of RBT and hybrids in the Gros Ventre River, Wyoming over a 15 year period resulted in a reduction in individual and population level pRBT (Kovach et al. 2018a). Reducing the abundance of non-natives where they are most abundant (mainstem) will reduce propagule pressure and in turn allow mechanisms like assortative mating to reinforce resistance in sites where propagule pressure is currently high.

The capacity for genotypes to assortative mate has been overlooked as a native salmonid conservation tool. Streams with ample, well-distributed spawning habitats, unimpacted hydrologic regimes, and migratory life histories will promote the strongest assortative mating. This may help explain the remnant migratory WCT life history and persistence of non-hybridized sites. Alternatively, changes to hydrologic regimes that compress spawn timing, losses of spawning habitat and migratory life histories will reduce assortative mating. In some cases, streams that are naturally limited in spawning habitat (e.g., natural barrier, intermittent flows) may lack strong assortative mating. These types of systems may still show lower resistance after propagule pressure reduction and selective passage barriers coupled with removals may be appropriate.

Anthropogenic changes have dramatically altered freshwater fish communities and will likely continue to change them in unexpected ways (Kuczynski et al. 2018) and climate change is expected to favor non-native expansion in the intermountain West (Muhlfeld et al. 2014, 2017; Al-Chokhachy et al. 2016). We provide another management tool for managers prioritizing conservation of native salmonids in large, connect systems that are threatened with hybridization. More broadly, we’ve synthesized several crucial aspects of fish biology and microevolutionary forces to work toward a more wholistic understanding of hybridization in fishes.

Table 3.1. Microevolutionary forces from Eq. 1 used to address objectives 2 and 3, measured variables for each mechanism, predictions, and literature supporting predictions.

Study Objective	Hypothetical Mechanisms	Variable	Prediction	Other Studies
<u>Objective 2</u>: Environmentally mediated selection given propagule pressure	Extrinsic or Environmentally mediated selection (s)	Mean August Temp	If environmental selection is acting against hybrids then, hybridization should be reduced as site temperatures below 11°C	Rassmusen et al. 2012; Yau and Taylor 2013; Isaak et al. 2015; Young et al. 2016a, 2017a; McKelvey et al. 2016
		Discharge at base flow	Higher base flow (higher stream order) and higher percentage of fines sediment should indicate better habitat for *Tubifex spp.* and prevalence of whirling disease which should decrease hybridization.	Sauter and Güde 1996; de la Hoz Franco and Budy 2004; Hallet and Bartholomew 2008; Granath and Vincent 2010; Carim et al. 2015.
		% Fines		
	Dispersal/Propagule Pressure (m)	Distance from source	If propagule pressure is acting on hybridization, then we expect to it to be strongest near the contemporary highest source of non-native admixture.	Rubidge and Taylor 2005; Boyer et al. 2008a; Gunnell et al. 2008; Muhlfeld et al. 2009b, 2009c, 2017; Bennett et al. 2010; Marie et al. 2012; Yau and Taylor 2013; Kakareko et al. 2016; Heim et al. 2020
		Historic Stocking	Streams that received greater amounts of RBT stocking will be more hybridized.	Loxterman et al. 2014; Muhlfeld et al. 2017; Mandeville et al. 2019
<u>Objective 3</u>: Microevolutionary forces of localized resistance	Dispersal/Propagule Pressure (m)	Distance from source	If dispersal is acting on hybridization, then we expect to see higher resistance sites further from the source of non-natives as frequency of encounters with heterospecifics decrease.	Willis et al. 2011; Atwell and Wagner 2014; Hinton et al. 2018
		Home range	If dispersal is strongly related to the landscape pattern of hybridization (i.e., declining admixture as distance from source increases), then we would expect that home range of RBT should be reduced compared to RBTxWCT and RBT.	Avise and Saunders 1984; Wirtz 1999; Willis et al. 2011; Atwell and Wagner 2014; Hinton et al. 2018
	Assortative Mating (A)	Spawning location	Sites with higher resistance should show less spatial overlap.	Ostberg et al. 2004; Buehrens et al. 2013

		Spawn timing	Sites with higher resistance should show less temporal overlap.	McMillan et al. 2007
		Discharge at base flow	Sites with higher resistance should have higher discharge at base flow. Positive assortative mating will break down in smaller streams as spatial overlap and encounter rates with heterospecifics increases.	Jansson and Öst 1997; Ostberg et al. 2004; Thériault et al. 2007; Buehrens et al. 2013
		Total length (mm)	In non-migratory populations, sites with higher resistance will have larger bodied resident WCT.	Foote and Larkin 1988; Aspinwall et al. 1993; Fleming and Gross 1994; Auld et al. 2019
		Migratory Abundance	In migratory populations, sites with higher resistance will have more migratory WCT and few or no migratory RBTxWCT.	Kanda et al. 2002; Ostberg et al. 2004
		Female Choice (Not tested in this study)	If female choice for conspecifics is strong, then we expect to see directional hybridization toward the more common genotype.	Wirtz 1999; Peters et al. 2017
	Intrinsic or Environmentally independent selection (s)	(Not tested in this study)	Selection against non-native alleles will lead to a basin wide reduction in hybrids regardless of local conditions.	Muhlfeld et al. 2009a; Mavarez et al. 2009; Bierne et al. 2011; Kovach et al. 2015, 2016
	Genetic drift associated with small population size (N)	Catch per unit effort (CPUE)	If genetic drift is acting on hybrid populations, then smaller populations should have increased variability in site level admixture.	None found, but extensive population genetic theory.

Table 3.2. Changes in pRBT and hybrid individuals over time at 14 sites in Rock Creek, MT. RM is the distance upstream of the mouth of the waterbody. All current samples were analyzed with 39 markers. P-values were calculated using Fisher's exact test.

Waterbody	RM	Historic pRBT (No. Markers)	Current pRBT	pRBT Change	Years Between Samples	Fisher Exact p-value
Alder Creek	*0.2*	*0.13 (14)*	*0.35*	*+0.22*	*11*	*<0.01**
Bear Creek	*2.3*	*0.14 (14)*	*<0.01*	*-0.14*	*11*	*<0.01**
Brewster Creek	4.4	0.00 (14)	0.00	0.00	11	1.00
Brewster Creek	1.4	0.15 (14)	0.14	-0.01	11	0.65
Camp Creek	0.1	0.00 (18)	0.01	+0.01	10	0.40
Cowan Gulch	2.7	0.00 (5)	<0.01	<0.01	27	1.00
Meadow Creek	3.5	0.00 (18)	0.00	0.00	11	1.00
North Fork Rock Creek	2.8	0.00 (14)	0.00	0.00	11	1.00
Sand Basin Creek	0.1	0.00 (14)	0.00	0.00	11	1.00
Stony Creek	*0.3*	*0.33 (5)*	*0.12*	*-0.21*	*38*	*<0.01**
Upper Willow Creek	13.0	0.00 (5)	<0.01	<0.01	23	1.00
Welcome Creek	*0.3*	*0.06 (5)*	*0.61*	*+0.55*	*37*	*<0.01**
West Fork Rock Creek	1.9	0.00 (5)	0.02	+0.02	28	0.16
Windlass Gulch	*0.6*	*0.52 (14)*	*0.36*	*-0.16*	*13*	*<0.01**

Table 3.3. Model comparison of models testing alternative hypotheses to distance from source. * indicates a significant p-value.

Model	**AICc**	**ΔAICc**	**BIC**
pRBT ~ Distance*	10266.6	0.0	10281.8
pRBT ~ Distance* + Historic Stocking	10268.3	1.7	10288.5
pRBT ~ Distance* + Mean Aug Temp	10268.4	1.8	10288.7
pRBT ~ Distance* + Discharge + Fines	10270.3	3.7	10295.6
Hybrid ~ Distance*	988.6	0.0	1003.8
Hybrid ~ Distance* + Mean Aug Temp	990.3	1.7	1010.5
Hybrid ~ Distance* + Historic Stocking	990.4	1.8	1010.7
Hybrid ~ Distance* + Discharge + Fines	992.0	3.4	1017.3

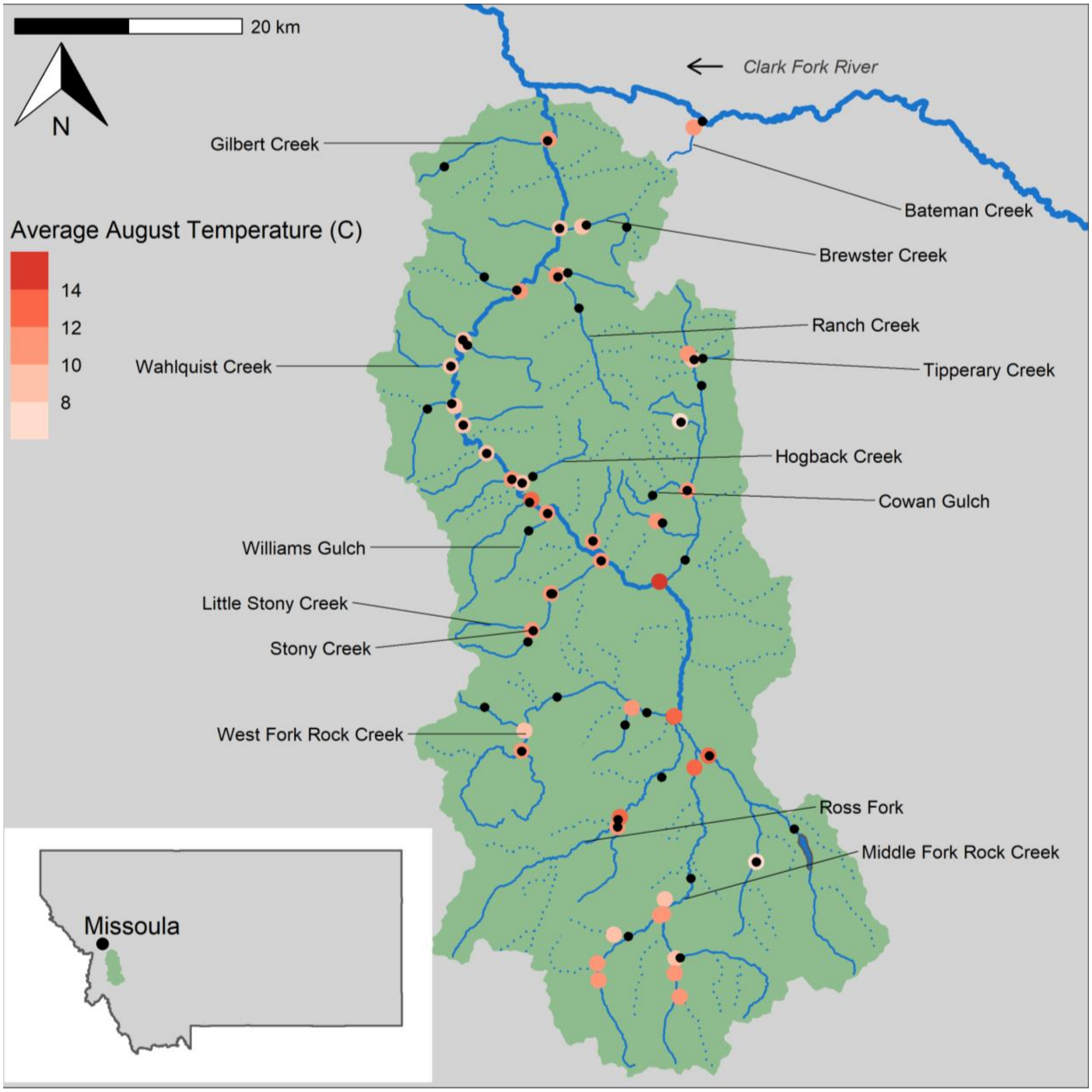


Figure 3.1. Map of the Rock Creek drainage and study area in western Montana with location of temperature loggers (color) and electrofishing and genetic collection sites (black). Named tributaries are where we observed telemetered migratory *Oncorhynchus spp.* spawning. Dotted tributaries are small or ephemeral streams that were not sampled.

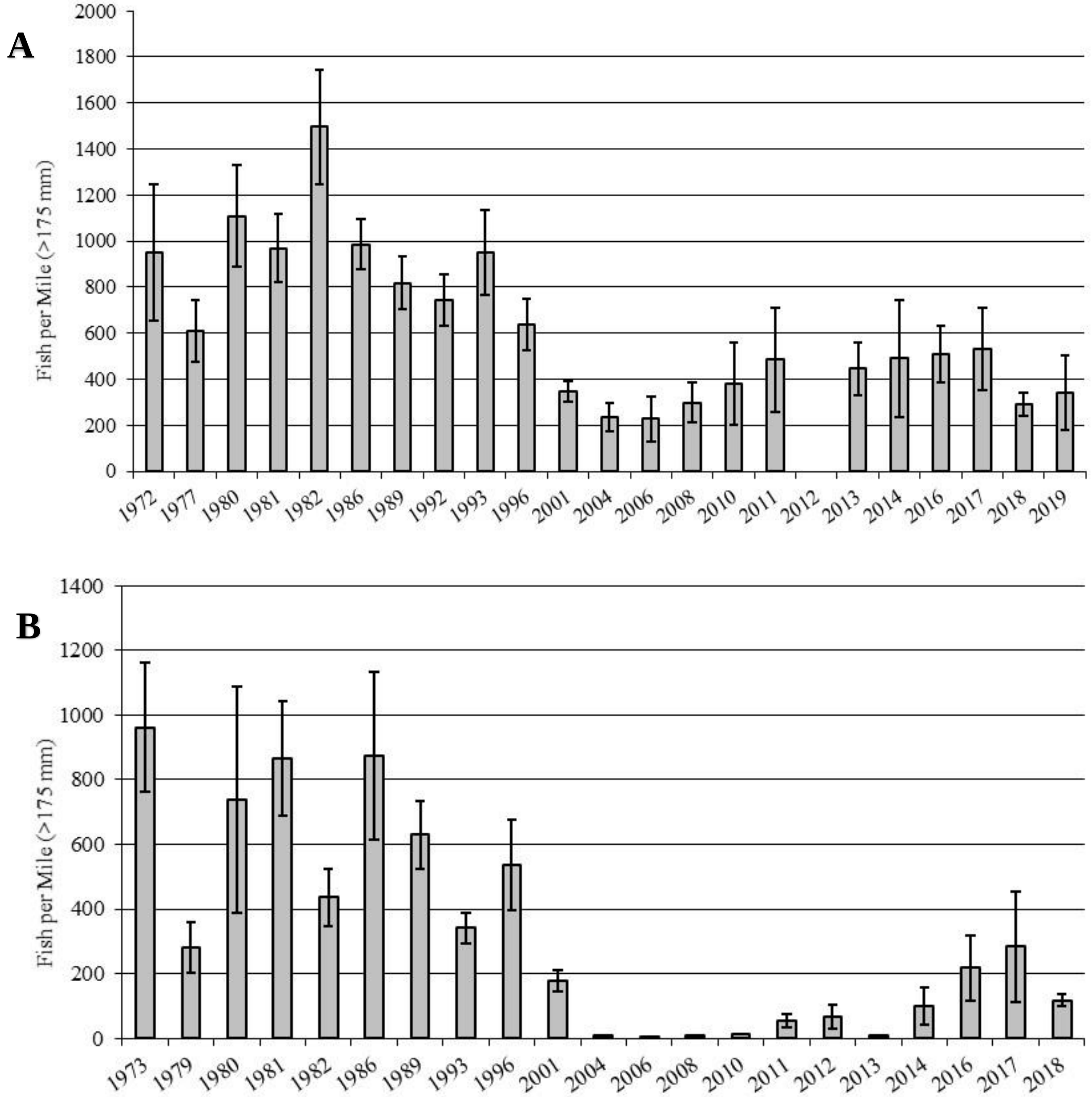


Figure 3.2. Population estimates and 95% confidence intervals for RBT in the lower (A) and middle (B) sections of Rock Creek over the past 45 years. Whirling disease was detected in Rock Creek in 1996 and is likely the mechanism behind the decline in RBT abundances in the early 2000s (Liermann 2021).

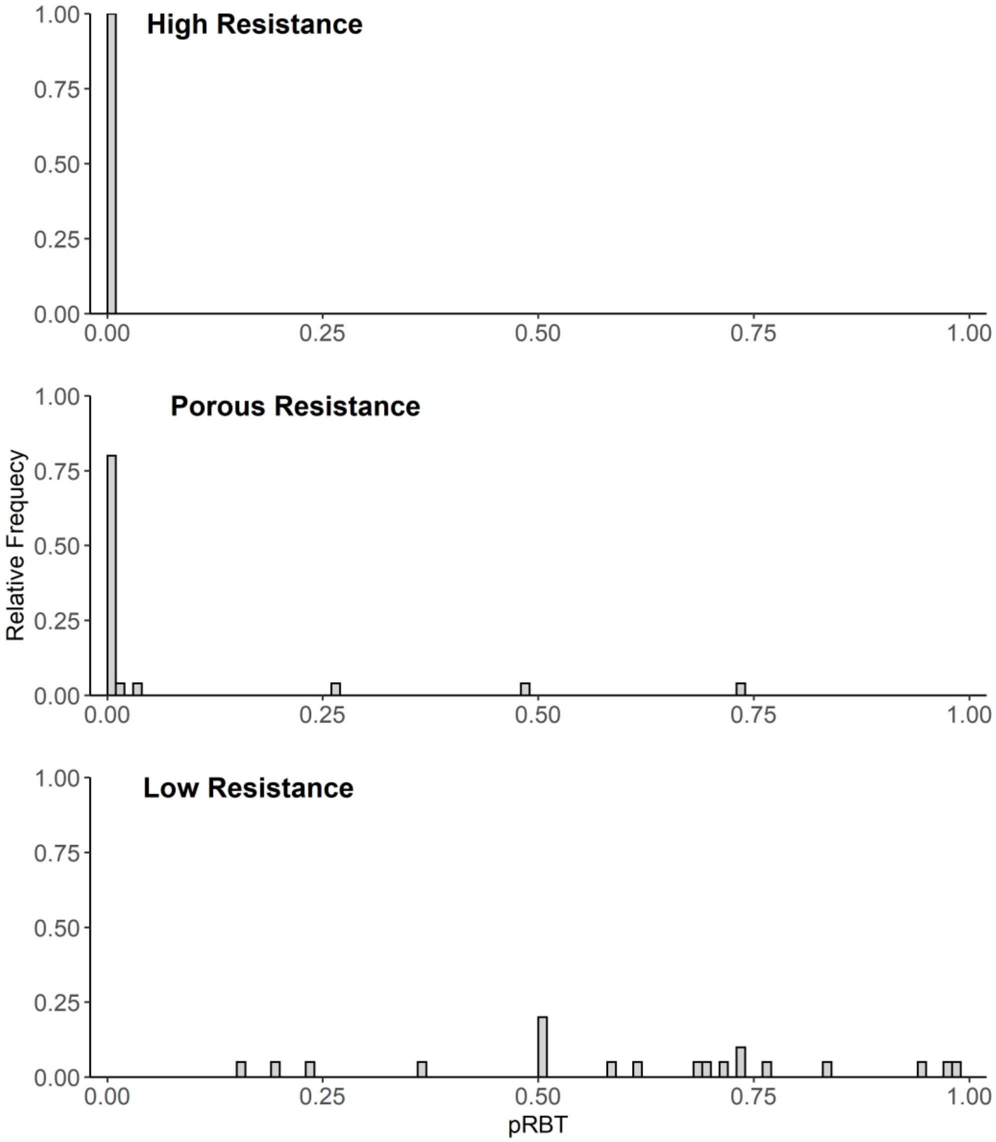


Figure 3.3. Histograms of example distribution of individual pRBT at three typified hybrid sites in Rock Creek, MT 2019-2020. pRBT values of 0.00 are parental WCT and 1.00 are RBT.

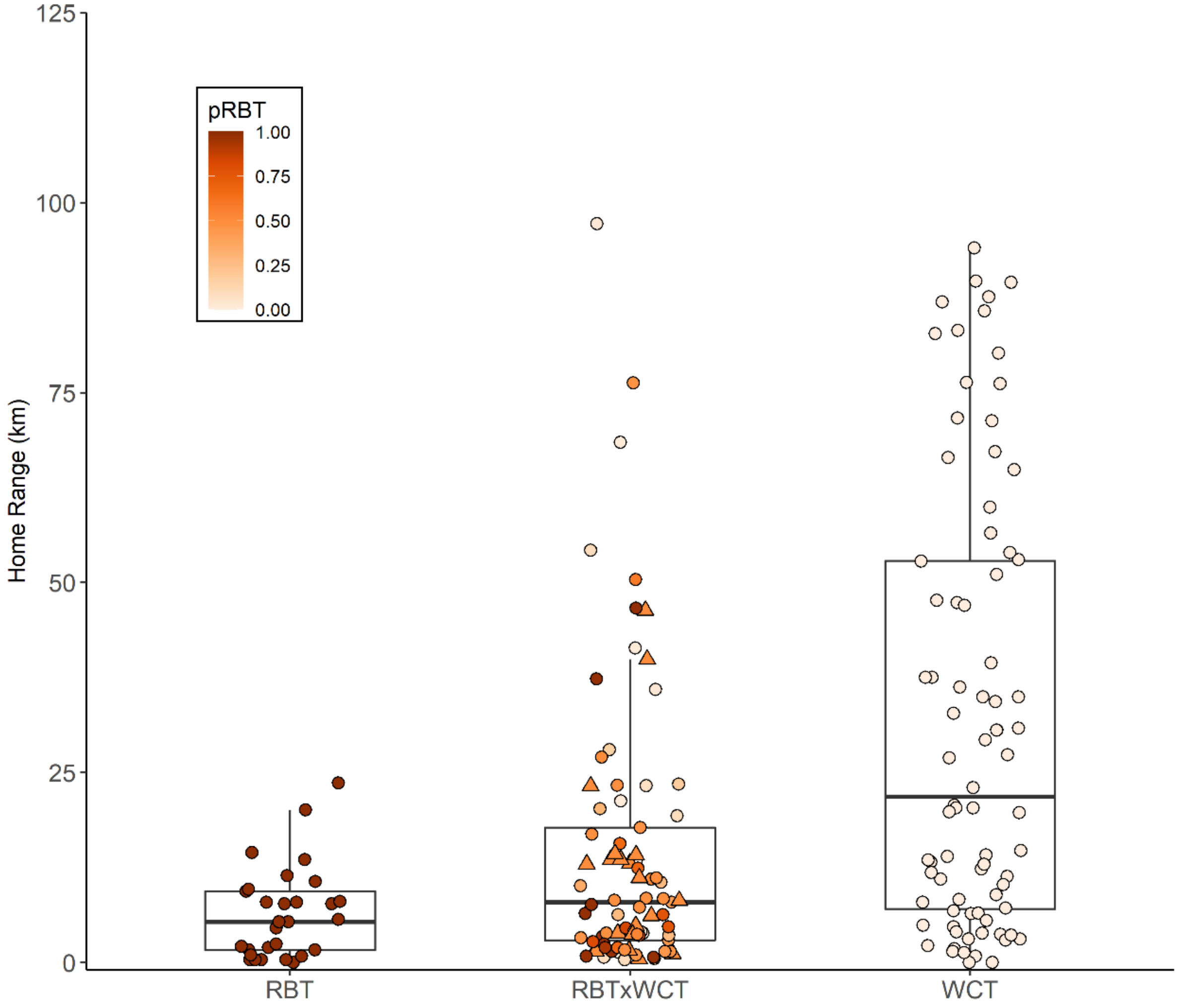


Figure 3.4. Home ranges of telemetered individual RBT (n = 29), RBTxWCT (n = 81), and WCT (n = 80) in Rock Creek, MT 2018-2021. Triangles indicate first generation hybrids.

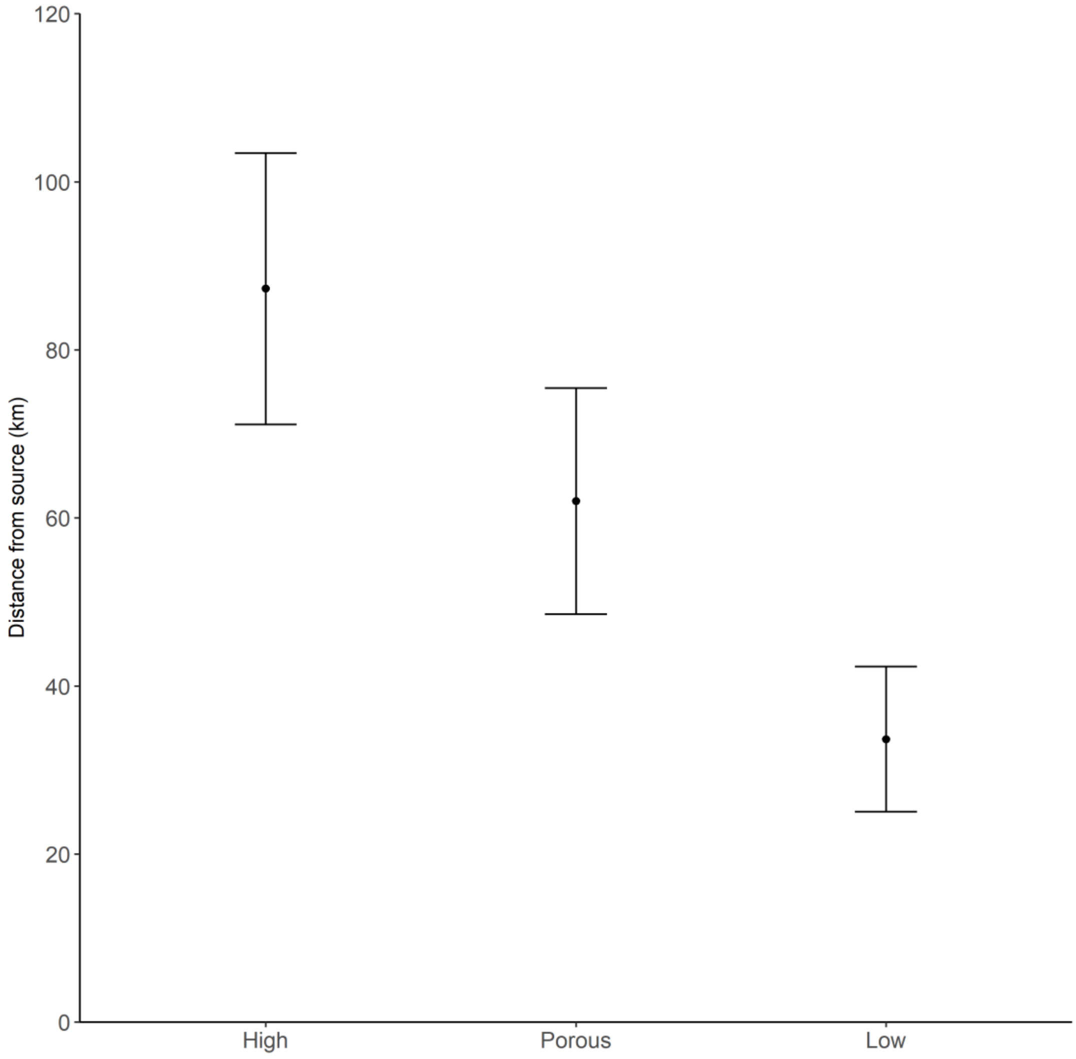


Figure 3.5. Comparison of mean values and 95% confidence intervals of distance from source across different levels of site level resistance in Rock Creek, MT 2019-2020.

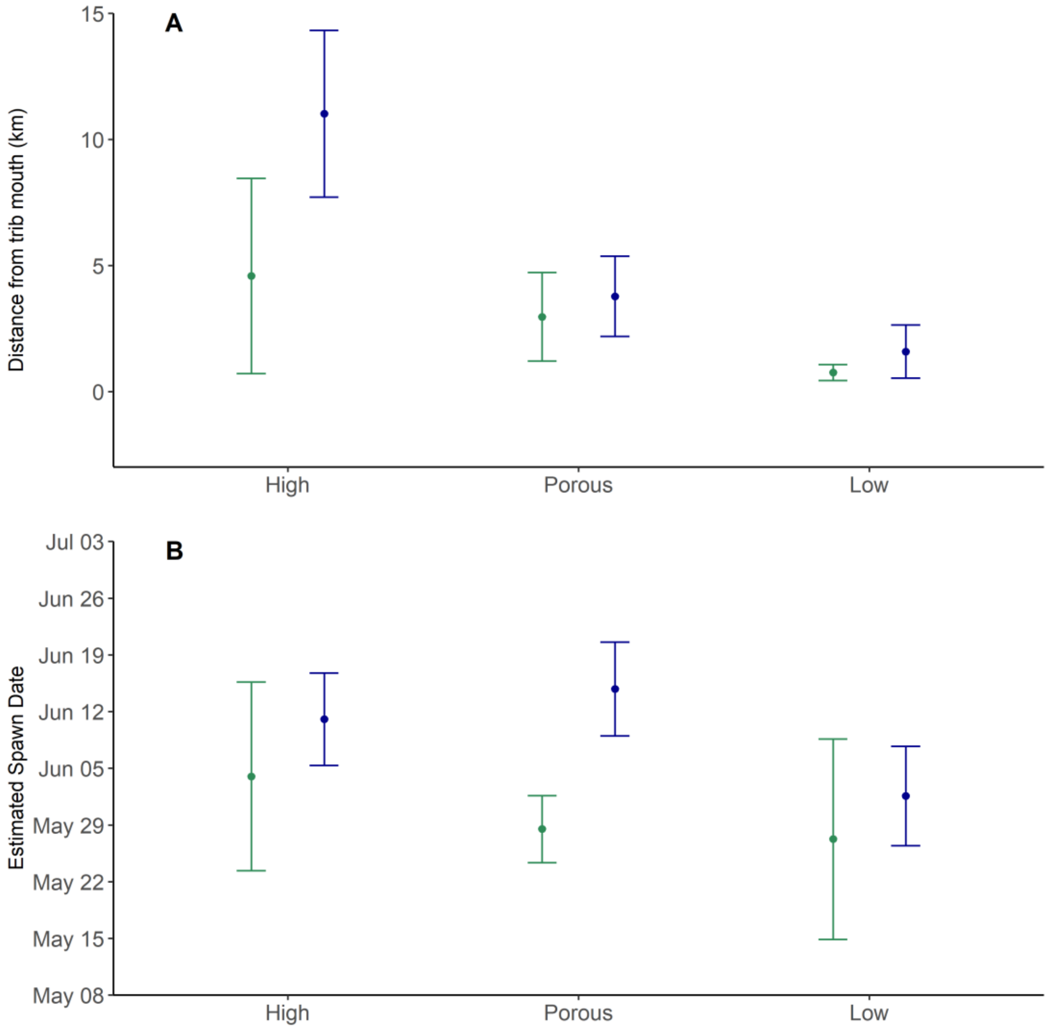


Figure 3.6. Comparison of mean values and 95% confidence intervals for spawning tributary overlap (**A**) and spawn timing (**B**) between WCT (blue) and RBTxWCT (green) across different hybridization resistance levels in Rock Creek, MT 2018-2021.

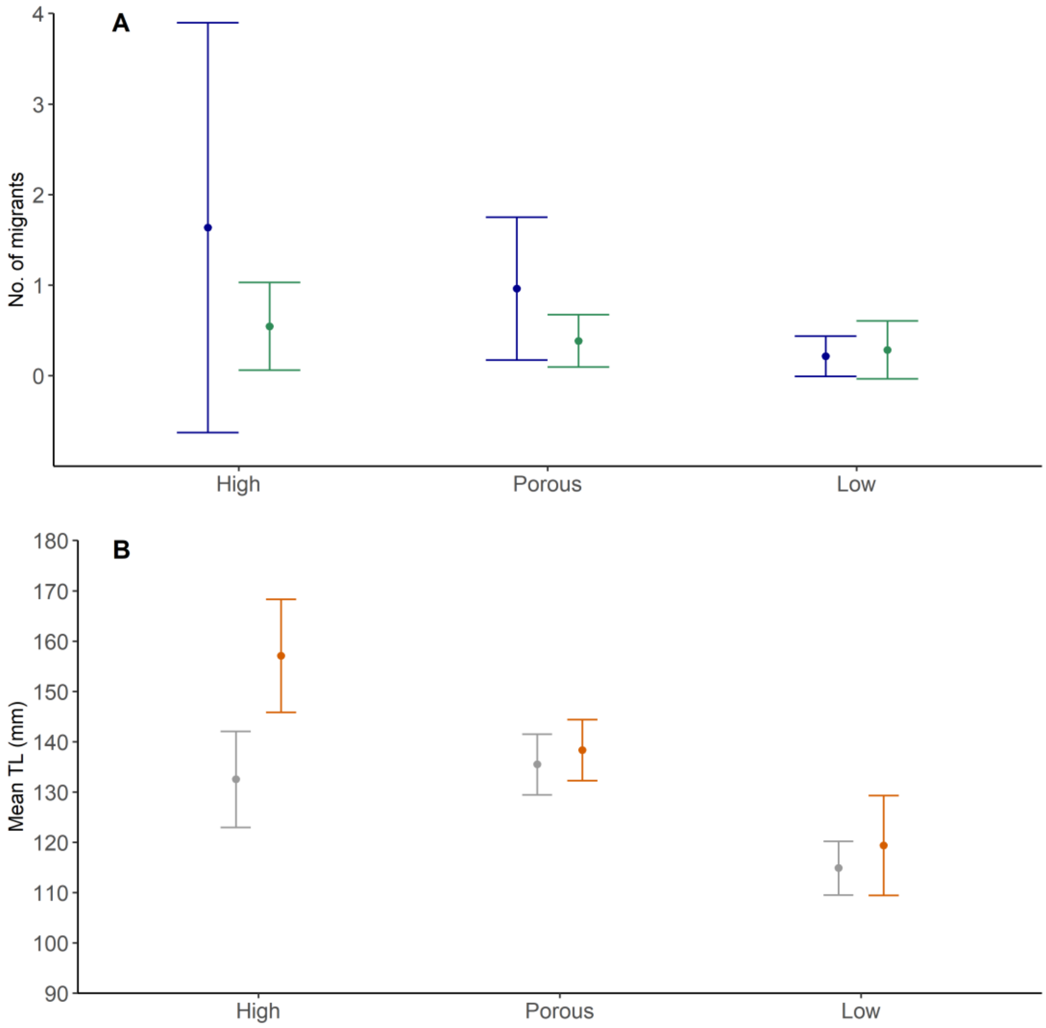


Figure 3.9. Comparison of mean values and 95% confidence intervals of (**A**) number of telemetered WCT (blue) and RBTxWCT (green) and (**B**) mean total lengths of resident individuals (75 – 330 mm TL) for migratory (orange) and non-migratory (grey) streams across different levels of hybridization resistance in Rock Creek, MT 2018-2021.

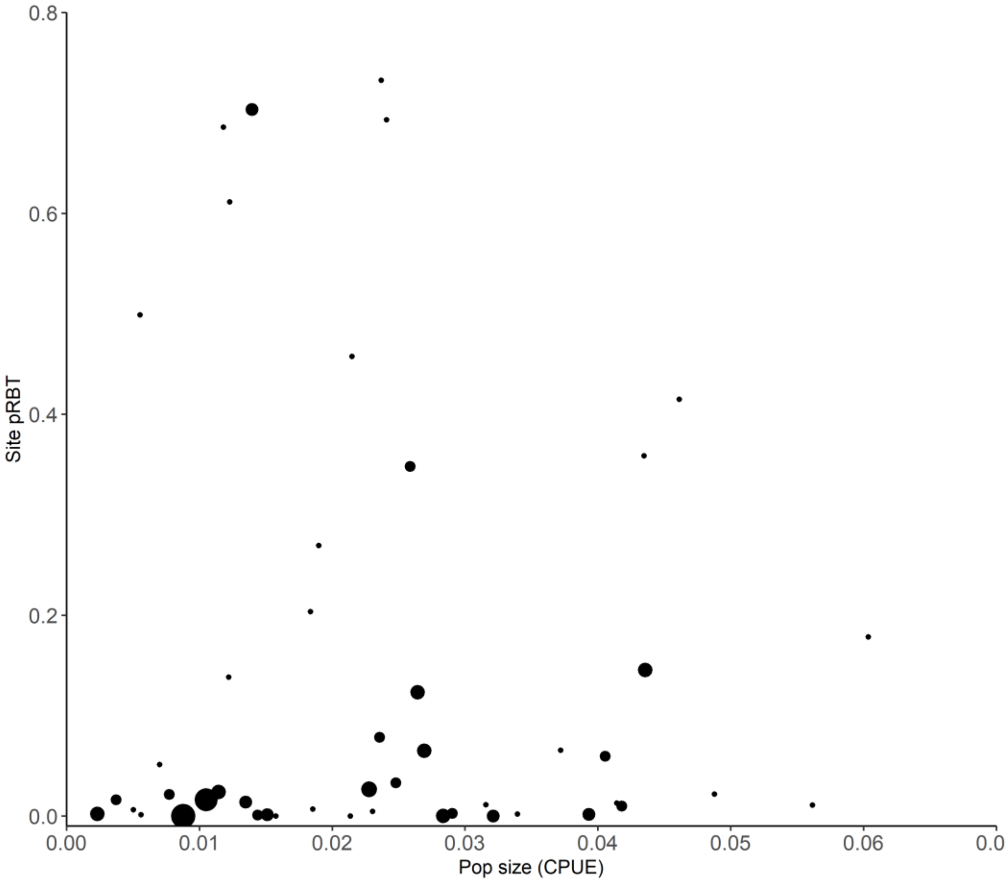


Figure 3.8. Plot of relative densities of *Oncorhynchus spp.* by site level pRBT in Rock Creek, MT 2019-2020. Larger dots indicate high abundance of migratory WCT and RBTxWCT.

APPENDIX A

TABLES

Table A.1. Summary of capture and tagging of *Oncorhynchus spp.* for radio telemetry by year, river section (river kilometer, RKM), and species for Rock Creek, MT 2018-2021.

Year	**Capture Location (RKM)**	**WCT**	**RBxWCT**	**RBT**
2018	CFR (0)	0	0	0
	Tamarack (8)	5	3	0
	Wahlquist (45)	9	1	0
	Hogback (51)	0	0	0
	Stony (69)	14	1	0
	Forks (80)	0	0	0
2018 Total		*28*	*5*	*0*
2019	CFR (0)	4	2	0
	Tamarack (8)	9	7	7
	Wahlquist (45)	3	10	2
	Hogback (51)	0	0	0
	Stony (69)	8	12	2
	Forks (80)	7	0	0
2019 Total		*31*	*31*	*11*
2020	CFR (0)	4	3	0
	Tamarack (8)	11	2	0
	Wahlquist (45)	5	2	0
	Hogback (51)	0	17	7
	Stony (69)	1	11	2
	Forks (80)	0	0	0
2020 Total		*21*	*35*	*9*
2021	CFR (0)	0	3	3
	Tamarack (8)	0	7	6
	Wahlquist (45)	0	0	0
	Hogback (51)	0	0	0
	Stony (69)	0	0	0
	Forks (80)	0	0	0
2021 Total		*0*	*10*	*9*
Grand Total		80	81	29

Table A.2. Habitat variables for 52 reaches across 38 tributaries within the Rock Creek drainage 2019-2020. Variables were used in univariate analysis and as covariates in the linear discriminant function analysis to investigate the relationship between natal streams characteristics and migratory categories. Bonferroni corrected p-values for each ANOVA test. * indicates significant (p-value < 0.05) difference between migratory categories for untransformed variables.

Variable	**Description**	**Mean**	**SD**	**Min**	**Max**	**p-val**
Mean Aug Temp	Mean stream temperature (°C) for the month of August	10.4	1.6	7.1	14.6	0.986
Growing Degree Days	Number of days where stream temperature was greater than 4°C	658.8	342.3	293.3	2033.2	1.000
Elevation	Elevation (m) at the upstream end of reach	1558.1	245.3	1133.0	2140.0	1.000
Landscape Position	Distance (km) of tributary confluence from mouth of Rock Creek	54.2	30.1	6.8	92.9	0.560
Base Flow	Discharge (m^3/s) at base flow	0.26	0.35	0.02	1.57	0.006*
Large Woody Debris	Number of large woody debris observed within the reach	6.2	4.5	0.0	16.0	1.000
Overwinter Pools	Number of pools deeper than 0.5 m per m^2	0.005	0.005	0.0	0.019	1.000
Adult Biomass	Total biomass (g) of fish greater than 150 mm TL divided by reach area (m^2)	28.4	46.0	0.0	236.0	0.030*
Juvenile Biomass	Total biomass (g) of fish less than 150 mm TL divided by reach area (m^2)	6.1	5.7	0.8	26.8	1.000
Brook Trout Density	Number of Brook Trout per m^2	0.08	0.20	0.0	0.98	1.000
Fines	Estimated % of fine (< 2.5 mm) substrate within the reach	22.1	20.8	1.0	97.5	1.000

FIGURES

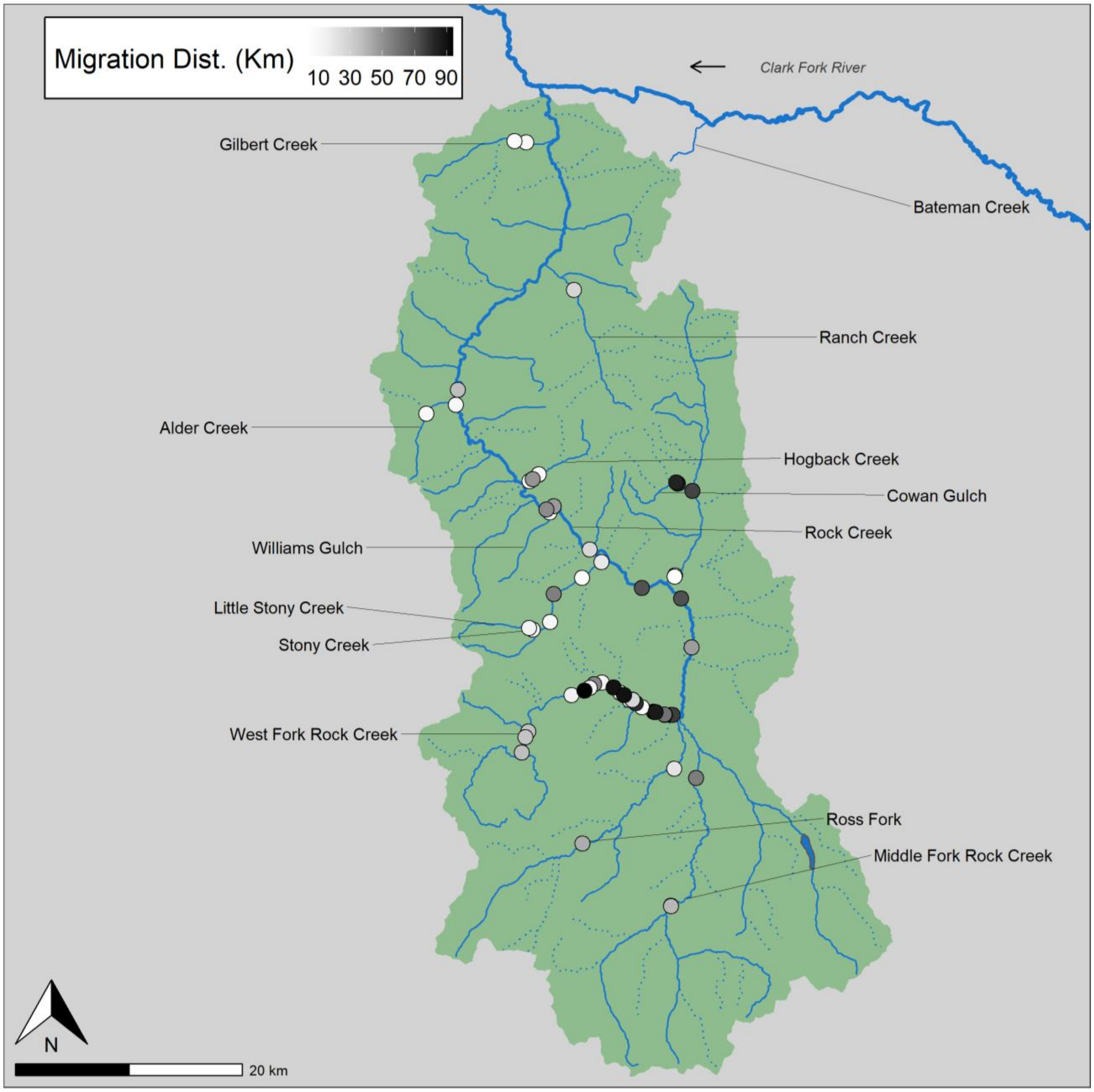


Figure A.1. Spawning locations and migration distances of telemetered WCT in Rock Creek, MT 2018-2020.

APPENDIX B

TABLES

Table B.1. Table of site level metrics for 51 sites sampled in Rock Creek, MT 2019-2020. * indicates temperatures that were not directly measured. Estimate of genetic mixing (m_d) between RBT and WCT using Kalinowski and Powell's (2015) mixing parameter. m_d is undefined when only one parental form exists.

Waterbody	**RKM**	**Mean Aug. Temp (°C)**	**Discharge at base flow (m^3sec^{-1})**	**Fines (%)**	**Sample Size**	**No. Hybrids**	**Mean pRBT**	**Genetic mixing (m_d)**	**Resistance**
Alder Creek	0.3	9.5	0.16	1	24	2	0.033	0.60	Low
Alder Creek	2.7	8.8*	0.12	5	21	15	0.348	0.56	Low
Bateman Creek	0.2	10.7	0.02	23	30	18	0.013	1.00	Porous
Bear Creek	3.7	7.5	0.02	45	22	4	0.004	0.99	Porous
Beaver Creek	1.9	11.3	0.04	35	24	1	0.001	1.00	Porous
Big Spring Creek	0.2	11.8	0.05	5	30	22	0.178	0.77	Low
Brewster Creek	7.1	7.4*	0.11	15	30	0	0.000	-	High
Brewster Creek	2.3	9.3	0.12	40	20	6	0.138	0.57	Porous
Butte Cabin Creek	0.5	9.2	0.18	3	22	22	0.499	0.83	Low
Camp Creek	0.2	11.7	0.02	13	30	6	0.011	0.86	Porous
Carpp Creek	0.5	9.6	0.34	5	20	1	0.001	0.99	Porous
Cinnamon Bear Creek	0.2	9.9	0.06	30	22	22	0.733	0.70	Low
Copper Creek	4.5	11.1	0.82	20	21	1	0.006	0.88	Porous
Cougar Creek	0.2	8.8	0.13	1	22	21	0.686	0.58	Low
Cowan Gulch	0.5	11.5	0.16	43	25	11	0.065	0.71	Porous
Cowan Gulch	4.3	9.6*	0.02	80	22	3	0.002	1.00	Porous
Eagle Creek	0.2	9.0	0.02	1	30	28	0.415	0.77	Low

Waterbody	RKM	Mean Aug. Temp (°C)	Discharge at base flow (m^3sec^{-1})	Fines (%)	Sample Size	No. Hybrids	Mean pRBT	Genetic mixing (m_d)	Resistance
East Fork Rock Creek	1.4	12.4	0.54	15	7	3	0.088	0.60	Porous
Gilbert Creek	0.3	10.3	0.18	3	21	21	0.703	0.75	Low
Gilbert Creek	10.5	8.8*	0.10	15	25	1	0.009	0.77	Porous
Grizzly Creek	1.0	9.2	0.08	20	30	6	0.065	0.60	Porous
Hogback Creek	1.4	8.3*	0.23	10	21	10	0.145	0.69	Porous
Hogback Creek	0.3	8.3	0.24	5	18	8	0.299	0.17	Low
Little Stony Creek	0.3	10.1	0.21	13	25	0	0.000	-	High
Meadow Creek	5.6	7.1	0.21	43	21	0	0.000	-	High
Middle Fork Rock Creek	17.9	13.5	1.57	23	21	2	0.002	0.99	Porous
Moose Meadow Creek	0.2	10.3	0.12	55	20	0	0.000	-	High
North Fork Rock Creek	4.5	9.5	0.02	1	25	0	0.000	-	High
Ranch Creek	1.9	10.2	0.52	5	25	5	0.062	0.50	Porous
Ranch Creek	5.5	10.2*	0.76	4	24	3	0.014	0.73	Porous
Ross Fork	14.3	13.4	0.48	41	18	0	0.000	-	High
Ross Fork	7.1	15.8	0.76	30	11	0	0.000	-	High
Sand Basin Creek	0.2	11.2	0.15	63	25	0	0.000	-	High
Sawmill Creek	0.2	9.7	0.09	10	20	20	0.693	0.69	Low
Scotchman Gulch	4.0	11.1	0.03	98	27	13	0.022	0.95	Porous
Stony Creek	0.5	11.2	0.91	0	19	11	0.130	0.61	Low
Stony Creek	5.6	10.0*	1.13	3	20	4	0.027	0.85	Porous
Stony Creek	10.8	9.5*	0.13	1	20	0	0.000	-	High
Tipperary Creek	0.3	8.9	0.01	35	23	2	0.001	1.00	Porous
Tipperary Creek	1.1	8.9*	0.02	30	20	0	0.000	-	High
Upper Willow Creek	3.5	15.6	0.99	78	19	9	0.017	0.98	Porous
Upper Willow Creek	20.9	11.1	0.31	18	25	4	0.003	1.00	Porous

Waterbody	**RKM**	**Mean Aug. Temp (°C)**	**Discharge at base flow (m^3sec^{-1})**	**Fines (%)**	**Sample Size**	**No. Hybrids**	**Mean pRBT**	**Genetic mixing (m_d)**	**Resistance**
Wahlquist Creek	0.5	9.5	0.04	5	27	21	0.204	0.92	Porous
Welcome Creek	0.5	10.4	0.36	20	20	20	0.611	0.76	Low
Welcome Creek	4.0	10.4*	0.22	4	27	2	0.002	0.99	Porous
West Fork Rock Creek	12.9	12.8*	1.06	1	20	0	0.000	-	High
West Fork Rock Creek	3.1	13.9	1.21	20	20	2	0.017	0.69	Porous
Williams Gulch	0.5	11.7	0.06	13	26	10	0.060	0.66	Porous
Williams Gulch	2.9	11.7*	0.03	33	21	8	0.011	0.99	Porous
Windlass Gulch	1.0	10.6	0.02	18	30	26	0.359	0.68	Low
Wyman Gulch	0.3	12.4	0.20	13	29	25	0.458	0.65	Low

Table B.2. Mean variable values and 95% confidence intervals for variables used to assess forces of localized resistance in Objective 3 across the different levels of resistance to hybridization in Rock Creek, MT 2018-2021.

Variable	**High Resistance (n =11)**	**Porous Resistance (n=26)**	**Low Resistance (n=14)**
Distance from source (km)	87.3 (71.1, 103.0)	62.0 (48.6, 75.5)	33.7 (25.0, 42.3)
CPUE (fish/shock sec)	0.016 (0.012, 0.020)	0.027 (0.023, 0.032)	0.027 (0.021, 0.032)
Spawning location (WCT) (km)	11.0 (7.7, 14.3)	3.8 (2.2, 5.4)	1.6 (0.5, 2.6)
Spawning location (RBTxWCT) (km)	4.6 (0.7, 8.5)	3.0 (1.2, 4.47)	0.8 (0.4, 1.1)
Spawn timing (WCT)	Jun. 11th (Jun. 5th, Jun. 15th)	Jun. 14th (Jun. 9th, Jun. 20th)	Jun. 1st (May 26th, Jun. 7th)
Spawn timing (RBTxWCT)	Jun. 4th (May 23rd, Jun. 15th)	May 28th (May 24th, Jun. 1st)	May 27th, (May 26th, Jun. 1st)
Discharge at base flow (m^{3}sec^{-1})	0.30 (0.10, 0.40)	0.36 (0.19, 0.53)	0.19 (0.07, 0.31)
Migratory Abundance (WCT)	1.6 (0.0, 3.9)	0.9 (0.2, 1.8)	0.2 (0.0, 0.4)
Migratory Abundance (RBTxWCT)	0.5 (0.1, 1.0)	0.4 (0.1, 0.7)	0.3 (0.0, 0.6)
Resident Size - Migratory streams (mm TL)	157 (146, 168)	138 (132, 144)	119 (109, 129)
Resident Size – Non-migratory streams (mm TL)	133 (123, 142)	136 (129, 142)	115 (110, 120)

FIGURES

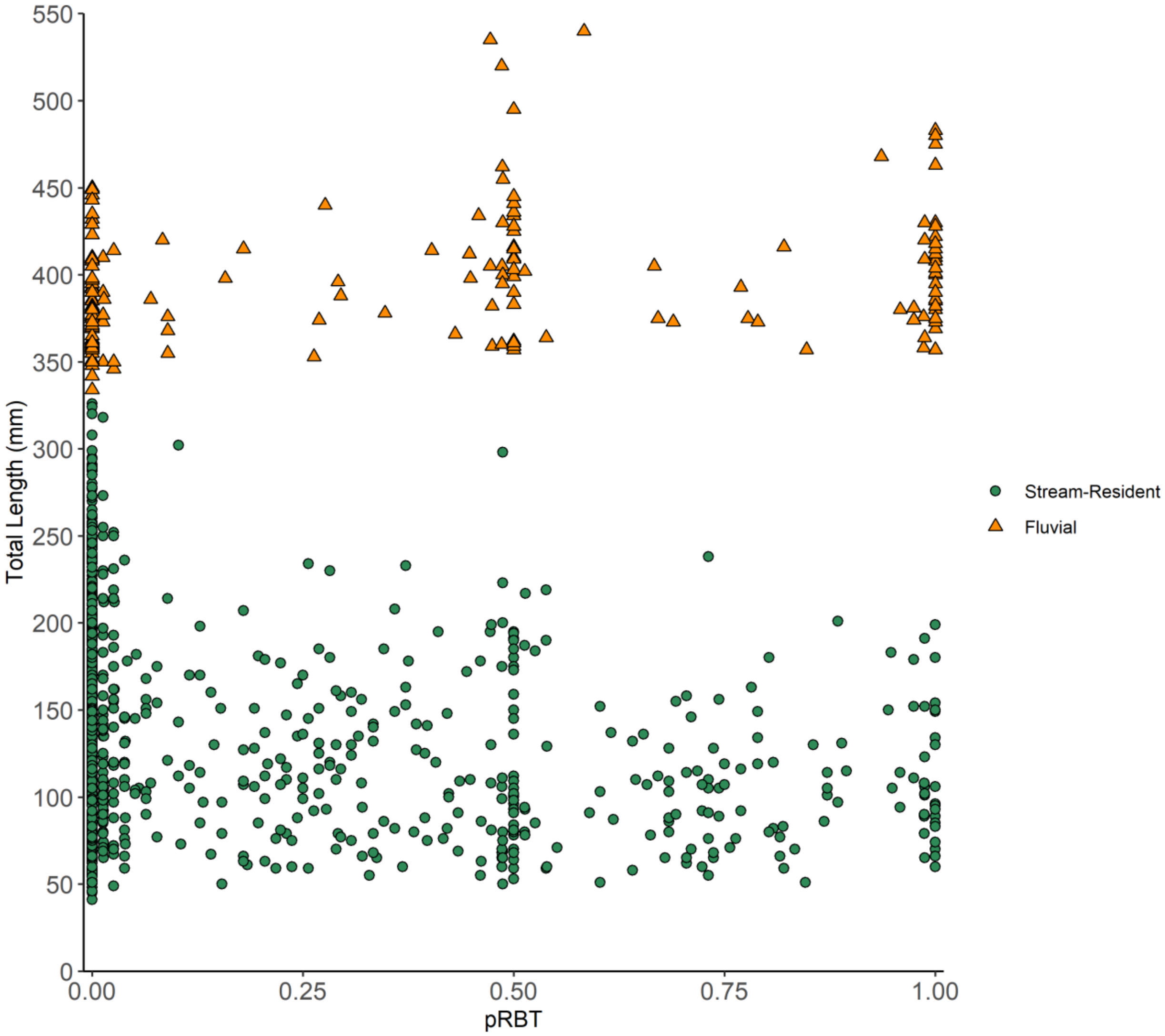


Figure B.1. Individual pRBT and corresponding total lengths for *Oncorhynchus spp*. (n = 1,369) in Rock Creek, MT 2019-2020. Dark green circles are resident fish, and orange triangles are migratory or fluvial fish.

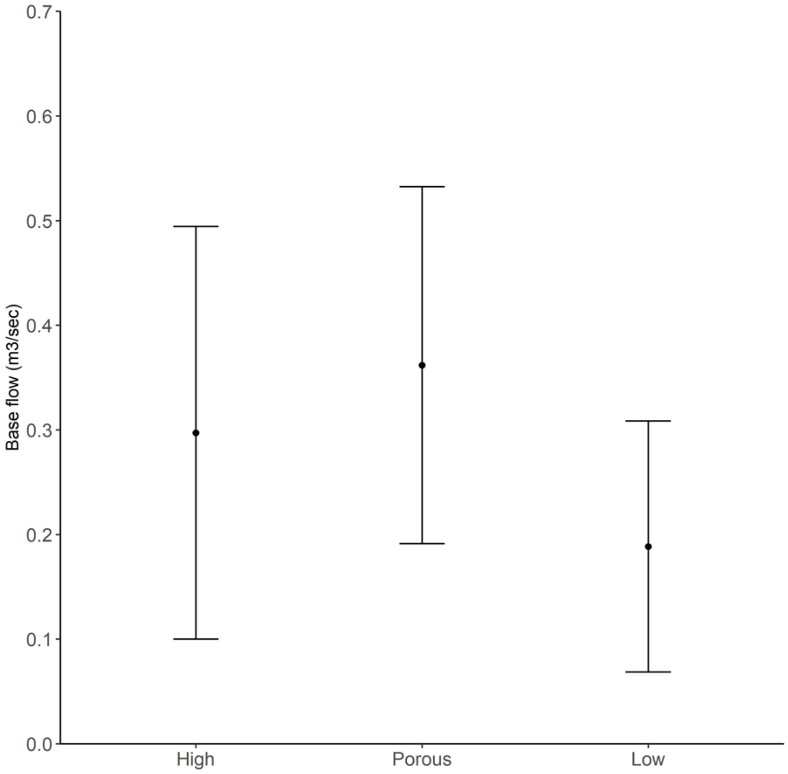


Figure B.2. Mean discharge at base flow and 95% confidence intervals for 51 streams in Rock Creek, MT 2020 for three different levels of site level resistance.

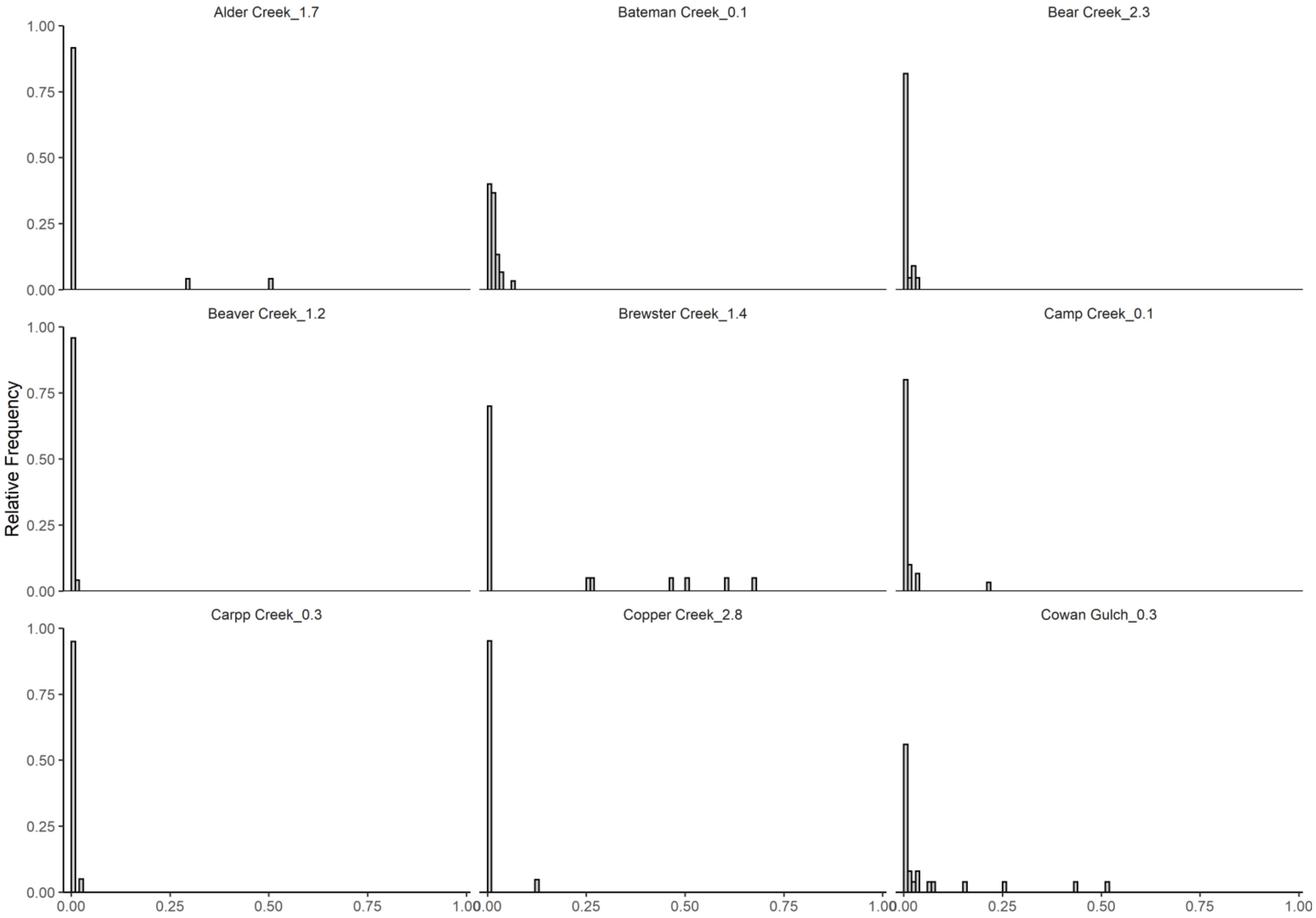
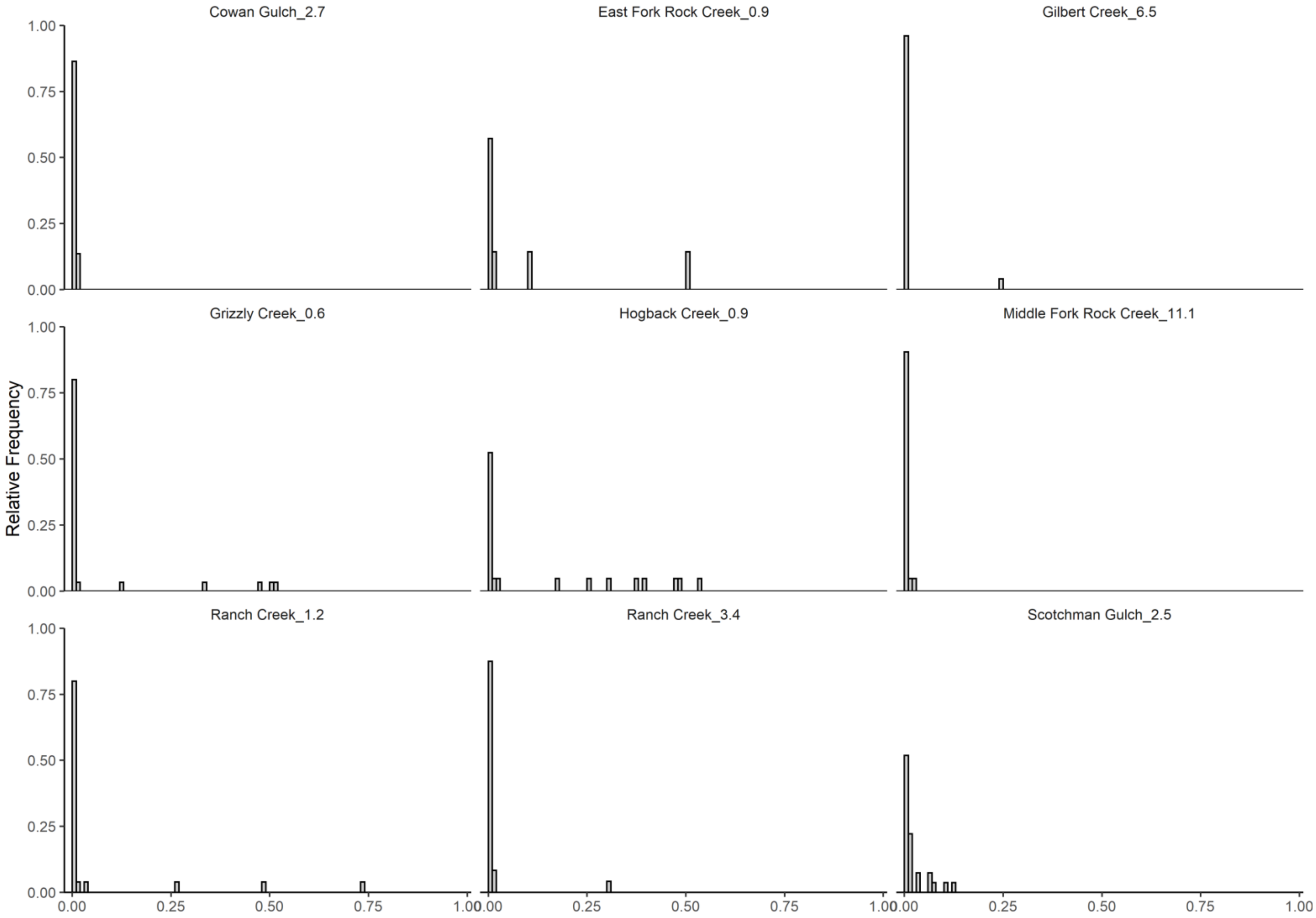


Figure B.3. Hybrid distributions for sites classified as porous resistance from Rock Creek MT 2019-2020. These sites were classified based on (1) the majority (> 50%) of fish samples had pRBT less than 0.01, (2) individuals greater than 0.50 were rare (< 15 %) and (3) no parental RBT were detected.



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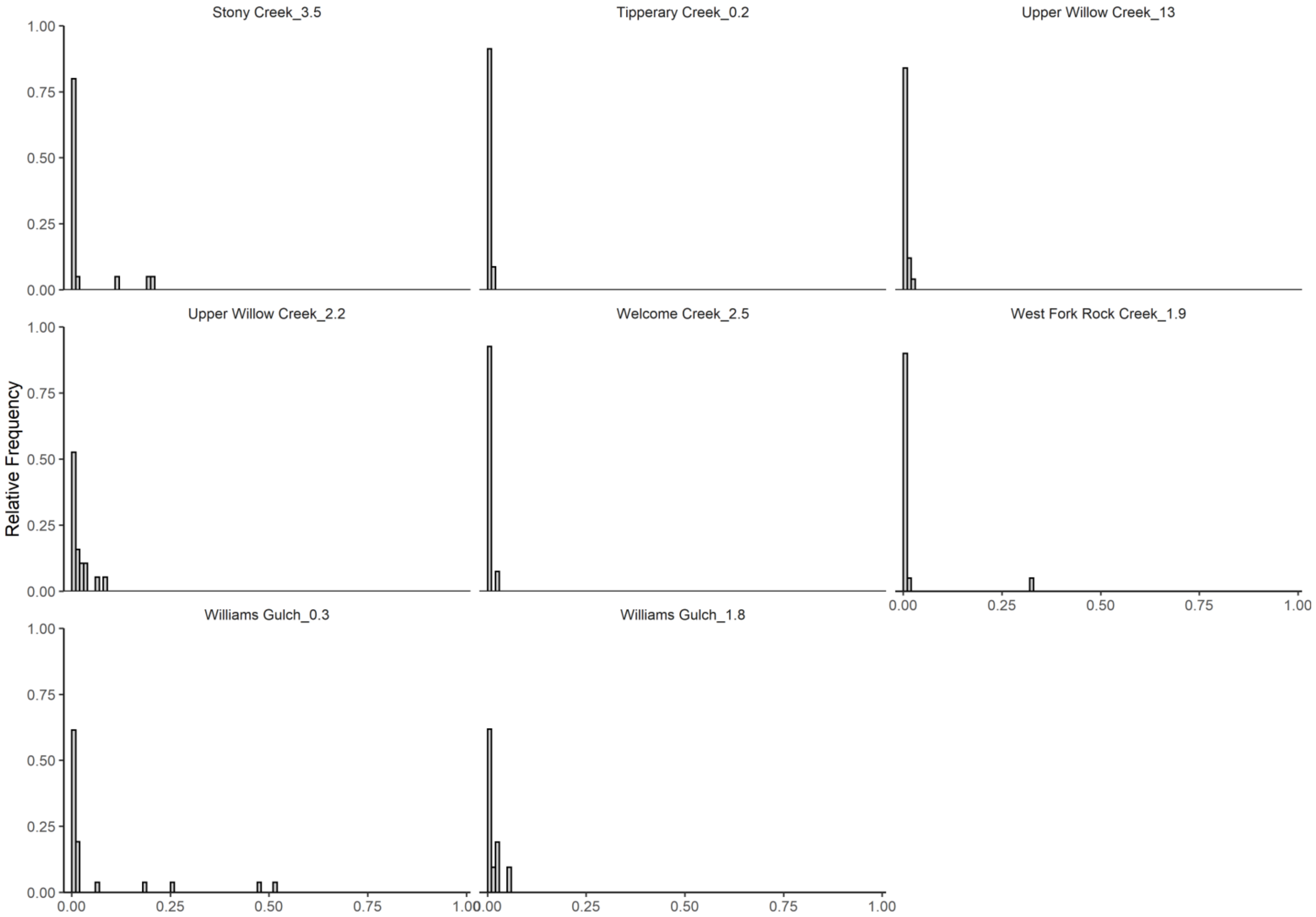

Figure B.3. Hybrid distributions for sites classified as porous resistance from Rock Creek MT 2019-2020. These sites were classified based on (1) the majority (> 50%) of fish samples had pRBT less than 0.01, (2) individuals greater than 0.50 were rare (< 15 %) and (3) no parental RBT were detected.

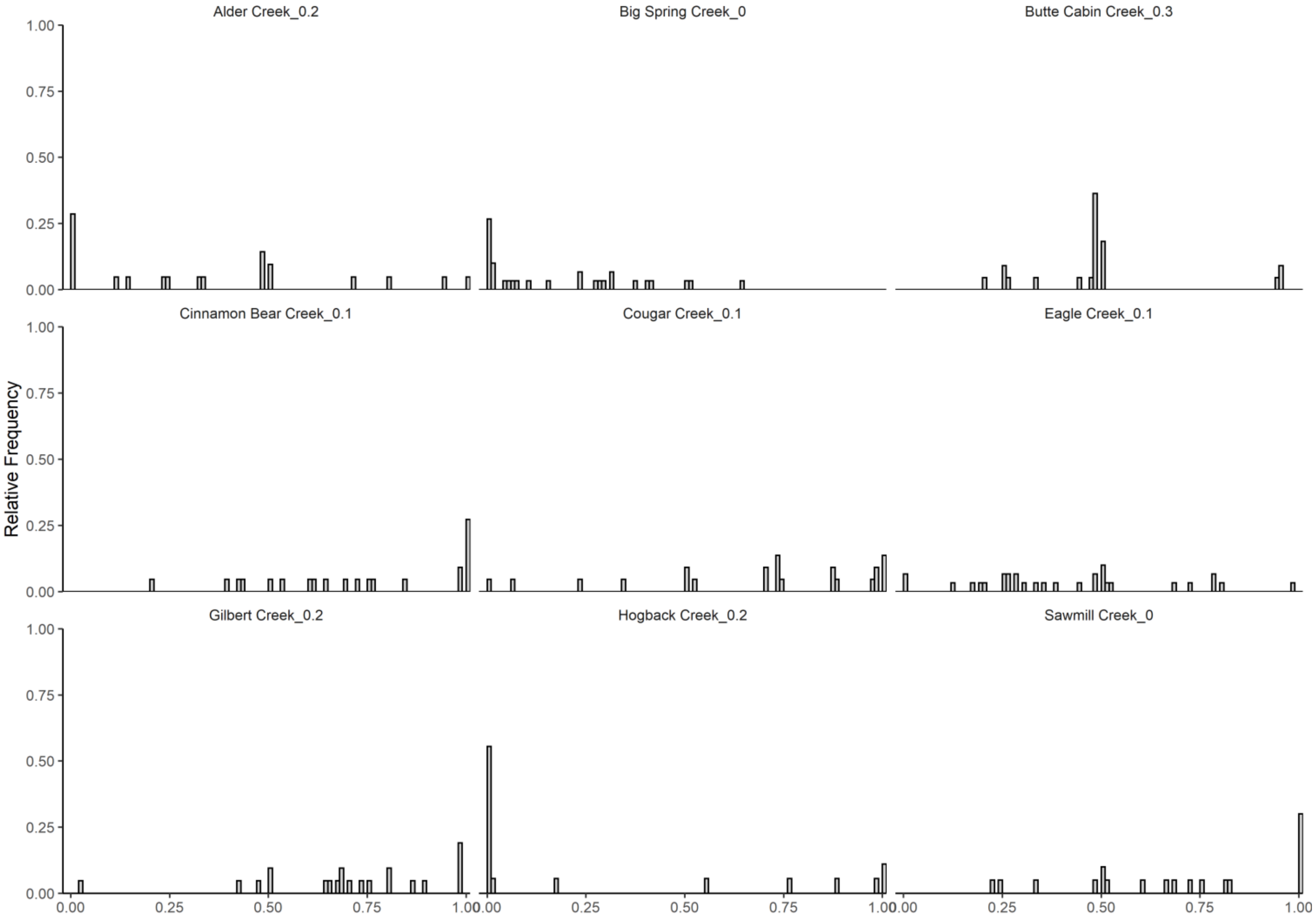

Figure B.4. Hybrid distributions for sites classified as low resistance from Rock Creek MT 2019-2020. These sites were classified as either (1) The majority (> 50%) of fish samples had pRBT greater than 0.01, (2) or parental RBT were observed.

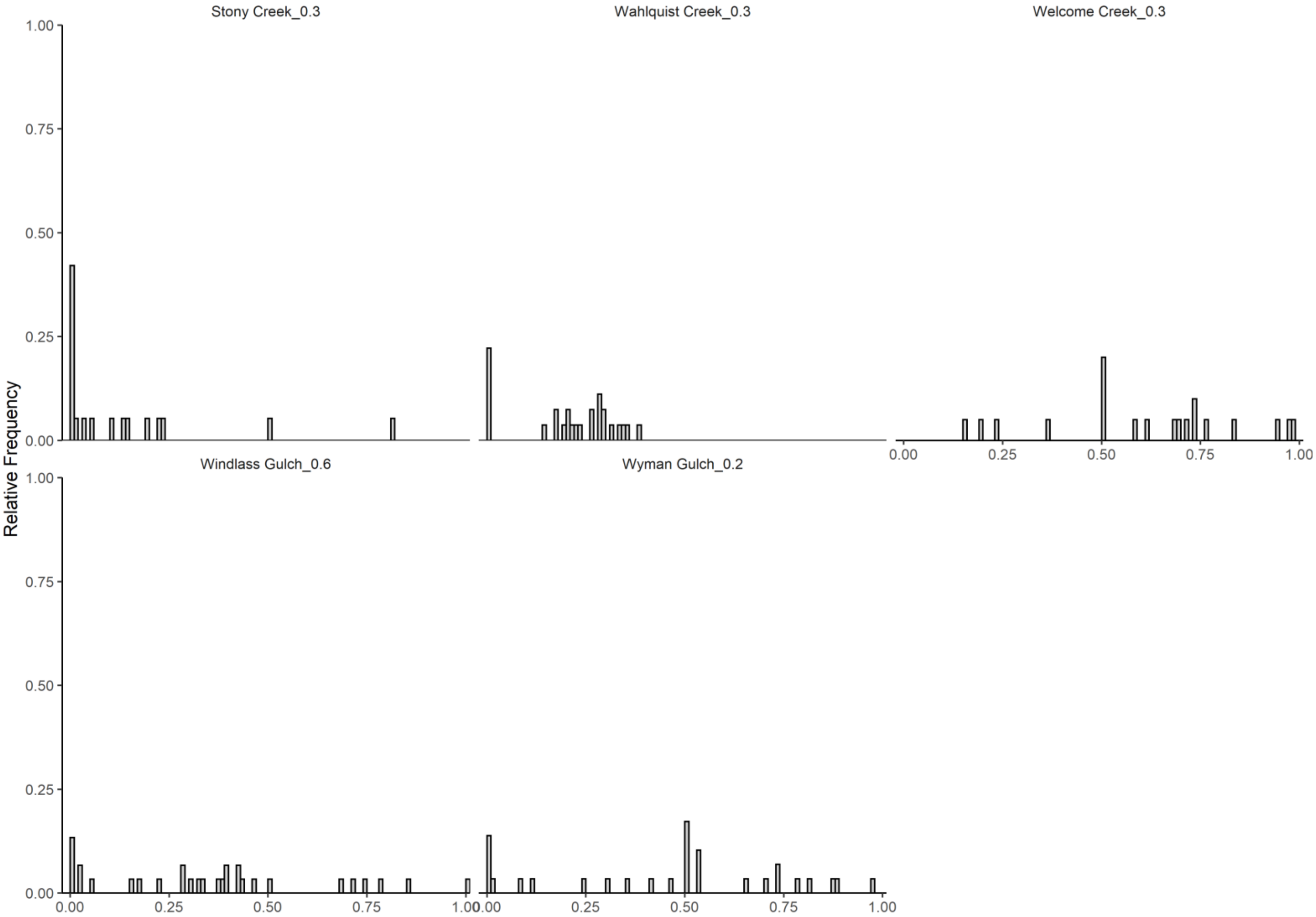

Figure B.4. Hybrid distributions for sites classified as low resistance from Rock Creek MT 2019-2020. These sites were classified as either (1) The majority (> 50%) of fish samples had pRBT greater than 0.01, (2) more than one individual was greater than 0.50 or (3) parental RBT were observed.

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