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EVALUATING THE MANAGEMENT AND CONSEQUENCES OF HYBRIDIZATION BETWEEN NONNATIVE RAINBOW TROUT AND NATIVE WESTSLOPE CUTTHROAT TROUT

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EVALUATING THE MANAGEMENT AND CONSEQUENCES OF HYBRIDIZATION BETWEEN NONNATIVE RAINBOW TROUT AND NATIVE WESTSLOPE CUTTHROAT TROUT

By

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ABSTRACT

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Evaluating the management and consequences of hybridization between nonnative rainbow trout and native westslope cutthroat trout

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The introduction of nonnative fish is a major driver in the decline of native fish species. Nonnative rainbow trout (*Oncorhynchus mykiss*; RBT) introduced into the native range of westslope cutthroat trout (*O. clarkii lewisi*; WCT) have led to the introgressive hybridization between these two species. This widespread hybridization is a primary threat to the long-term persistence of WCT as it can cause population-level genomic extinction. Since there are no set management solutions for hybridization, there is a need to evaluate the different conservation approaches to ensure the persistence of WCT populations. Additionally, beyond propagule pressure, the array of drivers that form hybridization landscape patterns are equivocal. This study focused on evaluating management actions and furthering our understanding of the potential mechanisms providing resistance to the spread of hybridization. We conducted a Before-After-Control-Impact study to evaluate the accuracy of selective passage of phenotypic WCT above barriers and the resulting impact on hybridization in the Jocko River Watershed, Montana. Our results showed phenotypic-based passage was generally successful; of the fish passed above the barrier, 82% had a proportion of RBT admixture < 0.01. We saw no significant increase in hybridization metrics in the above barrier populations over 9-14 years, while populations below the barrier had a significant increase in RBT admixture. Second, we validated the use of otolith microstructure to estimate hatch date in WCT with hatchery origin WCT. We than evaluated the effect of RBT admixture on age-0 *Oncorhynchus* hatch date and growth in the Rock Creek and Rattlesnake Creek Watersheds. Within sites, there was high variation in hatch date and individual growth rates. In the two streams where WCT were present, they had a significantly higher growth rates than hybrids. Our findings show promise for using barriers to manage the spread of RBT hybridization while maintaining the migratory WCT life history. We add support to previous research that found selection against RBT alleles is occurring at the early life stage, which provides valuable information on the potential mechanisms limiting the spread of RBT hybridization.

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CHAPTER 1

INTRODUCTION

Human actions driving global decline in biodiversity is exacerbated in freshwater ecosystems (Revenga et al. 2005; Butchart et al. 2010). The major drivers of this decrease in freshwater ecosystems are human-mediated habitat degradation, overexploitation, and the introduction of nonnative species (Reid et al. 2019). The intentional and nonintentional introduction of nonnative fish adversely affects native species through multiple mechanisms, including predation, competition, and hybridization (Allendorf and Lundquist 2003; Vitule et al. 2009; Cucherousset and Olden 2011). Closely related fish species have an increased risk of hybridization due to their spawning behavior and genetic similarities (Hubbs 1955; Scribner et al. 2001). Although naturally-occurring hybridization can lead to the rapid evolution of species (Abbott 1992; Hedrick 2013), hybridization can be detrimental to native species when caused by anthropogenic factors (Allendorf et al. 2001). The increase in human-mediated hybridization is a growing conservation concern as the consequences of hybridization and how to manage this threat effectively are primarily undetermined (Allendorf et al. 2001; Ottenburghs 2021).

Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) were once the most widely distributed subspecies of cutthroat trout in the Intermountain West (Shepard et al. 2005). A major driver of the WCT decline in their distribution is the hybridization with nonnative rainbow trout (*O. mykiss*; RBT), the most widely stocked fish species in the world (Halverson 2010). Currently, nonhybridized WCT are estimated to inhabit ~10% of their historical distribution and are continually threatened by the expansion of RBT hybridization (Hitt et al. 2003; Shepard et al. 2005; Muhlfeld et al. 2017). Widespread RBT hybridization leading to population-level genomic extinction is a primary conservation concern to the persistence of

WCT (Allendorf et al. 2004). Throughout their native range, WCT are highly regarded for their cultural and economic significance amongst tribes, anglers, and managers alike. Montana Fish, Wildlife, and Parks, tribes, landowners, and researchers have set forth three goals for the management and conservation of WCT in Montana.

> *"1) ensure the long-term, self-sustaining persistence of each subspecies distributed across their historical ranges as identified in recent status reviews (Shepard et al. 2003; Shepard et al. 2005; May et al. 2003)*
>
> *2) maintain the genetic integrity and diversity of non-introgressed populations, as well as the diversity of life histories, represented by remaining cutthroat trout populations*
>
> *3) protect the ecological, recreational, and economic values associated with each subspecies."* (Montana Department of Fish, Wildlife, and Parks 2007)

As we work to conserve WCT against the ongoing threat of RBT hybridization, we need to evaluate management actions to achieve those goals. Although limited in its application, suppression of local RBT sources to reduce the abundance of RBT and highly hybridized fish has been effective when a distinct source can be identified (Al-Chokhachy et al. 2014; Meyer et al. 2017a; Kovach et al. 2018). At times, managers will implement or retain barriers to isolate a population from nonnative species (Harig and Fausch 2002; Fausch et al. 2009). However, isolation increases the risk of localized extinction within smaller populations due to the loss of gene flow from migratory individuals (Liknes and Graham 1988; Novinger and Rahel 2003). A potential management tool that could restore migratory life history is the selective passage at barriers based on phenotypic characteristics (Ardren and Bernall 2017). Further research of the management strategies to limit the spread of RBT hybridization and maintain diversity of life histories is necessary for the conservation of WCT. The goal of Chapter 2 was to collaborate with Confederated Salish Kootenai Tribes (CSKT) tribal biologists to evaluate their management

actions involving selective passage at existing barriers with the goals of constraining the spread of hybridization and maintaining WCT migratory life history.

Throughout the WCT native range, we lack a comprehensive understanding of the mechanisms driving spatiotemporal patterns of RBT hybridization (Muhlfeld et al. 2017). There is strong evidence that the primary force behind the spread of hybridization in Montana is the dispersal of RBT and highly hybridized fish from historic RBT stocking locations (Boyer et al. 2008; Kovach et al. 2015; Muhlfeld et al. 2017). Additionally, research has shown a shift in genotypes from spawning adults to juvenile fish in admixed populations that suggests strong selection against individuals with higher levels of admixture successfully reproducing (Kovach et al. 2015). The age-0 stage could be critical in determining the level of admixture in a population, yet little is known about the effects of hybridization at this stage for fish in the wild. Furthering our understanding of the mechanisms driving spatiotemporal patterns in hybridization is needed to provide key information as we develop conservation strategies for WCT populations. In collaboration with Montana Fish, Wildlife, and Parks, the goal of Chapter 3 was to understand the effects of RBT admixture on age-0 growth of WCT, RBT, and their hybrids.

Chapter 2 evaluated the accuracy of phenotype-based migratory fish identification and effects of selective passage on hybridization metrics above the barrier in the Jocko River Watershed. CSKT has collected a long-term hybridization dataset consisting of 20 sample sites above and below the barriers, before and after the beginning of selective passage, allowing us to use a Before-After-Control-Impact (BACI) study framework. The Before samples were collected from 2005-2007. Selective fish passage began in 2010 at two irrigation diversions, and the After samples were collected from 2016-2019. Sites below the furthest downstream barrier, open to the mainstem (source of RBT), served as Control, and those influenced by the selective passage of

fish above the barrier were Impact sites. Selective passage based on phenotype was fairly accurate; 82% of the fish passed above the barrier had a proportion of RBT admixture (pRBT) less than 0.01. We used three hybridization metrics to assess the site-level temporal changes in hybridization, including change in the mean pRBT, change in the proportion of individuals with pRBT > 0.10 (a conservation threshold used by Montana Fish, Wildlife & Parks), and change in the mean run of admixture length. There was no significant increase in the hybridization metrics upstream of the barrier, but all three metrics significantly increased in Control Sites. The site located closest downstream of the barrier had the most substantial increase; for example, the site pRBT increased by 578 % (Before 0.049, After 0.332). Our results suggest selective passage could promote migratory life history and not further jeopardize WCT conservation populations with preexisting low levels of admixture.

In Chapter 3, we validated the use of otolith microstructure to estimate hatch dates and growth rates in WCT using hatchery origin WCT. We evaluated the effect of RBT admixture on age-0 WCT hatch date and growth using otolith microstructure across six wild populations in Rock Creek and Rattlesnake Creek Watersheds. We calculated hatch date, length at hatch, and growth rate for 122 fish using otolith microstructure. Within two sample sites we found a negative relationship between RBT admixture and growth in two sample sites. In these sites, WCT had a significantly higher growth rate than hybrids. Our findings of WCT having higher growth rates than hybrids despite later hatch dates and being exposed to the same stream environment suggests WCT have countergradient variation in growth. The high growth rates resulted in WCT reaching a similar length by mid-August sampling to RBT and hybrids. Our results suggest that WCT countergradient variation in growth may provide a selective advantage at this early life stage.

This thesis highlights a possible management action to limit the spread of RBT hybridization while maintaining WCT migratory life history. We caution that phenotypic-based passage is not a highly accurate way to prevent above barrier hybridization because of the challenges surrounding the visual assessment of migratory individuals. Therefore, this management action should be carefully considered and not applied when the above barrier populations are nonhybridized. Additionally, we add support to previous research on the mechanisms driving selection against RBT alleles in the early life stage (Kovach et al. 2015). Our findings show evidence of countergradient variation in growth in WCT that may provide a selective advantage at this life stage. We suggest further research should focus on sampling older age-0 individuals to see if the variation in growth rates is sustained between RBT, WCT, and hybrids and ultimately influence admixture in adult populations. Our findings highlight the usefulness of selective passage as an effective management tool to balance the need for life history diversity while limiting the spread of RBT hybridization and adding to the growing literature on understanding the mechanisms influencing the spread of hybridization.

References

Abbott, R.J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends in Ecology & Evolution **7**(12): 401–405. doi:10.1016/0169-5347(92)90020-C.

Al-Chokhachy, R., Muhlfeld, C.C., Boyer, M.C., Jones, L.A., Steed, A., and Kershner, J.L. 2014. Quantifying the effectiveness of conservation measures to control the spread of anthropogenic hybridization in stream salmonids: a climate adaptation case study. North American Journal of Fisheries Management **34**(3): 642–652. doi:10.1080/02755947.2014.901259.

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Lundquist, L.L., and Spruell, P. 2004. Intercrosses and the U.S. Endangered Species Act: Should hybridized populations be included as westslope cutthroat trout? Conservation Biology **18**(5): 1203–1213. doi:10.1111/j.1523-1739.2004.00305.x.

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution **16**(11): 613–622. doi:10.1016/S0169-5347(01)02290-X.

Allendorf, F.W., and Lundquist, L.L. 2003. Introduction: Population biology, evolution, and control of invasive species. Conservation Biology **17**(1): 24–30. doi:10.1046/j.1523-1739.2003.02365.x.

Ardren, W.R., and Bernall, S.R. 2017. Dams impact westslope cutthroat trout metapopulation structure and hybridization dynamics. Conserv Genet **18**(2): 297–312. doi:10.1007/s10592-016-0906-6.

Boyer, M.C., Muhlfeld, C.C., and Allendorf, F.W. 2008. Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Can. J. Fish. Aquat. Sci. **65**(4): 658–669. doi:10.1139/f08-001.

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K.E., Carr, G.M., Chanson, J., Chenery, A.M., Csirke, J., Davidson, N.C., Dentener, F., Foster, M., Galli, A., Galloway, J.N., Genovesi, P., Gregory, R.D., Hockings, M., Kapos, V., Lamarque, J.-F., Leverington, F., Loh, J., McGeoch, M.A., McRae, L., Minasyan, A., Morcillo, M.H., Oldfield, T.E.E., Pauly, D., Quader, S., Revenga, C., Sauer, J.R., Skolnik, B., Spear, D., Stanwell-Smith, D., Stuart, S.N., Symes, A., Tierney, M., Tyrrell, T.D., Vié, J.-C., and Watson, R. 2010. Global Biodiversity: Indicators of recent declines. Science **328**(5982): 1164–1168. doi:10.1126/science.1187512.

Cucherousset, J., and Olden, J.D. 2011. Ecological impacts of nonnative freshwater fishes. Fisheries **36**(5): 215–230. doi:10.1080/03632415.2011.574578.

Drinan, D.P., Webb, M.A.H., Naish, K.A., Kalinowski, S.T., Boyer, M.C., Steed, A.C., Shepard, B.B., and Muhlfeld, C.C. 2015. Effects of hybridization between nonnative rainbow trout and native westslope cutthroat trout on fitness-related traits. Transactions of the American Fisheries Society **144**(6): 1275–1291. doi:10.1080/00028487.2015.1064475.

Fausch, K.D., Rieman, B.E., Dunham, J.B., Young, M.K., and Peterson, D.P. 2009. Invasion versus Isolation: Trade-offs in managing native salmonids with barriers to upstream movement. Conservation Biology **23**(4): 859–870. doi:10.1111/j.1523-1739.2008.01159.x.

Halverson, A. 2010. An entirely synthetic fish: how rainbow trout beguiled America and overan the world. Yale University Press, New Haven and London.

Harig, A.L., and Fausch, K.D. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. Ecological Applications **12**(2): 535–551. doi:10.1890/1051-0761(2002)012[0535:MHRFET]2.0.CO;2.

Hedrick, P.W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol Ecol **22**(18): 4606–4618. doi:10.1111/mec.12415.

Hitt, N.P., Frissell, C.A., Muhlfeld, C.C., and Allendorf, F.W. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi* , and nonnative rainbow trout, *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. **60**(12): 1440–1451. doi:10.1139/f03-125.

Hubbs, C.L. 1955. Hybridization between Fish Species in Nature. Systematic zoology **4**(1): 1–20.

Kovach, R.P., Al-Chokhachy, R., and Stephens, T. 2018. Proactive rainbow trout suppression reduces threat of hybridization in the upper Snake River Basin. North Am J Fish Manage **38**(4): 811–819. doi:10.1002/nafm.10177.

Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Lowe, W.H., Allendorf, F.W., and Luikart, G. 2015. Dispersal and selection mediate hybridization between a native and invasive species. Proc. R. Soc. B. **282**(1799): 20142454. doi:10.1098/rspb.2014.2454.

Liknes, G.A., and Graham, P.J. 1988. Westslope cutthroat trout in Montana: Life history, status and management. Status and management of interior stocks of cutthroat trout. American Fisheries Society Symposium **4**(53–60).

Meyer, K.A., Kennedy, P., High, B., and Campbell, M.R. 2017. Distinguishing Yellowstone Cutthroat Trout, Rainbow Trout, and Hybrids by Use of Field-Based Phenotypic Characteristics. North American Journal of Fisheries Management **37**(2): 456–466. doi:10.1080/02755947.2017.1280572.

Montana Department of Fish, Wildlife, and Parks. 2007. Memorandum of understanding and conservation agreement for Westslope Cutthroat Trout and Yellowstone Cutthroat Trout in Montana.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. **5**(3): 328–331. doi:10.1098/rsbl.2009.0033.

Muhlfeld, C.C., Kovach, R.P., Al-Chokhachy, R., Amish, S.J., Kershner, J.L., Leary, R.F., Lowe, W.H., Luikart, G., Matson, P., Schmetterling, D.A., Shepard, B.B., Westley, P.A.H., Whited, D., Whiteley, A., and Allendorf, F.W. 2017. Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout. Glob Change Biol **23**(11): 4663–4674. doi:10.1111/gcb.13681.

Novinger, D.C., and Rahel, F.J. 2003. Isolation Management with Artificial Barriers as a Conservation Strategy for Cutthroat Trout in Headwater Streams. Conservation Biology **17**(3): 772–781. doi:10.1046/j.1523-1739.2003.00472.x.

Ottenburghs, J. 2021. The genic view of hybridization in the Anthropocene. Evol Appl **14**(10): 2342–2360. doi:10.1111/eva.13223.

Rasmussen, J.B., Robinson, M.D., Hontela, A., and Heath, D.D. 2012. Metabolic traits of westslope cutthroat trout, introduced rainbow trout and their hybrids in an ecotonal

hybrid zone along an elevation gradient: metabolic traits of trout hybrids. Biological Journal of the Linnean Society **105**(1): 56–72. doi:10.1111/j.1095-8312.2011.01768.x.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., and Cooke, S.J. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev **94**(3): 849–873. doi:10.1111/brv.12480.

Revenga, C., Campbell, I., Abell, R., de Villiers, P., and Bryer, M. 2005. Prospects for monitoring freshwater ecosystems towards the 2010 targets. Phil. Trans. R. Soc. B **360**(1454): 397–413. doi:10.1098/rstb.2004.1595.

Scribner, K.T., Page, K.S., and Bartron, M.L. 2001. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and Fisheries (10): 293–323.

Shepard, B.B., May, B.E., and Urie, W. 2005. Status and conservation of westslope cutthroat trout within the Western United States. North American Journal of Fisheries Management **25**(4): 1426–1440. doi:10.1577/M05-004.1.

Vitule, J.R.S., Freire, C.A., and Simberloff, D. 2009. Introduction of non-native freshwater fish can certainly be bad. Fish and Fisheries **10**(1): 98–108. doi:10.1111/j.1467-2979.2008.00312.x.

CHAPTER 2

EVALUATING THE EFFECTS OF SELECTIVE PASSAGE OF MIGRATORY WESTLOPE CUTTHROAT TROUT ON NONNATIVE ADMIXTURE

Abstract

Hybridization with nonnative rainbow trout (*Oncorhynchus mykiss*; RBT) is a primary threat to the persistence of westslope cutthroat trout (*O. clarkii lewisi*; WCT). Managers concerned with conserving WCT in the presence of RBT often face the predicament of tolerating the spread of hybridization or isolating WCT populations. Selective passage of migratory WCT above existing barriers is a management approach with potential to limit hybridization while minimizing population impacts of barriers. We conducted a Before-After-Control-Impact study to evaluate a phenotype-based selective passage protocol for migratory WCT in the Jocko River, Montana. Of the 364 genotyped individuals passed upstream of the barrier, 82% had a proportion of RBT admixture (pRBT) < 0.01. Over 9-14 years, there was no significant increase in hybridization metrics upstream of the barrier, but metrics increased within Control sites. This increase was strongest at a site just downstream from the barrier, suggesting hybrids and RBT blocked by the barrier might have dispersed into this tributary. Our results suggest selective passage could promote migratory life history and maintain WCT conservation populations with low-level admixture.

Introduction

The widespread introduction, establishment, and expansion of nonnative species is a prominent biodiversity threat (Vitousek et al. 1997; Clavero and Garcia-Berthou 2005). Many nonnative populations have adversely affected native species through multiple mechanisms, including predation, competition, and hybridization (Rhymer and Simberloff 1996; Allendorf and Lundquist 2003; Britton et al. 2011). Although hybridization can create novel evolutionary outcomes, there are often negative consequences when caused by anthropogenic factors (Grant and Grant 1994; Hedrick 2013). In a recent review, Ottenburghs (2021) found that 80% of recent anthropogenic hybridization events led to hybridization beyond the first generation. Anthropogenic hybridization tends to occur on a short evolutionary time scale and is often associated with detrimental fitness effects (Epifanio and Philipp 2000; Allendorf et al. 2001). Furthermore, repeated backcrossing among hybridizing taxa can result in localized genomic extinctions (Allendorf and Leary 1988; Epifanio and Philipp 2000; Todesco et al. 2016).

Anthropogenic hybridization resulting from the historic, long-term stocking of nonnative rainbow trout (*Oncorhynchus mykiss*, RBT) throughout western North America threatens native westslope cutthroat trout (*O. clarkii lewisi*, WCT) populations (Shepard et al. 2005). The lack of reproductive barriers between WCT and RBT has led to widespread hybridization and population-level genomic extinction throughout the native range of WCT (Allendorf and Leary 1988; Muhlfeld et al. 2017). Currently, non-hybridized populations of WCT occupy approximately ten percent of their historical distribution (Shepard et al. 2005).

A common cause of the contemporary spread of admixture appears to be the dispersal of RBT and hybrids from established populations in historic mainstem stocking locations (Boyer et al. 2008; Muhlfeld et al. 2009b; Kovach et al. 2015). To mitigate the effects of dispersal,

managers may intentionally isolate WCT populations to prevent the immigration of RBT and hybrids. However, this management strategy creates isolated populations that are at increased risk of local extirpation (Harig and Fausch 2002; Fausch et al. 2009) because immigration and size-dependent fecundity tend to buffer demographic stochasticity (Liknes and Graham 1988). One management approach to balance this risk is selective passage of migratory WCT upstream into spawning areas based on phenotypic characteristics. Selective passage of phenotypic WCT above a barrier is a rarely used management tool that has the potential to support above-barrier population viability. However, given that phenotype can be a poor predictor of genotype, especially in individuals with low admixture (Leary et al. 1984; Weigel et al. 2002), selective passage could lead to increased hybridization in the above-barrier populations. Thus, evaluating the accuracy of phenotypic-based selective passage and watershed-level effects on spatiotemporal hybridization patterns is needed to verify the effectiveness of this management strategy.

A WCT selective passage program based on phenotype and migration timing was instituted by the Confederated Salish and Kootenai Tribes (CSKT) in the Jocko River and offers a rare opportunity to test the efficacy of this management approach. Located in western Montana on the Flathead Indian Reservation (FIR), this watershed provides a unique opportunity to link historical landscape hybridization patterns between WCT and RBT with long-term monitoring of selective passage. WCT are found throughout the watershed in nearly all fish-bearing streams, and RBT were historically stocked throughout the Jocko River stream network. Two diversion structures associated with irrigation canals have been barriers to upstream fish passage since the early 20th century, except under high-flow conditions. An initial assessment of watershed-wide hybridization in the early to mid-2000s revealed that fish with higher RBT ancestry were

established in the mainstem, and most tributary populations had low RBT admixture (< 1%; Corsi 2011). To maintain migratory WCT and protect WCT conservation populations above these diversion structures, CSKT began selectively passing later migrating fish deemed phenotypically WCT in 2010 at the two primary barriers.

In this paper, we evaluated the accuracy of phenotype-based identification and the effects of selective passage on above-barrier populations using a Before-After-Control-Impact (BACI) framework applied to the Jocko River Watershed. We addressed two primary questions: 1) What was the genetic ancestry associated with selective passage decisions based on migration timing and phenotype? 2) What are the effects of selective passage on the hybridization of 'Impact' populations upstream of a barrier compared to 'Control' populations? Our investigation of this long-term watershed-scale management action provides valuable insights for limiting hybridization in partially isolated populations while maintaining the demographic benefits of the WCT migratory life history form.

Methods

Study area

The Jocko River is a tributary of the Flathead River located in northwest Montana. The 979 km^2 watershed consists of Finley Creek, Valley Creek, Big Knife Creek, and the Jocko River's North, Middle, and South Forks (Figure 2.1). The watershed lies entirely within the FIR and is managed by the CSKT. Land use surrounding the meandering low-elevation stream types is mixed and consists of agriculture, rural subdivision, developed transportation corridors, and, more recently, riparian conservation areas. Higher elevation streams, many with steeper gradients, lie within a mix of managed and protected forestland. Since the 20th century, an

extensive irrigation system has influenced stream flows and fish movements throughout much of the landscape.

The Jocko River Watershed supports the only remaining migratory fluvial populations of WCT and native bull trout (*Salvelinus confluentus*) on the FIR. The nonnative fish assemblage within the watershed consists of RBT, brown trout (*Salmo trutta*), and brook trout (*S. fontinalis*). Hybridization between RBT and WCT occurs throughout the watershed, with generally low proportions of RBT admixture (pRBT < 0.1) at sites higher in the basin; admixture increases with proximity to the main-stem Jocko River (Corsi 2011). Additionally, there is a low-density population of RBT located in Liberty Creek, a tributary in the headwaters of the South Fork Jocko River. No stocking records exist for this population, but it has been present for decades. Stream habitat conditions and low abundances appear to limit emigration and the influence of RBT from Liberty Creek into the South Fork Jocko River (Craig Barfoot, unpublished).

The K and S Canal Irrigation Diversions (hereafter the K and S Canals) have been barriers to fish movement into the three forks of the Jocko River for over a century. Both diversions were retrofitted (1996 at K Canal and 2002 at S Canal; Figure 2.1) with fish ladders and trap boxes to monitor and pass bull trout upstream into spawning and rearing habitats above the diversions. The K Canal is a pin-and-plank style diversion. It is located furthest downstream and limits passage into all three Jocko forks. When checked for irrigation, this diversion is a near-complete barrier to fish passage; however, fish may pass during high-flow events, especially when the structure is unchecked. The S Canal is located upstream of the K Canal and restricts fish passage into the Middle and South Fork of the Jocko River (Figure 2.1). The S Canal is also porous to fish passage at high flows.

In 2010, CSKT began using the K and S Canal fish traps to capture and selectively pass WCT during the spring spawning migration (April – June). The management goal of selective passage was to maintain life history diversity and productivity of WCT while limiting the spread of RBT hybridization into populations above the barriers. The availability of hybridization data collected before selective passage began (Corsi 2011) allowed us to apply a BACI study design in our evaluation. Finley, Valley, and Big Knife Creeks are open to movement from the main-stem Jocko River and served as Control tributaries (Figure 2.1). The three forks of the Jocko River (North, Middle, and South) received selectively passed migratory individuals from the main-stem Jocko River and served as Impact tributaries (Figure 2.1).

Selective passage criteria

Fish were passed at the K and S Canal Diversions based on arrival time at the fish traps and phenotypic characteristics. Corsi et al. (2013) found that highly hybridized individuals migrated earlier in the season before peak spring runoff within the Jocko River. The median time of WCT migration occurred later and on the descending limb of the snowmelt-dominated hydrograph. Phenotypic characteristics used in this study were similar to those described in (Ardren and Bernall 2017), and included slash intensity, body spotting intensity and location, and body and fin coloration. Individuals migrating on the hydrograph's descending limb and phenotypically resembled WCT were passed upstream of the K Canal Diversion. Individuals that phenotypically resembled RBT, hybrids, or uncertain phenotypes were released downstream of the K Canal Diversion or were removed from the system. Individuals passed at the K Canal Diversion and recaptured at the upstream S Canal Diversion were passed upstream of the S Canal Diversion.

Sampling of fish captured at diversions

From 2010 through 2019, all captured fish at each diversion were measured (total length; TL mm), and a caudal fin sample was collected for genetic analysis. Of the individuals phenotypically identified as WCT and passed upstream of the diversions (K Canal Diversion, n = 509; S Canal Diversion, n = 279), we genetically analyzed a subsample from each week from March through June across every year (n = 364, Figure 2.2). From 2010 through 2019, 330 fish were phenotypically identified as RBT or hybrids and were not passed at the K Canal Diversion. We genetically analyzed a smaller subsample of these individuals between 2010 and 2019 (n = 64).

Sampling of fish within Control and Impact sites

Longitudinal sampling throughout the Jocko River Watershed was performed by CSKT technicians using a backpack electrofisher. Sample sites were a minimum of 152 m long with a target sample size of 25 individuals. Once captured, fish were measured (TL, mm), and a fin sample was collected and stored in 95% ethanol.

The Before sampling in the BACI framework occurred from 2005 through 2007, and the After sampling occurred from 2016 through 2019. The After sample occurred 6-9 years after the passage of migratory fish began at K Canal Diversion in 2010. There were eight Control sites throughout Big Knife Creek, Finley Creek, and Valley Creek drainages (Table 2.1, Figure 2.1). There were twelve Impact sites upstream of the K Canal Diversion (the North, Middle, and South Forks of the Jocko River, Table 2.1, Figure 2.1).

Genetic analyses

All individuals were genotyped using a RAD-Capture panel with 796 RBT species diagnostic loci (Amish et al. 2012; Hohenlohe et al. 2013; Ali et al. 2016). Genetic samples were prepared, sequenced, and genotyped following the laboratory and bioinformatic methods described in Ali (2016) and Strait (2021). Bioinformatic filtering was based on allele balance, read depth, and genotype missingness. We filtered individuals based on the number of RBT diagnostic loci amplified; individuals were required to be amplified at 398 RBT loci (50%). We examined sensitivity to this amount of missing diagnostic-locus genotypes by performing analyses with 40% and 60% missing genotypes and found only minor differences in our results (see supplemental S1 for bioinformatics methods).

We calculated two individual-level hybridization metrics to examine changes in admixture over time. First, we calculated the proportion of RBT diagnostic alleles present divided by two times the total number of successfully genotyped alleles within each individual, termed proportion of RBT admixture (pRBT). Second, we used mapped genome locations based on the RBT genome (Pearse et al. 2019) for RBT diagnostic loci to calculate the distance along chromosomes containing consecutive RBT diagnostic loci, here termed runs of admixture (ROA). ROA was defined as two or more consecutive (adjacent on a chromosome) RBT homozygote or heterozygote genotypes. A ROA ended once a WCT homozygote allele or more than one missing genotype was present at a ROA-adjacent locus. We summed ROA length across chromosomes within each individual and measured the length in a million base pairs (Mb; for more information, see supplemental S1).

We measured site-level changes in mean admixture (pRBT), the proportion of fish with > 0.10 pRBT, and mean ROA length to evaluate the effects of selective passage on Impact sites. The proportion of fish with > 0.10 pRBT is based on a management threshold used to define a conservation population of WCT in Montana (Montana FWP, 2007). We included both metrics because predictions differ somewhat for each metric in our Impact sites. In this study, during the initial period (Before) prior to the start of selective passage, all sites had low levels of admixture (mean pRBT = 0.019, SD = 0.036; S1, S2), only 5.7% of individuals had a pRBT > 0.10, and overall mean ROA length was low (0.16 Mb, SD = 0.46 Mb; Figure S2.3, S2.4). The time interval for our study is approximately ten years or 2-3 generations for WCT (Corsi et al. 2013). In the absence of propagule pressure (dispersal) from highly admixed individuals over this short period, we expected either small declines or no changes in pRBT and proportion of fish with > 0.10 pRBT, depending on the influence of selection against RBT alleles (Kovach et al. 2015, 2016; Muhlfeld et al. 2017). Therefore, in our BACI, at the Impact sites, we predict that we would observe either small declines or no changes in mean pRBT and that the proportion of fish with pRBT > 0.10 would decrease following the selective passage treatment because the influx of RBT or highly admixed fish was limited, and the sites had low baseline admixtures. In contrast, if RBT or highly hybridized fish immigrated, as could occur in our fully open Control sites, then we expected an increase in one or both of these metrics because dispersal and propagule pressure would overwhelm signals of selection (Kovach et al. 2015, 2016; Table 2.2).

The inclusion of ROAs is a novel approach that has the potential to provide additional information about the dynamics of hybridization and admixture. The tributary populations had a low mean ROA length at the initial period (Before), so we focused our predictions for change based on that initial state (Figure S2.4). In the absence of immigrating fish with longer ROA

lengths, we expected a 'ratchet' effect to decrease ROA length over time due to recombination (Figure S2.5) in a manner analogous to Runs of Homozygosity (ROH; Kardos et al. 2016) or Admixture Tracts (Liang and Nielsen 2014), possibly aided by selection against RBT alleles (Kovach et al. 2015, 2016). We expected this effect to be small (i.e., no or small decreases in ROAs) compared to the effect of gene flow (Kardos et al. 2016). Thus, we expected that immigration of RBT or highly admixed individuals would increase the mean ROA length between two time periods. In our BACI study, we predicted that mean ROA length would decrease following the selective passage treatment because the influx of RBT or highly admixed fish was limited, and sites had a low admixture baseline (Table 2.2).

Data analyses

For our first question, we examined pRBT and ROA length for fish with different selective passage decisions (passed upstream or not passed) at the K and S Canal Diversions to test protocols based on migration timing and phenotype. For our second question, we compared the three site-level admixture metrics within our BACI design to test the effects of selective passage on spatiotemporal patterns of admixture in the Jocko River Watershed. Each site had a minimum sample size of ten or more genotyped individuals during each sampling period. We used a hierarchical bootstrap approach to compare the change in site-level response metrics between the Before and After sampling periods. Individuals were sampled with replacement during a single bootstrap to calculate the response metric for each time period at the site level. The difference between sampling periods was calculated by subtracting each site's After value from the Before value. We used 95% confidence intervals to examine the significance of these changes between our Control (open, no barrier) and Impact (upstream of selective passage)

treatment groups. Within each treatment category, we also compared differences among drainages.

Results

Selective passage

The selective passage protocol based on migration timing and phenotype accurately distinguished WCT and low-admixture individuals from RBT and high-admixture individuals. The mean pRBT of subsampled fish passed upstream of the K and S Canal Diversions was 0.011 (range 0 - 0.490) compared with 0.327 (range 0 - 1.000, Figure 2.3A) for fish released downstream. The mean individual ROA length was 0.067 Mb (range 0 - 12.441 Mb) for fish passed upstream compared with a mean ROA length of 8.864 Mb (range 0 - 23.036 Mb) for fish not passed (Figure 2.3B).

Spatiotemporal patterns of admixture

Across the three hybridization metrics, we observed no significant increase in admixture within Impact sites. We observed a significant increase in Control sites, which was driven by a single site, Big Knife Creek.

There was no significant change in pRBT at Impact sites, whereas pRBT significantly increased at Control sites. The mean increase in pRBT of 0.031 (0.010 - 0.052, 95% CI) for Control sites was six times that of Impacted sites (mean 0.006 (-0.005 - 0.016, 95% CI); Figure 2.4A). This result was strongly influenced by Big Knife Creek, which had the largest increase (mean 0.281 [0.184 - 0.379, 95% CI]) in site mean pRBT (Figure 2.5A). No other drainage within the Control treatment had a significant increase.

The proportion of individuals with pRBT > 0.10 significantly increased at Control sites between sampling periods (mean increase 0.068 [0.011 - 0.124, 95% CI]), while there was no change in Impact sites (mean increase 0.002 [-0.023 - 0.027, 95% CI]; Figure 2.4B). Within Control sites, the drainage with the maximum increase was Big Knife Creek, where individuals with a pRBT > 0.10 increased by 63% (Figure 2.5B). The only other drainage with a significant increase in the proportion of individuals with pRBT > 0.10 was the North Fork Jocko (7% increase; Figure 2.5B).

We observed a significant increase in mean individual ROA in Control sites (0.819 Mb [0.246 - 1.392 Mb, 95% CI]) and no significant change in Impact sites (0.195 Mb, [-0.046 - 0.437 Mb, 95% CI]) between the two sample periods (Figure 2.4C). Big Knife Creek had significantly increased mean individual ROA length (6.836 Mb, [3.349 - 10.323 Mb, 95% CI]; Figure 2.5C). There were no other significant changes in mean individual ROA length.

Discussion

Our results suggest that the selective passage of WCT in the Jocko River Watershed was successful. We observed substantial disrupted propagule pressure of individuals dominated by RBT ancestry attempting to migrate into Impact drainages. This illustrates the threat of hybridization to populations upstream of the K Canal Diversion and the need for selective passage. The protocol for selective passage based on migration timing and phenotypic assessment of migratory WCT individuals allowed hundreds of migratory WCT access to spawning habitat with no significant increase in hybridization metrics in above barrier populations. Using a genomic approach with an adequate number (n = 796) of species diagnostic

markers, we were able to confidently estimate admixture at the individual level. We observed consistency across three metrics of hybridization that summarize admixture across the genome. These results suggest that selective passage might be an effective tool for managing WCT populations and maintaining life history diversity and productivity in other watersheds with low above-barrier admixture.

Hybridization metrics for Control sites varied and did not always significantly differ from Impact sites. This likely reflects variation in propagule pressure of RBT and hybrids dispersing into each drainage from the Jocko River mainstem. Propagule pressure was only directly measured for fish captured at the canal diversions attempting to enter Impact sites. Therefore, it most directly reflects propagule pressure for the North, Middle, and South Fork of the Jocko River. Propagule pressure from highly admixed fish and RBT may be lower in the Control sites, except for Big Knife Creek (see below). Additionally, the lack of change in hybridization metrics at other Control sites is most likely explained by drainage slope, the change in elevation from RBT source to a sample site divided by the distance between source and sample site. Within the Jocko River watershed, Corsi (2011) found a strong association between site pRBT and slope in the baseline assessment of hybridization.

The success of selective passage at K and S Canal Diversions suggests that this management approach could benefit conservation populations isolated by barriers in the presence of a nearby source of highly hybridized fish. Even though the selective passage protocol in this study was generally accurate, some fish with relatively high pRBT were passed upstream. This risk was expected as phenotypic assessment of WCT becomes less accurate at low levels of admixture (Leary et al. 1984; Weigel et al. 2002; Ardren and Bernall 2017). The risk of passing low-level hybrids was deemed acceptable given that the populations above the barrier already

had low levels of admixture (< 0.10 pRBT) before selective passage began. If selective passage is being considered for core populations with no hybridization, the protocol should be adjusted to identify WCT using genetic data in combination with phenotype assessment. The rapid genetic assignment is performed on migrating bull trout to pass them over dams and into natal spawning tributaries (DeHaan et al. 2011), illustrating the potential for selective passage to incorporate genetic information quickly. Advancements in genomic techniques will likely allow for the detection of hybridization with a faster turnaround capacity, making this a more practical consideration for future applications. With this possibility, managers would be able to combine phenotypic information and rapid genomic testing for barrier management across the landscape, as is done for rapid bull trout population assignments (Bohling et al. 2021).

The increase in hybridization metrics in Big Knife Creek emphasizes the risk of hybridization from individuals that are not being passed or are blocked by the barrier. This may have unintentionally created a nearby source of RBT or highly hybridized individuals. The mouth of Big Knife Creek is located approximately 100 meters downstream of the K Canal Diversion. The forced dispersal of blocked RBT and hybrids most likely explains the increase in hybridization in Big Knife Creek. Similar barrier-induced dispersal to nearby downstream spawning sites was observed for rainbow trout at multiple dams in northern Idaho (Ardren and Bernall 2017). Big Knife Creek now appears to represent a new 'hotspot' for highly hybridized fish, which is a concern because straying from localized sources of highly admixed fish is a major driver in the spread of RBT hybridization in some river systems (Boyer et al. 2008; Muhlfeld et al. 2009c). Previous research has shown that RBT and RBT hybrids migrate on the ascending limb of the hydrograph and during peak flow, both generally (Muhlfeld et al. 2009b) and, more specifically at the Jocko River K Canal Diversion (Corsi et al. 2013). Additionally,

straying of RBT and hybrids during high peak flow conditions when the K Canal Diversion is occasionally passable most likely explains the significant increase in the proportion of individuals with pRBT > 0.10 in the North Fork Jocko River, which is downstream of the S Diversion and the first of the three major forks upstream of K Diversion. Thus, the establishment of a nearby RBT hotspot near the K Canal Diversion should be taken into consideration as managers weigh the risk of potential upstream movement over the K Canal Diversion at high peak streamflow.

Recent advancements in genomics allowed us to gain novel insights into hybridization dynamics between WCT and RBT, specifically through our novel application of Runs of Admixture (ROA). Admixture tracts have been used in population genetics to examine the temporal dynamics of gene flow (Liang and Nielsen 2014; Avadhanam and Williams 2022). Further, Runs of Homozygosity, or tracts of contiguous homozygous genotypes, have become widely used to examine inbreeding in a conservation context (Kardos et al. 2016). We used ROAs to evaluate likely sources of RBT chromosomal segments and drivers of temporal change in hybridization. The latter was possible because our study design included estimates of mean ROA length from before passage began (2005-2007) to after passage (2016-2019) across all twenty sample sites (Table 2.1; Figure S2.3; S2.4). Using a combination of baseline data and estimates of ROA length in fish captured at the diversions, we could make directional predictions about the change in mean ROA length over time based on the RBT source.

Conclusion

We found that phenotype-based passage of WCT under the threat of RBT hybridization successfully promoted the migratory life history without increasing above barrier hybridization.

We recommend that consideration of phenotypic-based passage be restricted to above-barrier populations with preexisting low admixture. Although generally quite accurate, visual assessment of hybridization status for migratory fish based on phenotype and run timing was not entirely failsafe. For that reason, the passage of low-admixture fish would pose risks if non-hybridized populations of high conservation value occur above a barrier. The ongoing threat of RBT hybridization to WCT has led to the need to consider and evaluate a variety of management actions, including suppression (Al-Chokhachy et al. 2014; Meyer et al. 2017; Kovach et al. 2018), isolation (Harig and Fausch 2002; Fausch et al. 2009), and selective passage to conserve WCT populations (Ardren and Bernall 2017). Our work suggests that selective passage holds promise in situations where the maintenance of the migratory life history is one of the competing goals.

References

Al-Chokhachy, R., Muhlfeld, C.C., Boyer, M.C., Jones, L.A., Steed, A., and Kershner, J.L. 2014. Quantifying the effectiveness of conservation measures to control the spread of anthropogenic hybridization in stream salmonids: a climate adaptation case study. North American Journal of Fisheries Management **34**(3): 642–652. doi:10.1080/02755947.2014.901259.

Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., and Miller, M.R. 2016. RAD Capture (Rapture): Flexible and efficient sequence-based genotyping. Genetics **202**(2): 389–400. doi:10.1534/genetics.115.183665.

Allendorf, F.W., and Leary, R.F. 1988. Conservation and Distribution of Genetic Variation in a Polytypic Species, the Cutthroat Trout. Conservation Biology **2**(2): 170–184. doi:10.1111/j.1523-1739.1988.tb00168.x.

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution **16**(11): 613–622. doi:10.1016/S0169-5347(01)02290-X.

Allendorf, F.W., and Lundquist, L.L. 2003. Introduction: Population biology, evolution, and control of invasive species. Conservation Biology **17**(1): 24–30. doi:10.1046/j.1523-1739.2003.02365.x.

Amish, S.J., Hohenlohe, P.A., Painter, S., Leary, R.F., Muhlfeld, C., Allendorf, F.W., and Luikart, G. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. Molecular Ecology Resources **12**(4): 653–660. doi:10.1111/j.1755-0998.2012.03157.x.

Ardren, W.R., and Bernall, S.R. 2017. Dams impact westslope cutthroat trout metapopulation structure and hybridization dynamics. Conserv Genet **18**(2): 297–312. doi:10.1007/s10592-016-0906-6.

Avadhanam, S., and Williams, A.L. 2022. Simultaneous inference of parental admixture proportions and admixture times from unphased local ancestry calls. preprint, Genetics. doi:10.1101/2022.01.05.475139.

Bohling, J., Von Bargen, J., Piteo, M., Louden, A., Small, M., Delomas, T.A., and Kovach, R. 2021. Developing a standardized single nucleotide polymorphism panel for rangewide genetic monitoring of bull trout. N American J Fish Manag **41**(6): 1920–1931. doi:10.1002/nafm.10708.

Boyer, M.C., Muhlfeld, C.C., and Allendorf, F.W. 2008. Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Can. J. Fish. Aquat. Sci. **65**(4): 658–669. doi:10.1139/f08-001.

Britton, J.R., Gozlan, R.E., and Copp, G.H. 2011. Managing non-native fish in the environment: Managing non-native fishes. Fish and Fisheries **12**(3): 256–274. doi:10.1111/j.1467-2979.2010.00390.x.

Clavero, M., and Garcia-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology & Evolution **20**(3): 110–110. doi:10.1016/j.tree.2005.01.003.

Corsi, M.P. 2011. Management and life history consequences of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*). Graduate Student Theses, Dissertations, & Professional Papers. 640, University of Montana. Available from https://scholarworks.umt.edu/etd/640.

Corsi, M.P., Eby, L.A., and Barfoot, C.A. 2013. Hybridization with rainbow trout alters life history traits of native westslope cutthroat trout. Can. J. Fish. Aquat. Sci. **70**(6): 895–904. doi:10.1139/cjfas-2012-0312.

DeHaan, P.W., Bernall, S.R., DosSantos, J.M., Lockard, L.L., and Ardren, W.R. 2011. Use of genetic markers to aid in re-establishing migratory connectivity in a fragmented metapopulation of bull trout (*Salvelinus confluentus*). Can. J. Fish. Aquat. Sci. **68**(11): 1952–1969. doi:10.1139/f2011-098.

Epifanio, J., and Philipp, D. 2000. Simulating the extinction of parental lineages from introgressive hybridization: the effects of fitness, initial proportions of parental taxa, and mate choice. Reviews in Fish Biology and Fisheries **10**(3): 16.

Fausch, K.D., Rieman, B.E., Dunham, J.B., Young, M.K., and Peterson, D.P. 2009. Invasion versus Isolation: Trade-offs in managing native salmonids with barriers to upstream movement. Conservation Biology **23**(4): 859–870. doi:10.1111/j.1523-1739.2008.01159.x.

Grant, P.R., and Grant, B.R. 1994. Phenotypic and genetic effects of hybridization in Dawrwin's' finches. Evolution **48**(2): 297–316. doi:10.1111/j.1558-5646.1994.tb01313.x.

Hand, B.K., Hether, T.D., Kovach, R.P., Muhlfeld, C.C., Amish, S.J., Boyer, M.C., O'rourke, S.M., Miller, M.R., Lowe, W.H., Hohenlohe, P.A., and Luikart, G. 2015. Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. Current Zoology **61**(1): 146–154. doi:10.1093/czoolo/61.1.146.

Harig, A.L., and Fausch, K.D. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. Ecological Applications **12**(2): 535–551. doi:10.1890/1051-0761(2002)012[0535:MHRFET]2.0.CO;2.

Hedrick, P.W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol Ecol **22**(18): 4606–4618. doi:10.1111/mec.12415.

Hohenlohe, P.A., Day, M.D., Amish, S.J., Miller, M.R., Kamps-Hughes, N., Boyer, M.C., Muhlfeld, C.C., Allendorf, F.W., Johnson, E.A., and Luikart, G. 2013. Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. Mol Ecol **22**(11): 3002–3013. doi:10.1111/mec.12239.

Kardos, M., Taylor, H.R., Ellegren, H., Luikart, G., and Allendorf, F.W. 2016. Genomics advances the study of inbreeding depression in the wild. Evol Appl **9**(10): 1205–1218. doi:10.1111/eva.12414.

Kovach, R.P., Al-Chokhachy, R., and Stephens, T. 2018. Proactive rainbow trout suppression reduces threat of hybridization in the upper Snake River Basin. North Am J Fish Manage **38**(4): 811–819. doi:10.1002/nafm.10177.

Kovach, R.P., Hand, B.K., Hohenlohe, P.A., Cosart, T.F., Boyer, M.C., Neville, H.H., Muhlfeld, C.C., Amish, S.J., Carim, K., Narum, S.R., Lowe, W.H., Allendorf, F.W., and Luikart, G. 2016. Vive la résistance: genome-wide selection against introduced alleles in invasive hybrid zones. Proc. R. Soc. B. **283**(1843): 20161380. doi:10.1098/rspb.2016.1380.

Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Lowe, W.H., Allendorf, F.W., and Luikart, G. 2015. Dispersal and selection mediate hybridization between a native and invasive species. Proc. R. Soc. B. **282**(1799): 20142454. doi:10.1098/rspb.2014.2454.

Leary, R.F., Allendorf, F.W., Phelps, S.R., and Knudsen, K.L. 1984. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. Proceedings of the Montana Academy of Sciences **43**: 1–18.

Liang, M., and Nielsen, R. 2014. The lengths of admixture tracts. Genetics **197**(3): 953–967. doi:10.1534/genetics.114.162362.

Liknes, G.A., and Graham, P.J. 1988. Westslope cutthroat trout in Montana: Life history, status and management. Status and management of interior stocks of cutthroat trout. American Fisheries Society Symposium **4**(53–60).

Meyer, K.A., Kennedy, P., High, B., and Campbell, M.R. 2017. Purifying a Yellowstone Cutthroat Trout Stream by Removing Rainbow Trout and Hybrids via Electrofishing. Transactions of the American Fisheries Society **146**(6): 1193–1203. doi:10.1080/00028487.2017.1362470.

Montana Department of Fish, Wildlife, and Parks. 2007. Memorandum of understanding and conservation agreement for Westslope Cutthroat Trout and Yellowstone Cutthroat Trout in Montana.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009a. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. **5**(3): 328–331. doi:10.1098/rsbl.2009.0033.

Muhlfeld, C.C., Kovach, R.P., Al-Chokhachy, R., Amish, S.J., Kershner, J.L., Leary, R.F., Lowe, W.H., Luikart, G., Matson, P., Schmetterling, D.A., Shepard, B.B., Westley, P.A.H., Whited, D., Whiteley, A., and Allendorf, F.W. 2017. Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout. Glob Change Biol **23**(11): 4663–4674. doi:10.1111/gcb.13681.

Muhlfeld, C.C., McMahon, T.E., Belcer, D., and Kershner, J.L. 2009b. Spatial and temporal spawning dynamics of native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, introduced rainbow trout, *Oncorhynchus mykiss*, and their hybrids. Can. J. Fish. Aquat. Sci. **66**(7): 1153–1168. doi:10.1139/F09-073.

Muhlfeld, C.C., McMahon, T.E., Boyer, M.C., and Gresswell, R.E. 2009c. Local habitat, watershed, and biotic factors influencing the spread of hybridization between native westslope cutthroat trout and introduced rainbow trout. Transactions of the American Fisheries Society **138**(5): 1036–1051. doi:10.1577/T08-235.1.

Ottenburghs, J. 2021. The genic view of hybridization in the Anthropocene. Evol Appl **14**(10): 2342–2360. doi:10.1111/eva.13223.

Pearse, D.E., Barson, N.J., Nome, T., Gao, G., Campbell, M.A., Abadía-Cardoso, A., Anderson, E.C., Rundio, D.E., Williams, T.H., Naish, K.A., Moen, T., Liu, S., Kent, M., Moser, M., Minkley, D.R., Rondeau, E.B., Brieuc, M.S.O., Sandve, S.R., Miller, M.R., Cedillo, L., Baruch, K., Hernandez, A.G., Ben-Zvi, G., Shem-Tov, D., Barad, O., Kuzishchin, K., Garza, J.C., Lindley, S.T., Koop, B.F., Thorgaard, G.H., Palti, Y., and Lien, S. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. Nat Ecol Evol **3**(12): 1731–1742. doi:10.1038/s41559-019-1044-6.

Rhymer, J.M., and Simberloff, D. 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. **27**(1): 83–109. doi:10.1146/annurev.ecolsys.27.1.83.

Scribner, K.T., Lowe, W.H., Landguth, E., Luikart, G., Infante, D.M., Whelan, G.E., and Muhlfeld, C.C. 2016. Applications of Genetic Data to Improve Management and Conservation of River Fishes and Their Habitats. Fisheries **41**(4): 174–188. doi:10.1080/03632415.2016.1150838.

Shepard, B.B., May, B.E., and Urie, W. 2005. Status and conservation of westslope cutthroat trout within the Western United States. North American Journal of Fisheries Management **25**(4): 1426–1440. doi:10.1577/M05-004.1.

Strait, J.T., Eby, L.A., Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Amish, S.J., Smith, S., Lowe, W.H., and Luikart, G. 2021. Hybridization alters growth and migratory life-history expression of native trout. Evol Appl **14**(3): 821–833. doi:10.1111/eva.13163.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G., and Rieseberg, L.H. 2016. Hybridization and extinction. Evol Appl **9**(7): 892–908. doi:10.1111/eva.12367.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., and Melillo, J.M. 1997. Human Domination of Earth's Ecosystems. **277**: 7.

Weigel, D.E., Peterson, J.T., and Spruell, P. 2002. A Model using phenotypic characteristics to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. Transactions of the American Fisheries Society **131**(3): 389–403. doi:10.1577/1548-8659(2002)131<0389:AMUPCT>2.0.CO;2.

Tables

Table 2. 1: Summary of site-level rainbow trout hybridization metrics and sample size for 21 sample sites in the Jocko River Watershed, Montana. Shown are the proportion of rainbow trout admixture (pRBT), the proportion of individuals with pRBT > 0.10, and the mean site Run of Admixture ROA length. Treatment is based on the BACI framework of this study. The sites open to all migratory individuals are Control sites, and those upstream of the K and S Canal Diversions are Impact sites. The Before sampling period occurred before fish passage at the K and S Canal Diversions during 2005-2007. The After sampling period occurred during 2016-2019 after fish passage began in 2010. Site numbers correspond with site labels in Figure 2.1.

Site #	Drainage	Treatment	Sampling Period	Sample Size	Mean Site pRBT (*min - max*)	Proportion Individuals With pRBT > 0.10	Mean Site ROA Length (*min - max*)
1	Big Knife Creek	Control	Before	16	0.049 (0.002 - 0.276)	0.186	258,351 (0.003 - 1,675,150)
			After	22	0.332 (0.006 - 0.632)	0.818	7,048,227 (0.001 - 20,287,959)
2	Valley Creek	Control	Before	21	0.116 (0.002 - 0.479)	0.238	1,379,843 (0.005 - 12,809,545)
			After	22	0.025 (0 - 0.270)	0.046	128,359 (0 - 1,898,282)
3	Valley Creek	Control	Before	10	0.0004 (0 - 0.001)	0	0.001 (0 - 0.002)
			After	12	0.0003 (0 - 0.001)	0	0.001 (0 - 0.001)
4	Valley Creek	Control	Before	24	0.004 (0 - 0.055)	0	13,192 (0 - 201,995)
			After	22	0.078 (0 - 0.367)	0.273	375,530 (0 - 1,863,823)
5	Valley Creek	Control	Before	27	0.0007 (0 - 0.003)	0	0.001 (0 - 0.004)
			After	18	0.0004 (0 - 0.001)	0	0.001 (0 - 0.003)
6	Finley Creek	Control	Before	24	0.014 (0 - 0.177)	0.083	49,175 (0 - 515,813)
			After	24	0.008 (0 - 0.039)	0	19,894 (0 - 105,784)
7	Finley Creek	Control	Before	28	0.108 (0 - 0.998)	0.321	1,273,943 (0 - 22,782,994)
			After	22	0.098 (0 - 0.592)	0.227	1,885,390 (0 - 20,296,680)
8	Finley Creek	Control	Before	28	0.003 (0 - 0.030)	0	4,005 (0 - 82,548)
			After	25	0.007 (0 - 0.047)	0	15,671 (0 - 178,315)
9	North Fork	Impact	Before	12	0.011 (0 - 0.047)	0	25,196 (0 - 148,595)
			After	22	0.072 (0 - 0.879)	0.182	1,149,031 (0 - 21,812,237)
10	North Fork	Impact	Before	13	0.002 (0 - 0.013)	0	4,012 (0 - 26,564)
			After	22	0.047 (0 - 0.994)	0.045	1,035,143 (0 - 22,727,023)
11	North Fork	Impact	Before	22	0.003 (0 - 0.011)	0	5,139 (0 - 45,359)
			After	13	0.0006 (0 - 0.006)	0	697 (0 - 9,071)

Table 2.1: Continued

Site #	Drainage	Study Status	Sampling Period	Sample Size	Mean Site pRBT (*min - max*)	Proportion Individuals With pRBT > 0.10	Mean Site ROA Length (*min - max*)
12	North Fork	Impact	Before	23	0.002 (0 - 0.036)	0	4,029 (0 - 61,730)
			After	24	0.001 (0 - 0.010)	0	1,652 (0 - 27,527)
13	Middle Fork	Impact	Before	26	0.002 (0 - 0.020)	0	4,457 (0 - 58,580)
			After	23	0.002 (0 - 0.013)	0	4,172 (0 - 27,102)
14	Middle Fork	Impact	Before	21	0.004 (0 - 0.021)	0	6,384 (0 - 55,282)
			After	23	0.003 (0 - 0.010)	0	5,468 (0 - 32,169)
15	Middle Fork	Impact	Before	26	0.003 (0 - 0.058)	0	7,963 (0 - 168,717)
			After	25	0.002 (0 - 0.010)	0	1,756 (0 - 27,473)
16	South Fork	Impact	Before	21	0.006 (0 - 0.056)	0	14,378 (0 - 157,737)
			After	19	0.002 (0 - 0.006)	0	2,874 (0 - 38,100)
17	South Fork	Impact	Before	15	0.024 (0 - 0.155)	0.133	103,550 (0 - 838,834)
			After	25	0.020 (0 - 0.474)	0.040	402,090 (0 - 10,027,018)
18	South Fork	Impact	Before	18	0.023 (0 - 0.259)	0.111	1,00,050 (0 - 1,418,349)
			After	19	0.003 (0 - 0.021)	0	2,740 (0 - 40,564)
19	South Fork	Impact	Before	10	0.001 (0 - 0.008)	0	0.003 (0 - 0.016)
			After	18	0.0004 (0 - 0.003)	0	174 (0 - 3,142)
20	South Fork	Impact	Before	19	0.007 (0 - 0.037)	0	18,249 (0 - 112,178)
			After	24	0.001 (0 - 0.007)	0	2,422 (0 - 34,552)
21	Liberty Creek	Potential Source	Before	15	0.945 (0.920 - 0.975)	1.00	20,094,170 (17,130,121 - 22,601,562)
			After	20	0.949 (0.927 - 0.977)	1.00	18,877,626 (12,478,965 - 23,125,743)

Table 2. 2: Summary of predictions for site-level hybridization response metrics for different baseline hybridization and propagule pressure scenarios. The first metric is the mean proportion of rainbow trout admixture (pRBT), which is the summary of pRBT for all individuals within site. The second metric is based on the state of Montana's threshold of a population mean > 0.10 pRBT to determine a conservation population of westslope cutthroat trout. The third metric is the site mean of Runs of Admixture (ROA) length for all individuals within a site. All scenarios assume a baseline with low admixture (low pRBT) and vary in the number of immigrants with admixed ancestry (propagule pressure) inferring populations following a fish passage treatment like the one in this study. The combination of low pRBT baseline and low propagule pressure resembles the Impact sites in our study, the combination of low pRBT baseline and moderate to high propagule pressure of fish with high RBT genetic ancestry likely resembles the Control sites in our study.

	Changes in Site Level Metrics (After Fish Passage – Before Fish Passage)		
Scenarios	Mean Proportion of RBT admixture (pRBT)	Proportion Individuals with pRBT > 0.10	Mean ROA Length
Low pRBT baseline No Propagule Pressure	NC, ↓	↓	↓
Low pRBT baseline Low Propagule Pressure (*Impact Sites*)	NC, ↓	↓	NC, ↓
Low pRBT baseline Moderate/High Propagule Pressure (*Control Sites*)	↑	↑	↑

Note: NC = No change, ↑ = Increase in metric between sampling periods, ↓ = Decrease in metric between sampling periods.

Figures

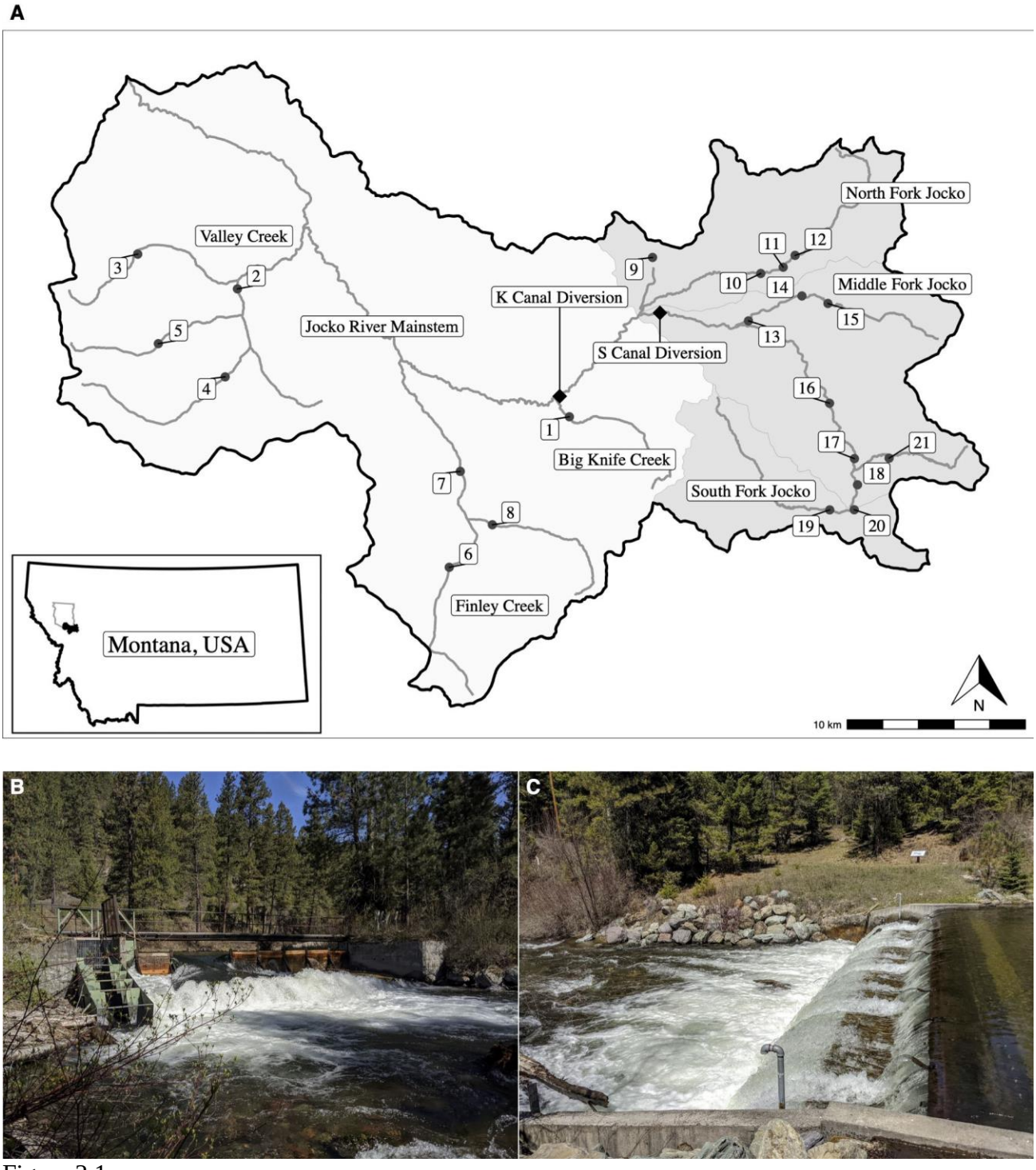
Figure 2. 1: (A) Map of Jocko River Watershed on the Flathead Indian Reservation, MT. The K and S Canals are located on the mainstem Jocko River. Dots indicate sample sites and their corresponding site numbers are shown in Table 2.1. The gray background indicates Impact drainages in our BACI design, which were sites above selective passage at the K Canal Diversion. The white background indicates Control drainages. The insert shows Montana and the Flathead Indian Reservation with the Jocko River Watershed in black. Pictures of the K Canal Irrigation Diversion (B) and the S Canal Irrigation Diversion (C) during typical spring stream flows (Photo Credit: Anthony Dangora 5/6/21).

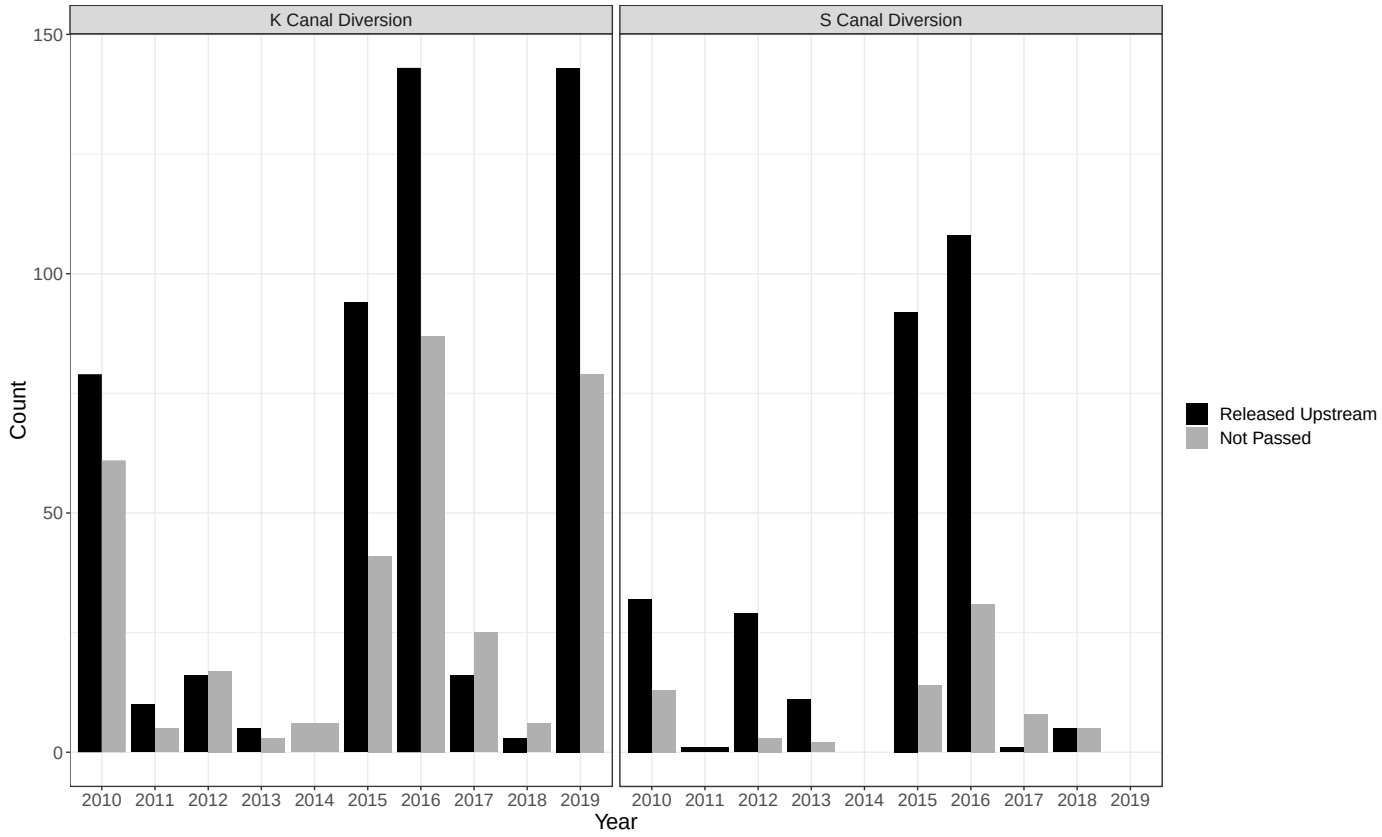
Figure 2. 2: Count of phenotypic migratory westslope cutthroat trout individuals released upstream (black) of the K and S Canal Diversions and phenotypic rainbow trout or hybrid individuals not passed (grey) at the K and S Canal Diversions on the Jocko River, Montana for each year between 2010 and 2019.

Figure 2. 3: (A) The distribution of the proportion of RBT admixture (pRBT) for subsampled individuals not passed at the K Canal Diversion and individuals passed upstream at the K and S Canal Diversions in the Jocko River Watershed, MT. (B) The distribution of mean Run of Admixture (ROA) length for RBT diagnostic loci of genotyped individuals at K and S Canal Diversions.

Figure 2. 4: (A) Bootstrapped estimates of the change in the mean site proportion of rainbow trout admixture (pRBT) from Before selective passage at the K and S Canal Diversion began (2005-2007) and After (2016-2019) sampling periods in the Control and Impact sites located in the Jocko River Watershed, Montana. (B) Bootstrapped estimates of the site change in the proportion of individuals with pRBT > 0.10 between the Before and After sampling periods in both Control and Impact sites. (C) Bootstrapped estimates of site change in mean Runs of Admixture (ROA) length for Control and Impact sites. The black dot represents the mean bootstrapped estimate of each metric, and the black line represents the minimum and maximum 95% confidence intervals for each estimate.

Figure 2. 5: (A) Bootstrapped estimates of the change in the mean site proportion of rainbow trout admixture (pRBT) from Before selective passage at the K and S Canal Diversion (2005-2007) and After selective passage (2016-2019) sampling periods across each major Control and Impact drainage located in the Jocko River Watershed, Montana. (B) Bootstrapped estimates of the site change in the proportion of individuals with pRBT > 0.10 between the Before and After sampling periods in all study drainages. (C) Bootstrapped estimates of site change in mean Runs of Admixture (ROA) length between the Before and After sampling periods in all study drainages. The black dot represents the mean bootstrapped estimate of each metric, and the black line represents the minimum and maximum 95% confidence intervals for each estimate.


A
North Fork Jocko
Valley Creek
Middle Fork Jocko
K Canal Diversion
Jocko River Mainstem
S Canal Diversion
Big Knife Creek
South Fork Jocko
Finley Creek
Montana, USA
N
10 km
B
C
Figure 2.1


K Canal Diversion
S Canal Diversion
Count
150
100
50
0
2010
2011
2012
2013
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2015
2016
2017
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2019
Year
Released Upstream
Not Passed

Figure 2.2

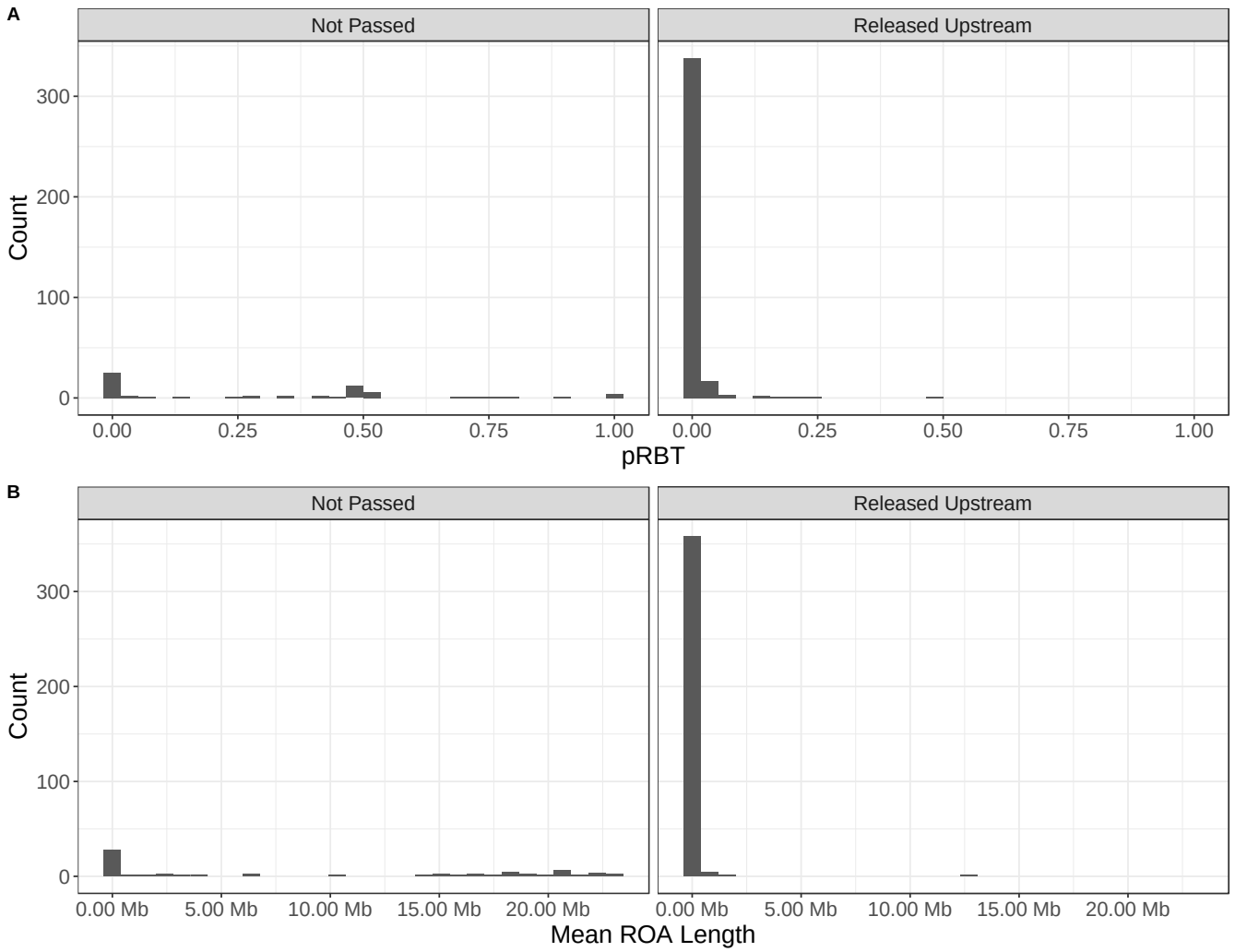

A
Not Passed
Released Upstream
Count
300
200
100
0
0.00
0.25
0.50
0.75
1.00
pRBT
B
Not Passed
Released Upstream
Count
300
200
100
0
0.00 Mb
5.00 Mb
10.00 Mb
15.00 Mb
20.00 Mb
Mean ROA Length

Figure 2.3

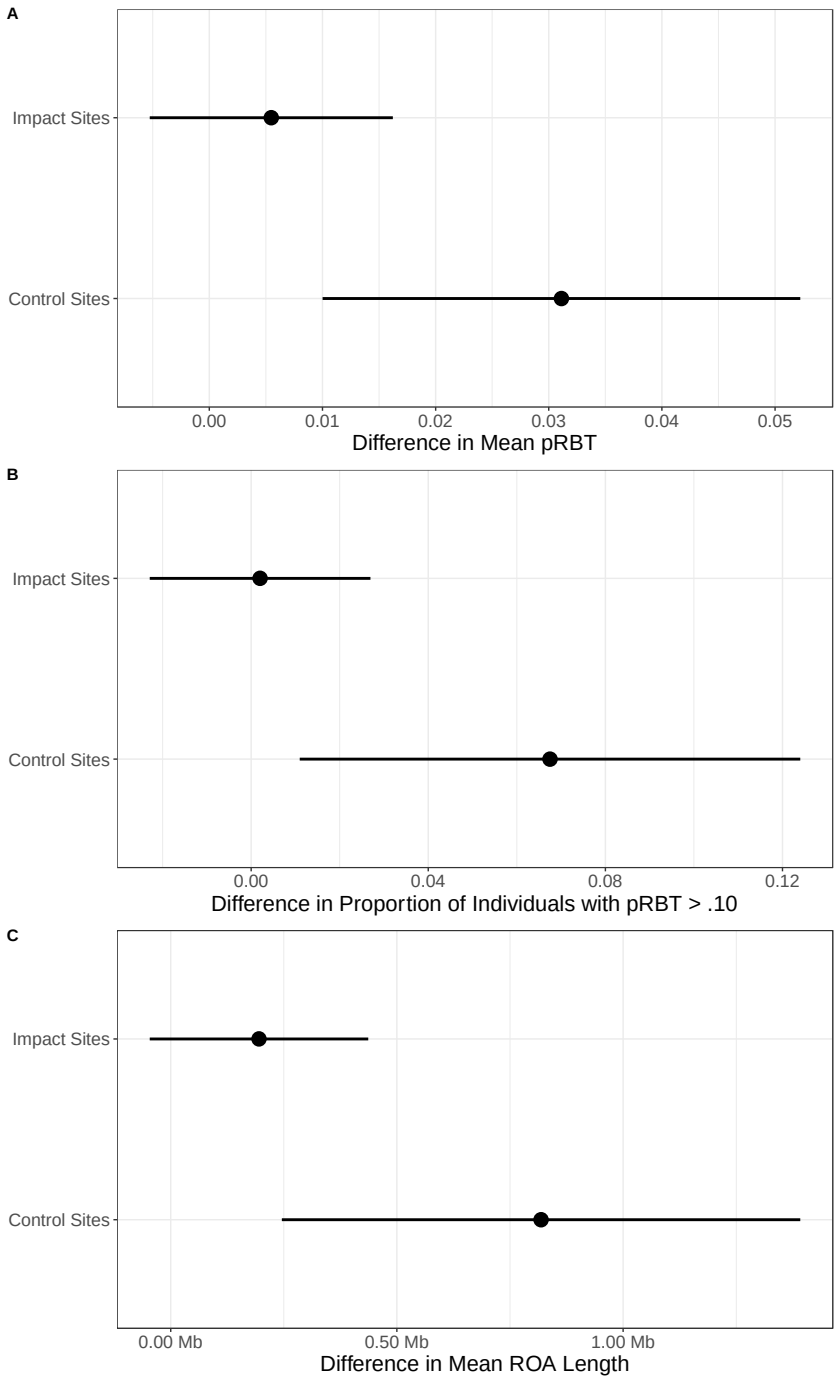

A
Impact Sites
Control Sites
0.00
0.01
0.02
0.03
0.04
0.05
Difference in Mean pRBT
B
Impact Sites
Control Sites
0.00
0.04
0.08
0.12
Difference in Proportion of Individuals with pRBT > .10
C
Impact Sites
Control Sites
0.00 Mb
0.50 Mb
1.00 Mb
Difference in Mean ROA Length

Figure 2.4

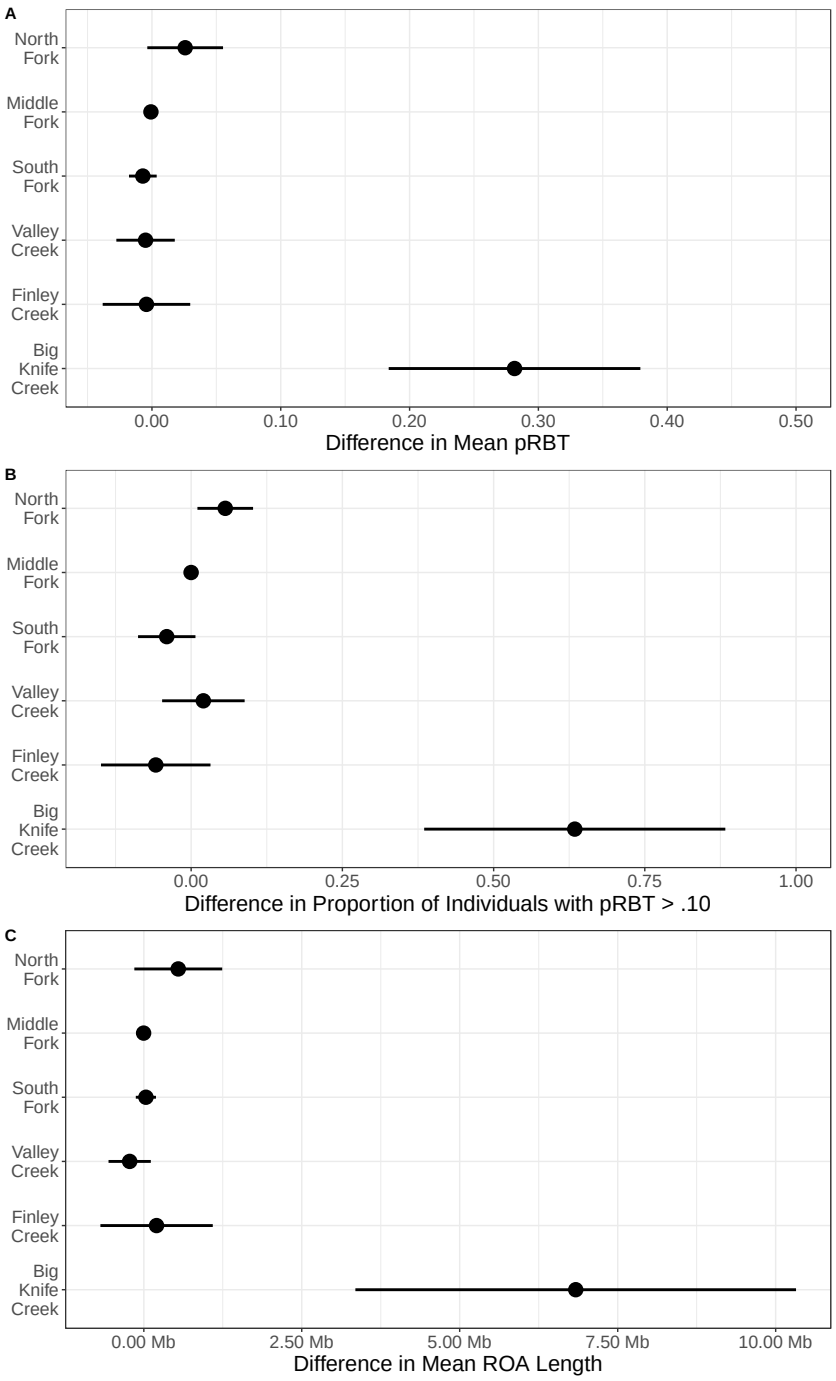

A
North Fork
Middle Fork
South Fork
Valley Creek
Finley Creek
Big Knife Creek
0.00
0.10
0.20
0.30
0.40
0.50
Difference in Mean pRBT
B
North Fork
Middle Fork
South Fork
Valley Creek
Finley Creek
Big Knife Creek
0.00
0.25
0.50
0.75
1.00
Difference in Proportion of Individuals with pRBT > .10
C
North Fork
Middle Fork
South Fork
Valley Creek
Finley Creek
Big Knife Creek
0.00 Mb
2.50 Mb
5.00 Mb
7.50 Mb
10.00 Mb
Difference in Mean ROA Length

Figure 2.5

Supplemental

Appendix 2.1 - Methods

a) Genetic analyses

DNA for all 'Before' samples from 2005-2007 were extracted using isopropyl extraction protocol described by Muhlfeld (2009) and Corsi (2011). For the After genetic samples (2010 to 2019), DNA was extracted using SPRI bead extraction protocol described in Ali (2016). After extraction, individual DNA concentration was measured using QuantIT Picogreen assays (Thermo Fisher Scientific, Waltham, Massachusetts) at a 1:20 dilution. We followed the bestRAD and Rapture (RAD-Capture) protocols described by Ali (2016) to prepare our libraries for sequencing. All sequencing was done on an Illumina HiSeq X by Novogene Corporation. The RAD-Capture panel includes previously identified and established RAD loci containing WCT, RBT, and Yellowstone cutthroat trout species diagnostic SNPs evenly distributed across the assembled RBT genome (Amish et al. 2012; Hohenlohe et al. 2013; Hand et al. 2015; Ali et al. 2016). All samples were genotyped using a preestablished pipeline described by Strait (2021).

b) Locus Missingness

For this study, 1,257 individuals were genotyped with 796 RBT diagnostic loci. We tested the sensitivity of proportion RBT admixture (pRBT) and run of admixture (ROA) length estimates at 40%, 50%, and 60% diagnostic RBT loci missingness to establish the acceptable minimum number of diagnostic loci. We calculated the percent change for sample size and hybridization metrics from 50% missingness (398 RBT diagnostic loci), compared to 40% (477 RBT diagnostic loci) and 60% missingness (318 RBT diagnostic loci) at the Control and Impact sites from Before fish passage and After fish passage (Table S2.1). We found that 50%

missingness (minimum of 398 RBT diagnostic loci) was an adequate rate, as it allowed for sufficient sample size with minimal change across hybridization metrics.

c) Runs of Admixture

Runs of admixture (ROA) is a novel approach to evaluating RBT admixture at both the individual and population level to further our understanding of the spread of hybridization (Figure S2.5). All RBT-specific diagnostic loci from the RAD-Capture panel are mapped to the RBT genome (Pearse et al. 2019). The known position of each diagnostic loci allowed us to calculate the distance along chromosomes containing consecutive RBT diagnostic loci by the number of base pairs between loci. On each chromosome, when present, we measured the distance from the RBT homozygote or heterozygote to the next RBT homozygote or heterozygote genotype. All ROAs ended if the WCT homozygote allele was present. If a RBT homozygote or heterozygote genotype was preceded and followed by a WCT homozygote genotype, we still incorporated them as a ROA with a length of one base pair (a singleton). We had two rules to deal with missing genotypes. The first rule was that an ROA ended if there were two adjacent missing genotypes. Second, we allowed for multiple singular missing genotypes in ROAs if the following genotype was RBT homozygote or heterozygote. All WCT homozygote genotypes were considered to have a run length of zero to maintain proportionality. We averaged all RBT runs, singletons, and WCT homozygote genotypes to calculate the mean individual ROA.

References

Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., and Miller, M.R. 2016. RAD Capture (Rapture): Flexible and efficient sequence-based genotyping. Genetics **202**(2): 389–400. doi:10.1534/genetics.115.183665.

Amish, S.J., Hohenlohe, P.A., Painter, S., Leary, R.F., Muhlfeld, C., Allendorf, F.W., and Luikart, G. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. Molecular Ecology Resources **12**(4): 653–660. doi:10.1111/j.1755-0998.2012.03157.x.

Corsi, M.P. 2011. Management and life history consequences of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*). Graduate Student Theses, Dissertations, & Professional Papers. 640, University of Montana. Available from https://scholarworks.umt.edu/etd/640.

Hand, B.K., Hether, T.D., Kovach, R.P., Muhlfeld, C.C., Amish, S.J., Boyer, M.C., O'rourke, S.M., Miller, M.R., Lowe, W.H., Hohenlohe, P.A., and Luikart, G. 2015. Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. Current Zoology **61**(1): 146–154. doi:10.1093/czoolo/61.1.146.

Hohenlohe, P.A., Day, M.D., Amish, S.J., Miller, M.R., Kamps-Hughes, N., Boyer, M.C., Muhlfeld, C.C., Allendorf, F.W., Johnson, E.A., and Luikart, G. 2013. Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. Mol Ecol **22**(11): 3002–3013. doi:10.1111/mec.12239.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. **5**(3): 328–331. doi:10.1098/rsbl.2009.0033.

Pearse, D.E., Barson, N.J., Nome, T., Gao, G., Campbell, M.A., Abadía-Cardoso, A., Anderson, E.C., Rundio, D.E., Williams, T.H., Naish, K.A., Moen, T., Liu, S., Kent, M., Moser, M., Minkley, D.R., Rondeau, E.B., Brieuc, M.S.O., Sandve, S.R., Miller, M.R., Cedillo, L., Baruch, K., Hernandez, A.G., Ben-Zvi, G., Shem-Tov, D., Barad, O., Kuzishchin, K., Garza, J.C., Lindley, S.T., Koop, B.F., Thorgaard, G.H., Palti, Y., and Lien, S. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. Nat Ecol Evol **3**(12): 1731–1742. doi:10.1038/s41559-019-1044-6.

Strait, J.T., Eby, L.A., Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Amish, S.J., Smith, S., Lowe, W.H., and Luikart, G. 2021. Hybridization alters growth and migratory life-history expression of native trout. Evol Appl **14**(3): 821–833. doi:10.1111/eva.13163.

Appendix 2.2 – Tables and Figures

Supplemental Tables

Table S2. 1: The percent change of sample size, mean runs of admixture (ROA) length, and proportion of rainbow trout admixture (pRBT) based on loci missingness for Control and Impact sites Before Passage and After Passage in the Jocko River, Montana. Percent change was calculated by taking the metric measurement difference at 50% loci missingness (398 diagnostic RBT loci) from the metric measurement at 40% loci missingness (477 diagnostic RBT loci) and dividing it by the measurement at 50% loci missingness. This process is the same at 60% loci missingness (318 diagnostic RBT loci).

Study Sites	Sampling Period	N (Percent Change at 40% RBT Loci)	N (Percent Change at 60% RBT Loci)	Mean ROA Length (Percent Change at 40% RBT Loci)	Mean ROA Length (Percent Change at 60% RBT Loci)	pRBT (Percent Change at 40% RBT Loci)	pRBT (Percent Change at 60% RBT Loci)
Control	Before Fish Passage	5.01 %	- 3.93 %	1.55 %	3.78 %	5.11 %	3.57 %
	After Fish Passage	1.80 %	- 0.60 %	- 1.83 %	0.60 %	- 1.68 %	0.57 %
Impact	Before Fish Passage	4.42 %	- 5.75 %	- 3.68 %	5.44 %	- 3.22 %	4.76 %
	After Fish Passage	2.33 %	- 4.28 %	- 2.34 %	0.69 %	- 2.26 %	- 3.86 %

Supplemental Figures

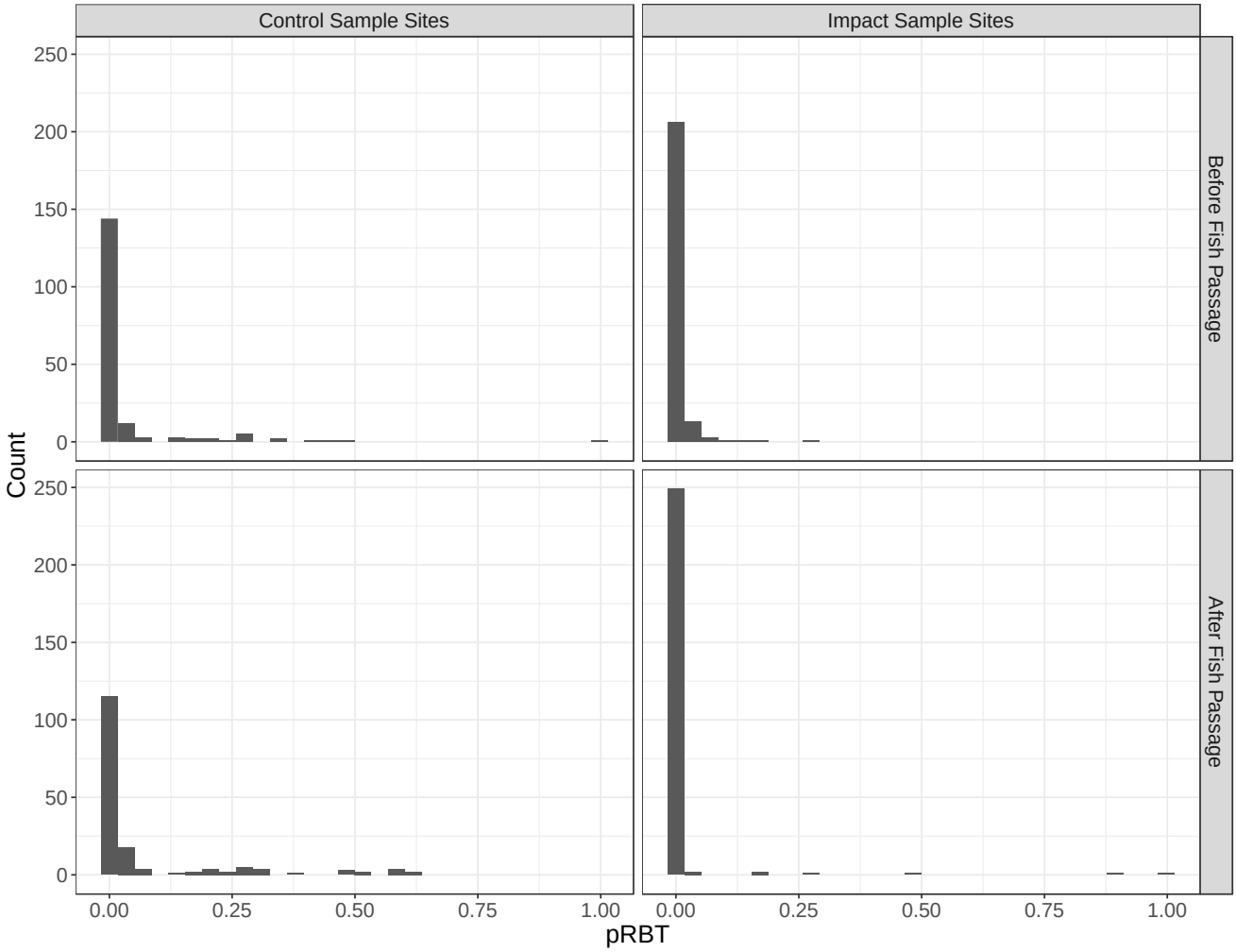


Figure S2. 1: Distributions of the proportion of rainbow trout admixture (pRBT) for all individuals in Control and Impact sites in the Jocko River Watershed, Montana. The Before Fish Passage is from 2005-2007 before fish passage at the K and S Canal Diversions began. The After Fish Passage sampling occurred during 2016-2019.

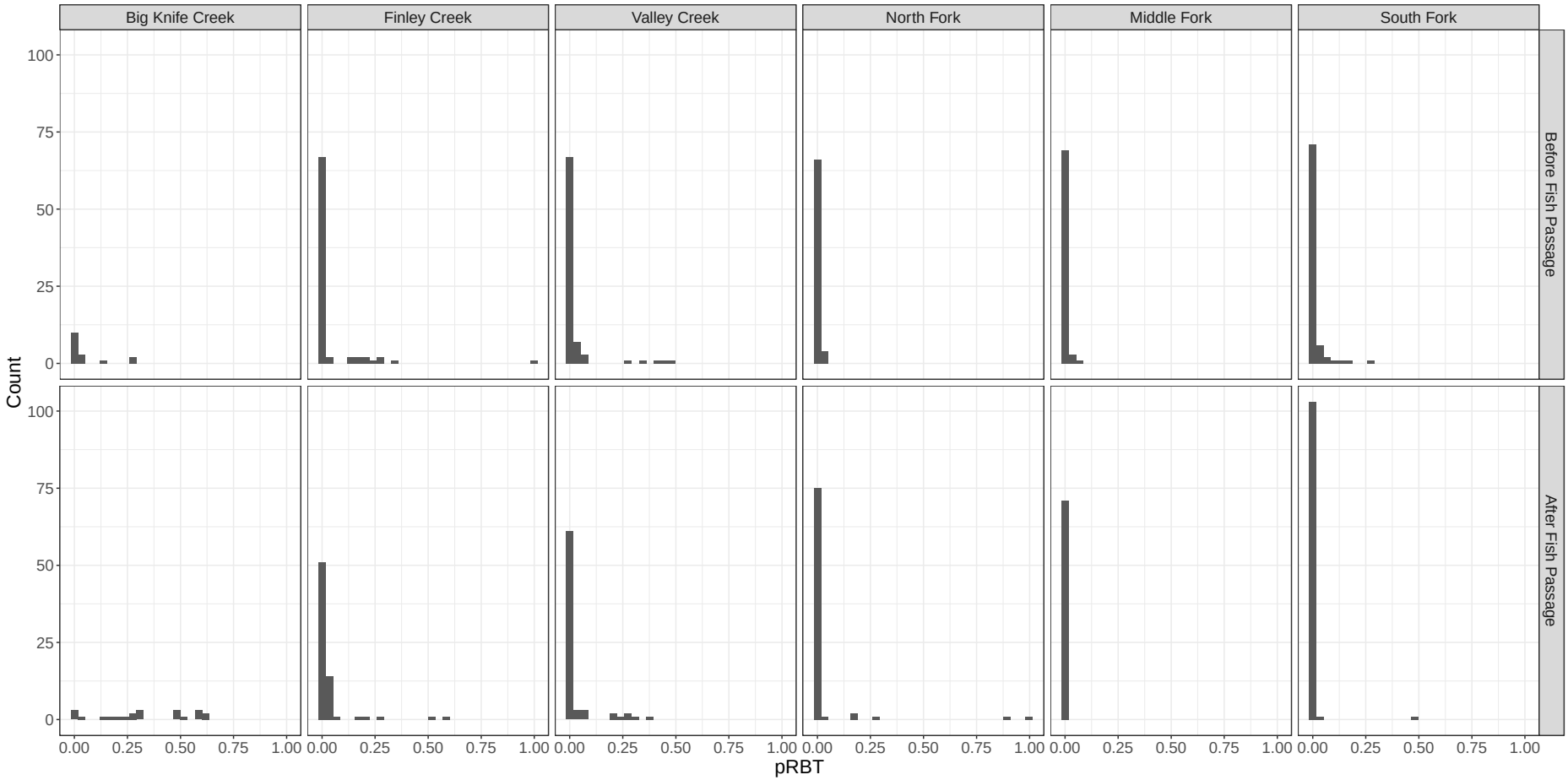


Figure S2. 2: Distributions of the proportion of rainbow trout admixture (pRBT) for all individuals across all major study drainages in the Jocko River Watershed, Montana. The Before Fish Passage sampling is from 2005-2007 before fish passage at the K and S Canal Diversions began. The After Fish Passage sampling occurred from 2016-2019.

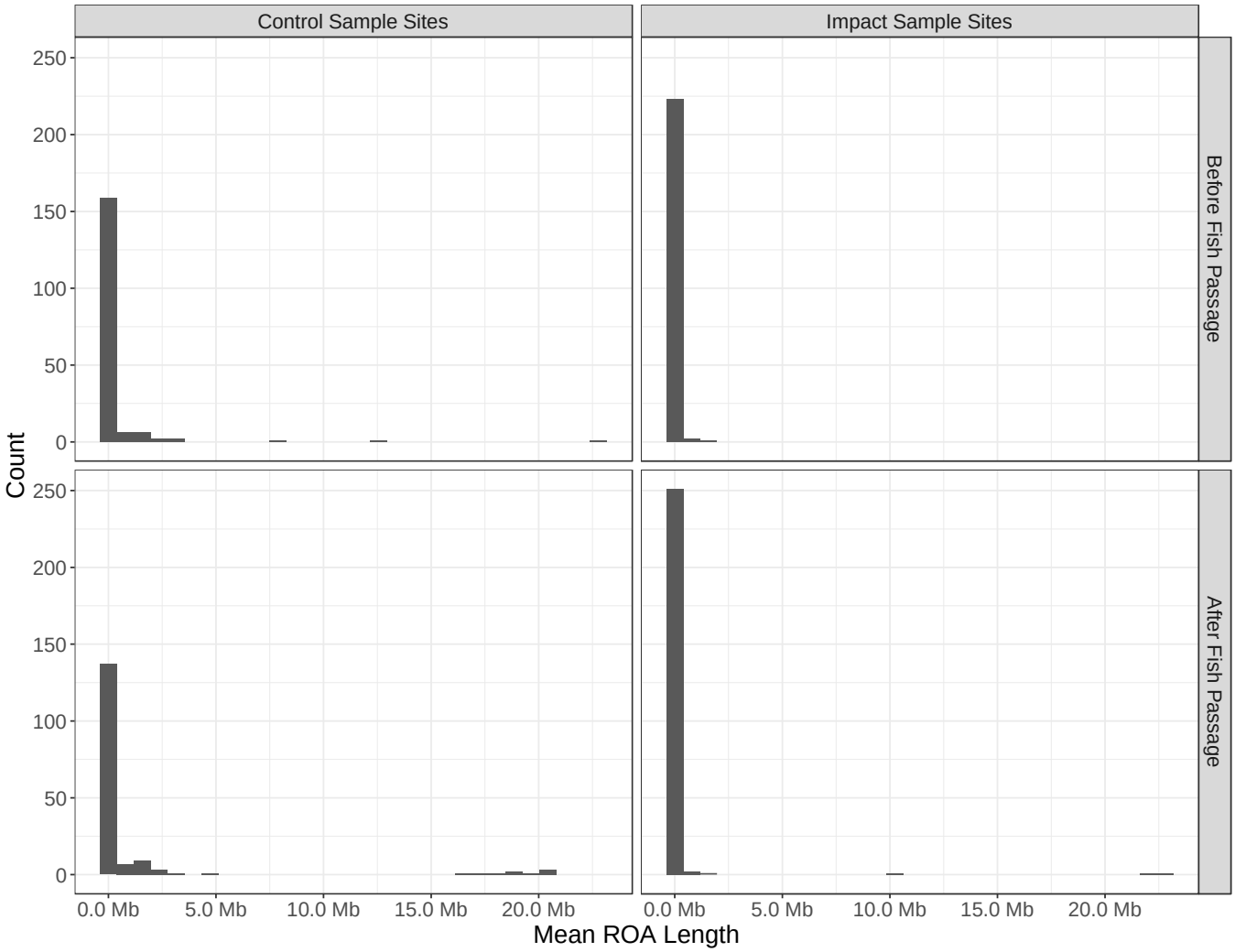


Figure S2. 3: Distribution of mean Run of Admixture (ROA) length for RBT diagnostic loci of genotyped individuals in the Control and Impact sample sites in the Jocko River Watershed, Montana. The Before Fish Passage is from 2005-2007 before fish passage at the K and S Canal Diversions began. The After Fish Passage is from 2016-2019.

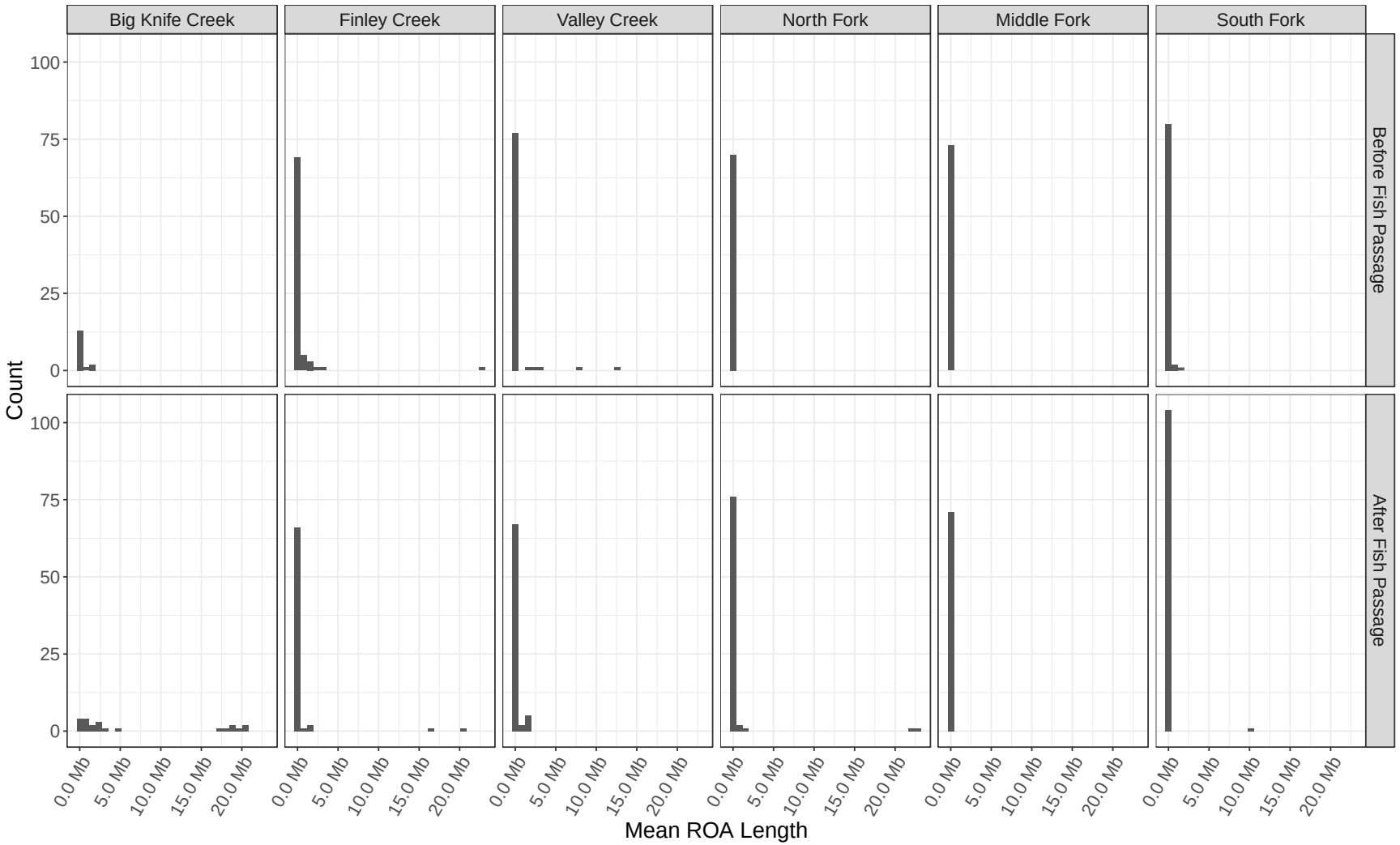


Figure S2. 4: The distribution of mean Run of Admixture (ROA) length for RBT diagnostic loci of genotyped individuals across all major study drainages in the Jocko River Watershed, Montana. The Before Fish Passage is from 2005-2007 before fish passage at the K and S Canal Diversions began. The After Fish Passage is from 2016-2019.

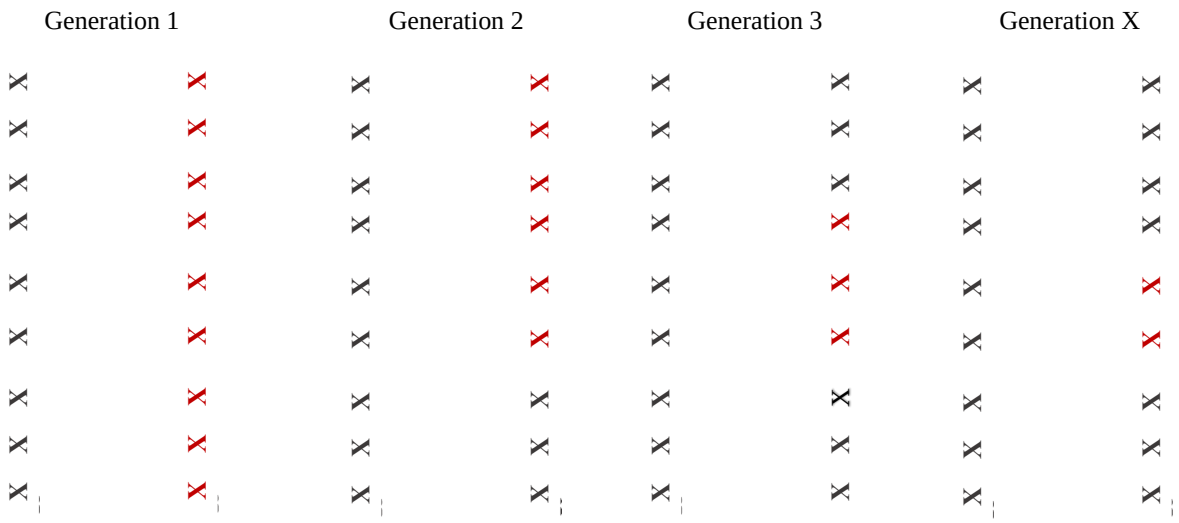


Figure S2. 5: Diagram of rainbow trout hybridization in an individual fish along a single pair of chromosomes. Using multiple generations to visualize the decrease in rainbow trout Runs of Admixture (ROA) length over time. The bars represent a chromosome in an individual, with the color depicting species ancestry (gray = westslope cutthroat trout and red = rainbow trout). The X marks are an example of diagnostic loci located across the entire chromosome, representative of the diagnostic loci on the RAD-Capture panel used in this study. The X marks are colored by genotype; westslope cutthroat trout alleles are colored gray, and rainbow trout alleles are colored red. Using genomic data, we can calculate the ROA length; for example, we have the known chromosome position of locus. We can then calculate the distance from the first red X mark to the last red X mark to measure an individual's ROA length. In Generation 1, the hybrid individual contains a complete set of rainbow trout (red) and westslope cutthroat trout (gray) chromosomes. Generation 2 is an example of when the individual from Generation 1 backcrosses with a non-hybridized westslope cutthroat trout, resulting in the portion of the chromosome from rainbow trout ancestry (ROA) decreasing over time. This backcrossing will result in the ROA maintaining a large block of genes within the chromosome. The continuation of Generation 2 and Generation 3 mating with a non-hybridized westslope cutthroat trout will decrease ROA length over time and lead to a small ROA length during succeeding generations (Generation X).

CHAPTER 3

OTOLITH MICROSTRUCTURE REVEALS DIFFERENCE IN AGE-0 HATCH DATE AND GROWTH RATES OF WESTSLOPE CUTTHROAT TROUT, RAINBOW TROUT, AND HYBRIDS IN WILD POPULATIONS

Abstract

Previous research has indicated strong selection against hybrids between Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi,* WCT) and Rainbow Trout (*Oncorhynchus mykiss,* RBT) occurs between the spawning adult and juvenile life stages. Due to the earlier spawning migration timing of RBT, potential selection pressures in their first few months across individuals with varying admixtures have been suggested to help explain the spread of hybridization. Yet, there is limited knowledge on the early life stages of *Oncorhynchus,* specifically hatch date and early growth. Otolith microstructure has not been published for WCT, so we first validated the occurrence of otolith microstructure to calculate a hatch date using hatchery origin fish. We then genotyped and aged 122 larval fish from six sites in western Montana's Rock Creek and Rattlesnake Creek watersheds to examine variation in hatch date and larval growth in relation to genetic ancestry. We calculated hatch date, and length at hatch using the Dahl-Lea method and estimated the growth rate from hatch to capture. Within sites, there was high variation in hatch date and individual growth rate that was not associated with genetic ancestry. Interestingly, in the two streams where WCT were present, WCT had significantly higher growth rates than hybrids. Growth rate differences were consistent with WCT having higher growth rates in the same stream environment, despite hatching later than hybrids. Larval WCT reached a similar length by mid-August compared to RBT and hybrids. To determine if

this resulted in a selective advantage, sampling older age-0 into the fall is needed to determine whether growth rate differences persist and result in higher overwinter survival of WCT.

Introduction

Natural hybridization can lead to novel genomic combinations and is beneficial when it leads to adaptive introgression and speciation (Grant and Grant 1994; Hedrick 2013). Anthropogenic hybridization resulting from human-mediated actions occurring on a rapid evolutionary time scale often leads to negative conservation outcomes (Rhymer and Simberloff 1996; Allendorf et al. 2001). Hybridization caused by the introduction of nonnative species can be detrimental to native species, as it can lead to the disruption of locally adapted gene complexes and localized genomic extinction (Rhymer and Simberloff 1996; Allendorf et al. 2001; Todesco et al. 2016). Anthropogenic hybridization appears to be increasing (Ottenburghs 2021) and presents a challenging conservation and management issue (Allendorf et al. 2001; Laikre et al. 2010).

The stocking of Rainbow Trout (*Oncorhynchus mykiss*; RBT) throughout the native range of Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*; WCT) has led to widespread hybridization (Allendorf and Leary 1988; Shepard et al. 2005). Spatiotemporal patterns of RBT hybridization are highly variable throughout the WCT native range (Muhlfeld et al. 2017). Much of this variation can be explained by propagule pressure from RBT and hybrids dispersing into WCT spawning areas (Boyer et al. 2008; Kovach et al. 2015; Muhlfeld et al. 2017). Additionally, evidence of fitness differences has been demonstrated in the lab (Leary et al. 1995; Yau and Taylor 2014; Drinan et al. 2015) and field settings (Muhlfeld et al. 2009a; Kovach et al. 2015, 2016). For example, RBT hybridization can significantly reduce reproductive success compared

to WCT (Muhlfeld et al. 2009a), and RBT alleles are selected against across environments in admixed populations (Kovach et al. 2016). These patterns could be driven by selection against RBT hybridization occurring in the early life stages (Kovach et al. 2015). Lab-based research has shown that RBT admixture negatively affects traits such as growth, survival, and swimming endurance (Leary et al. 1995; Drinan et al. 2015) at early life stages. Despite lab studies highlighting the potential effects of hybridization on age-0 fish growth, there are no field studies examining if these differences contribute to the patterns of selection seen on the landscape (Kovach et al. 2015).

The spawning migration phenology of RBT, hybrids, and WCT could provide a framework for different selective pressures across the landscape. Previous studies have shown that RBT and fish with high levels of admixture tend to migrate on the ascending limb of the hydrograph before WCT, who migrate on the descending limb, while hybrids migrate throughout (Muhlfeld et al. 2009b; Corsi et al. 2013; Figure 3.1). If this translates to earlier spawn timing and hatch dates, we might expect an extended window of age-0 growth for RBT and early spawning hybrids (Crisp 1990), where fish experience different extrinsic selective pressures associated with genetic ancestry. Recently, the validation and use of otolith microstructure (defined as daily rings) has revealed information on age-0 life stages, such as hatch date, age, and daily growth (Campana and Moksness 1991; Moyano et al. 2012). Most migration and spawn timing studies in our *Oncorhynchus* study system have been based on tracking migratory fish, however, there are no known studies using otolith microstructure to investigate the hatch date of RBT, hybrids, and WCT in the wild.

In addition to intrinsic selective forces (e.g., due to structural chromosomal differences), extrinsic forces can be key factors for growth and survival in early life stages. Several studies of

headwater salmonids have revealed a relationship at the age-0 life stage between fish growth and water temperature, where warmer temperatures cause earlier hatching, with subsequent longer age-0 growth periods leading to higher growth rates (Crisp 1990; Sloat et al. 2005; McGrath et al. 2008). In age-0 salmonids, overwinter survival has been documented to be size-dependent (Smith and Griffith 1994; Sogard 1997). Therefore, earlier summer hatch dates that lead to higher growth rates and larger body sizes in the fall could contribute to survival in this critical early life stage. Understanding the influence of genetic ancestry on age-0 growth could help explain the selective forces driving hybridization patterns across the landscape.

In this study, we first validated the use of otolith microstructure with hatchery origin WCT. Next, we examined the influence of RBT admixture on hatch date and growth in admixed WCT populations. We sampled age-0 individuals at six sites across two watersheds in western Montana and used otolith microstructure and fish length at capture to determine hatch date and growth rate. We addressed two questions 1) Do individuals with higher RBT admixture have an earlier hatch date? 2) Does RBT admixture influence the growth rate of age-0 individuals? Our findings provide insight into the consequences of RBT admixture on age-0 growth and potential selection occurring in the early life stage in wild populations furthering our understanding of the mechanisms behind the variation in spatiotemporal patterns of RBT hybridization with WCT.

Methods

Study Area

This study was conducted in two western Montana watersheds, Rock Creek and Rattlesnake Creek (Figure 3.2). Rock Creek and Rattlesnake Creek are 5th and 3rd order tributaries to the Clark Fork River, respectively. Confined valley channels and mixed land use

characterize the 1,425 km^2 Rock Creek Watershed and 210 km^2 Rattlesnake Creek Watershed. The two watersheds share many characteristics, such as historical whirling disease (*Myxobolus cerebralis*), nonnative salmonids, and partial migratory *Oncorhynchus.* Historically, both watersheds were WCT and Bull Trout (*Salvelinus confluentus*) fisheries, but the current salmonid assemblage includes nonnative Brown Trout (*Salmo trutta*), Brook Trout (*Salvelinus fontinalis*), and RBT. The historic stocking of RBT has led to the establishment of naturally reproducing populations of RBT in the lower main river sections of both watersheds and the widespread distribution of hybrid fish. This study consists of six sites, two located on the mainstem Rattlesnake Creek and four on different tributaries within lower Rock Creek (Figure 3.2). Previous information on hybridization for all sites indicated variation in genetic ancestry among individuals (Ryan Kovach, MFWP, unpublished data). All sites are accessible by migratory *Oncorhynchus* and have a resident component.

Field Sampling

Age-0 *Oncorhynchus* were sampled via backpack electrofishing in August 2019 and 2020. We sampled a minimum of 100 meters and continued as needed until 30 individuals were captured. We dispersed sampling in an attempt to avoid capturing individuals from the same family. Once captured, fish were measured (total length, mm), sacrificed, and preserved in individual vials with 95% ethanol for future otolith and genetic extraction. Temperature loggers (HOBO® Pendant MX2201, HOBO® Pendant UA-001-64) were deployed across all sites from pre-spawning (May) to the last sampling period (August) to record water temperature in 30-minute intervals. We calculated the mean daily temperature during the growth period, which was estimated based on the capture and estimated hatch date for every individual (see Otolith Age

and Growth). The two Rattlesnake Creek sample sites were the warmest throughout the growth period with similar temperatures (Table 3.1, Figure S3.1). Within the Rock Creek drainage, there was a range of cooler temperatures across sites. The coldest sample site was Alder Creek, and the warmest was Stony Creek (Table 3.1, Figure S3.1).

Genetic Analyses

Caudal fin clips of all individuals were genotyped using a RAD-Capture panel with species-specific diagnostic loci for WCT and RBT (Amish et al. 2012; Hohenlohe et al. 2013; Ali et al. 2016). Genetic samples were prepared, sequenced, and genotyped following the laboratory and bioinformatic methods described in Ali (2016) and Strait (2021). Initial filtering was carried out to remove thirteen potential non-*Oncorhynchus* individuals that had high locus missingness (> 40%) and whose genotypes at RBT diagnostic loci were concordant with patterns observed in control *Salvelinus (S. confluentus* and *S. fontinalis)* samples. After bioinformatic filtering, our dataset contained 823 RBT diagnostic loci, with a median of 467 loci per individual and a minimum of 257 RBT diagnostic loci. For every individual, we estimated the proportion of RBT admixture (pRBT) as the number of RBT alleles present divided by two times the number of RBT diagnostic loci successfully genotyped in each individual.

Otolith Microstructure Validation

To validate the occurrence of otolith microstructure in WCT, we used fish from Montana Fish, Wildlife & Parks Washoe Fish Hatchery in Anaconda, Montana. During the Spring and Summer of 2020, we had four sampling events where 30 fish were collected from 14 to 70 days post-swim-up. Otoliths were prepared and aged as described below (see *Otolith Preparation and*

Imaging & *Otolith Age and Growth).* We used hatchery fish with known ages and hatch dates to confirm the occurrence of a visually identifiable 'check' at hatch. Using a linear regression, we compared the observed number of daily rings (age) to the expected number of days since hatch across the four sampling events.

Otolith Preparation and Imaging

Sagittal otoliths were removed using a low-powered dissection microscope (30-40x magnification; model Leica S8APO) for all individuals genotyped. Otoliths were extracted from the ventral surface of the skull using both forceps and a dissection probe and stored in individually marked vials until mounting. Otoliths were rinsed and cleaned of debris before being mounted onto a glass microscope slide using Crystalbond 509® adhesive. Depending on otolith size, they were polished using 1500-2000 grit sandpaper on both the distal and proximal sides to improve readability. Once polished, otoliths were submerged in mineral oil and viewed under reflected light at 20 x 1.5-micron magnification. Each otolith was photographed using a Lumenera® camera mounted to a Micro-Optical Solutions® compound microscope. All images were cataloged and analyzed using Image-Pro 10® Insight software (MediaCybernatics, Rockville, MD, USA).

Otolith Age and Growth

Individuals were aged following protocols described by Stevenson and Campana (1992). *Oncorhynchus* otolith microstructure consists of multiple primordia (nucleus of otolith; Figure 3.3A) and a distinctive feature (what we refer to as a check) defining hatch as described by Moyano (2012; Figure 3.3B). Age was determined by counting increments from hatch to the

outermost edge of the otolith along the posterior axis of the sagittal plane, opposite of the rostrum (Figure 3.3; Campana and Neilson 1985; Mugiya and Oka 1991). All fish were aged at least twice, and we calculated the coefficient of variation (CV) to evaluate the precision of age estimates (Chang 1982; Campana 2001). The mean CV for this study was 7.2 %. Otoliths with a CV > 10 % were reexamined by both agers and removed from the dataset if a consensus age could not be achieved.

Length at hatch and daily growth were calculated for every individual. We confirmed a strong linear relationship (R^2 = 0.90, Figure S3.2) between total otolith size and fish total length before calculating length at hatch (Campana 1990). Additionally, Moyano (2012) demonstrated that the otolith-length/fish-length relationship was linear, and otolith growth was proportional to fish growth for *Oncorhynchus mykiss.* We calculated length at hatch using the Dahl-Lea method originally defined by Lea (1910) for scales:

$$\textbf{Eq 1. } L_i = \frac{R_i}{R_C} L_C$$

Where L_i is the fish length at the time of formation of the i^{th} primary increment, for example, the hatch check. R_i is the radius of the otolith from the outermost primordia to the i^{th} increment (e.g., hatch check). R_C is the radius of the otolith at capture measured from the outermost primordia to the outer edge of the otolith. L_C is the total fish length at capture. All measurements were to the nearest micron using calibrated Image Pro software. As described in Lugert (2016), we calculated the absolute growth rate (*G;* mm/d) as:

$$\textbf{Eq. 2 } G = \frac{L_C - L_H}{A}$$

L_C is the individual length at capture, and L_H is the individual length at hatch estimated from the Dahl-Lea method. *A* is the age (in days), measured by the number of increments counted from the hatch check to the outermost edge of the otolith (capture).

Hatch Date and Growth Analyses

For each watershed, we conducted an analysis of covariance (ANCOVA) to test if hatch date and growth were described by the interaction of pRBT and sample site. If the interaction of pRBT and sample site was significant at the watershed level, we conducted a separate linear regression for each sample site to test the relationship between pRBT and hatch date and pRBT and growth. Our analysis was carried out at the site level to control for among site variation and differences within site. Additionally, a one-way analysis of variance (ANOVA) was used to test if fish total length at capture differed across sample sites in the Rock Creek Watershed.

Results

We reliability detected the hatch check in the WCT otolith microstructure within hatchery fish. The hatch check was often characterized by 1 to 2 dark zones followed by a wider light zone (Figure 3.3B). Within the hatchery fish, we saw a strong positive relationship between the number of increments and the number of days since hatch (linear regression, $p < 0.05$, $R^2 = 0.983$; Figure S3.3).

Within the Rattlesnake Creek and Rock Creek sample sites, we only observed a significant effect of RBT admixture (pRBT) on hatch date at three Rock Creek sites. The relationship between pRBT and hatch date was not dependent on the sample site within

Rattlesnake Creek, which consisted of only hybrid individuals (ANCOVA, F = 3.323, df = 1, 54, p =0.074; Table 3.2A, Figure 3.4A). Within Rock Creek, the relationship between pRBT and hatch date was dependent on sample site (ANCOVA, F = 10.560, df = 3, 56, p < 0.001; Table 3.2B, Figure 3.4B). Hatch date was significantly earlier with pRBT in both Brewster Creek (linear regression, $p < 0.001$, $R^2 = 0.887$; Figure 3.4B) and Stony Creek (linear regression, $p < 0.001$, $R^2 = 0.656$; Figure 3.4B). RBT had earlier hatch dates, while WCT hatched later. The opposite relationship appeared in Alder Creek, where RBT individuals hatched later than hybrids (linear regression, $p = 0.032$, $R^2 = 0.163$; Figure 3.4B). There was no relationship between individuals within Gilbert Creek and hatch date or pRBT.

Across all sites in the two watersheds, we only observed a significant effect of RBT admixture (pRBT) on larval growth at two sample sites in Rock Creek. These were the only sample sites in this study where WCT were captured (Table 3.1) and the same sites with a significant relationship between pRBT and hatch date (Brewster and Stony Creeks). The relationship between pRBT and age-0 growth was not significantly dependent on sample site in the Rattlesnake Creek, where there were only hybrid individuals (ANCOVA, $F = 0.031$, df = 1, 54, $p = 0.861$; Table 3.3A, Figure 3.5A). In Rock Creek, the relationship between pRBT and age-0 growth significantly depended on sample site (ANCOVA, $F = 3.097$, df =3, 56, $p = 0.034$, Table 3.3B, Figure 3.5B). Growth rate decreased significantly with pRBT in both Brewster Creek (linear regression, $p = 0.008$, $R^2 = 0.719$; Figure 3.5B) and Stony Creek (linear regression, $p = 0.005$, $R^2 = 0.383$; Figure 3.5B). In Brewster Creek, for every 0.10 increase in pRBT, growth declined by 0.017 mm/day (-0.025, -0.008; 95% CI). For every 0.10 increase in pRBT at Stony Creek, growth declined by 0.036 mm/day (-0.058, -0.014; 95% CI). Gilbert Creek and Alder

Creek individuals only included hybrid and RBT individuals, and we did not see a significant effect of pRBT on growth.

Discussion

Our study validated the occurrence of reliable hatch checks within otolith microstructure for WCT and examined the effects of RBT hybridization on age-0 hatching phenology and growth. Where we captured WCT in our sample at Brewster and Stony Creeks, RBT admixture had a significant negative effect on age-0 growth in WCT. WCT had later hatch dates and higher growth rates than RBT and hybrids. The hybrid individuals that hatched earlier had a lower growth rate than WCT that hatched later. Despite later hatch dates, WCT were similar in length at capture to hybrids in early August. These results suggest that selection for a faster growth rate associated with later hatching could have led to a pattern of countergradient variation in growth rates at the age-0 life stage in WCT.

We did not see a distinct hatch phenology that mirrored the generalized migration phenology of RBT, WCT, and hybrids either within or among sample sites. The expectations from the generalized migration phenology would result in a gradient of environmental conditions (and selective pressures) based on hatch times. We primarily examined the contribution of genetic ancestry within a sampling area as these fish have access to broadly similar environmental conditions, such as growing degree days. Within systems dominated by hybrids (no WCT or RBT), there were no consistent or significant trends in hatch dates. This might be expected based on the expected wide range of spawning migration timing of hybrids (Muhlfeld et al. 2009b; Corsi et al. 2013). In creeks with RBT individuals present, RBT were both earlier or later hatching fish in the sample. For example, in Alder Creek, RBT and highly hybridized

individuals had the latest hatch dates in our study, although WCT were absent from this site (Figure S3.4). Our results highlight the potential for high variation among and within tributaries in hatch dates for highly admixed individuals and RBT. A potential source of the high variation in hatch dates in this study could be the presence of resident and migratory fish throughout our sampling sites, as spawning phenology of resident life history in inland *Oncorhynchus* is not well described. In creeks with WCT, we did see WCT hatch later as expected based on expected spawning phenology. Interestingly these individuals reached a similar size at capture as hybrids in the same sampling area.

Countergradient variation occurs when individuals are locally adapted to have a high growth rate to counteract variation caused by environmental conditions (i.e., cold stream temperature) and has been documented across several fish species (Conover 1990; Conover and Schultz 1995; Chavarie et al. 2010). Fish that hatched later in our study showed evidence for countergradient variation in growth, indicated by their higher growth rate in a similar environment despite a shorter growth period for hybrid age-0 fish (Figure S3.5, S3.6, S3.7). This relationship occurred in Stony and Brewster Creeks where there was later hatching WCT (Figure S3.5). Although, there was a similar nonsignificant trend of faster growth in late hatching hybrid fish at Alder and Gilbert Creeks, where WCT were absent (Figure S3.6). Variation in growth rate across all sites was high and our within site sample sizes were not large. Yet the sites with WCT present in a population demonstrated a different and significant pattern in age-0 growth across genetic ancestry, suggesting the potential for different selective mechanisms to act. WCT are adapted for growth at a lower critical thermal minimum than RBT and hybrids (Yau and Taylor 2014). By early August, WCT were similar in length to RBT and hybrids despite later hatch dates (Figure 3.6). The high growth rate of WCT should benefit them throughout the rest of their

age-0 growing period, potentially providing an advantage for overwinter survival compared to slower-growing RBT and hybrids. Research has shown that size is a major driver of overwinter survival for age-0 salmonids (Smith and Griffith 1994; Sogard 1997; Meyer and Griffith 1997). In Brook Trout (*Salvelinus fontinalis*), there were no indications of size-dependent survival rates, but size differences established in a fish's early life stages persisted through the individual's life (Letcher et al. 2011). Similarly, Hawkins and Foote (1998) found that RBT hatch earlier but develop slower than Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*), likely limiting any RBT size advantage over coastal cutthroat. Our results provide further evidence from wild populations that RBT admixture has a negative effect on growth during the early life stage.

Even though we found evidence that RBT admixture negatively affected growth at the age-0 life stage, future research could improve upon our findings. First, we recommend increased sampling of wild populations to capture more individuals equally distributed across the range of admixture. Second, we suggest sampling wild populations across a gradient of stream temperatures to further our understanding of extrinsic sources of selection. Third, future sampling efforts should focus on older age-0 individuals to test for size differences closer to winter. Given that our sampling occurred in August, we could only investigate a short growth window in the early life stage. However, we caution the extension of sampling efforts for older age-0 fish as there could be complications in otolith readability for daily growth. Lastly, future research should further evaluate the influence of proximity to RBT sources when sampling wild populations to evaluate the impact of propagule pressure and dispersal, which will likely overwhelm selection (Kovach et al. 2015; Muhlfeld et al. 2017).

Conclusion

Our findings support previous research showing signals of selection favoring WCT at the early life stage (Kovach et al. 2015). Similar research has shown RBT admixture to have a negative effect on fitness traits at various larval stages (Leary et al. 1995; Drinan et al. 2015). The early life stage is an important driver of population dynamics for salmonids (Sogard 1997; Grant and Imre 2005). Our research indicates that WCT and their higher growth rates at cold stream temperatures will likely give them an advantage for overwinter survival, potentially influencing the distribution of hybridization in adult populations. We provide the first validation and use of otolith microstructure to show variation in hatch dates across RBT, hybrids, and WCT. We suggest future research at the age-0 life stage across wild populations with varying stream temperatures to further investigate this aspect of natural selection.

References

Ali, O. A., S. M. O'Rourke, S. J. Amish, M. H. Meek, G. Luikart, C. Jeffres, and M. R. Miller. 2016. RAD Capture (Rapture): Flexible and efficient sequence-based genotyping. Genetics 202(2):389–400.

Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2(2):170–184.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16(11):613–622.

Amish, S. J., P. A. Hohenlohe, S. Painter, R. F. Leary, C. Muhlfeld, F. W. Allendorf, and G. Luikart. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. Molecular Ecology Resources 12(4):653–660.

Boyer, M. C., C. C. Muhlfeld, and F. W. Allendorf. 2008. Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Canadian Journal of Fisheries and Aquatic Sciences 65(4):658–669.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47(11):2219–2227.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59(2):197–242.

Campana, S. E., and E. Moksness. 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES Journal of Marine Science 48(3):303–316.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42(5):1014–1032.

Chang, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences 39(8):1208–1210.

Chavarie, L., J. B. Dempson, C. J. Schwarz, J. D. Reist, G. Power, and M. Power. 2010. Latitudinal variation in growth among Arctic charr in eastern North America: evidence for countergradient variation? Hydrobiologia 650(1):161–177.

Conover, D. O. 1990. The relation between capacity for growth and length of growing season: Evidence for and implications of countergradient variation. Transactions of the American Fisheries Society 119(3):416–430.

Conover, D. O., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology & Evolution 10(6):248–252.

Corsi, M. P., L. A. Eby, and C. A. Barfoot. 2013. Hybridization with rainbow trout alters life history traits of native westslope cutthroat trout. Canadian Journal of Fisheries and Aquatic Sciences 70(6):895–904.

Crisp, D. T. 1990. Water temperature in a stream gravel bed and implications for salmonid incubation. Freshwater Biology 23(3):601–612.

Drinan, D. P., M. A. H. Webb, K. A. Naish, S. T. Kalinowski, M. C. Boyer, A. C. Steed, B. B. Shepard, and C. C. Muhlfeld. 2015. Effects of hybridization between nonnative rainbow trout and native westslope cutthroat trout on fitness-related traits. Transactions of the American Fisheries Society 144(6):1275–1291.

Grant, J. W. A., and I. Imre. 2005. Patterns of density-dependent growth in juvenile stream-dwelling salmonids. Journal of Fish Biology 67:100–110.

Grant, P. R., and B. R. Grant. 1994. Phenotypic and genetic effects of hybridization in Dawrwin's' finches. Evolution 48(2):297–316.
Hawkins, D. K., and C. J. Foote. 1998. Early survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead (*Oncorhynchus mykiss*), and reciprocal hybrids:8.
Hedrick, P. W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology 22(18):4606–4618.
Hohenlohe, P. A., M. D. Day, S. J. Amish, M. R. Miller, N. Kamps-Hughes, M. C. Boyer, C. C. Muhlfeld, F. W. Allendorf, E. A. Johnson, and G. Luikart. 2013. Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. Molecular Ecology 22(11):3002–3013.
Kovach, R. P., B. K. Hand, P. A. Hohenlohe, T. F. Cosart, M. C. Boyer, H. H. Neville, C. C. Muhlfeld, S. J. Amish, K. Carim, S. R. Narum, W. H. Lowe, F. W. Allendorf, and G. Luikart. 2016. Vive la résistance: genome-wide selection against introduced alleles in invasive hybrid zones. Proceedings of the Royal Society B: Biological Sciences 283(1843):20161380.
Kovach, R. P., C. C. Muhlfeld, M. C. Boyer, W. H. Lowe, F. W. Allendorf, and G. Luikart. 2015. Dispersal and selection mediate hybridization between a native and invasive species. Proceedings of the Royal Society B: Biological Sciences 282(1799):20142454.
Laikre, L., M. K. Schwartz, R. S. Waples, and N. Ryman. 2010. Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. Trends in Ecology & Evolution 25(9):520–529.
Lea, E. 1910. Contributions to the methodics in herring-investigations. ICES Journal of Marine Science s1(53):7–33.
Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native Fish. American Fisheries Society Symposium 15:91–101.
Letcher, B. H., J. A. Coombs, and K. H. Nislow. 2011. Maintenance of phenotypic variation: repeatability, heritability and size-dependent processes in a wild brook trout population: Maintenance of phenotypic variation. Evolutionary Applications 4(4):602–615.
Lugert, V., G. Thaller, J. Tetens, C. Schulz, and J. Krieter. 2016. A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in Aquaculture 8(1):30–42.
McGrath, K. E., J. M. Scott, and B. E. Rieman. 2008. Length variation in age-0 westslope cutthroat trout at multiple spatial scales. North American Journal of Fisheries Management 28(5):1529–1540.
Meyer, K. A., and J. S. Griffith. 1997. First-winter survival of rainbow trout and brook trout in the Henrys Fork of the Snake River, Idaho. Canadian Journal of Zoology 75(1):59–63.
Moyano, G., G. Plaza, and M. I. Toledo. 2012. Otolith micro structure analysis of rainbow trout alevins (*Oncorhynchus mykiss*) under rearing conditions. Latin American Journal of Aquatic Research 40(3):722–729.
Mugiya, Y., and H. Oka. 1991. Biochemical relationship between otolith and somatic growth in the rainbow-trout (*oncorhynchus-mykiss*) consequence of starvation, resumed feeding, and diel variations. Fishery Bulletin 89.2:239–245.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009a. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters 5(3):328–331.

Muhlfeld, C. C., R. P. Kovach, R. Al-Chokhachy, S. J. Amish, J. L. Kershner, R. F. Leary, W. H. Lowe, G. Luikart, P. Matson, D. A. Schmetterling, B. B. Shepard, P. A. H. Westley, D. Whited, A. Whiteley, and F. W. Allendorf. 2017. Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout. Global Change Biology 23(11):4663–4674.

Muhlfeld, C. C., T. E. McMahon, D. Belcer, and J. L. Kershner. 2009b. Spatial and temporal spawning dynamics of native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, introduced rainbow trout, *Oncorhynchus mykiss*, and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 66(7):1153–1168.

Ottenburghs, J. 2021. The genic view of hybridization in the Anthropocene. Evolutionary Applications 14(10):2342–2360.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27(1):83–109.

Shepard, B. B., B. E. May, and W. Urie. 2005. Status and conservation of westslope cutthroat trout within the Western United States. North American Journal of Fisheries Management 25(4):1426–1440.

Sloat, M. R., B. B. Shepard, R. G. White, and S. Carson. 2005. Influence of stream temperature on the spatial distribution of westslope cutthroat trout growth potential within the Madison River Basin, Montana. North American Journal of Fisheries Management 25(1):225–237.

Smith, R. W., and J. S. Griffith. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. Transactions of the American Fisheries Society 123(5):747–756.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of marine science 60.3:1129–1157.

Stevenson, D. K., and S. E. Campana. 1992. Otolith microstructure examination and analysis.

Strait, J. T., L. A. Eby, R. P. Kovach, C. C. Muhlfeld, M. C. Boyer, S. J. Amish, S. Smith, W. H. Lowe, and G. Luikart. 2021. Hybridization alters growth and migratory life-history expression of native trout. Evolutionary Applications 14(3):821–833.

Todesco, M., M. A. Pascual, G. L. Owens, K. L. Ostevik, B. T. Moyers, S. Hübner, S. M. Heredia, M. A. Hahn, C. Caseys, D. G. Bock, and L. H. Rieseberg. 2016. Hybridization and extinction. Evolutionary Applications 9(7):892–908.

Yau, M. M., and E. B. Taylor. 2014. Cold tolerance performance of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*) and its potential role in influencing interspecific hybridization. Canadian Journal of Zoology 92(9):777–784.

Tables

Table 3. 1: Table of site summary metrics of the six sample sites across Rattlesnake Creek and Rock Creek sampled in August 2019 and 2020 for age-0 Westslope Cutthroat Trout (WCT), Rainbow Trout (RBT), and hybrids. Site hybridization was measured by the mean of the proportion of RBT admixture (pRBT) for all individuals. Mean hatch date for the individuals captured at each site was estimated from age-0 otolith microstructure. Mean daily July temperature is reported for the same year as the sample from each site.

Watershed	**Sample Site**	**Sample Date**	**Sample Size**	**Mean pRBT (min, max)**	**Mean Total Length (mm)**	**Mean Hatch Date**	**Mean Growth (mm/day)**	**Mean July Temperature (ºC)**
Rattlesnake Creek	Site 1	8/23/19	35	0.508 (0.141, 0.827)	44.40	6/30/19	0.65	10.93
	Site 2	8/23/19	23	0.449 (0.225, 0.997)	37.13	7/10/19	0.63	10.86
Rock Creek	Alder Creek	8/12/20	23	0.766 (0.377, 1)	27.73	7/12/20	0.51	7.99
	Brewster Creek	8/4/20	8	0.483 (0, 0.856)	26.50	7/2/20	0.49	8.27
	Gilbert Creek	8/3/20	14	0.868 (0.492, 1)	27.64	6/25/20	0.47	9.45
	Stony Creek	8/4/20	19	0.081 (0, 0.542)	26.32	7/10/20	0.69	9.82

Table 3. 2**:** Results of ANCOVA for age-0 hatch date in Rattlesnake Creek (A) and Rock Creek (B). An asterisk indicates significance ($p < 0.05$). The model equation was hatch date = pRBT + Sample Site + pRBT:Sample Site.

(A) Rattlesnake Creek

Effect Test: hatch date	**df**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr (>F)**
pRBT	1	160.7	160.7	3.050	0.086
Sample Site	1	1377.1	1377.1	26.129	< 0.001 *****
pRBT:Sample Site	1	175.1	175.1	3.323	0.073
Residuals	54	2846.0	52.7		

(B) Rock Creek

Effect Test: hatch date	**df**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr (>F)**
pRBT	1	467.4	467.4	20.80	< 0.001 *****
Sample Site	3	2417.0	805.7	35.86	< 0.001 *****
pRBT:Sample Site	3	711.6	237.2	10.56	< 0.001 *****
Residuals	56	1258.1	22.5		

Table 3. 3: Results of ANCOVA test for age-0 growth in Rattlesnake Creek (A) and Rock Creek (B). An asterisk indicates significance ($p < 0.05$). The model equation was growth = pRBT + Sample Site + pRBT:Sample Site.

(A) Rattlesnake Creek

Effect Test: growth	**df**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr (>F)**
pRBT	1	0.0078	0.0078	1.288	0.261
Sample Site	1	0.0032	0.0032	0.530	0.470
pRBT:Sample Site	1	0.0002	0.0002	0.031	0.861
Residuals	54	0.3274	0.0061		

(B) Rock Creek

Effect Test: growth	**df**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr (>F)**
pRBT	1	0.5047	0.5047	131.838	< 0.001 *****
Sample Site	3	0.1015	0.0338	8.835	< 0.001 *****
pRBT:Sample Site	3	0.0356	0.0119	3.097	0.034 *****
Residuals	56	0.2144	0.0038		

Figures

Figure 3. 1: A simplified distribution of migration timing for Rainbow Trout (gray), Westslope Cutthroat Trout (dark gray), and hybrids (light gray) commonly documentated in Montana. Dashed line represents hydrograph during spring snowmelt runoff. As shown by Muhlfeld et al. (2009) and Corsi et al. (2013).

Figure 3. 2: Map of the Rattlesnake Creek and lower Rock Creek study areas in western Montana, USA. Points are labeled sampling locations where backpack electrofishing was used to collect age-0 Oncorynchus. Mainstem Rock Creek and Rattlesnake Creek are represented by solid lines, Rock Creek sampling tributaries by dashed lines. Both watersheds are a tributary to the Clark Fork river labeled on the map. The insert shows Montana and the two study watersheds in black.

Figure 3. 3: Otolith microstructure of saggittae otolith from wild age-0 Oncorhynchus collected in Rock Creek Watershed, Montana, 2020. All aging and otolith length measurements were collected from the outermost primordium (A). Fish were aged from double banded hatch check (B) to the post-rostrum outer edge (C). Otolith was imaged at 20 x 1.5-micron magnification using a Lumenera® camera and Image-Pro 10® Insight software (MediaCybernatics, Rockville, MD, USA).

Figure 3. 4: The effect of individual-level proportion Rainbow Trout admixture (pRBT) on hatch date (Julian Day) within (A) Rattlesnake Creek and (B) Rock Creek. Individuals with sample sites are differentiated by shape. pRBT values range from 0.00 (Westslope Cutthroat Trout) to 1.00 (Rainbow Trout). Linear relationships are depicted for each sample site within a watershed. Significant linear relationships ($p < 0.05$) are shown in black, non-significant in gray. The influence of pRBT on growth depended on sample site ($p < 0.001$) in Rock Creek (B). Hatch date was earliest for individuals with increased pRBT at both Brewster Creek ($p < 0.001$, $R^2 = 0.887$) and Stony Creek ($p < 0.001$, $R^2 = 0.656$). Hatch Date was latest for individuals with increased pRBT at Alder Creek ($p = 0.032$, $R^2 = 0.163$).

Figure 3. 5: The effect of individual-level proportion Rainbow Trout admixture (pRBT) on growth rate (millimeter/day) within (A) Rattlesnake Creek and (B) Rock Creek. Individuals within sample sites are differentiated by shape. pRBT values range from 0.00 (Westslope Cutthroat Trout) to 1.00 (Rainbow Trout). Linear relationships are depicted for each sample site within a watershed. Significant linear relationships ($p < 0.05$) are shown in black, non-significant in gray. The influence of pRBT on growth depended on sample site ($p = 0.034$) in Rock Creek (B). Growth decreased significantly with pRBT at both Brewster Creek ($p = 0.008$, $R^2 = 0.673$) and Stony Creek ($p = 0.005$, $R^2 = 0.347$).

Figure 3. 6 Boxplots of age-0 total fish length at capture measured in millimeters across four sample sites in Rock Creek, Montana, 2019-2020. Each dot represents an individual fish with color corresponding to an individual-level proportion of Rainbow Trout admixture (pRBT). White dots are Westslope Cutthroat Trout and color increases with pRBT, with red depicting Rainbow Trout.

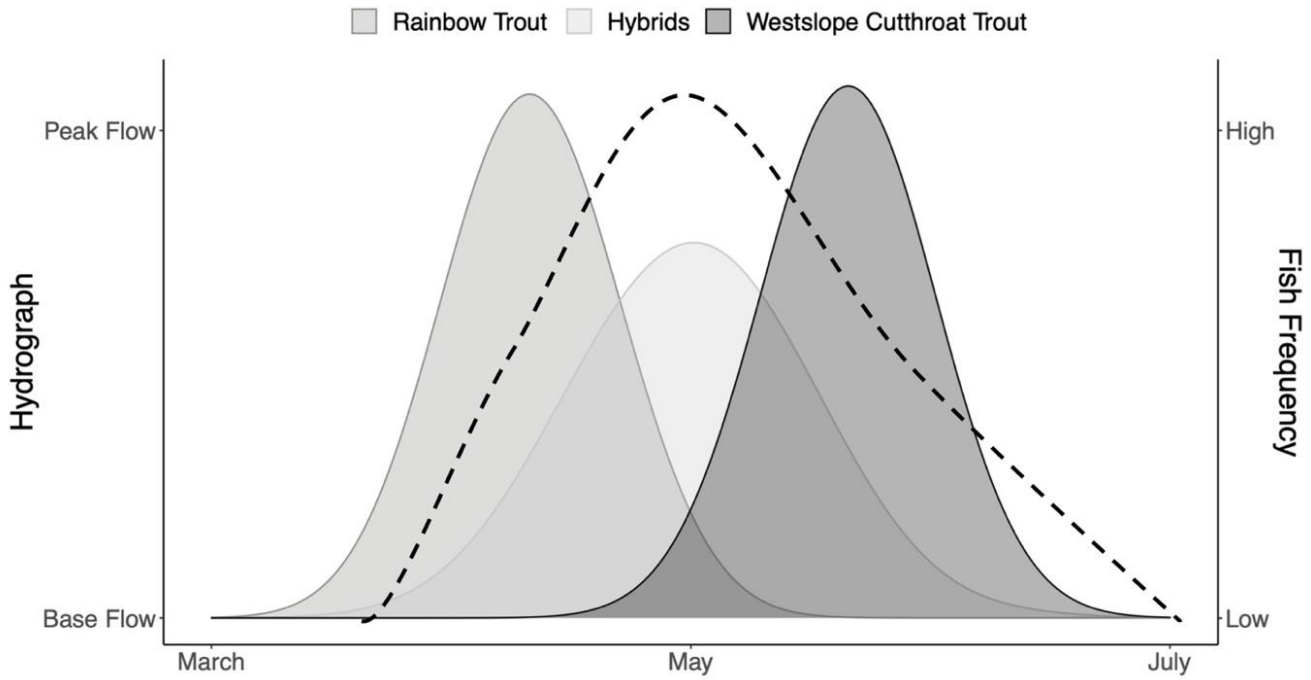
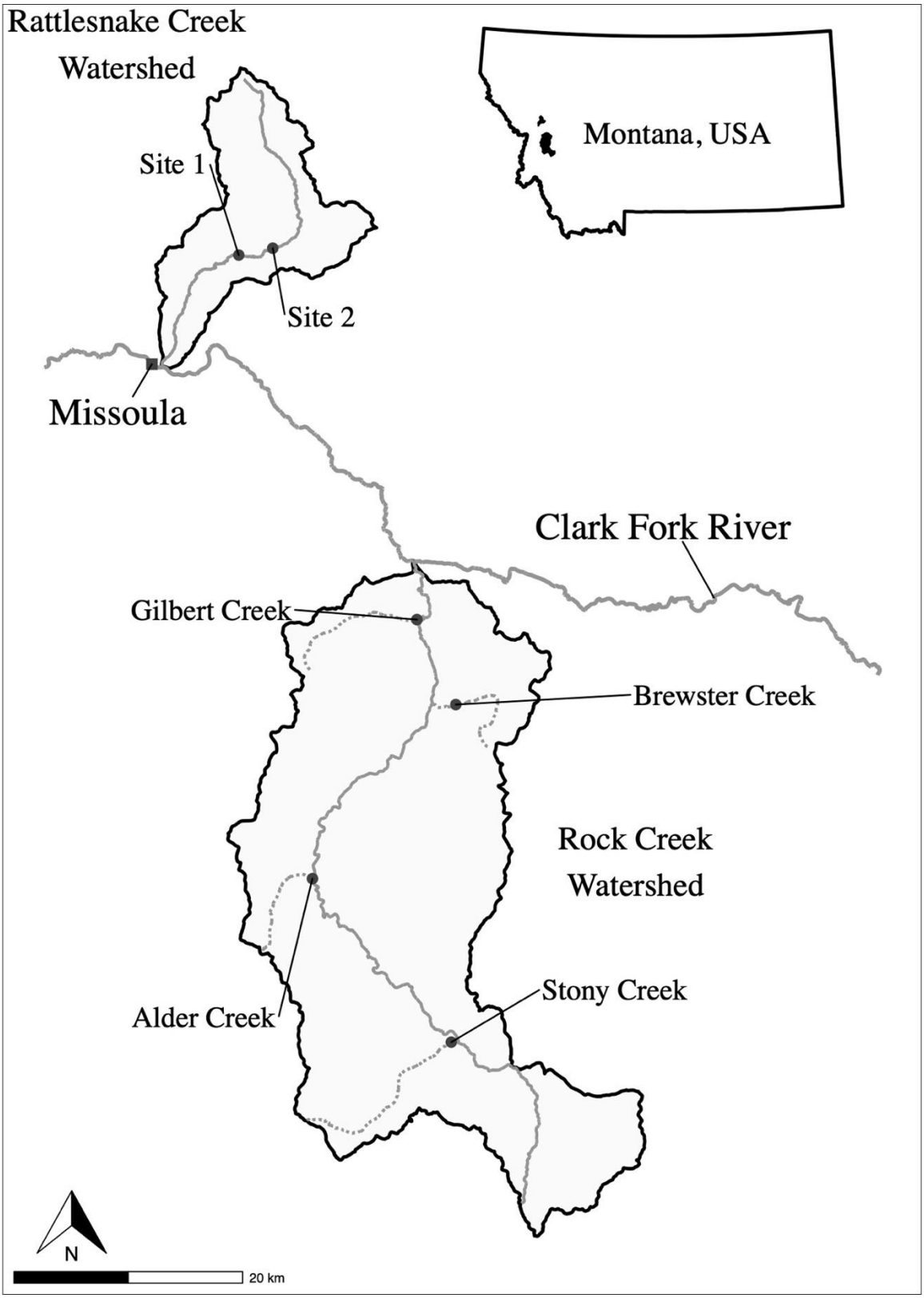

Rainbow Trout
Hybrids
Westslope Cutthroat Trout
Peak Flow
Base Flow
Hydrograph
High
Low
Fish Frequency
March
May
July

Figure 3.1


Rattlesnake Creek
Watershed
Site 1
Site 2
Missoula
Montana, USA
Clark Fork River
Gilbert Creek
Brewster Creek
Rock Creek
Watershed
Alder Creek
Stony Creek
N
20 km
Figure 3.2

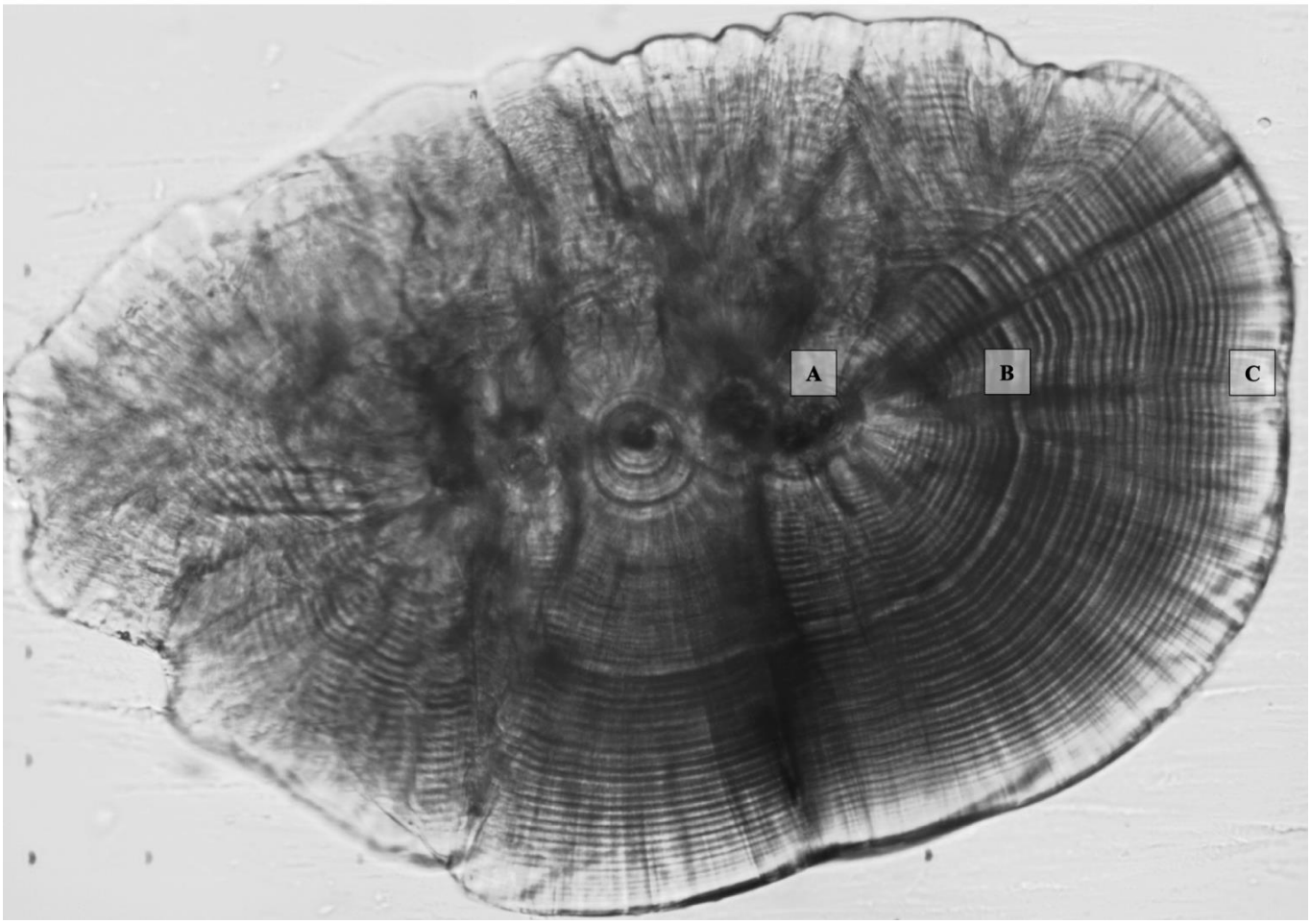


Figure 3.3

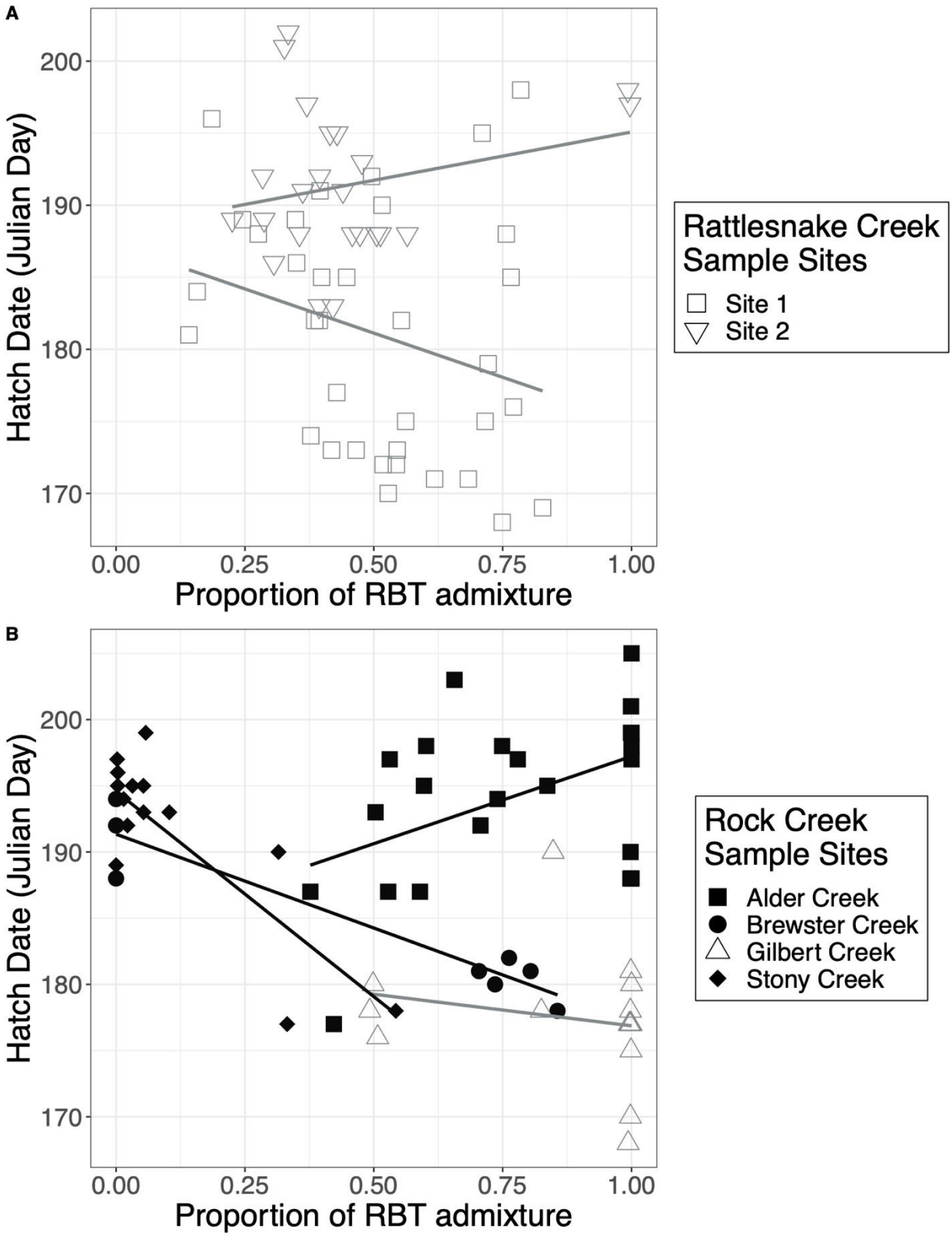

A
Hatch Date (Julian Day)
200
190
180
170
0.00
0.25
0.50
0.75
1.00
Proportion of RBT admixture
Rattlesnake Creek
Sample Sites
Site 1
Site 2
B
Hatch Date (Julian Day)
200
190
180
170
0.00
0.25
0.50
0.75
1.00
Proportion of RBT admixture
Rock Creek
Sample Sites
Alder Creek
Brewster Creek
Gilbert Creek
Stony Creek

Figure 3.4

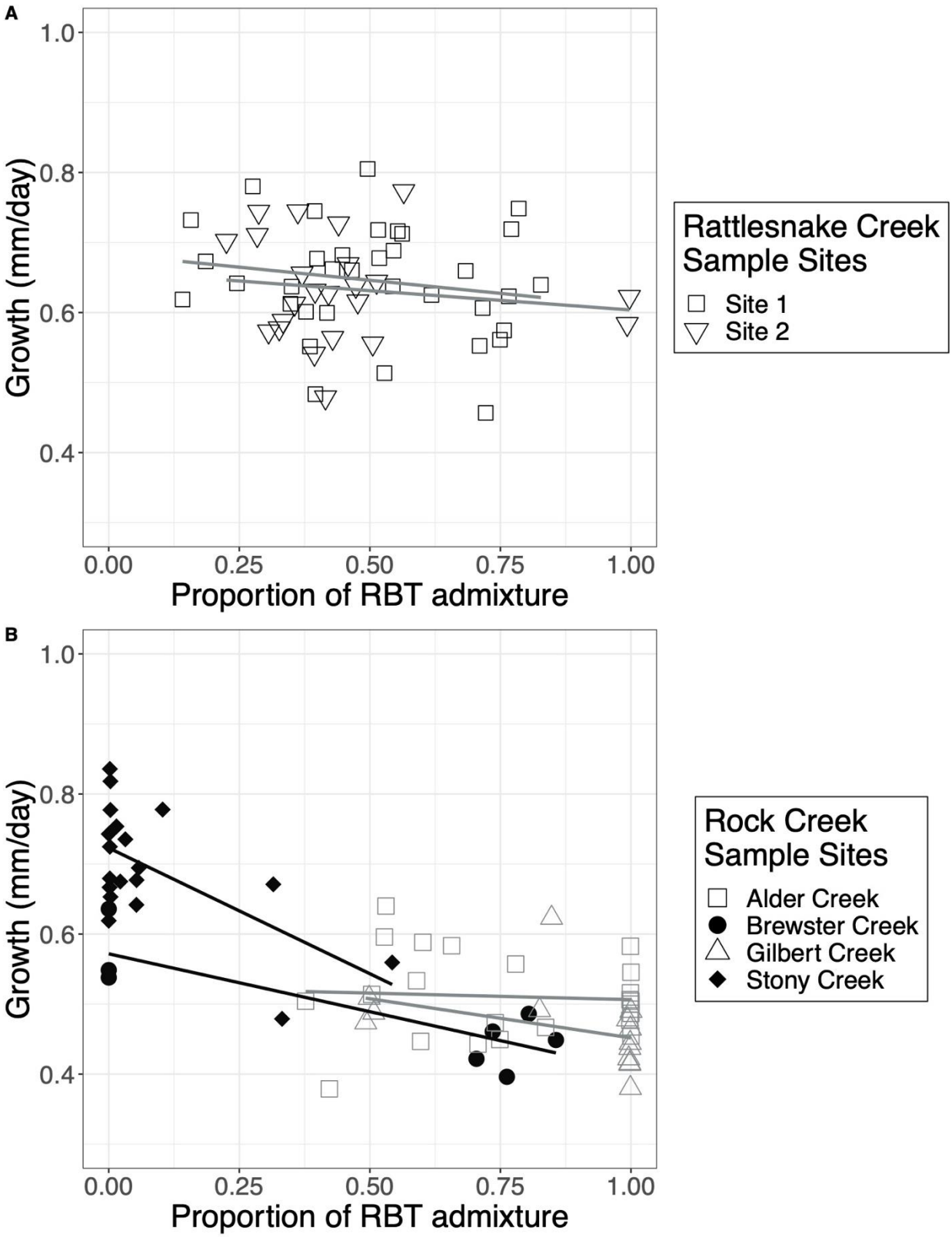

A
1.0
0.8
0.6
0.4
Growth (mm/day)
0.00
0.25
0.50
0.75
1.00
Proportion of RBT admixture
Rattlesnake Creek
Sample Sites
Site 1
Site 2
B
1.0
0.8
0.6
0.4
Growth (mm/day)
0.00
0.25
0.50
0.75
1.00
Proportion of RBT admixture
Rock Creek
Sample Sites
Alder Creek
Brewster Creek
Gilbert Creek
Stony Creek

Figure 3.5

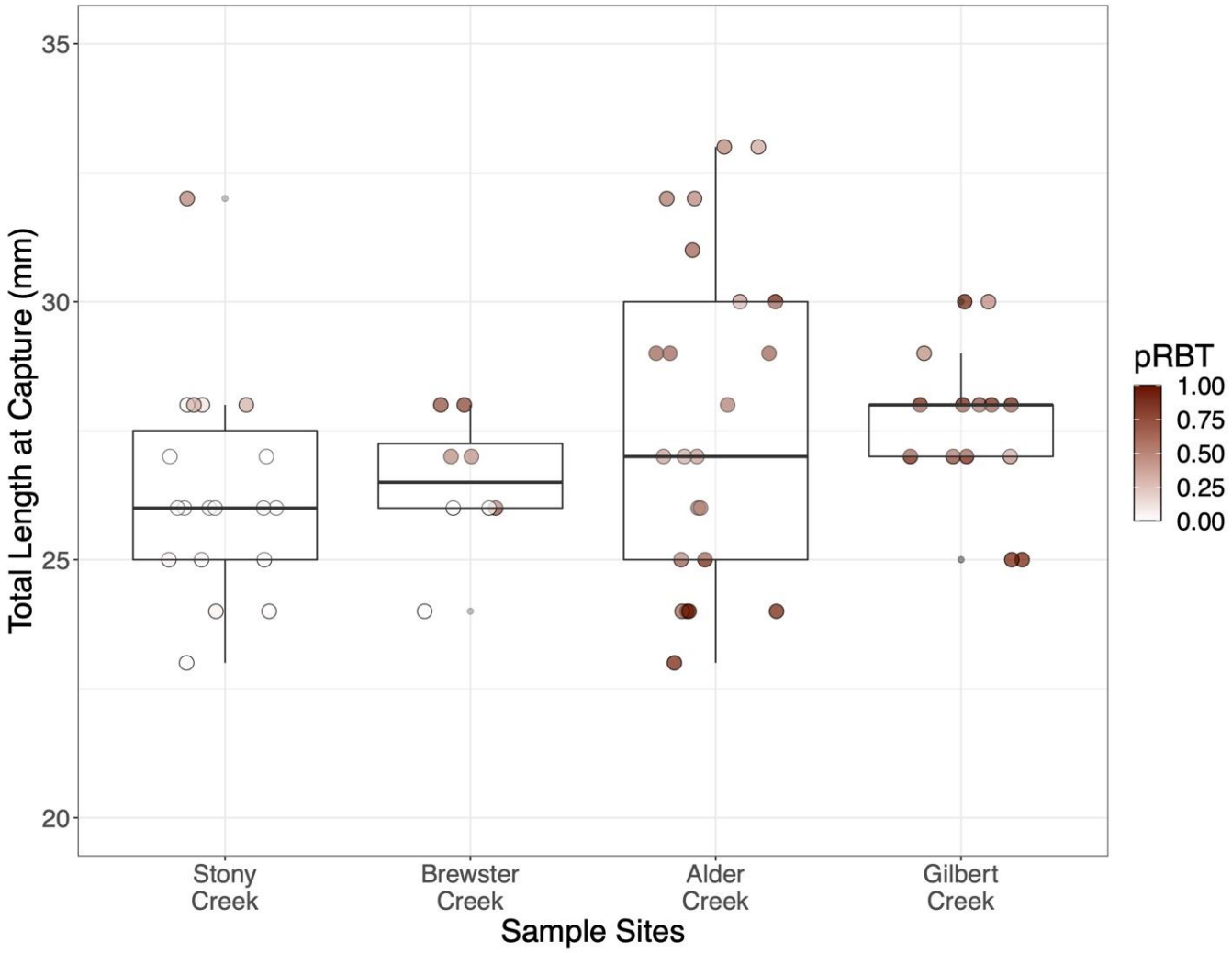

Total Length at Capture (mm)
35
30
25
20
Stony Creek
Brewster Creek
Alder Creek
Gilbert Creek
Sample Sites
pRBT
1.00
0.75
0.50
0.25
0.00

Figure 3.6

Supplemental

Appendix 3.1 – Supplemental Figures

Supplemental Figures

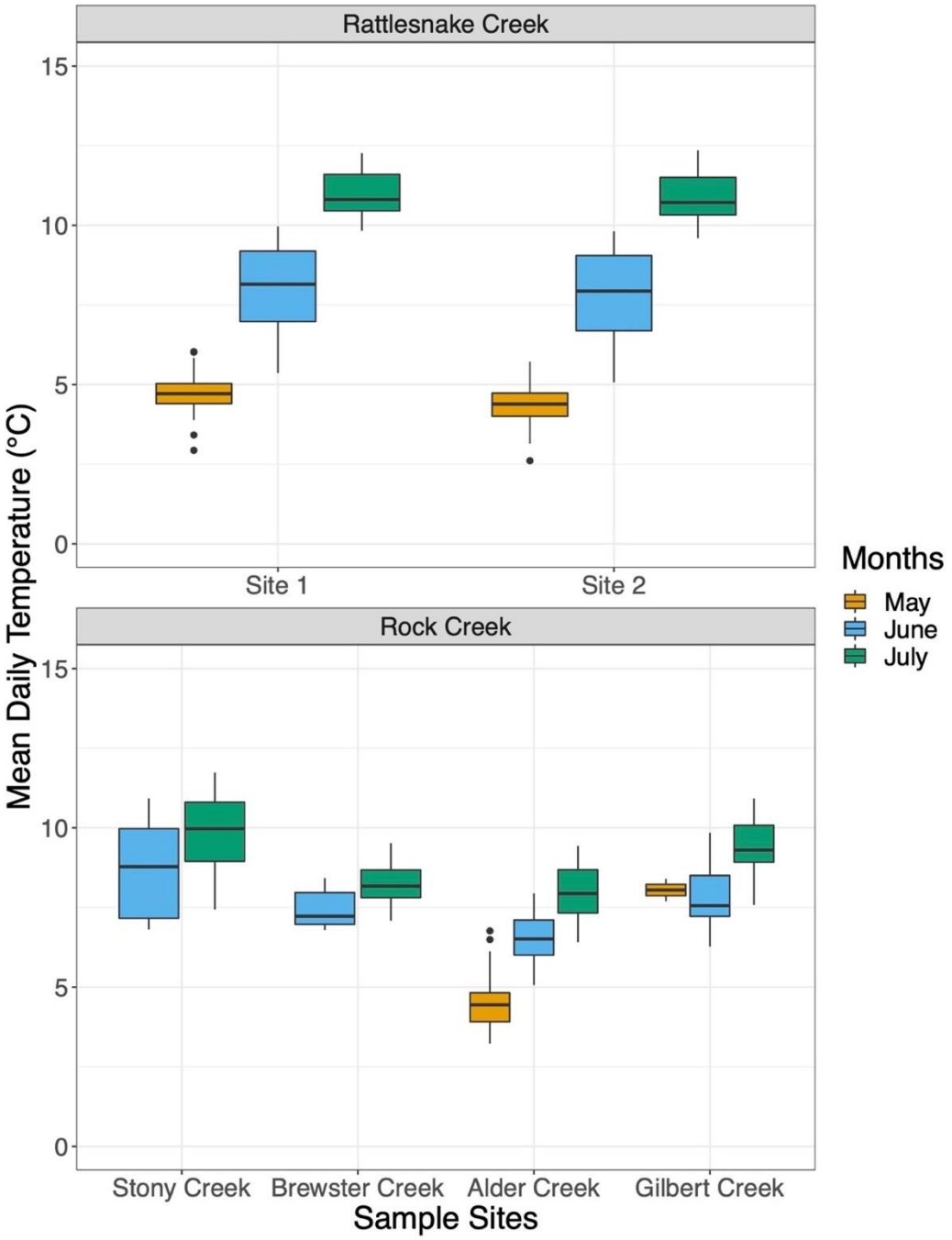


Figure S3. 1: Boxplots of mean daily temperature in Celsius at age-0 Oncorhynchus sample sites in Rock Creek and Rattlesnake Creek Watersheds. Months are differentiated by boxplot color.

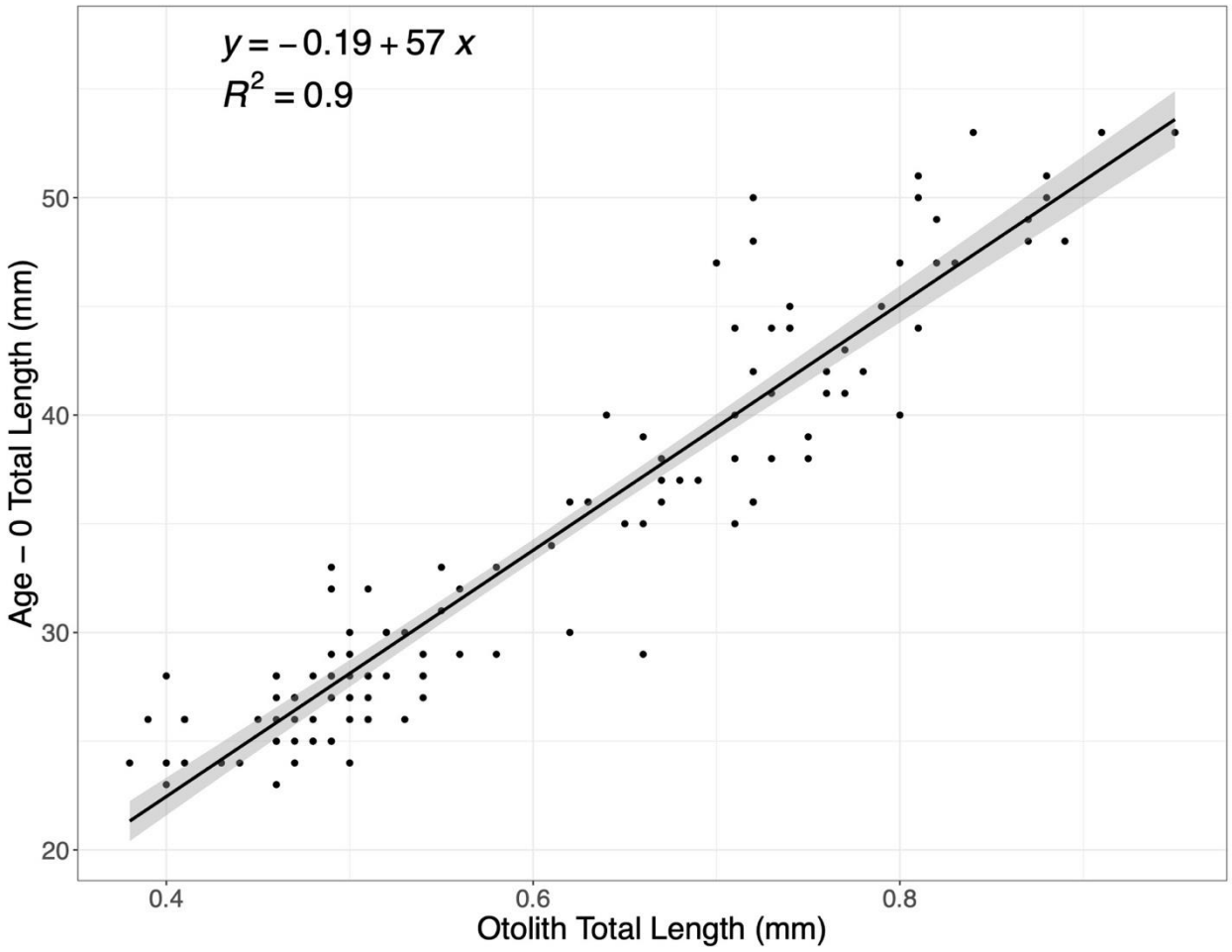


Figure S3. 2: Linear regression between age-0 Oncorhynchus fish total body length in millimeters and sagittae otolith total length in millimeters from Rock Creek and Rattlesnake Creek. Line is defined as y = -0.19 + 57 x, R^2 = 0.90.

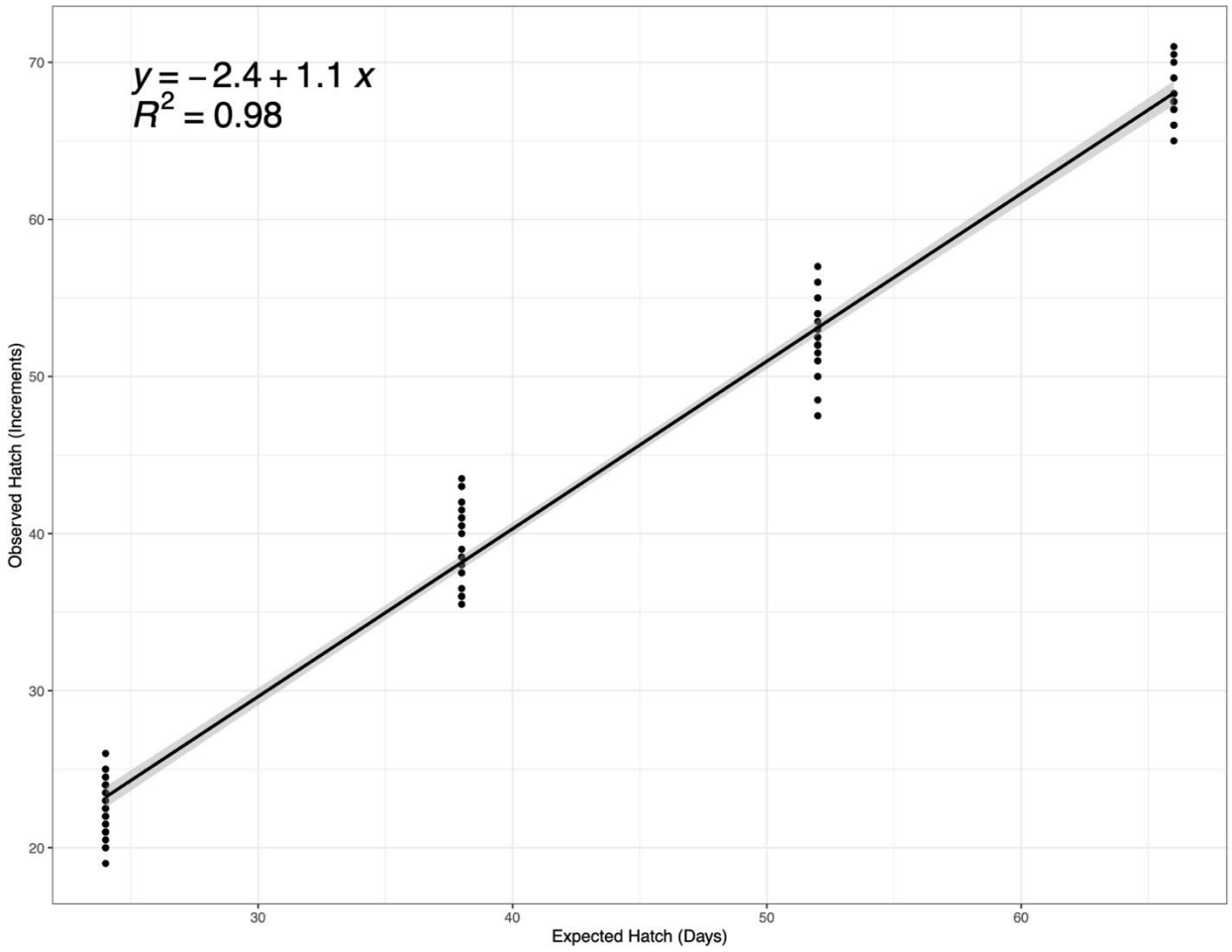


Figure S3. 3: Linear regression between expected number of days and observed number of increments from hatch to capture in age-0 hatchery Westslope Cutthroat Trout. Line is defined as $y = -2.4 + 1.1 x$, $R^2 = 0.98$.

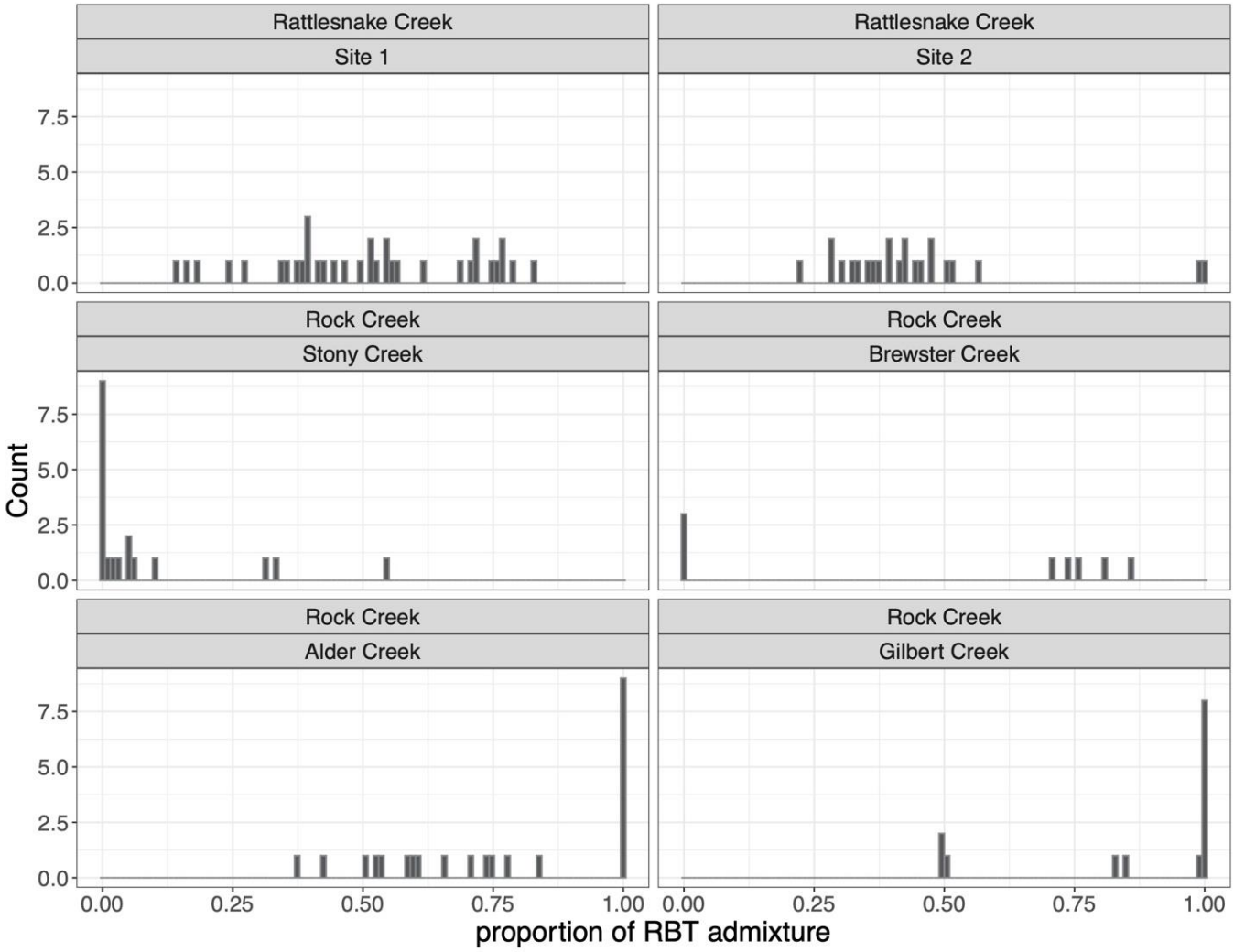


Figure S3. 4: Histograms of distribution of individual proportion of Rainbow Trout admixture for age-0 fish across six sample sites in Rock Creek and Rattlesnake Creek Watersheds. All sites were sampled in August 2019 and 2020. Proportion of RBT admixture values of 0.00 are Westslope Cutthroat Trout, and 1.00 are Rainbow Trout.

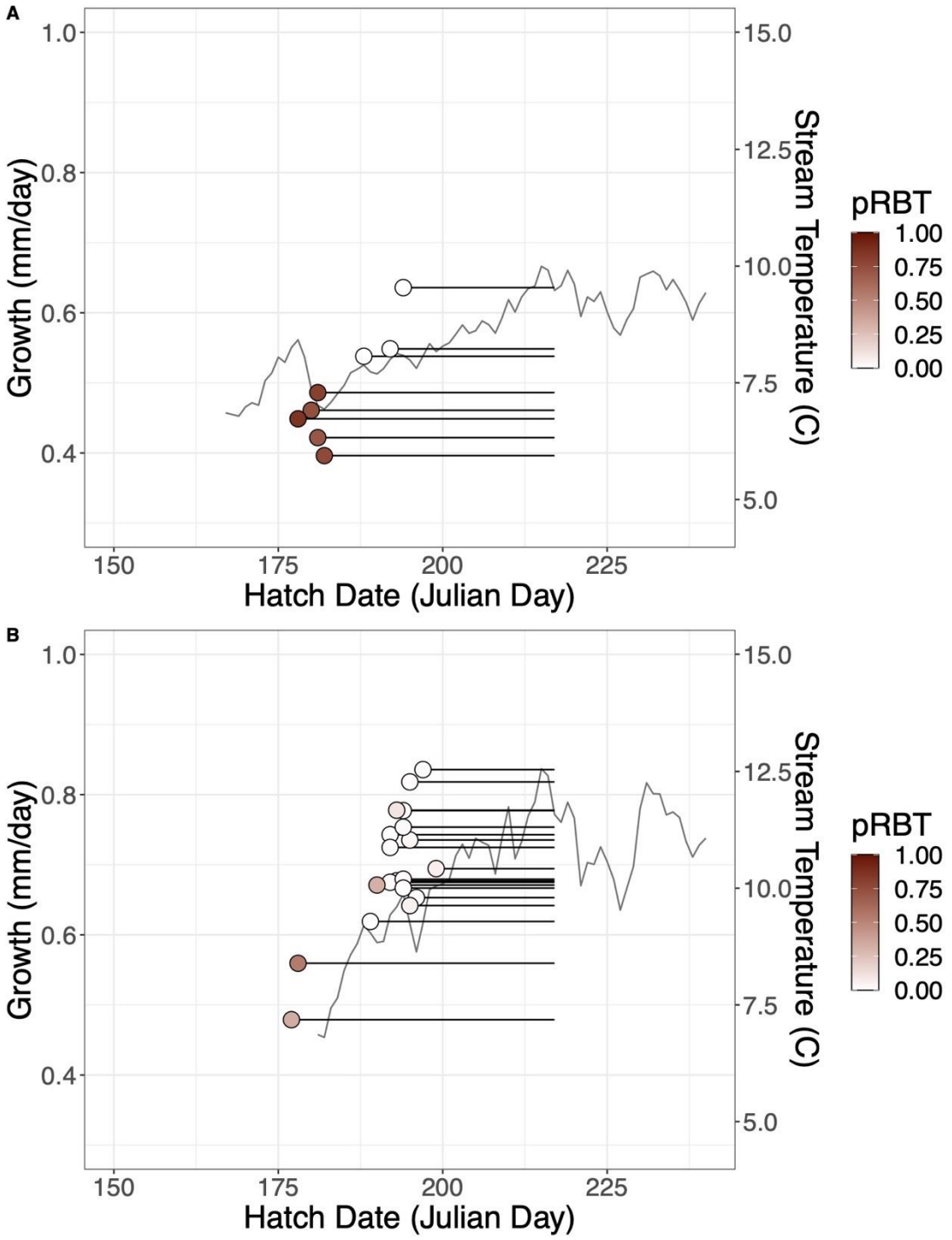


Figure S3. 5: Individual's average growth rate (mm/day) in relation to stream temperature in Brewster Creek (A) and Stony Creek (B), the two Rock Creek sample sites with a significant relationship between growth and proportion of Rainbow Trout admixture (pRBT). Each dot represents an individual fish and its estimated hatch date, individuals are color coded to correspond with estimated pRBT. White dots are Westslope Cutthroat Trout. Red dots are Rainbow Trout. The black lines represent an individual's growth window until capture. The gray line represents mean daily stream temperature (Celsius).

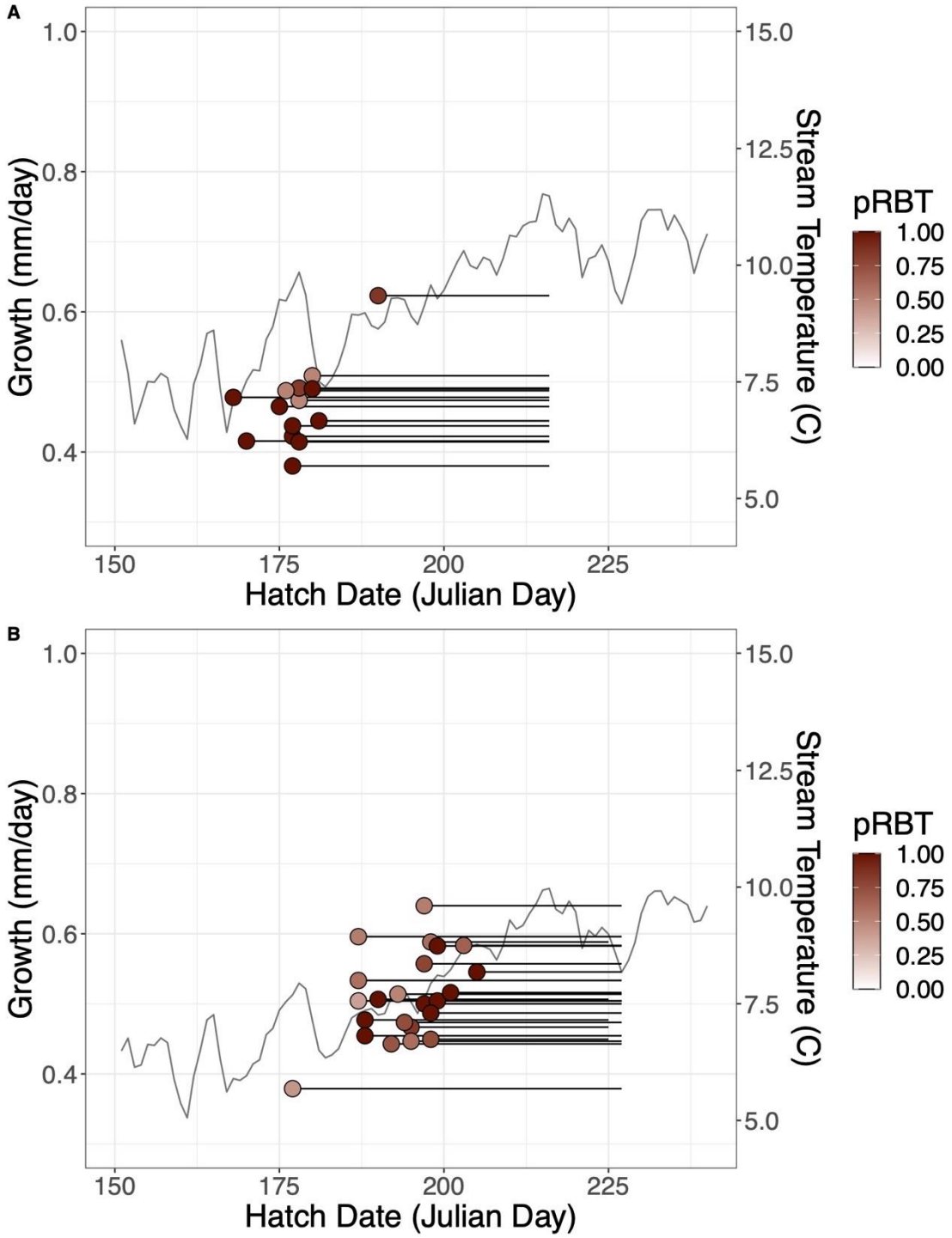


Figure S3. 6**:** Individual's average growth rate (mm/day) in relation to stream temperature in Gilbert Creek (A) and Alder Creek (B). Each dot represents an individual fish and its estimated hatch date, individuals are color coded to correspond with estimated pRBT. White dots are Westslope Cutthroat Trout. Red dots are Rainbow Trout. The black lines represent an individual's growth window until capture. The gray line represents mean daily stream temperature (Celsius).

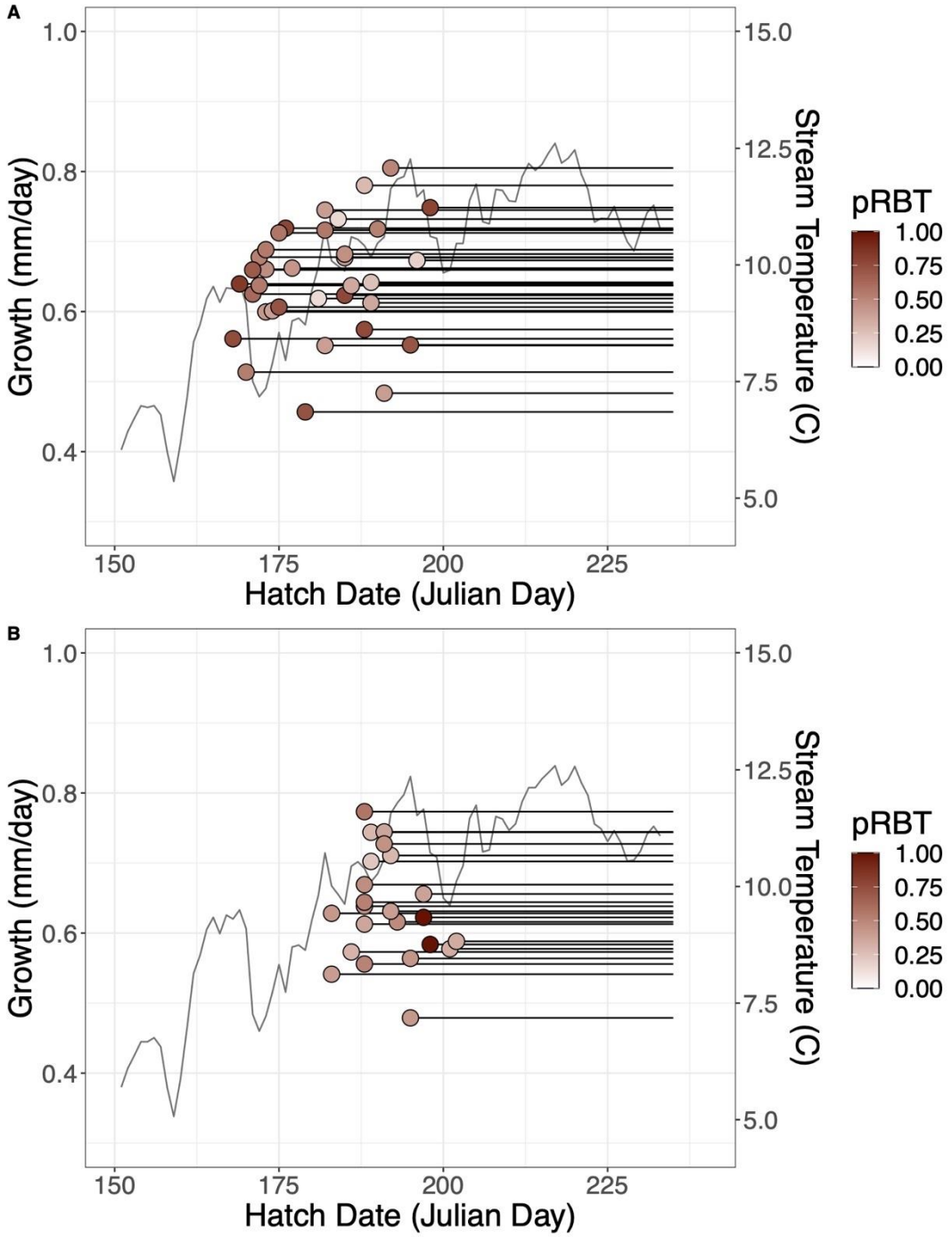


Figure S3. 7**:** Individual's average growth rate (mm/day) in relation to stream in Rattlesnake Creek Site 1 (A) and Rattlesnake Creek Site 2 (B). Each dot represents an individual fish and its estimated hatch date, individuals are color coded to correspond with estimated pRBT. White dots are Westslope Cutthroat Trout. Red dots are Rainbow Trout. The black lines represent an individual's growth window until capture. The gray line represents mean daily stream temperature (Celsius).