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MANAGEMENT AND LIFE HISTORY CONSEQUENCES OF HYBRIDIZATION BETWEEN WESTSLOPE CUTTHROAT TROUT (ONCORHYNCHUS CLARKII LEWISI) AND RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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MANAGEMENT AND LIFE HISTORY CONSEQUENCES OF HYBRIDIZATION BETWEEN WESTSLOPE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKII LEWISI*) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

By

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ABSTRACT

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Management and life history consequences of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*)

Chairperson: Dr. Lisa Eby

The role of introductions of nonnative fishes in the decline of native fishes cannot be overstated. Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT) are a salmonid native to the northern Rocky Mountains. These trout hybridize with rainbow trout (*O. mykiss*, RBT) where they are sympatric; however, in portions of the WCT range where RBT have been introduced, hybridization appears to spread rapidly and threatens continued existence of WCT. The conservation value of these hybridized populations is equivocal, and a better knowledge of the ecological, demographic, behavioral, and genetic consequences of hybridization is needed to better inform conservation strategies. I investigated three related questions: specifically, what landscape factors are associated with estimates of introgression; what are life history differences between WCT and hybrids; and what are tradeoffs in restoring migratory life history in populations threatened by hybridization?

The riverscape context plays a substantial role in the distribution of hybrids, as estimates of introgression declined with increases in stream slope, elevation, and distance from a primary source of RBT, three correlated landscape variables. Spatial variation in patterns of hybridization suggests clarifying objectives for sampling and careful designs are necessary to adequately understand the status of populations. Variation in location may relate to some of the ecological differences, such as growth, among fish with different levels of introgression. In the Jocko River, hybrids with ancestry > 20% RBT demonstrated higher growth, earlier migration, increased egg size, and lower fecundity versus WCT. These lines of evidence demonstrate the importance of limiting further hybridization even in populations that already have low levels of hybridization. Given that many unaltered populations currently reside in isolated habitat fragments, I evaluated several demographic tradeoffs of restoring a migratory life history weighed against the risks of increased potential for hybridization from removing barriers or selectively passing migratory fish above the barriers using both matrix and genetic population models. Restoration of migratory life history substantially increases population viability; however, hybridization in above-barrier population increases predictably relative to hybridization status of below-barrier population, which may reduce viability if vital rates are reduced in hybrids. Hybridization creates a challenging set of management problems, but this research adds several important pieces to the puzzles to help develop and evaluate conservation strategies.

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CHAPTER 1

INTRODUCTION AND OVERVIEW

Anthropogenic activities have influenced nearly every ecosystem worldwide (Vitousek et al. 1997). Freshwaters and the species they support are among the most imperiled of Earth's ecosystems because of their vulnerability to pollutants, demands for water, hydroelectric development, habitat alteration, and a suite of other factors (Ricciardi and Rasmussen 1999; Revenga et al. 2005). One important way humans have forever altered freshwater ecosystems is through the intentional and accidental introductions of nonnative fishes. Nonnative fish introductions have been implicated in the declines of native fishes, alteration of fish assemblages, and modification to aquatic food webs through several avenues, including competition, predation, and hybridization (Rahel 2000; Kolar and Lodge 2002; Perry et al. 2002; Eby et al. 2006). Hybridization between native and nonnative fishes represents a major challenge to conservation efforts because the results of hybridization are often irreversible (Rhymer and Simberloff 1996). When hybridization results in fertile offspring that mate with parental types (introgression[1]), it represents not only an invasion of habitat by the nonnative species, but also an invasion of locally adapted genomes by nonnative genes (Allendorf et al. 2001).

Westslope cutthroat trout (WCT, *Oncorhynchus clarkii lewisi*) are native to the interior of northwestern North America and are considered a species of concern by states and other entities throughout their range (UDWR 2000; Behnke 2002). They were

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[1] Refer to the Glossary for definitions of terms related to hybridization that are used throughout this dissertation.

petitioned for listing under the Endangered Species Act (ESA) in 1997. The cutthroat trout subspecies was precluded from listing on the basis that populations that were morphologically similar to the accepted taxonomic description for WCT existed in a large portion of their historic range (USFWS 2003). Hybridization with introduced rainbow trout (RBT, *O. mykiss*) is considered the primary threat to the conservation of WCT (Rubidge et al. 2001; Allendorf et al. 2004; Muhlfeld et al. 2009a and others). RBT are native to the western portion of North America; however, their range does naturally overlap with WCT in several large drainage basins. Hybridization between WCT and nonnative RBT is an interesting example of introgressive hybridization between fishes because both taxa have high socioeconomic value, resulting in a burgeoning body of scientific work that endeavors to better understand the processes and consequences of hybridization. Limited evidence suggests that where they are naturally sympatric, WCT and RBT have evolved isolating mechanisms that maintain species identity (e.g. Leary et al. 1995; Kozfkay et al. 2007), but where they evolved in allopatry, RBT introductions have lead to introgressive hybridization and hybrid swarms (Leary et al. 1984; Allendorf and Leary 1988; Leary et al. 1995). Current estimates suggest that non-hybridized populations of WCT occupy only 21% of their native range (Shepard et al. 2005). In addition, evidence is accumulating that hybridization is spreading rapidly through many drainages in the West (Rubidge et al. 2001; Hitt et al. 2003; Boyer et al. 2008).

There has been substantial debate regarding the conservation and evolutionary value of populations of WCT that have hybridized with populations of RBT (Allendorf et al. 2001; Allendorf et al. 2004; Allendorf et al. 2005; Campton and Kaeding 2005; Shepard et al. 2005). Natural hybridization is an essential component of the evolutionary process (Taylor 2004), but anthropogenic introgressive hybridization can result in the genomic extinction (Rhymer and Simberloff 1996) of the native taxon in as few as five generations (Epifanio & Philipp 2000). Allendorf et al. (2001) suggest the conservation value of hybridized populations increase as the number of remaining pure populations decrease. Peacock and Kirchoff (2004) made a similar conclusion when they recommended conservation of populations of Lahontan cutthroat trout (*O. c. henshawi*) with limited introgression because there were few remaining pure populations in an isolated basin. The states of Colorado, Idaho, Montana, Nevada, New Mexico, Utah and Wyoming classify cutthroat trout populations with no detectable RBT introgression as core conservation populations and populations with < 10% RBT introgression as conservation populations (UDWR 2000).

Much of the debate over the conservation value of hybridized WCT populations has been the result of various interpretations of the statement in Allendorf et al. (2001), “An argument can be made that any admixture should preclude a population from being protected [when introgression is facilitated by anthropogenic activities].” Campton and Kaeding (2005) defend the policy of the U.S. Fish and Wildlife Service (USFWS) that hybrid individuals are to be considered as WCT if they are (1) morphologically indistinguishable and (2) < 20% of their genes are derived from another taxon (USFWS 2003). Their rationale is that individuals with morphological characteristics of WCT are likely to be ecologically and behaviorally similar to WCT. Likewise, USFWS assumes some degree of genetic similarity confers a high degree of ecological and behavioral similarity. Allendorf et al. (2004) assert these USFWS criteria will protect populations that do not belong to the evolutionary lineage of WCT. They argue that hybridized populations meeting USFWS criteria will subsequently become source populations for further hybridization that will disrupt local adaptation and lead to outbreeding depression.

Clarification of this debate depends on knowledge of the ecological, demographic, behavioral, and genetic consequences of hybridization (Figure 1.1). Continued introgression and spreading hybrid swarms may lead to the loss of native genotypes (genomic extinction). It is unknown if hybridization leads to the formation of evolutionarily novel (Ellstrand and Schierenbeck 2000) hybrid trout that do not function in a manner ecologically equivalent to the native WCT (ecological extinction), but swimming stamina and competitive differences in Yellowstone cutthroat trout (*O. c. bouvieri*) X RBT hybrids (Seiler and Keeley 2007, 2009) and potential dispersal differences in WCT X RBT hybrids versus WCT (Hitt et al. 2003; Boyer et al. 2008) have been documented. In addition, hybrid swarms may not be viable (demographic extinction) during periods of environmental stress (Allendorf et al. 2004), but no empirical evidence directly relating WCT population decline to hybridization exists.

The following three chapters describe research I conducted to further our understanding of the patterns and consequences of hybridization between WCT and RBT from a fishery management and conservation perspective[2]. Management options for reducing or eliminating introgression from nonnative species generally involve fairly drastic actions, such as use of barriers, eradications of nonnative fish and hybrids, and reintroduction of fish from nearby populations or conservation hatchery stock (Leary et al. 1995). We are beginning to develop an understanding of how hybrids are distributed

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[2] Each chapter is written as a stand-alone paper to facilitate future publication. I use a first person plural writing style throughout the dissertation to reflect the fact that the research described in each chapter had multiple contributors who will serve as coauthors when these chapters are submitted for publication

across landscapes (e.g. Rubidge et al. 2001; Boyer et al. 2008) and how the structure of hybrid zones may vary with biotic and abiotic features of those landscapes (e.g. Rubidge and Taylor 2005; Muhlfeld et al. 2009c). There has, however, been little discourse about the consistency of those patterns across landscapes and the implications of that variation for how we sample for conservation planning and monitoring. At the same time, we are developing conservation strategies for WCT based on assumptions of morphological, life history, and behavioral differences between WCT and hybrid trout, but with little empirical evidence of whether or not those differences occur (but see Boyer et al. 2008; Muhlfeld et al. 2009a; Muhlfeld et al. 2009b). Additionally, many conservation strategies being considered for conservation populations of WCT, especially barrier placement, may result in the reduction or loss of population connectivity and migratory life history types (Fausch et al. 2006). The scientific literature is currently lacking an empirical examination of the tradeoffs of loss of fluvial migratory life history types versus increased introgression. Such an examination may become important, not just to inform WCT conservation, but also to inform management of other hybridizing freshwater taxa (see Perry et al. 2002).

This dissertation has three primary objectives. The first objective (Chapter 2) was to describe the distribution of hybridization between WCT and RBT in the Jocko River, MT, and determine which landscape factors correlated with patterns of introgression observed. In addition, we described how spatial variation in estimated rates of introgression has implications for sampling. The second objective (Chapter 3) was to test for equivalency in migratory behavior (life history) and demographic metrics related to fitness (such as growth and fecundity) between WCT and hybrid trout. The final objective (Chapter 4) of this study was to use models to examine the tradeoffs associated with selective or complete passage of migratory WCT when hybridized trout populations are present below the barrier. We used a demographic model to test the importance of migratory life history to population viability and a population genetic model to test the hybridization consequences of passing hybrid trout above a barrier.

Chapter 2 results suggested there are strong associations between estimated rates of RBT introgression (pRBT) and stream slope, the number of barriers, elevation, and distance from the primary RBT source (three strongly correlated variables on the landscape) in the Jocko River system. These results are consistent with other landscape studies of hybridization between cutthroat trout subspecies and RBT (e.g. Rubidge and Taylor 2004; Boyer et al. 2008; Rasmussen et al. 2010), suggesting RBT and WCT actually form broad hybrid zones that may be structured by environmental resistance. Even though RBT have been established in the Jocko River for at least 70 years, parental-type RBT and WCT still occurred in high densities at low and high elevations, respectively. While distributions of RBT alleles across individuals within samples suggested hybrid swarms were common, we only detected one hybrid swarm with pRBT > 0.05. This is further evidence indicating that at the landscape scale, while there is introgression, the two parental types are maintaining some reproductive isolation (Jiggins and Mallet 2000). While there was an overarching landscape pattern, at small spatial scales (1-20km) within a single tributary there was site to site variation in pRBT and the number of highly hybridized individuals detected. That variation may have important ramifications for sampling. We wanted to simulate different sampling approaches in tributaries to examine the potential for missing important information due to that variation. Resampling a spatially extensive dataset from the South Fork Jocko River suggested current sampling norms of collecting 25-30 fish at a single sample site are sufficient for estimation of pRBT for a stream, but these samples were inadequate for detecting individuals with high RBT ancestry. These individuals, while potentially rare in WCT conservation populations, represent vectors of continued introgression between RBT and WCT and could be vectors for broader dispersal of RBT genes (Boyer et al. 2008). Fewer samples statistically conformed to a hybrid swarm when the number of individuals sampled was increased; this is a result of the likelihood that capturing rare individuals with relatively high RBT admixture increases with sample size. Increasing the length of the sampling frame by resampling from more sites also improved detection of rare high admixture individuals, because they were patchy (concentrated at middle elevation sites) in the South Fork Jocko River and a longer sampling frame increased the likelihood of sampling a site with these individuals. Understanding that introgression is consistently associated with landscape variables, such as elevation, will help managers prioritize steams and reaches for conservation actions. Developing sampling schemes that are not only effective at quantifying pRBT, but also detecting rare, highly hybridized individuals will help managers better classify priority areas as core or conservation populations prior to deciding upon the appropriate management action.

In addition to understanding what might be influencing introgression on the landscape, understanding how individuals with varying ancestry may differ is critical for testing assumptions underlying policy as well as predicting the production and persistence of introgressed populations (even those managed for sport fishing). We compared life history metrics among classes of migratory individuals estimated to have

WCT parental genotypes, individuals estimated to have less than 20% of their ancestry from RBT, and individuals estimated to have greater than 20% of their ancestry from RBT. Individual levels of RBT ancestry were classified by using a hybrid index score (HI), which is the number of RBT alleles amplified from an individual divided by the total number of alleles amplified across all loci for that individual. Hybrid index scores of zero indicate a WCT genotype, and scores of 1 indicate an RBT genotype. Individuals collected for Chapter 3 were sampled in upstream migration traps at irrigation diversion dams during spring spawning timeframes and generally exhibited a fluvial migratory life history type.

We compared hybrid classes using length at age, fecundity and egg size relationships, and migration timing. Individuals with more than 20% RBT ancestry had significantly higher growth, lower fecundity, and larger egg size. Additionally, they migrated earlier, at lower stream discharges, and at lower stream temperatures than individuals with less than 20% RBT ancestry. Interestingly, the first major jump in the hydrograph associated with the spring freshet signaled the end of nearly all migration of individuals with more than 20% RBT ancestry. Individuals with low levels of RBT alleles likely come from populations that are spawning at higher elevations relative to those individuals that are more highly hybridized (Chapter 2). These environmental differences in rearing habitat may explain life history differences related to growth. However, it is reasonable to expect these differences are also a function of local adaptation, and further introgression from the high hybrid category into the zero and low hybrid categories may result in outbreeding depression. Furthermore, following outmigration of fluvial cutthroat trout phenotype juveniles, it is likely there is greater overlap in rearing habitat use by high, low, and zero hybrid category fish.

Many WCT core and conservation populations are upstream of complete and incomplete barriers in relatively little habitat (median stream length occupied = 8.6km; Shepard et al. 2005), and many managers are debating removing or reinforcing these barriers to protect fish from introgression. At the same time, researchers are demonstrating the importance of maintaining a diverse ecological and life history portfolio for the sustainability of stocks and persistence of native fishes (Schindler et al. 2010; Piccolo 2011). Having a formal framework to begin to assess these tradeoffs is timely and critical as decisions are constantly being made. In Chapter 4, using two model frameworks, we assessed the tradeoffs associated with restoring migratory life histories above two complete barriers to movement. On one hand, allowing escapement of migratory individuals (either by selective passage or complete removal of the barrier) has potential to increase population productivity and viability. On the other hand, mistakenly passing migratory hybrid individuals above the barriers may increase introgression and have subsequent consequences on the viability of upstream populations.

We used a stochastic, multiple life history matrix population model to test scenarios with various levels of escapement by migratory fish into an otherwise resident population. Restoration of migratory fish into the resident population dramatically improved population viability and potential productivity. However, when juvenile survival was reduced according to recent estimations by Muhlfeld et al. (2009a) or fecundity was decreased according to Chapter 3 for populations comprised of highly hybridized individuals, we observed substantial declines in viability and population productivity. That said, there have been no reports of rapid population crashes in hybridized populations with RBT introgression. Reduced fecundity (egg number) displayed by trout with greater than 20% RBT ancestry may be a life history tradeoff for larger egg size (which was demonstrated in Chapter 3), rather than evidence for reductions in fitness. Further, in Chapter 3 we observed a lower (but not statistically significant) age at migration in the high hybrid category relative to the low and zero categories. If this is equivalent to an earlier age at maturity for high category hybrids, the resultant increased survival to maturity may outweigh the effects of reduced fecundity. The results of an elasticity analysis demonstrated that changes in survival of subadults to adulthood can have an equal or greater influence on population growth compared to reductions in fecundity. Achieving a better understanding of how tradeoffs in life history may mitigate apparent reductions in vital rates such as fecundity is an important avenue for future research to better understand the true demographic consequences of hybridization between WCT and RBT.

Results of the genetic model indicate that with barrier removal and random mating, estimates of pRBT in the above barrier population approach, but do not achieve, the pRBT of the below barrier population after 4-10 generations. The results of this chapter indicate above barrier populations are substantially more demographically viable with migrants, but selective passage and barrier removal are not viable strategies for maintaining unhybridized core conservation populations, unless the downstream population is also a core population, or WCT can be identified and selectively passed without error, which is unlikely. On the other hand, if the management situation deems population connectivity and life history diversity to be of greater importance than genetic purity, there is potential to maintain conservation population status (pRBT < 0.10) using selective passage with reasonable error. Because this model assumed random mating and no reproductive isolation between WCT and hybrids, it represents a worst-case introgression scenario. Further examination of selection against hybrid fish and mating systems in hybridized populations, as well as better understanding of overlap in spawning habitat for WCT and hybrids are needed before we can more accurately predict increases in introgression caused by restoration of migratory life histories and connectivity.

The results of this dissertation provide further evidence that WCT are at risk from genomic extinction (Figure 1.1), as we did observe hybrid swarms, though WCT genotypes are dominant in the Jocko River system and only 12 of 61 population samples had pRBT > 0.10 (see Chapter 2). Thus, in this case, the term "genomic extinction" describes a situation where the local genome is forever altered, but much of the native genetic variation may be conserved. The results of Chapter 3 indicate there may be some important life history differences between WCT and hybrids with RBT ancestry greater than 20%. We observed differences in mean and median migration conditions among hybrid categories, but the overlap we observed certainly sets the stage for further introgression. Our sample sizes were limited, so it is still uncertain whether or not there are ecological differences between WCT and individuals with 10-20% RBT ancestry. We found no evidence that individuals with RBT ancestry less than 10% had different life history from individuals with a WCT genotype. There may be some potential for ecological differences of hybridized WCT populations but likely only at relatively high (pRBT > 0.20) rates of admixture. Other studies have documented ecological differences in cutthroat trout and hybrids at varying degrees of RBT admixture including significant results for hybrids with as little as 10% RBT ancestry at different measures of behavior, performance, and vital rates (e.g. Muhlfeld et al. 2009a; Muhlfeld et al. 2009b; Seiler and Keeley 2007, 2009). More studies in a variety of habitats are needed before we fully understand life history and ecological and fitness differences between WCT and hybrids.

In populations where putative fitness consequences of hybridization, such as those described by Muhlfeld et al. (2009a), are modeled as decreases in population vital rates, there is the potential for demographic extinction due to hybridization (see Chapter 4). It is important to note, however, there have been no descriptions of such occurrences in nature. Studies measuring vital rates in populations in varying habitats and times since invasion by RBT would be highly beneficial to our understanding of true consequences of hybridization over short and long time scales. The elasticities of the matrix model in Chapter 4 suggest young of year survival and the transition of subadult residents to adult residents (probability of maturity) are the vital rates that have the most influence on population growth, and should therefore receive the first in-depth investigations by researchers.

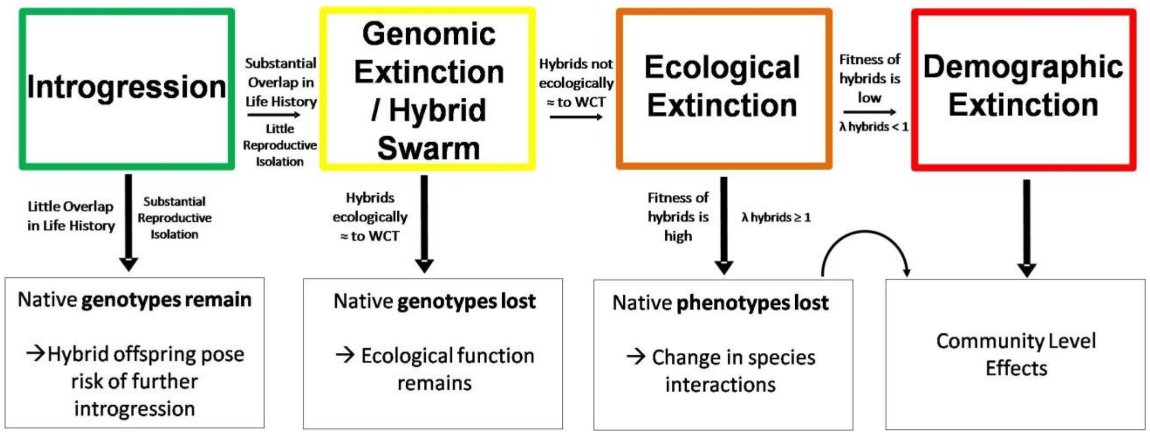

Figure 1.1. Hypothetical consequences of introgressive hybridization leading to the formation of a hybrid swarm. For the purposes of this study, ecological equivalence is defined as equivalence in behavior and life history, but it could also represent community or food web interactions. λ_{hybrids} represents the population growth rate. $\lambda_{\text{hybrids}} = 1$ implies a stable population.

CHAPTER 2

DISTRIBUTION OF HYBRIDIZATION IN A MONTANA RIVER WITH IMPLICATIONS FOR CONSERVATION AND SAMPLING DESIGN TRADEOFFS

Abstract

Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT) populations are classified for conservation according to extent of introgression with rainbow trout (*O. mykiss*, RBT). Understanding the patterns of introgression across the landscape and how that pattern may influence sampling considerations is becoming increasingly important for cutthroat conservation and management. We collected 61 samples (median sample size = 29 individuals), which includes every fish-bearing stream in the Jocko River watershed, MT. We detected RBT alleles in all but 10 population samples; however, only 12 samples had a proportion of rainbow trout alleles (pRBT) greater than 0.10, and WCT genotypes were dominant in the system. We evaluated the relative ability of a series of linear regression models with independent variables including distance from source population, elevation, slope, number of barriers to fish movement, and road densities to describe patterns in introgression. Because trout with the highest levels of introgression were found in the lowest Jocko River sites, distance from source, elevation, and slope were all strongly correlated, but the model with slope and barriers had the best fit. We subsampled 334 individuals from 11 sites in the South Fork Jocko River in different combinations to examine how sampling tradeoffs in number of sites versus individuals per site influenced common hybridization metrics given our landscape

patterns in introgression. There was no difference in detection of hybrids (presence) across the simulated designs with different tradeoffs. Detection of individuals with hybrid index scores greater than 0.20 improved with number of individuals and number of sites (lengthening the sampling frame). Subsampling commonly resulted in a slight overestimation of pRBT (typically < 0.02). Samples statistically conformed to a hybrid swarm in 28-46% of simulated samples with 30 fish and in 1-22% of simulated samples with 60 or 90 fish. Thus, the appropriate field sampling design depends on the hybridization metric of greatest interest to practitioners.

Introduction

Hybridization with introduced salmonids is considered a primary threat to the conservation of native salmonids throughout the world (Rhymer and Simberloff 1996; Allendorf et al. 2004). This has particularly been the case when considering hybridization between cutthroat trout (*Oncorhynchus clarkii spp.*) and introduced rainbow trout (*O. mykiss*, RBT). There are multiple management approaches to reducing or eliminating hybridization between salmonids. These include placement (or maintenance) of barriers to invasion, eradication of hybrid and non-native individuals followed by reintroduction of unhybridized fish, and adjustments to angling regulations to promote harvest of hybrid and non-native individuals (Novinger and Rahel 2003; Allendorf et al. 2005; Meyer et al. 2006; Fausch et al. 2009).

Due to conflicting management and conservation values, these approaches can be politically, ecologically, and financially risky and as a result, determining the status and distribution of hybridized fish populations has become increasingly important for conservation and management (see Clarkson et al. 2005; Pister 2010). For example, efforts to restore greenback cutthroat trout *O. c. stomias* populations in Colorado have been stymied by inadequate description of hybrid zones between greenback cutthroat trout and Colorado cutthroat trout *O. c. pleuriticus*. Poor understanding of hybrid distributions has resulted in misidentification of putatively pure greenback cutthroat populations and accidental use of hybridized populations as conservation broodstock (Metcalf et al. 2007). Installation of artificial barriers to protect native salmonids is an expensive endeavor with potential negative long-term consequences of isolation for the local populations (Kruse et al. 2001; Fausch et al. 2009). Thus, ensuring the best possible understanding of the distribution of hybrids prior to barrier installation is important.

Current management plans in Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming categorize populations of westslope cutthroat trout (*O. c. lewisi*, WCT), Yellowstone cutthroat trout (*O. c. bouvieri*) and other cutthroat trout subspecies into core, conservation, and sports fishing populations depending primarily upon the extent of introgression within and among local populations (UDWR 2000; MTFWP 2007). For management purposes, extent of introgression is typically quantified as a proportion estimated by the total number of nonnative alleles in a population sample divided by the total alleles amplified across all individuals in the sample (pRBT, UDWR 2000). A population with pRBT ≤ 0.10 is defined as a conservation population, and any population with no detectable RBT introgression is a core conservation population (UDWR 2000). Thus, managers use the presence (P/A) of hybrids as well as extent of introgression to determine the conservation status of populations when making management recommendations. Additionally, the presence of first generation hybrids or relatively recent backcrosses to WCT is also useful information because it may indicate recent introgression events in populations where levels of introgression are very low or not previously detected (Boyer et al. 2008; Neville et al. 2009). Linking landscape factors that may drive the distribution of hybrids and influence these metrics across the landscape can help us understand potential natural limits to hybridization and design effective sampling programs.

Several studies have examined the influences of landscape factors on the distribution of hybrids and extent of introgression within a population. Distance from established RBT source populations or stocking locations has been the most consistently described factor explaining extent of introgression (Rubidge et al. 2001; Hitt et al. 2003; Rubidge and Taylor 2005; Boyer et al. 2008; Muhlfeld et al. 2009c). Elevation, which is correlated with stream temperature and other habitat gradients, has also been described as a potentially important factor driving rates of RBT introgression in cutthroat trout populations (Weigel et al. 2003; Boyer et al. 2008; Rasmussen et al. 2010). Muhlfeld (2009c) found that anthropogenic disturbance, as measured by the number of stream road crossings, was positively associated with presence of hybridization in a stream system. Additionally, life history factors, such as migration timing, spawning timing, and spawning habitat preferences are influenced by temperature, hydrology, and other gradients related to the landscape, and could influence the degree of reproductive isolation between RBT and cutthroat trout (Henderson et al. 2000; Muhlfeld et al. 2009b; Rasmussen et al. 2010). Testing the relationships between hybridization and landscape factors across multiple basins is needed to determine the generality of patterns.

In some systems where WCT and RBT are naturally sympatric, or where RBT have been stocked, substantial reproductive isolation has been maintained between species (Ostberg et al. 2004; Young et al. 2004; Gunnell et al. 2007; Kozfkay et al. 2007; Ostberg and Rodriguez 2006; DeRito et al. 2010). In other systems, there appears to be a complete breakdown of reproductive isolation with the formation of hybrid swarms (Leary et al. 1995; Bettles et al. 2005). Researchers speculate that the formation of hybrid swarms could disrupt local adaptations and coadapted gene complexes when nonnative alleles are randomly distributed throughout the genome (Rhymer and Simberloff 1996; Allendorf et al. 2001). To improve our understanding of the consequences of hybridization, we need to understand where populations are maintaining some reproductively isolated sources, forming hybrid swarms, or some combination of the two. Frequency distributions of a hybrid index, where minimum and maximum values represent unhybridized parental types have been used to examine this issue (Jiggins and Mallet 2000; Rubidge and Taylor 2004). Generally, bimodal distributions are indicative of two species with partial reproductive isolation. Unimodal distributions are typically dominated by hybrid genotypes (or distributions that conform to a binomial distribution) and are representative of a complete breakdown of reproductive isolation (Jiggins and Mallet 2000; Allendorf et al. 2001; Boyer et al. 2008).

Given the multitude of landscape factors that could influence the distribution of hybrids, the extent of introgression, and potential formation of hybrid swarms, researchers and managers need to be cognizant of the spatial patterns of hybridization and potential implications of the sampling design. Genetic analyses are expensive, and management actions that affect the native population could be irreversible, so evaluation of the influence of a sampling scheme on the description of hybrid populations is vital. In typical sampling efforts, samples are often collected from sites most readily accessible because of financial and logistical constraints. There are always tradeoffs in the number of individuals sampled per site and the number of sites (spatial extent) sampled. It is common for biologists to use a sample, composed of approximately 30 individuals, from a single stream reach, to classify a stream according to its hybridization status. As biologists design both field sampling plans and management strategies, it is important to determine how tradeoffs in sampling could affect hybridization metrics.

Confederated Salish and Kootenai Tribe (CSKT) biologists in the Jocko River basin in northwestern Montana have been monitoring WCT populations since the early 1980s. The montane reaches of the upper Jocko River had long been thought to be a stronghold of native WCT, despite the fact that major RBT populations existed in the basin. We completed a genetic survey to describe the distribution of hybrids in the Jocko River from 2005-2009. This study had two primary objectives. First, we determined if distribution of hybrids in the Jocko River basin was associated with the same landscape factors (distance from source, elevation, average stream slope, barriers, and road density) that had been observed in other studies to establish whether those patterns are generally consistent among landscapes. Second, we examined how sample design tradeoffs in the number of individuals per site versus the number of sample sites influenced hybridization metrics in a major tributary.

Study Site

This study was conducted in the Jocko River system, a 979 km^2 basin that is a tributary of the Flathead River in northwestern Montana. The entirety of the basin lies within the boundaries of the Flathead Indian Reservation. Natural resources, including fisheries, are managed by the Confederated Salish and Kootenai Tribes (CSKT). The North, Middle, and South Forks of the Jocko River comprise the upper reaches of the Jocko River (Figure 2.1). The Finley Creek and Valley Creek drainages comprise the remainder of the large tributary systems to the main-stem Jocko River. In general, the streams in the Jocko River system are high gradient, confined channels at high elevations transitioning to lower gradient reaches at low elevations.

There are several natural and artificial barriers to fish movement distributed throughout the Jocko River system (Figure 2.1). The basin has a large irrigation project that was developed in the early 20th century. This irrigation system has likely played a substantial role in structuring WCT populations since its implementation. Diversions and the associated canal network on the upper Jocko River created partial and full barriers to fish movement in most cases and may have artificially connected some streams in others. For example, there is an irrigation diversion that moves water and entrains fish from the Middle Fork Jocko River into the North Fork Jocko River (CSKT unpublished data). Two major diversions (K-Canal and S-Canal, Figure 2.1) in the upper main-stem Jocko River were fitted with ladders for selective passage of bull trout (*Salvelinus confluentus*). However, since construction of the ladders in 1996 (K-Canal) and 2002 (S-Canal), CSKT fisheries managers have not passed *Oncorhynchus spp.* at these diversions in order to reduce the potential for introgression of upstream westslope cutthroat trout. Some fish, however, may occasionally pass the K-Canal diversion when irrigation managers remove check boards when the river is at bank-full flows or higher.

The salmonid assemblage of the Jocko River basin is comprised of a mix of native and introduced fishes. Non-native species including brown trout (*Salmo trutta*) and trout with RBT phenotypes are abundant in the main-stem Jocko River below K-Canal diversion. Putative RBT and brook trout (*Salvelinus fontinalus*) are present in several tributaries throughout the system. Native trout with WCT phenotypes and mountain whitefish (*Prosopium williamsoni*) are also abundant in the main-stem Jocko River, and

WCT phenotypes are present at various abundances in nearly all fish-bearing tributaries. The three forks of the upper Jocko River support the largest populations of native WCT and bull trout in the drainage.

Methods

Fish Sampling and Tissue Collection

To examine hybridization and associated landscape factors, we sampled at least one site from every salmonid bearing stream in the basin over a period from 2005 to 2009, including multiple samples distributed longitudinally in Valley Creek, Finley Creek, and the three forks of the upper Jocko River (Table 2.1). Samples in the upper Jocko River were collected as part of a systematic survey and used to examine fine-scale distribution of hybrid trout above the K-Canal diversion. In the upper Jocko River, CSKT personnel established sampling sites at 1 km intervals beginning at the mouth of each of the North, Middle, and South Forks. In 2009, we included a sample from a fluvial RBT population that spawns in the lower main-stem Jocko River.

At all sampling sites, we set up a block net on the downstream end of the site and then determined the upper boundary of the sample section by using a hip-chain to measure a 152 m reach. We then sampled by making a downstream pass with one backpack electrofisher in small streams or with two backpack electrofishers operated in tandem on larger streams (e.g. Jocko River and its major forks); after finishing the electrofishing pass, we carefully examined the block net and captured any fish present. We sampled most locations at or near base flows. We measured total length (TL) of each fish to the nearest mm, and a small section of paired or caudal fin was collected and stored in 95% ethanol. At all sampling sites, we attempted to collect at least 30 individuals. Due to low fish abundances in the lower North Fork Jocko River, we extended the sampling site by 200 m and/or aggregated adjacent samples in an effort to collect tissues from 30 individuals.

DNA Extraction and Hybrid Analyses

We extracted and amplified DNA in 10 ul reactions following the standard QIAGEN Microsatellite protocol. We used two different PCR profiles. Multiplex 1 consisted of Ogo8 (Olsen et al. 1998), Omm1019, Omm1050, Omm1060 (Rexroad III et al. 2002), and Omy 0004 (Holm and Brusgaard 1999). Multiplex 2 consisted of Omy1001 (Spies et al. 2005), and Sfo8 (Small et al. 1998). All markers are diagnostic for WCT and RBT for most watersheds tested in Montana (Sally Painter, Montana Conservation Genetics Laboratory, personal communication). We used a touchdown profile for Multiplex 1 with an initial annealing temperature of 58°C stepping down to 48°C, and we used a typical profile for multiplex 2 with an annealing temperature of 59°C. We used an ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) to visualize PCR products. We used the ABI GS600LIZ ladder (Applied Biosystems Inc., Foster City, CA) to determine allele sizes, and we viewed and analyzed chromatogram output using GeneMapper version 3.7 (Applied Biosystems Inc., Foster City, CA).

For each sample, we measured introgression by calculating pRBT (the number of RBT alleles detected divided by the total number of alleles amplified in a sample). We estimated individual levels of hybridization by calculating a hybrid index score (HI), which we reported as the total number of RBT alleles observed in an individual divided by the total number of alleles amplified for each individual across all diagnostic loci. We summarized the hybridization status of each sample by reporting the pRBT, the number of individuals in the sample with at least one RBT allele, and the maximum individual HI in the sample. Similar to Boyer et al. (2008), we tested each population sample to determine if it was a hybrid swarm by comparing the observed distributions of individual HI to those expected from a binomial distribution with a probability of success equal to the observed pRBT. Significance was determined using a χ^2 test and $\alpha = 0.05$. Significant results indicate RBT alleles are non-randomly distributed across individuals. Non-significant results indicate a completely admixed hybrid swarm where there is random mating among parental types and there has been sufficient time for decay of linkage disequilibrium. Finally, we determined whether or not each sample was in Hardy-Weinberg proportions using exact tests in GENEPOP 4.0 (Raymond and Rousset 1995, Appendix A)

Landscape Factors Influencing Introgression

We used a stream layer from the National Hydrography Dataset and a digital elevation model (from nris.mt.gov) to derive landscape factors using ArcGIS 9.3.1 (Environmental Systems Research Institute, Inc., Redlands, CA). We assumed the RBT source was the main-stem population and measured fluvial distances to each site from the main-stem. Thus, the distance from RBT source was measured from the most downstream point sampled in the main-stem Jocko River to the downstream point of all other sampling locations. We obtained elevations for each sampling location from the digital elevation model. We calculated a slope variable as the change in elevation from the RBT source to a sample site divided by the distance between the RBT source and a sample site. The slope variable accounts for average gradient over the whole distance between a sample site and the source. We counted the number of barriers between a sample site and the RBT source (Figure 2.1).   We did not have quantitative fish passage information for each barrier, so all putative barriers were treated equally in this study as providing equivalent resistance to potential RBT introgression. We included road density in the watershed above a sample site to address the influence of land use on pRBT, as we expected it to be associated with access for RBT stocking (Weigel et al. 2003) and anthropogenic disturbance that may promote conditions for hybridization (Hitt et al. 2003, Rasmussen et al. 2010). Road densities were calculated for the watershed above a pour point defined by the bottom of each sample site.

We developed a series of linear models to describe patterns in pRBT using slope, elevation, or distance from a putative RBT source, along with the number of barriers below a sample site, and road density upstream of a sample site. We expected slope, distance, elevation, and barriers to be negatively associated with pRBT and road density to be positively associated with pRBT. Elevation, distance, and slope were never used in the same model because we wanted to compare the relative fit of models that included these variables. Because we only used two additional explanatory variables for this modeling exercise, *a priori* candidate models included all possible combinations of road density and barrier variables in models with distance, elevation, or slope. We log (base 10) transformed distance and elevation for analyses, as preliminary data analyses suggested non-linear relationships with pRBT. We logit transformed pRBT in order to overcome violations of normality. Logit transformations were performed using the "logit" function in R (R Core Development Team 2010), which remaps the pRBT dataset to a minimum of 0.025 and maximum of 0.975 to overcome inability to logit transform zeros and ones. We used an information theoretic approach to weigh the relative fit of the candidate models to the data and used corrected AIC values (AIC_c) for small sample sizes (Burnham and Anderson 1998). Models were determined to be equally plausible if they were within 2.0 ΔAIC_c of the top model (Burnham and Anderson 1998).

Influence of Sampling Design Tradeoffs on Hybridization Metrics

In order to examine the relationship of spatially structured hybridization patterns and field sampling designs at the tributary scale, we examined the robustness of hybridization metrics (pRBT, P/A hybrids, P/A high-HI hybrids, hybrid swarm test) to variations in sampling design by resampling the South Fork Jocko River dataset. Sample design variations included tradeoff of number of individuals per site as well as number and spatial extent of sites within a tributary. We resampled the dataset from the South Fork Jocko River (11 sample sites, 334 individuals) to complete this analysis because it represented the drainage with the longest sampling frame, the most sites, and the most individuals from which we could subsample.

We simulated alternative field sampling scenarios in R by resampling our South Fork spatially-explicit dataset under various scenarios. We resampled the South Fork dataset with sample sizes of 30, 60, and 90 individuals (N_{ind}) across either 1, 3, and 6 sites (N_{sites}) (median distance between sites = 1.6km). We sampled without replacement within a field sample (there was replacement between resampling events), so none of the 1 sample site scenarios had greater than 30 fish (number collected in field) in a subsample. For each sample, a site from the dataset was randomly chosen. If the sample included more than one site, individuals were collected from the neighboring upstream sites to simulate datasets obtained by a field crew collecting individuals systematically from sites upstream until the targeted sample size was complete. We divided the number of individuals (30, 60, or 90) to be randomly sampled by the number of sites (1, 3, or 6) to be sampled (N_{ind}/N_{sites}) to determine the number of individuals sampled at each site. To compare scenarios with a random sample of all fish from the South Fork, we completed another set of simulations by randomly sampling 30, 60, and 90 individuals from the entire South Fork dataset. We replicated 500 samples for each sampling scenario.

For each sample, we calculated pRBT, determined P/A hybrids, and P/A high-HI hybrids (HI ≥ 0.20). We also determined if RBT alleles were randomly distributed across individuals by examining the frequency distribution of HI. Often researchers examine the frequency distribution of HIs from a sample when the sample does not conform to a hybrid swarm to assess whether there is evidence for an underlying hybrid swarm with recent immigrants with HIs that fall outside of expectations under a binomial distribution (Robb Leary, Montana Fish, Wildlife, and Parks, personal communication). For the purposes of this exercise, we did not differentiate these situations, and simply reported whether or not a sample conformed to binomial expectations under a hybrid swarm. We used HI ≥ 0.20 to categorize high-HI hybrids because the U.S. Fish and Wildlife Service determined populations with ≤ 0.20 pRBT would be morphologically indistinguishable from WCT, and therefore would qualify as WCT populations (USFWS 2003). To summarize our findings, we calculated the proportions of samples under each scenario that detected hybrids, high-HI hybrids, and hybrid swarms. We also calculated the mean pRBT across the 500 samples under each scenario.

We do not address tradeoffs involving number of loci amplified. Increasing the number of loci tested per individual will increase power to detect hybridization at the individual level, and it will reduce the number of individuals that need to be tested for detection of hybridization and pRBT. Increasing the number of diagnostic loci does not eliminate the need to examine the interaction between hybrid metrics, sampling design, and spatially structured populations with different levels of introgression.

Results

We collected samples at 63 sites, 24 of which were part of the systematic sampling effort in the North, Middle, and South Forks of the Jocko River. The remaining 39 samples were collected in the main-stem Jocko River and in tributaries throughout the basin. Due to small sample sizes in the lower North Fork Jocko River, we combined tissue samples from 4 sampling sites into two samples, resulting in 61 total genetic samples (Table 2.1). Individuals were kept in the sample unless they amplified at less than two loci. Proportion of missing alleles in a sample ranged from 0 to 0.29 (only one sample failed to amplify at two microsatellite loci), and median proportion missing alleles was 0.04, as missing alleles were rare in most samples (see Appendix A for details). Sampled fish ranged from 31 mm to 508 mm in length TL, and the mean TL was 145.5 mm (SD = 65.8).

Distribution of Hybrids and Patterns of Introgression

We detected RBT alleles at all but 10 sites; however, only 12 sites had pRBT > 0.10, and WCT genotypes were dominant in the system (Figure 2.2). The lower main-stem Jocko River sample (site 1) and nearby sample sites in Spring Creek (site 2) and Valley Creek (site 3) had the highest pRBTs (0.92, 0.97, and 0.98, respectively) (Table 2.1, Figure 2.2, also see Appendix A), which supports our assumption that downstream populations in that area represent the source of RBT alleles in the Jocko River basin. The main-stem or lowest site in most sampled tributaries was commonly the sample site with the highest pRBT. Liberty Creek (site 60, pRBT = 0.88), a tributary to the South Fork Jocko River, and Pistol Creek (site 27, pRBT = 0.42) were the only samples where we detected pRBT > 0.10 at greater than average distance from the putative main-stem source (hereafter referred to as distance). Thirty-one of the 61 population samples had unimodal distributions of HIs that conformed to a binomial distribution (hybrid swarms), but only one of these samples had a pRBT of > 0.05 (site 15, see Appendix A for details). Estimates of pRBT were strongly correlated with other signals of introgression, including proportion of individuals with HI > 0.2 in a sample. Furthermore, sample sites that had a bimodal distribution of hybrid index scores always had other evidence of introgression. For example in the upper main-stem Jocko River (site 28), we detected (with one exception) intermediate or high HI individuals along with low, non-zero HIs (≤ 0.14).

Thus, presence of individuals with high HI values had very little influence on the magnitude of pRBT measured in a sample.

With the exception of Pistol Creek and Liberty Creek, RBT and high-HI individuals were found primarily at lower elevation sites and in relatively close proximity to the lower main-stem Jocko River populations (Figure 2.3). Of the twelve samples with pRBT > 0.10, only Pistol Creek and Liberty Creek occurred higher than the average sample site elevation (1248m) for this study (Figure 2.3). Even though these sites initially appeared to be potential high elevation sources of RBT alleles (Figure 2.3), these samples were located above barrier falls and appeared to have limited influence on local introgression. Therefore, these sites were removed from further landscape-scale analyses.

Landscape Factors Influencing Introgression

We used pRBT as the response variable in the landscape models as it was correlated with other indicators of introgression (see above) and would allow us to compare these results with published studies. We observed a strong linear relationships with significant negative slopes between logit transformed pRBT and log transformed distance (R^2=0.44, $F_{1,56}$=43.1, p<0.001), log transformed elevation (R^2=0.43, $F_{1,56}$=43.2, p<0.001), and slope (R^2=0.47, $F_{1,56}$=49.8, p<0.001) (Figure 2.4). The best fitting multivariate regression model included slope and the number of barriers (Akaike *w* = 0.74, Table 2.2). The second best fitting model (ΔAIC_c = 2.1) was the model with slope, number of barriers, and road densities (*w* = 0.26). Even though this model including both barriers and road densities was near 2.0 ΔAIC_c of the top model, the coefficient for road density was not significant in any model. In the top model, the coefficients for both variables were significant and negative (Slope coeff = -0.36, S.E. = 0.06, t = -6.3, $p < 0.001$; Barr coeff = -0.52, S.E. = 0.13, t = -3.9, $p < 0.001$). There were few differences among models with log(distance) and log(elevation) (Table 2.2).

Influence of Sample Design Tradeoffs on Hybridization Metrics

When all samples sites from the South Fork Jocko River were treated as if they were a single random sample, pRBT was 0.014 and we determined it was not a hybrid swarm. Five of the 11 sample sites in the South Fork Jocko River statistically conformed to a hybrid swarm when those samples were examined individually. In the resampling effort, the presence of hybridization was similar across all scenarios, but the likelihood of detecting a highly hybridized fish increased with sample size and spatial extent (number of sites). Hybridization (P/A hybrids) was detected in >89% of subsamples across all sampling scenarios (Table 2.3). More than 99% of the samples with 60 or 90 fish detected the presence of hybrids (Table 2.3). Detecting high-RBT hybrids in >80% of samples required at least 60 fish samples, and high-RBT hybrids were detected in at least 95% of samples with 90 fish (Table 2.3). Increasing the number of sites sampled improved detection rates for high-HI hybrids relative to the one sample site scenarios.

Resampling the dataset often resulted in a slight over-estimation of pRBT relative to the baseline value created by treating the entire South Fork Jocko River dataset as a single random sample (pRBT = 0.014 using entire dataset, mean pRBTs range from 0.013 to 0.020, see Figure 2.6 for distributions of pRBT from resampling). The overestimation of pRBT was exacerbated by sampling across more sites.

The sample size influenced whether one would conclude the sample is a hybrid swarm. Samples statistically conformed to a hybrid swarm in 28-46% of subsamples with 30 fish, and in 1-22% of subsamples with 60 or 90 fish. We observed a substantial increase in the percentage of simulations (8-13%) conforming to hybrid swarms in the subsamples where all samples were pooled relative to all other scenarios with 60 or 90 fish (Table 4). Samples did not conform to a hybrid swarm due to either the presence of high-HI hybrids in the sample, or the complete absence of hybrids in the sample.

Discussion

At a landscape scale, we observed strong patterns of RBT introgression in the Jocko River basin that were significantly and negatively associated with elevation, distance from source and a composite variable of slope (Δ elevation/distance from source). All of these explanatory variables are highly correlated and the effects of each cannot be separated. Populations of parental-type RBT occurred at low elevation sites and parental-type WCT occur at high elevation sites. Separation of these parental types has been influenced by the presence of barriers that provide resistance to hybrid and RBT dispersal. However, WCT parental types were found in some streams where there were no barriers, such as in Hewolf Creek (sites 7, 8) and South Fork Valley Creek (site 10).

Liberty Creek and Pistol Creek were outliers to these generalizations. Although a few high-HI hybrid individuals were found in other South Fork Jocko River sample sites (eight individuals in the main-stem South Fork, two individuals in tributaries), maximum pRBT in the remainder of that system was low (0.071). Of the eight high-HI hybrids detected in the main-stem South Fork Jocko River, five were detected within 1.5km of Liberty Creek, indicating that any introgression influence from Liberty Creek fish was highly localized relative to the influence of the main-stem Jocko River and had a minor influence on the broader landscape patterns. There are steep cascades and falls that are likely migration barriers near the mouths of Liberty Creek, as well as at Pistol Creek, which also had high pRBT. There are no official records for RBT stocking in these populations, and anecdotal evidence suggests these systems may have been fishless prior to unauthorized RBT introductions upstream of barrier falls as many as 70 years ago (Craig Barfoot, personal communication). Pistol Creek appeared to be a very small population, as only five individuals could be captured after extensive sampling.

The observed patterns of introgression indicate sampling design is important when attempting to fully understand distributions of hybrids and status of conservation populations. When sampling in putative conservation populations, researchers will need to complete sampling with large sample sizes collected in a spatially extensive manner in order to have a high probability of detecting high HI hybrids where introgression is spatially structured. For example, in subsamples, we observed 100% detection with 90 fish collected at 3 sites (separated by 1-2km). Overall, tradeoffs in sample design depend on the question being asked. In this study, sample sizes of 30 individuals, collected across all site number scenarios, were adequate for detecting hybridization, and obtaining a reasonable estimate of pRBT. Kanda et al. (2002) developed hybrid detection probabilities that suggested 30 fish was a sufficient sample size to have a 0.95 probability of detecting at least 0.01 pRBT when ≥ 5 diagnostic markers are used and sample sizes of 25-30 fish are commonly reported in the literature. Detection of high-HI hybrids as evidence of long-distance dispersal or recent introgression depended strongly on sample size. Detection of hybrid swarms also depended strongly on sample size and is associated with detection of rare, high-HI individuals; detection of a hybrid swarm became less likely with an increase in sample size, and an increase in the length of the sampling frame. So, while a sample size of 30 individuals is adequate for detecting hybridization, more samples are required to accurately describe the presence of high-HI hybrids, which are important evidence of continued introgression into low pRBT hybrid swarms.

Following the criteria outlined by UDWR (2000), introgressed WCT populations in tributaries in the Jocko River system would generally be considered “conservation populations” (<10% pRBT, WCT phenotypes) but not “core populations” (no detectable introgression), with a few exceptions. Although evidence of introgression in our study was ubiquitous, levels were generally low and resulted from a few individuals in a sample having only one or two rainbow trout alleles. While this finding was slightly biased by increased sampling effort in the upper Jocko River, the general conclusion that most samples had a low pRBT would hold even if we collapsed multiple samples in the North Fork Jocko River above site 35, and in the Middle and South Forks. Samples with no detectable introgression were collected in 10 locations throughout the Jocko River Basin, primarily in the highest elevation sites in a watershed (Figure 2.2; Table 2.1). However, three of these samples were collected at sites where hybrids were detected at adjacent sites upstream and downstream. Rainbow trout alleles were very rare in those adjacent sites, suggesting that we did not detect hybridization at the sites in question simply by chance. Likely exceptions to this include Agency Creek (sample 20) and

South Fork Jocko River (sample 55), where putative barriers separate these sites from other sites having RBT alleles (Figures 2.1 and 2.2)

Landscape Patterns in Introgression

In this study, average stream slope, which is a composite variable of distance and change in elevation from source, was the best fit model for explaining patterns of introgression (Figure 2.4). The analysis of these landscape variables do not tell us what the mechanism is that is driving “resistance” of introgression; however, given the consistencies observed among this and other studies, it clear that these variables should give important clues. Gradient is believed to provide some resistance to invasion of non-native species, especially brook trout (Fausch et al. 2007, Peterson et al. 2008). Muhlfeld et al. (2009c) did not find that gradient was associated with RBT introgression, but they measured gradient at the reach scale, rather than the total gradient between the RBT source and sample sites. Slope and elevation are likely important variables because they incorporate distance and other effects, such as change in temperature and stream size that have been demonstrated to be associated with RBT naturalization (Paul and Post 2001) and introgression (Rasmussen et al. 2010). Those same gradients have also been linked to distribution patterns of RBT, hybrids, and WCT (Weigel et al. 2003). Rasmussen et al. (2010) provided evidence that elevation was also related to life history differences between WCT and RBT. In a telemetry study, Muhlfeld et al. (2009b) found RBT preferentially spawned in low elevation reaches, WCT in headwater reaches, and hybrids in reaches intermediate to both. Boyer et al. (2008) demonstrated RBT and highly hybridized populations occurred in low elevation stream systems. In a related study, Muhlfeld et al. (2009c) established that logistic models, which included variables related to stream temperature, disturbance measures, and distance to source of RBT (all of which co-vary with elevation), best approximated the presence of hybridization in the North Fork Flathead River, MT. Similar patterns have been observed in a Yellowstone cutthroat trout and RBT system in the South Fork Snake River, where cutthroat trout spawned in high elevation reaches, RBT in low elevation reaches, and hybrids at intermediate elevations (Henderson et al. 2000). Genotypes in these streams suggest that introgression is related to elevation and distance to source (Gunnell et al. 2007).

In addition to elevation, distance from source of RBT has been a commonly described driver of introgression patterns by authors, and we observed a strong relationship between these variables in the Jocko River. Boyer (2008) described a strong negative correlation between proportion admixture and distance from the most likely source population of RBT. Spatial analysis of WCT x RBT hybrid zones in the upper Kootenay basin in British Columbia indicated decreasing hybridization with increasing distance from Koocanusa Reservoir, the most likely source of RBT (Rubidge and Taylor 2004). In addition, Weigel et al. (2003) and Gunnell et al. (2007) found evidence that distance from nearest stocking location influenced hybridization, but logistic regression models in Weigel et al. (2003) including this metric had relatively little support compared to models that incorporated elevation and stream width. This may indicate that historical stocking locations may not be the most appropriate choice when determining a RBT source. RBT were found in relatively low elevation sites in mountain streams in Alberta, despite stocking locations often occurring at higher elevations (Paul and Post 2001), indicating there is potential for habitat preferences drive establishment.

At first glance, it may seem counter-intuitive that distance would be strongly negatively correlated with pRBT, and simultaneously, slope is also strongly negatively correlated with pRBT, because when calculating slope, distance is in the denominator. In the Jocko River system, sites with relatively high pRBT were often found at relatively longer distances from the lower mainstem source of RBT when those sites were still in the low elevation, low gradient, valley bottom reaches of the stream network (e.g. sites 11, 28, 32), where we would expect a greater influence of RBT (Paul and Post 2001). Using slope as a variable to explain the major patterns of introgression better accounts for these sites relative to use of distance alone. As the stream network extends in to the more montane reaches, slope and distance have a stronger positive correlation due to the associated increase in elevation.

Certainly, barriers have played a role in maintaining some separation between species in the Jocko River Basin, as the K-Canal and S-Canal diversions have reduced or eliminated passage for 90-100 years into the upper Jocko River (with the exception of introduction of RBT in Liberty Creek). Still, some reproductive isolation has been maintained in South Fork Valley and Hewolf Creeks where there are no barriers. This finding indicates barriers are not required for maintaining reproductive isolation, because even locations with no barriers are demonstrating similar patterns of reproductive isolation and decreasing pRBT with increasing elevation or distance. Reproductive isolation has been observed in several other systems, particularly when RBT and cutthroat trout are naturally sympatric (Kozfkay et al. 2007; Ostberg et al. 2004), but also in cases where RBT are introduced (Ostberg and Rodriguez 2006).

Sample Design Tradeoffs

Results of the landscape scale analyses in this and other studies suggest that, in general, the spatial scale examined will have major influences on interpretation of patterns of hybridization. Parental type WCT and RBT still co-occur in systems that are resistant, but not completely closed to invasion. However, when analyzed at reach or even large tributary (such as the South Fork Jocko River) scale, many samples will appear to be hybrid swarms. Many population genetics studies on cutthroat trout suggest substantial population subdivision at relatively small spatial scales (e.g. Nielsen and Sage 2002; Taylor et al. 2003; Wofford et al. 2005) indicating reach or tributary scales may be more appropriate for understanding "population-level" hybridization dynamics. Often, strong patterns of differentiation described in such studies are driven by including isolated and resident populations in analyses.   However, connectivity and dispersal due to increased migratory life histories often reduce genetic variation among populations and result in population genetic structures consistent with an isolation-by-distance pattern, where population boundaries become less clear (Taylor et al. 2003; Neville et al. 2006). Thus, in systems such as the Jocko River, with connectivity and migratory life histories, it is prudent to consider patterns of introgression at multiple scales.

At the scale of a large tributary, such as the South Fork Jocko, the tradeoff in individuals and sites sampled depended on the metric of primary interest. A typical 30-fish sample from a single site is suitable for detecting hybridization and provides a reasonable estimate of pRBT for a stream, so long as it is reasonable to consider the whole stream a single population. Estimates of admixture (such as pRBT) assume a population is a hybrid swarm (Kanda et al. 2002). Given the sensitivity of detection of a hybrid swarm to sampling approach (Table 2.3, but also see below), managers should be cautious in using pRBT and hybrid swarm status to apply conservation value to populations without a rigorous examination of the distribution of hybrid alleles in the population sample to determine the relative number of hybrid genotypes and the degree to which backcrossing has occurred.

Samples with 30 fish detected high-RBT hybrids at relatively low rates (47-68% of samples) because these individuals were relatively rare. Samples with 60 or 90 fish performed substantially better at detecting high-RBT hybrids. While they are rare, high-RBT hybrids may be important individuals because they represent more recent introgression or potential introgression between parental types in a system where the sample distribution of HIs indicates introgression is old and/or primarily facilitated by hybrids backcrossing to WCT (Rubidge and Taylor 2004). Often, managers are interested in maintenance of current (usually low) levels of admixture. These high-RBT hybrids pose an important threat to conservation populations and have been shown to have potentially long dispersal distances relative to WCT (Boyer et al. 2008). Researchers and managers that compile a basin-wide dataset, such as the one described in this paper, with samples from all fish bearing streams, have the opportunity to determine the most likely sources of these high-HI hybrids. In this case, the rare high-HI hybrids in the South Fork Jocko River dataset are most likely the result of introgression from fish falling over the barrier at the mouth of Liberty Creek, or from long distance dispersers from the mainstem Jocko River.

Many more subsamples were hybrid swarms based on the binomial test when sample sizes were set at 30 versus 60 or 90 fish. This suggests that, in stream systems with low levels of hybridization, whether or not a population is called a hybrid swarm strictly based on a statistical test may be an artifact of low sample sizes and non-detection of rare high-RBT individuals (if they are present). In reality, these samples may represent underlying hybrid swarms with very low pRBT and high-HI individuals are evidence of recent movement from another population. Populations with low pRBT are likely very common across the range of WCT. Shepard et al. (2005) reported that ~41% of WCT populations (totaling 22,388 km of stream) are measured as unhybridized, are suspected to be unhybridized, or have ≤ 0.10 pRBT. In addition to detection issues related to rare high-HI individuals, we have demonstrated that spatial structuring of hybridization is a consistent pattern in multiple river systems, where hybridization is negatively correlated with both distance from the source of RBT and elevation. This indicates establishing a signal of random mating in a sample will be largely dependent on the spatial scale at which fish are collected, where the broader the sampling frame the less likely the samples will be collected from genetically similar individuals. In the Jocko River, hybrid swarms were detected at some of the sample sites, but only with low rates of introgression and many generations of backcrossing to WCT. Detailed understanding of the population genetic structure in a system would be useful for determining the scale at which individuals or population samples would be genetically similar, but typically this information is unavailable prior to hybrid distribution studies. In general, the number of samples that conformed to a hybrid swarm decreased with increases in the length of the sampling frame.

These results suggest researchers should be cautious when interpreting the results of a hybridization study using a single frequency distribution of hybrid individuals, as this model does not incorporate spatial structuring that seems to be common across hybridized systems. Hybrid zones, defined as areas where two distinct taxonomic groupings overlap and interbreed to produce hybrid offspring of mixed ancestry, commonly occur in many taxa (Arnold 1997), including fishes (Epifanio and Nielsen 2000; Perry et al. 2002). Stable hybrid zones generally rely on selection acting on hybrids, and selection may either be environmentally dependent (mosaic hybrid zone, bounded hybrid superiority zone) or environmentally independent (tension hybrid zone) (Taylor 2004). While authors have described expansion of hybridization between cutthroat trout and RBT as a function of time (Hitt et al. 2003), or simply as a function of space (Rubidge and Taylor 2005; Boyer et al. 2008), other authors (e.g. Weigel et al. 2003; Gunnell et al. 2007; Rasmussen et al. 2010) have suggested there may be important environmental limitations to introgression. Additionally, some authors have suggested there may be strong fitness consequences to hybridization between WCT and RBT (Allendorf et al. 2001; Muhlfeld et al. 2009a). A better understanding of the hybrid zone, its stability over time, and potential environmental and fitness limits to introgression are necessary as we design optimal sampling protocols and evaluate conservation efforts.

Study Limitations and Suggestions for Future Research

While we believe the results and conclusions of this study to be sound, there are some important limitations to this work that should be addressed. The most important of these limitations may be our inability to guarantee that every individual fish collected as part of a sample is a member of a local "population." However, given the goal of this study was to evaluate the change in magnitude of hybridization along landscape gradients, rather than develop a population-level point estimate of admixture, and pRBT was highly correlated with other hybridization metrics (see results above), we determined it to be an effective and appropriate metric. We recommend that researchers strongly consider the implications of sampling multiple populations at a single site to determine if sampling can be focused in reaches and during time periods that minimize this issue. In this study, we focused sampling efforts where a) resident life history individuals dominated the adult portion of the samples, or b) we could sample during spring spawning while adult fish were on redds. We clearly sampled individuals at some sample sites that could have been from distant populations given the exceptional size of these individuals or as evidenced by an exceptional HI relative to the rest of the sample. However, rather than assuming these individuals were not members of the local population and simply removing them from analysis, we made the assumption that these fish represented current or future hybridization potential for the local population and were therefore reflective of the patterns we were attempting to describe. It is important to note that in most samples hybrid individuals generally had a size distribution similar to other fish captured in the sample (see Appendix A for details).

The patterns of introgression we described in this study were well correlated with important master variables such as elevation, which should be highly correlated with habitat variables, such as stream temperature and size, reach-scale gradients, etc. However, the conclusions of this study are limited by the coarse scale of the data collected and by the fact that we use surrogate variables to describe habitat gradients, rather than using site-specific estimates of elevation, stream width, stream habitat types, etc. Furthermore, this study was not designed to establish the true mechanisms underlying the introgression patterns we observed. For example, we conclude in this study that patterns of introgression observed in this and other studies are concordant with hybrid zone models. As described above, the fitness of hybrids relative to parental types is likely to vary with habitat in mosaic or bounded hybrid superiority hybrid zones, thus driving landscape-scale introgression patterns. We recommend that future research efforts should attempt to relate fitness surrogates, such as survival, age at maturity, and reproductive success, to habitat factors that are likely to influence habitat choice, such as stream temperatures, physical habitat structure, and productivity.

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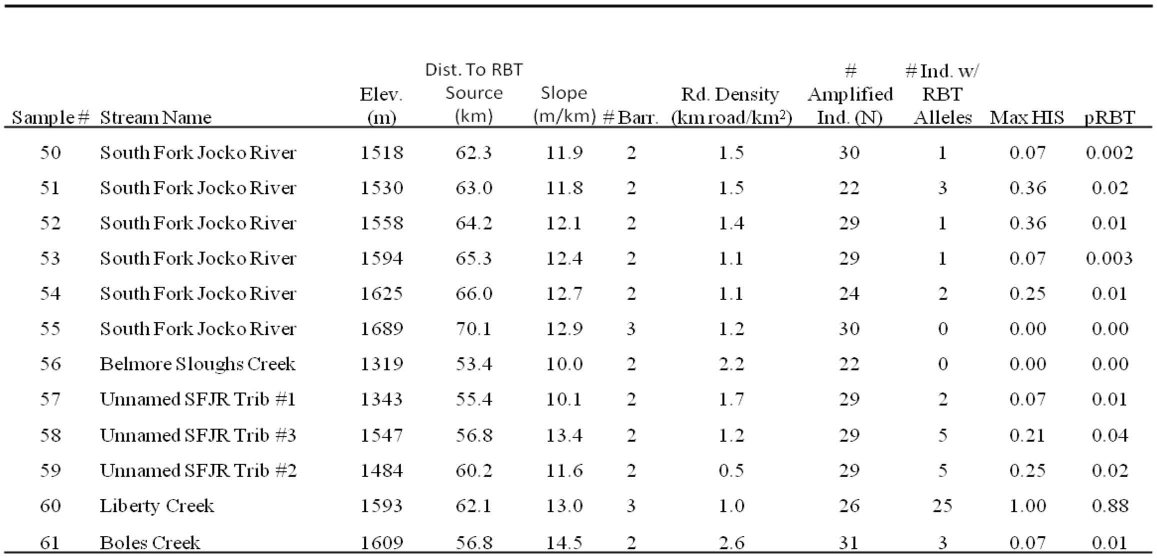
Table 2.1. Site code, along with landscape variables measured for this study, sample sizes, number of individuals with RBT alleles detected, maximum individual hybrid index score detected (Max HI), and sample proportion RBT alleles (pRBT). Sites correspond to labels in Figure 2.1.

Sample #	Stream Name	Elev. (m)	Dist. To RBT Source (km)	Slope (m/km)	# Barr.	Rd. Density (km road/km^2)	# Amplified Ind. (N)	# Ind. w/ RBT Alleles	Max HIS	pRBT
1	Jocko River	784	0.0	0.0	0	1.8	83	80	1.00	0.92
2	Spring Creek	847	14.7	4.3	0	2.1	23	23	1.00	0.97
3	Valley Creek	854	13.6	6.0	0	2.2	28	28	1.00	0.98
4	Valley Creek	879	16.0	5.6	0	2.2	13	13	1.00	0.86
5	Valley Creek	925	19.3	7.3	0	2.5	30	20	1.00	0.25
6	North Fork Valley Creek	1180	25.7	15.4	1	2.3	23	1	0.07	0.003
7	Hewolf Creek	1140	26.8	13.3	0	2.0	18	1	0.07	0.004
8	Hewolf Creek	1191	27.4	14.8	0	1.1	7	0	0.00	0.00
9	Hewolf Creek	1201	27.8	15.0	1	1.1	26	2	0.08	0.01
10	South Fork Valley Creek	1057	25.2	10.9	0	2.4	27	1	0.07	0.003
11	Finley Creek	964	28.9	6.2	0	2.4	35	35	1.00	0.85
12	Finley Creek	1007	31.2	7.1	0	2.3	29	14	0.93	0.13
13	Finley Creek	1012	31.7	7.2	0	2.3	25	9	0.50	0.07
14	Finley Creek	1186	38.5	10.4	1	3.1	30	3	0.17	0.01
15	Finley Creek	1185	39.3	10.2	1	3.1	21	12	0.36	0.08
16	Finley Creek	1191	40.0	10.2	1	3.1	17	3	0.10	0.01
17	Finley Creek	1256	45.3	10.4	5	3.8	30	0	0.00	0.00
18	Agency Creek	1107	37.3	8.7	3	0.7	29	1	0.21	0.01
19	Agency Creek	1131	37.4	9.3	4	0.6	30	0	0.00	0.00
20	East Fork Finley Creek	1157	36.3	10.3	2	0.5	22	2	0.10	0.01
21	Schley Creek	1135	36.3	9.7	2	0.3	30	0	0.00	0.00
22	Kitty Girl Creek	1304	35.5	14.7	1	3.2	18	3	0.07	0.01
23	Unnamed Finley Cr. Trib #6	1345	44.5	12.6	3	2.1	25	1	0.07	0.003
24	Frog Creek	1314	41.1	12.0	3	1.8	28	1	0.07	0.003
25	Big Knife Creek	1122	37.0	9.1	0	1.3	29	10	0.36	0.06
26	Cold Creek	1150	43.3	8.4	2	0.1	14	2	0.25	0.01
27	Pistol Creek	1269	45.7	10.6	2	3.5	5	5	0.60	0.42
28	Jocko River	1121	30.7	11.0	1	1.2	30	7	1.00	0.14
29	Jocko River	1125	31.7	10.9	1	1.2	30	1	0.07	0.002
30	Jocko River	1172	34.7	11.2	2	1.3	29	0	0.00	0.00
31	Kelly's Creek	1257	35.5	13.3	1	1.0	14	0	0.00	0.00
32	North Fork Jocko River	1147	33.4	10.9	1	0.8	6	3	1.00	0.32
33	North Fork Jocko River	1188	35.9	11.3	1	0.5	29	4	0.86	0.06
34	North Fork Jocko River	1220	39.2	11.1	1	0.3	28	3	0.07	0.01
35	North Fork Jocko River	1262	40.2	11.9	1	0.5	30	6	1.00	0.13
36	North Fork Jocko River	1277	41.2	12.0	2	0.3	26	0	0.00	0.00
37	North Fork Jocko River	1304	42.2	12.3	2	0.4	28	4	0.14	0.01
38	North Fork Jocko River	1373	43.2	13.6	3	0.4	31	3	0.14	0.01
39	North Fork Jocko River	1428	44.0	14.5	3	0.4	29	0	0.00	0.00
40	Middle Fork Jocko River	1221	37.5	11.6	2	0.7	29	7	0.08	0.02
41	Middle Fork Jocko River	1249	38.5	12.1	2	0.7	34	3	0.07	0.01
42	Middle Fork Jocko River	1246	39.5	11.7	2	0.6	30	3	0.14	0.01
43	Middle Fork Jocko River	1306	41.5	12.6	2	0.6	20	6	0.07	0.02
44	Middle Fork Jocko River	1342	43.6	12.8	2	0.6	38	2	0.14	0.01
45	South Fork Jocko River	1222	49.7	8.8	2	1.5	33	3	0.07	0.01
46	South Fork Jocko River	1280	52.3	9.5	2	1.5	29	2	0.07	0.005
47	South Fork Jocko River	1304	54.3	9.6	2	1.5	28	2	0.42	0.02
48	South Fork Jocko River	1357	57.3	10.0	2	1.4	30	4	0.07	0.01
49	South Fork Jocko River	1451	61.3	10.9	2	1.4	30	8	0.86	0.07

Table 2.1 Continued.



Sample #	Stream Name	Elev. (m)	Dist. To RBT Source (km)	Slope (m/km)	# Barr.	Rd. Density (km road/km^2)	# Amplified Ind. (N)	# Ind. w/ RBT Alleles	Max HIS	pRBT
50	South Fork Jocko River	1518	62.3	11.9	2	1.5	30	1	0.07	0.002
51	South Fork Jocko River	1530	63.0	11.8	2	1.5	22	3	0.36	0.02
52	South Fork Jocko River	1558	64.2	12.1	2	1.4	29	1	0.36	0.01
53	South Fork Jocko River	1594	65.3	12.4	2	1.1	29	1	0.07	0.003
54	South Fork Jocko River	1625	66.0	12.7	2	1.1	24	2	0.25	0.01
55	South Fork Jocko River	1689	70.1	12.9	3	1.2	30	0	0.00	0.00
56	Belmore Sloughs Creek	1319	53.4	10.0	2	2.2	22	0	0.00	0.00
57	Unnamed SFJR Trib #1	1343	55.4	10.1	2	1.7	29	2	0.07	0.01
58	Unnamed SFJR Trib #3	1547	56.8	13.4	2	1.2	29	5	0.21	0.04
59	Unnamed SFJR Trib #2	1484	60.2	11.6	2	0.5	29	5	0.25	0.02
60	Liberty Creek	1593	62.1	13.0	3	1.0	26	25	1.00	0.88
61	Boles Creek	1609	56.8	14.5	2	2.6	31	3	0.07	0.01

Table 2.2. Akaike Information Criterion (AIC) results for each of the candidate models in this study. Models were ranked by their ΔAIC_c values, likelihood of being the best model given the data (Lik.), and Akaike weights (w_i) (k is the number of parameters in the model). Model variable abbreviations included Elev (elevation), Barr (barriers), Dist (distance from source), and RdDens (road densities).

Model	k	AIC	AIC_c	ΔAIC_c	Lik.	w_i
Slope+Barr	3	170.69	171.1	0.0	1.0	0.74
Slope+Barr+RdDens	4	172.48	173.2	2.1	0.4	0.26
Slope	2	183.14	183.3	12.2	0.0	0.00
Slope+RdDens	3	183.98	184.4	13.3	0.0	0.00
log(Elev)+Barr	3	185.23	185.7	14.5	0.0	0.00
log(Dist)+Barr	3	186.33	186.8	15.6	0.0	0.00
log(Elev)	2	186.89	187.1	16.0	0.0	0.00
log(Dist)	2	186.93	187.1	16.0	0.0	0.00
log(Elev)+Barr+RdDens	4	186.48	187.2	16.1	0.0	0.00
log(Dist)+RdDens	3	187.61	188.0	16.9	0.0	0.00
log(Dist)+Barr+RdDens	4	187.39	188.1	17.0	0.0	0.00
log(Elev)+RdDens	3	187.73	188.2	17.0	0.0	0.00

Table 2.3. The number of subsamples out of 500 simulations detecting hybridization, a high RBT hybrid (HI ≥ 0.20), or a hybrid swarm. Under # sites, ASP represents the "all samples pooled" scenario.

Scenario		Samples detecting	Samples detecting	Samples detecting
Fish (N)	# Sites	hybrids (%)	high-HIS (%)	Hybrid swarms(%)
30	1	459 (91.8)	233 (46.6)	229 (45.8)
30	3	449 (89.8)	289 (57.8)	172 (34.4)
30	6	474 (94.8)	340 (68)	138 (27.6)
30	ASP	463 (92.6)	275 (55)	208 (41.6)
60	3	496 (99.2)	448 (89.6)	65 (13)
60	6	498 (99.6)	467 (93.4)	41 (8.2)
60	ASP	499 (99.8)	409 (81.8)	106 (21.2)
90	3	500 (100)	500 (100)	3 (0.6)
90	6	498 (99.6)	489 (97.8)	17 (3.4)
90	ASP	500 (100)	476 (95.2)	32 (6.4)

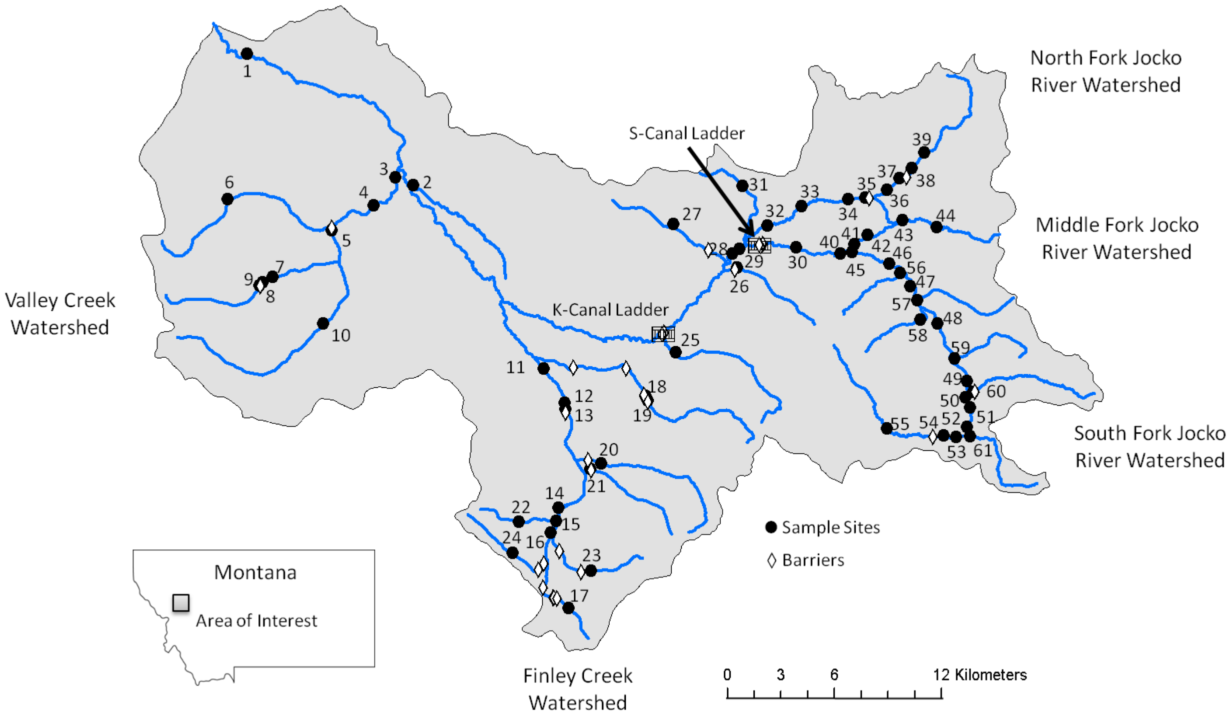


Figure 2.1. Sample locations and distribution of barriers in the Jocko River watershed, Montana. Map includes all fish bearing streams in the system.

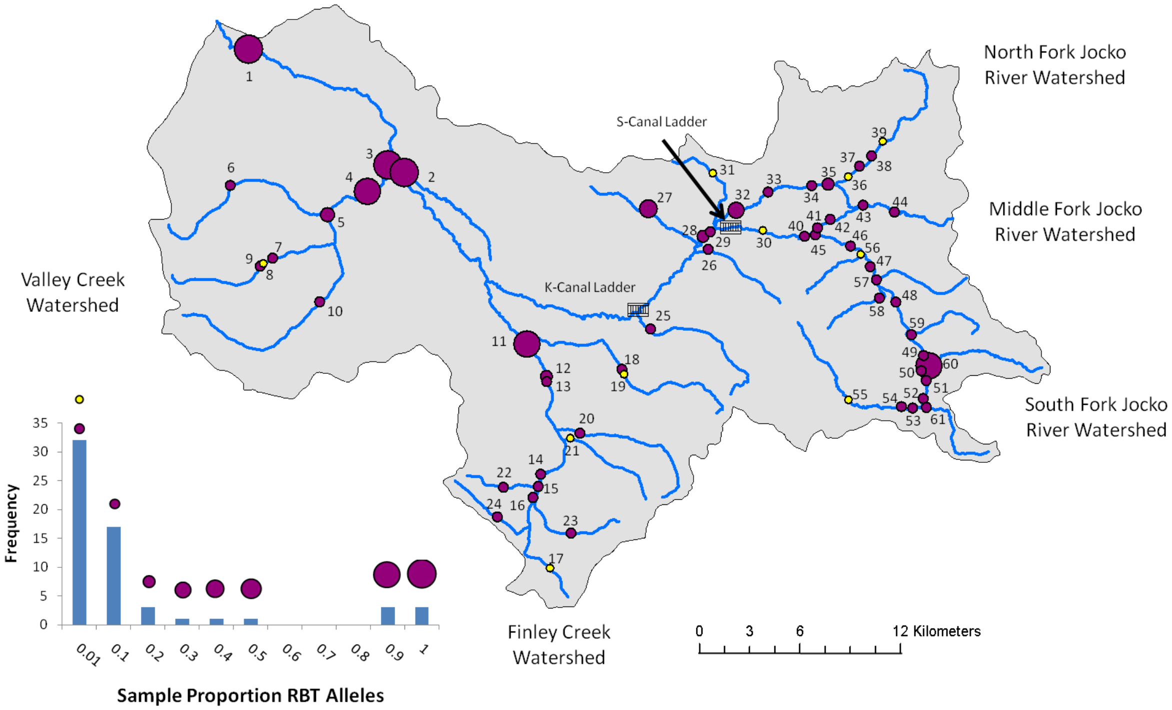


Figure 2.2. Distribution of sites where no hybridization was detected (yellow markers) and sites where at least one RBT marker in one individual was detected (purple markers). The size of the markers represents the pRBT estimated for each sample. The histogram inset describes the frequency distribution of pRBT in the Jocko River basin. The sizes of the markers positioned above the histogram bins correspond to the size of the makers on the map.

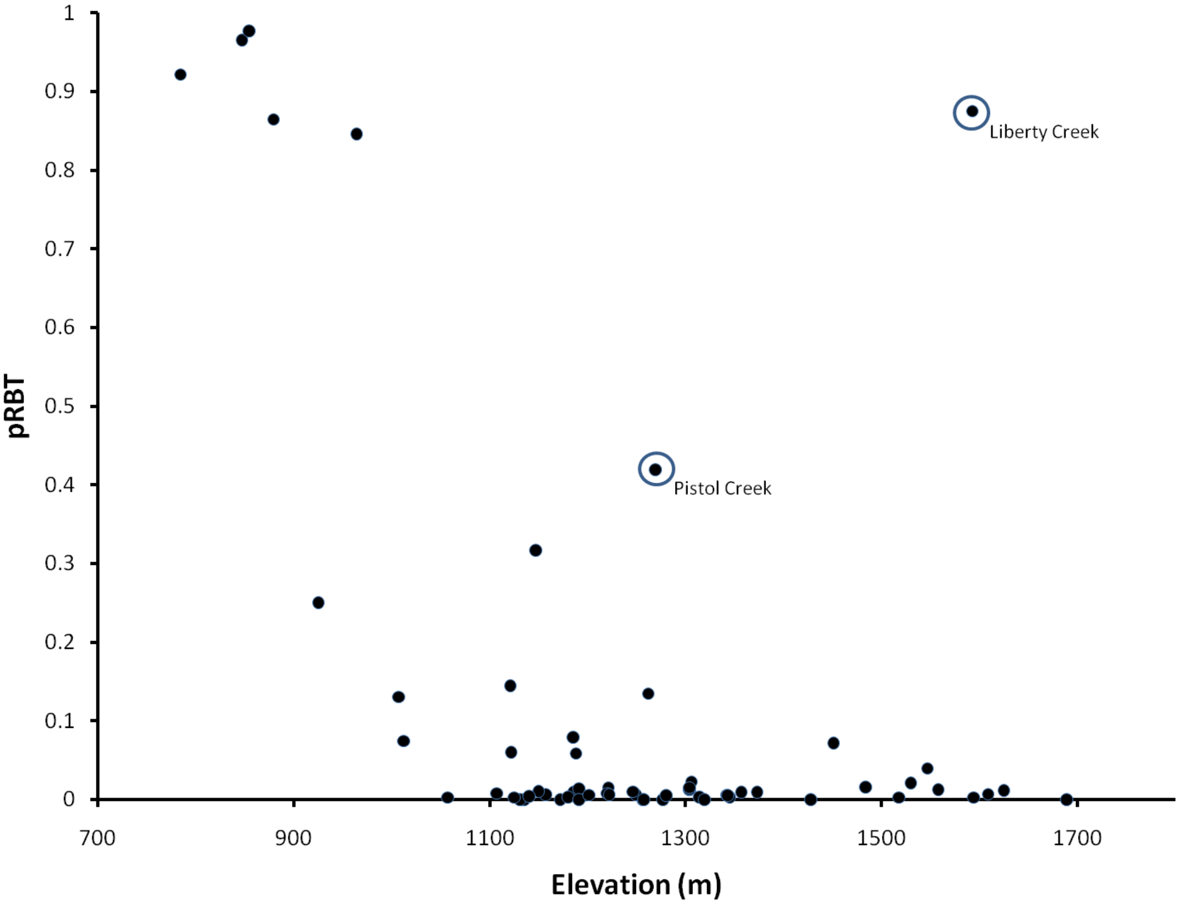


Figure 2.3. Relationship between elevation and pRBT. Liberty Creek and Pistol Creek were removed from subsequent analyses because they were both located above barrier waterfalls, and they did not appear to be contributing to introgression at neighboring sites.

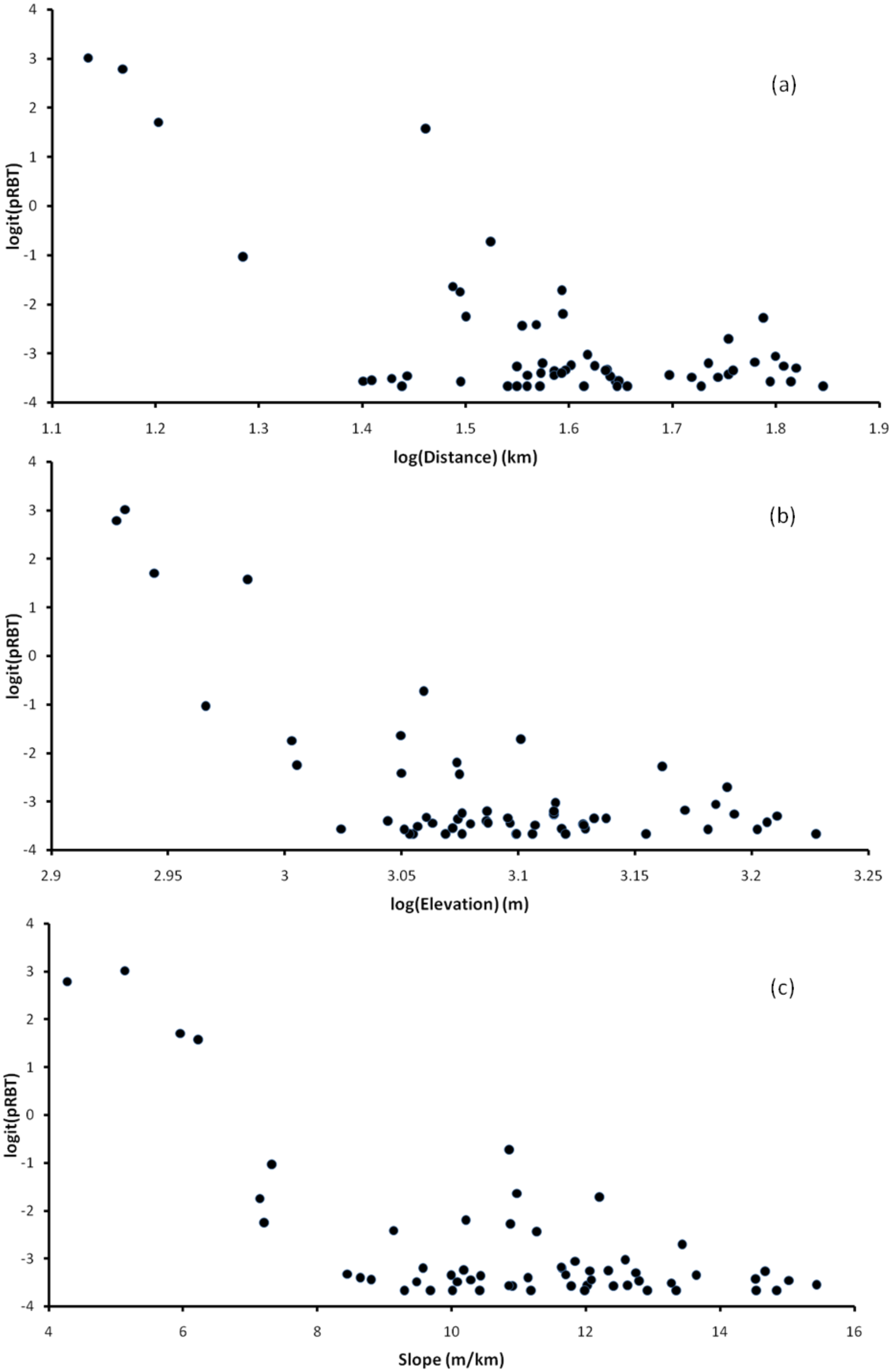


Figure 2.4. Relationships between pRBT and distance (a), elevation (b), and slope (c).

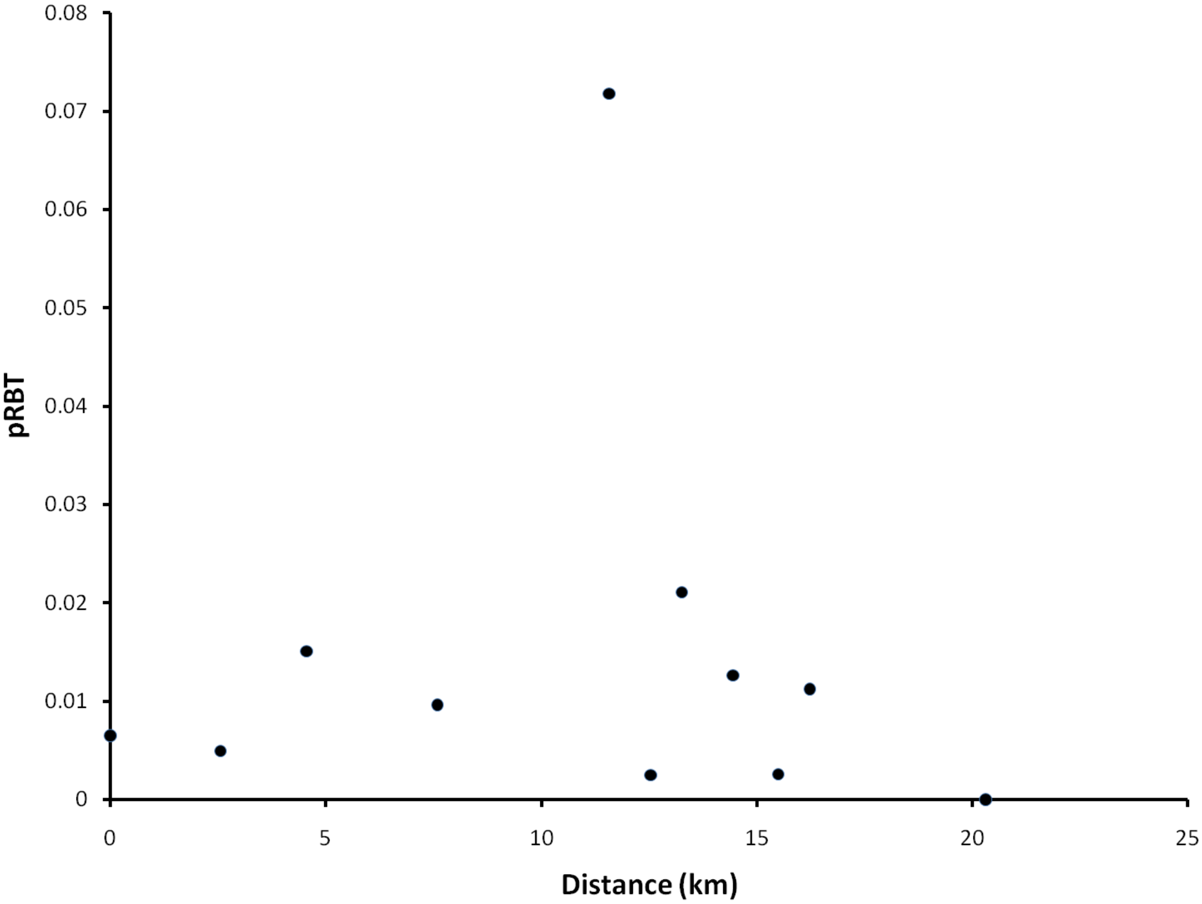


Figure 2.5. Spatial distribution and pRBT of 11 sample locations in the main-stem South Fork Jocko River that comprise the dataset used for sub-sampling simulations. The x-axis represents the distance from the confluence of the South and Middle Forks.

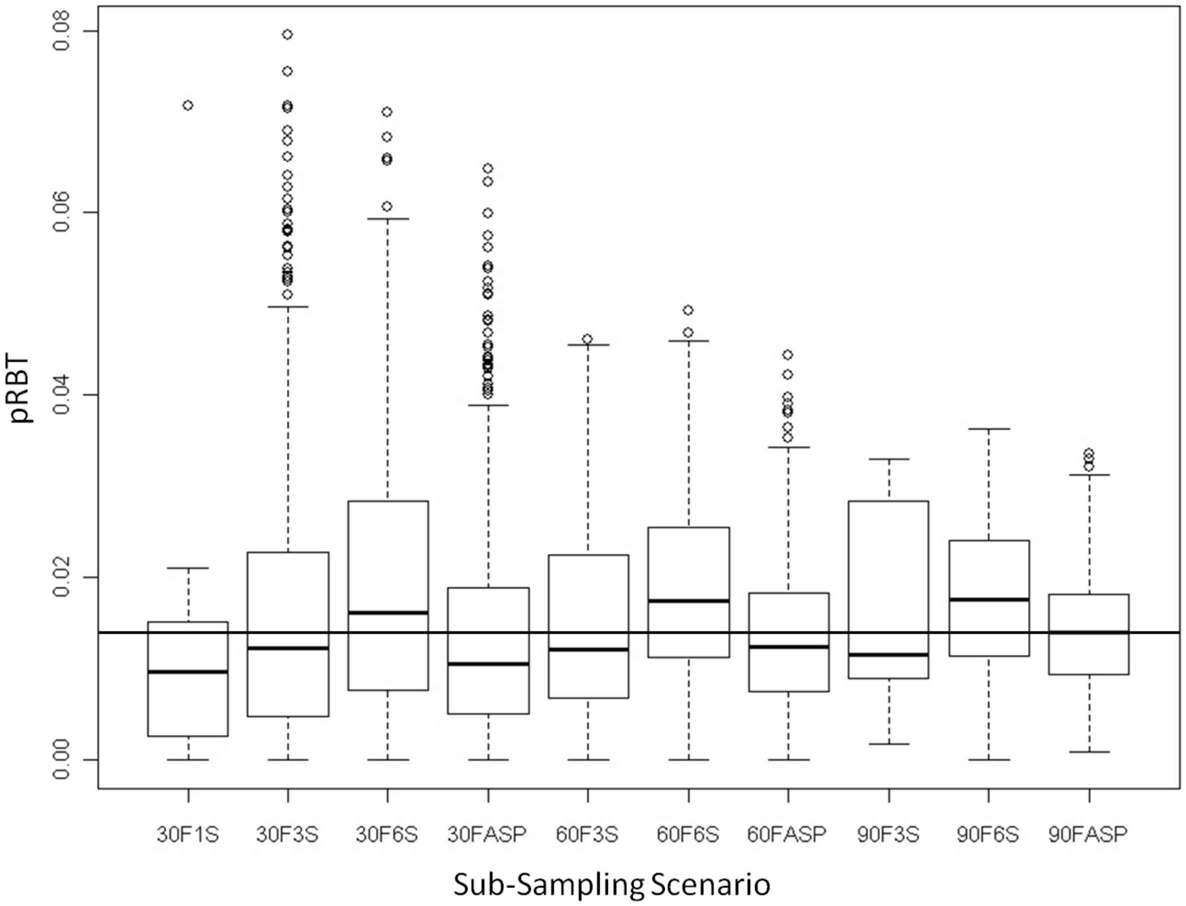


Figure 2.6. Distribution of estimated pRBT for each sub-sampling scenario. Abbreviations for scenarios indicate the number of fish (30F = 30 fish) and the number of sites sampled (1S = 1 site, ASP = all samples pooled). The reference line is the estimated pRBT (0.014) of the South Fork Jocko River, if every individual were included in a single sample.

CHAPTER 3

DIFFERENCES IN GROWTH, FECUNDITY, AND MIGRATION TIMING BETWEEN WESTSLOPE CUTTHROAT TROUT, AND WESTSLOPE CUTTHROAT TROUT X RAINBOW TROUT HYBRIDS

Abstract

Hybridization with rainbow trout (*O. mykiss*, RBT) has been described as a primary threat to the conservation of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, WCT). Conservation of WCT often hinges on the degree to which the populations are hybridized, yet little is known about differences in life history of individuals with varying degrees of rainbow trout ancestry. The primary goal of this project was to test for equivalency in growth, fecundity, and migratory behavior between WCT and hybrid trout in the migratory components of trout populations in the Jocko River, MT. We sampled trout at two fish ladders making upstream spawning migrations from 2006-2009. We estimated RBT ancestry for each individual using seven diagnostic microsatellite markers. We categorized individuals into three hybrid categories: zero (no RBT markers), low (< 20% RBT ancestry), and high (≥ 20% RBT ancestry). We fit growth models to back-calculated length at age data for each hybrid category. Von Bertanlanffy parameters were significantly different ($p \leq 0.001$), with high category hybrids exhibiting substantially higher growth than zero or low hybrids. Results of analyses of covariance suggest that, for a given length, high hybrids have significantly lower fecundity ($p < 0.001$), yet larger egg sizes ($p = 0.003$) than the zero category, but the low hybrid

category was not significantly different than the zero category. Similarly, hybrids with >20% RBT ancestry migrated earlier, at lower stream temperatures, and lower discharges than both the zero and low categories. We did not observe significant differences between individuals in the zero category and individuals in the low category in migration conditions. While there was overlap in migration timing among all hybrid categories, nearly all high hybrids had finished moving into the migration trap by when the first major increase related to spring runoff was observed. We conclude that, while there is substantial evidence indicating important life history differences between WCT and hybrids with more than 20% RBT ancestry, there is still enough uncertainty regarding differences of WCT and hybrids with less than 20% RBT ancestry to warrant further study.

Introduction

Introgressive hybridization with introduced species has been described as the greatest conservation threat to many native salmonid species worldwide (Rhymer and Simberloff 1996; Allendorf et al. 2004). Rainbow trout (RBT, *Oncorhychus mykiss*) have been introduced in nearly every suitable habitat worldwide (Lowe et al. 2000; Fausch et al. 2001). They interbreed and produce fertile offspring with cutthroat trout (*O. clarkii spp.*) in areas where they are naturally and anthropogenically sympatric (Leary et al. 1995; Behnke 2002; Kozfkay et al. 2007; Metcalf et al. 2007). Westslope cutthroat trout (WCT, *O. clarkii lewisi*) and RBT are naturally sympatric in certain portions of their range, and while hybridization and introgression does occur, the two species primarily remain reproductively isolated (Kozfkay et al. 2007). However, in areas where they evolved in allopatry, but are currently sympatric, little is known about the consequences of anthropogenic hybridization on phenotypic characteristics important to population productivity and/or persistence.

Due to uncertainty about the ecological and evolutionary consequences of anthropogenic hybridization between RBT and WCT, there has been controversy regarding the role of hybridized populations in conservation policies for native WCT in areas where RBT have been introduced. States with native cutthroat trout have adopted a conservation policy, where populations with <10% RBT introgression are protected as "conservation populations" and populations with <1% introgression receive special protection as "core populations" (UDWR 2000). The subspecies was precluded from listing under the Endangered Species Act (ESA) on the basis that populations that were morphologically similar to the accepted taxonomic description for WCT existed in a large portion of their historic range (USFWS 2003). The United States Fish and Wildlife Service (USFWS) established populations within the scientifically accepted morphological norms for the subspecies would be included as WCT populations, even if detectable hybridization was present. The USFWS further established that, based on the best available science, populations with as much as 20% RBT admixture should be morphologically indistinguishable from unhybridized WCT populations, and these hybridized populations would be included as WCT for ESA consideration (USFWS 2003). These policies are designed, in part, to preserve both the “historic genome,” as well as “unique genetic, ecological, and behavioral characteristics” (USFWS 2003). These policies have received substantial criticism for many reasons, among them is the sparse evidence regarding either behavioral differences or similarities between hybrids and WCT other than potential increased straying rates of hybrids (see Allendorf et al. 2005). Thus, understanding potential differences between individuals with varying ancestry is needed to provide guidance for refinement of current hybridization policies for WCT.

Few studies have attempted to test for differences in performance or fitness in hybridized and unhybridized populations of cutthroat trout. In laboratory studies, F1 hybrids performed better as coastal cutthroat trout (*O. c. clarki*) X RBT F_1 hybrids and Yellowstone cutthroat trout (*O. c. bouveri*) X RBT F_1 hybrids achieved higher swimming speeds than either parental form (Hawkins and Quinn 1996; Seiler and Keeley 2009). Additionally, F_1 Yellowstone cutthroat trout X RBT hybrids has been shown to decrease growth in Yellowstone cutthroat trout (Seiler and Keeley 2009). In contrast, Leary et al.

(1985) described developmental instability and high fluctuating asymmetry in lab-crosses of RBT with WCT, Yellowstone cutthroat trout, and coastal cutthroat trout. Field studies that have attempted to measure selection in wild populations of cutthroat trout have been largely inconclusive (Rubidge and Taylor 2004; Ostberg and Rodriguez 2006), with three notable exceptions. Muhlfeld et al. (2009a) demonstrated declines in reproductive success by as much as 50% with as little as 20% RBT admixture estimated at the individual level of WCT. In addition, possible selection against hybrid coastal cutthroat trout was suggested from a reduction in the frequency of hybrids in two populations on Vancouver Island over time, but no such pattern was observed in 11 others (Bettles et al. 2005). Finally, maintenance of high levels of gametic disequilibrium over time was described as evidence of weak selection acting against hybrids in several tributaries in the Strait of Juan de Fuca (Ostberg and Rodriguez 2004).

There have also been relatively few studies addressing differences in behavior between cutthroat trout and hybrids. In the Flathead River, MT, Boyer et al. (2008) estimated substantially lower straying rates of WCT using genetic distances among WCT populations compared with estimated straying from highly hybridized individuals observed in otherwise unhybridized WCT populations. In two river systems, separation in migration and spawning times and places have been observed with RBT migrating and/or spawning earlier and at low elevations than YCT and WCT, with hybrids having spatial and temporal overlap facilitating introgressive hybridization between parental types (Henderson et al. 2000; Muhlfeld et al. 2009b). Specifically, RBT and RBT backcrosses migrated before increases in peak flow, at lower flows, and cooler temperatures than WCT (Muhlfeld et al. 2009b). DeRito et al. (2010) established that radio-tagged Yellowstone cutthroat trout had moderate spatial overlap in spawning distributions, but very low temporal overlap in spawn timing with RBT.

Given published studies have shown differences in selection and behavior in some systems but not in others, there is a clear need to further identify whether there are facets of life history and behavior that are consistently different between different cutthroat subspecies, RBT, and their hybrids. Behavior related to life history and vital rates related to growth (such as fecundity) are among the most important factors influencing survival, reproduction, and fitness of individuals, and consequently, productivity, persistence, and evolution of populations (Hendry and Stearns 2004; Schaffer 2004).

The primary goal of this project was to evaluate the life history differences associated with introgressive hybridization in the Jocko River, MT. This study had three objectives: (1) To test for differences in growth between WCT and hybrids with varying RBT ancestry, (2) to determine if there were subsequent differences in fecundity and egg size relationships between WCT and hybrids, and finally (3) to describe differences in migratory behavior between WCT and hybrids. Our general approach was to contrast characteristics of fish with a range of RBT ancestries. We expected higher growth rates in individuals with high RBT ancestry, because RBT are typically the dominant species in low elevation, potentially warmer, more productive habitats (Paul and Post 2001; Rasmussen et al 2010; Chapter 2), and RBT have a higher growth capacity in these warmer habitats (Bear et al. 2007). Consequently, these fish may be expected to have more resources to allocate to reproduction and have greater fecundity and egg size. Similar to previous studies, we expected fish with higher RBT ancestry to migrate earlier than those with WCT ancestry.

Study Site

This study was conducted in the Jocko River, a 979 km^2 basin managed by the Confederated Salish and Kootenai Tribes (CSKT). There are several major tributaries to the Jocko River, including the North, Middle, and South Forks of the Jocko River, as well as the Finley and Valley Creek Drainages (Figure 3.1). In general, the streams in the Jocko River system are high gradient, confined channels at higher elevations transitioning to lower gradient reaches at lower elevations.

There is an extensive irrigation system throughout the drainage that influences fish movement by creating both barriers and artificial connections. K-Canal and S-Canal diversions are irrigation structures on the Jocko River that are complete barriers to movement and migration; although, fish can occasionally pass K-Canal diversion during bank-full or higher flow events when checks are removed from the diversion (Craig Barfoot, CSKT, personal communication). Both of these diversions have had fish ladders installed with a trap and a holding pen at the top of each ladder to pass bull trout (*Salvelinus confluentus*). Traps are checked regularly by CSKT personnel.

The salmonid assemblage of the Jocko River basin is comprised of native and introduced fishes. Introduced brown trout (*Salmo trutta*) and RBT phenotypes are abundant in the main-stem Jocko River below K-Canal diversion, and RBT phenotypes are present in several tributaries throughout the system. Native WCT and mountain whitefish (*P. williamsoni*) are present in the main-stem Jocko River, and WCT are present at various abundances in nearly all fish-bearing tributaries. The three forks of the upper Jocko River have been thought to be strongholds of native fishes including unhybridized WCT and bull trout. Fluvial life history forms of RBT, WCT, hybrids, brown trout, and bull trout utilize the main-stem Jocko River and Flathead River as foraging and overwintering habitat.

Methods

Fish Sampling and Tissue Collection

We sampled all fish entering the K-Canal and S-Canal ladder traps from 2006-2009. Fish were anesthetized, total length (L, mm) was measured for each fish in the field, and they were palpated and checked for secondary sexual characteristics to assess maturity and sex. Nonlethal fin clips were collected for genetic analyses from all fish. We began lethal sampling to collect gonads for fecundity estimates and otoliths for age and growth analyses during a subset of the migration in 2007 (4/24 – 5/18), and throughout the migration period in 2008-2009.

DNA Extraction and Hybrid Analyses

We used a subset of loci (7 diagnostic microsatellites) and extraction protocols as described in Muhlfeld et al. (2009a). Ogo8 (Olsen et al. 1998), Omm1019, Omm1050, Omm1060 (Rexroad III et al. 2002), Omy 0004 (Holm and Brusgaard 1999), Omy1001 (Spies et al. 2005), and Sfo8 (Small et al. 1998) are diagnostic markers for WCT and RBT in most tested Montana watersheds (Sally Painter, Montana Conservation Genetics Laboratory, personal communication). We amplified 4 additional microsatellite markers in an unsuccessful effort to assign individuals in this study to a population of origin (see Appendix B for details). We extracted and amplified DNA in 10 ul reactions following the standard QIAGEN Microsatellite protocol. We used two different PCR profiles. Multiplex 1 consisted of Ogo8, Omm1019, Omm1050, Omm1060, and Omy0004. Multiplex 2 consisted of Omy 1001 and Sfo8. We visualized PCR products on an ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). We determined allele sizes using an ABI GS600LIZ ladder (Applied Biosystems Inc., Foster City, CA). We viewed and analyzed chromatograms using GeneMapper version 3.7 (Applied Biosystems Inc., Foster City, CA). We estimated individual levels of hybridization by calculating a hybrid index score (HI). HI is the proportion of the total number of RBT alleles observed divided by the total number of alleles amplified for each individual across all diagnostic loci. We categorized individuals with a HI = 0 as "zero," individuals with 0 < HI < 0.20 as "low," and individuals with HI ≥ 0.20 as "high." We selected these categories to specifically evaluate the 0.20 admixture cutoff suggested by the USFWS to establish populations that are morphologically indistinguishable from WCT (an average HI in a population with 0.20 admixture hybrid swarm would be 0.20).

Age and Growth Analyses

We cleared otoliths by soaking them in clove oil for 10 days to examine annular rings. We took a digital image of the distal surface of each otolith at 20x magnification in a dissecting microscope under reflected light with a micrometer in the image for conversion of pixels to mm using SPOT Advanced version 4.7 (Diagnostic Instruments Inc., Sterling, MI). We aged fish using the digital images of otoliths examined by at least two independent readers, and a consensus age was determined for any individual for which there was disagreement. Periods of slow growth appear dark or clear under reflected light (Schill et al. 2010), and we assumed these regions corresponded to winter growth. We used the program Image J version 1.44c (National Institutes of Health, Bethesda, MD) to establish the otolith origin and an axis from the most distal point on the anterior end of the otolith through the origin. We established an increment measurement axis at a 45^{o} angle toward the ventral side of the otolith from the anterior-posterior axis. This measurement axis provided the most consistent readability across all otoliths. We marked annular increments and measured increment width.

We back-calculated total length at age for each aged individual using the direct proportion (Dahl-Lea) method because the otolith is present at hatching, and no adjustment for intercept is required (Klumb et al. 2001; Kruse et al. 1997). Length at age is given by,

$L_i = \frac{S_i}{S_c} L_c$ where

$L_i =$ back-calculated total length of the fish,

$L_c =$ total length of the fish at capture,

$S_i =$ radius of the otolith at capture, and

$S_c =$ radius of the otolith to the *i*th increment (Devries and Frie 1996).

We used a von Bertalanffy growth model (as described in Isely and Grabowski 2007) fit to back-calculated lengths at ages for each of the hybrid categories. The model is of the form,

$l_t = L_\infty(1 - e^{-K(t-t_0)})$ where

l_t is total length at time *t*,

L_∞ is the asymptotic length,

K is a growth curvature coefficient,

t is the time step (age in years in this case), and

t_0 is a time coefficient at which length would theoretically be 0 (Gross et al. 2002). Parameters for growth models were estimated from a curve fit using the non-linear regression function in R (R Core Development Team 2009). We examined the residuals of the growth model fit to the entire dataset versus HI to determine changes in growth with HI. Then we segregated the data into hybrid categories and used likelihood ratio tests (Cerrato 1990; Kimura 1980) to make statistical comparisons of model parameters (and thus different growth trajectories) among the categories using the vblrt function in the R package fishmethods (Nelson 2010). Likelihood ratio tests were completed in a pairwise fashion among the three hybrid categories. Determination of differences in von Bertalanffy parameters was made using a test that simultaneously compares all parameters for each growth model ($\alpha = 0.05$).

Fecundity Analyses

In the laboratory, we assessed maturity and sex of the individual and removed all eggs and ovarian tissue from females to estimate fecundity. For each female, a subsample of approximately 100 eggs was counted and placed in a 50ml graduate cylinder, pulverized, and the volume of the subsample was recorded. Then all eggs and ovarian tissue were placed in a 1000ml graduated cylinder with 200ml of water to measure the volume of water displaced by the ovaries. Estimated fecundity is given by $F = \frac{E_s}{V_s} \times V_o$ where

F is the estimated fecundity of the individual,

E_s is the number of eggs in the subsample,

V_s is the volume of the subsample in ml, and

V_o is the displaced volume of all ovarian tissue, including the subsample.

The estimation of egg volume for comparisons of egg size is given by

$E_{100} = \frac{V_s}{E_s} \times 100$ where

E_{100} is the standardized volume of 100 eggs, and

V_s and E_s are as above.

We used analysis of covariance (ANCOVA) to test for differences in fecundity and egg size among hybrid categories, after accounting for length. We log transformed fecundity and egg volume estimates, as well as total length measures to normalize the data. We used log(F) or log(E_{100}) as the response variables with hybrid category as a factor and log(length) as the covariate. We tested for significance of the interaction terms in saturated models to ensure models had equal slopes prior to making comparisons of fecundity relative to length for each hybrid category. All statistical analyses were completed in R (R Core Development Team 2010). To further examine differences between fecundity egg size and hybrid status, we developed regression equations for log fecundity (and log egg size) versus log length using data from all fish and plotted residuals versus HI.

Migration Timing

Potential cues for migration timing include Julian date (photoperiod), stream temperature, and discharge. We calculated mean daily water temperatures from hourly stream temperature data collected with HOBO data loggers (Onset Corporation, Pocasset, Massachusetts). Stream discharge data were collected at continuously recording measurement stations following overall procedures detailed in Rantz et al. (1982). Hydrographers from the CSKT measured discharge using methods in Nolan and Shields (2000). Rating curves and tables that defined the relationship between stream stage and measured discharge were developed and maintained following standard methods (Kennedy 1983). We tested whether HI, age, or sex best predicted conditions at the time of migration. Response variables (date, temperature, discharge) were correlated, so we tested for differences in hybrid category, age, and sex on all response variables simultaneously using multivariate analysis of variance (MANOVA).

We used individuals sampled at the K-Canal ladder for migration timing analyses, because we suspected lack of attracting flows to the S-Canal ladder might have caused biases in date of arrival in that trap. While we had data for date of arrival in trap and a HI for every individual sampled at the K-Canal trap from 2006-2007, only the 2008-2009 datasets were complete for hybrid category, sex, and age, because we did not start lethally sampling individuals throughout the migration period until 2008. Therefore, we used the 2008-2009 dataset to determine whether hybrid category, age, and sex explained a significant amount of the variance in the response variables. Additionally, we completed analyses of variance (ANOVA) to examine the differences in each response variable separately with respect to hybrid category, age, and sex. We also used the entire dataset to complete single factor ANOVA to test for differences in Julian date, discharge, and temperature at migration as a function of hybrid category. We used Tukey's post-hoc Honestly Significant Differences tests (Tukey's HSD) to detect significant differences among hybrid categories for each of the response variables. All analyses were completed in R (R Core Development Team 2010).

Results

We sampled and successfully amplified diagnostic microsatellite markers for 294 fish at the K-Canal and S-Canal ladders averaging 343mm in length and ranging in size from 93mm to 582mm. We collected otoliths for age and growth analyses from all individuals that were lethally sampled. Occasionally, we sampled fish that had already spawned, or were immature. Spent or immature fish were captured after or near the end of the spawning migration. These fish were eliminated from further analyses involving fecundity or migration timing because we only wanted to make comparisons using individuals actively migrating for spawning purposes. HI for sampled individuals ranged from 0 to 1, but WCT genotypes (HI = 0) dominated our samples, comprising 68% of all individuals sampled for this study. Individuals in the low hybrid category comprised 14%, and individuals in the high hybrid category comprised 18% of all individuals sampled (Figure 3.2).

Ages and Growth

We back-calculated length at age for 151 individuals collected in the K-Canal and S-Canal ladder traps that ranged from 93mm to 504mm in length, and from 1 to 7 years old in age. We classified 96 individuals into the zero category, 19 individuals into the low category, and 36 individuals into the high category. There was overlap in sizes among hybrid categories for all age classes, but von Bertalanffy curves demonstrated that high hybrids grow faster and generally achieved greater lengths at any given age (Figure 3.3). Tests of the equivalence of all von Bertalanffy parameters (Table 3.1) indicated significantly different growth between the zero and the high categories ($\chi^2 = 15.6$, d.f. = 3, $p = 0.001$), the low and the high categories ($\chi^2 = 25.3$, d.f. = 3, $p < 0.001$), but not the zero and the low category ($\chi^2 = 6.93$, d.f. = 3, $p = 0.074$). Asymptotic length (L_∞) was greater for the zero than the high category; however, caution should be taken when interpreting this result, as sample sizes at the oldest age classes were low, so estimates of L_∞ are suspect. Examination of the regression of residuals from the full von Bertanffy model versus HI indicated a significant, positive slope ($y = 19.98x - 0.1285$), substantial variation ($R^2=0.014$), but no apparent thresholds to indicate an HI at which growth changed substantially.

Fecundity and Egg Size

We estimated fecundity and egg size from 98 females collected at both the K-Canal and S-Canal Ladders in 2007-2009. Mature females ranged in length from 282 to 499mm. Estimated fecundities ranged from 221 to 3456 eggs per individual. E_{100} ranged from 3.96ml to 12.4ml. Of these females, 66 were classified as zero, 8 were classified as low, and 17 were classified as high. Interaction terms between hybrid category and log(length) in the saturated models were not significant, suggesting equal slopes models were adequate for determining the effect of hybrid category on F and E_{100} after accounting for the effects of log(length). The effect of hybrid category was significant for tests of differences in fecundity ($p = 0.016$, Table 3.2, Figure 3.4) and for egg size (p

< 0.001, Table 3.3). Results of the ANCOVA for fecundity revealed the high hybrid category had significantly lower $\log(F)$ than the zero category, but the low category and zero categories did not significantly differ (Table 3.4). On the other hand, results of the ANCOVA for egg size indicate a small, but significant increase in $\log(E_{100})$ for high hybrids relative to the zero category (Table 3.3). Similar to fecundity analyses, the zero category and the low category showed no differences in $\log(E_{100})$. To further explore these differences with hybridization, we examined the residuals of the log fecundity (and log egg size) regression with log length with hybrid index. The slope of the fecundity residuals versus HI was significant and negative (-0.46, $P<0.001$), while the slope of the egg volume residuals versus HI regression was significant ($P=0.002$) and positive (0.12). In both cases, there were no apparent break-points to indicate an HI at which major shifts in residuals occurred.

Migration Timing

We sampled 192 individuals (147 to 582 total length, mm) at the K-canal ladder that were making spawning migrations (mature individuals with developed gonads). The earliest migration date of these fish in any year was March 26, 2008 (Julian day 85). Two 2 year-old ripe males made the latest migrations of any fish in any year, also in 2008, on June 26 (Julian day 178) and June 30 (Julian day 181). Those individuals arrived in the trap 25 days after the remainder of the 2008 migrants and were removed from further analyses as they were highly influential outliers as the youngest, and by far, latest migrating individuals. Median ages of the zero, low, and high migrating fish in 2008-2009 were 5, 5, and 4, respectively. While there were no significant differences among the groups for age at migration, 14% of fish analyzed in the high category were 3 year old fish, versus 4% and 13% for the zero and low categories, respectively. In the 2008-2009 dataset, 71 individuals were classified as zero, 9 were classified as low, and 29 were classified as high. In the full dataset, 127 individuals were classified as zero, 26 individuals were classified as low, and 39 individuals were classified as high. Graphical analyses of the distribution of individuals across migration conditions (day, discharge, temperature) suggest that while there is substantial overlap between hybrid categories, there were only two individuals in the four-year data set in the high hybrid category that migrated after the first major increase in the hydrograph caused by spring runoff (Figure 3.5).

MANOVA results of the 2008-2009 data set suggested there were significant effects of hybrid category (Wilks' $\lambda = 0.632$, $F_{6,164} = 7.05$, $P < 0.001$) on the response variables (day, log[discharge], temperature). Age and sex were not significant in the MANOVA, but sex did explain a significant amount of variation in the responses Julian date (ANOVA, $P = 0.006$) and log[discharge] (ANOVA, $P = 0.030$), as males tend to migrate earlier than females, but with overlap. Hybrid category was significant across all response variables used in the MANOVA. These results were based on a model with no interaction between factors as the model with all two-way interactions suggested none of the interactions were significant.

Results from the MANOVA of the entire 2006-2009 data set also demonstrated significant differences (Wilks' $\lambda = 0.698$, $F_{6,\,374} = 12.3$, $P < 0.001$) in the conditions under which fish from different hybrid categories migrate. One-factor ANOVAs followed by Tukey's HSD tests indicated significant differences between the zero and the high category for Julian date ($P < 0.001$), log(discharge) ($P < 0.001$), and temperature ($P < 0.001$). The low and the high category were also significantly different for Julian date ($P < 0.001$), log(discharge) ($P = 0.005$), and temperature ($P = 0.001$). There were no significant differences across all the response variables for the zero versus low hybrid categories (Figure 3.6).

Discussion

Are WCT and Hybrids Equivalent?

This study demonstrated differences in important life history characteristics related to growth, fecundity, and migratory behavior between WCT with no evidence of RBT ancestry and RBT x WCT hybrids HIs > 0.20. Fish in the high hybrid category grew faster and had fewer, but larger eggs at a given length than fish in the low or the zero categories. While our sampling of upstream migrating fish could not resolve differences in actual spawn timing or location, there were differences in the environmental conditions during which fish migrate that could lead to separation in time and/or space in spawning. Individuals in the high hybrid category abruptly stopped arriving in the K-Canal trap as soon as the hydrograph began the most rapid increase of its rising limb (Figure 3.5). There did not appear to be as strong a threshold related to day or temperature conditions as that for a rapid increase in discharge (Figure 3.5). The differences observed in this study provide compelling evidence that WCT and hybrid individuals with HI > 0.20 are not equivalent in migratory behavior, growth, or fecundity.

This study did not provide any evidence that hybrids in the low category differed from individuals identified as unhybridized WCT. We may have observed this for several reasons. First, there may be few functional differences between WCT and low category hybrids. Second, it is likely several individuals classified in the zero category were actually hybrids. Genetic identification of an individual's HI was based on 7 codominant (14 total alleles per individual) diagnostic microsatellite markers. The probability of not detecting a hybrid marker in an individual with 10% RBT ancestry is $(pWCT)^{14} = (0.90)^{14} = 0.23$, where pWCT is the proportion of the individual's ancestry that is WCT (Kanda et al. 2002). Therefore, we likely overestimated the number of unhybridized individuals and underestimated the number of individuals in the low category. Given introgression is widespread in the Jocko River, but occurs at low rates ($pRBT < 0.05$) in most locations (Chapter 2), it is possible that many individuals genotyped as WCT had some very low level of RBT ancestry. Finally, we may not have observed differences between the zero and low categories because of the relatively small sample sizes in the low category. This small sample size in the low category made it impossible to evaluate differences between the <20% HI of the USFWS versus the <10% pRBT of many state policies (UDWR 2000).

Growth, Fecundity, and the Role of Growth Environments

We observed increased growth rates for individuals in the high hybrid category; however, given that the juvenile growth environments are likely disparate, there is no way to separate an effect of RBT ancestry from an effect of increased juvenile growth in a more productive environment. In the Jocko River system, individuals with $HI \geq 0.20$

are most likely to have been born in low elevation habitats, while individuals with WCT genotypes are most likely to be from high elevations (Chapter 2). Temperature and food availability are primary drivers of trout growth (e.g. Railsback and Rose 1999) that are highly correlated with elevation (Sloat et al. 2005; Rasmussen et al. 2010). Bear et al. (2007) demonstrated there was no significant difference in growth at the optimal growth temperature for WCT and RBT in laboratory studies. This may suggest the growth differences observed in this study are due to the differences in growth environments during juvenile life stages. Conversely, Seiler and Keeley (2009) suggests growth of juvenile Yellowstone cutthroat trout is impaired by competition with F1 Yellowstone cutthroat trout x RBT hybrids. It is uncertain how competitive interactions may influence growth of subadult individuals in the zero and low hybrid categories when they are present in the same habitats.

Muhlfeld et al. (2009a) found the median age of outmigration for fluvial WCT and hybrids was 2, suggesting that the beginning of the third year of life may mark the beginning of similar growth environments for WCT and high hybrid groups in the Jocko River. Modeled growth trajectories for zero and low hybrid categories remain below those for high hybrids across all age classes even when they are likely sharing similar low elevation habitats (Figure 3.3).

Fecundity, egg size, and growth are all positively related, and all are at least partially dependent on available resources and growth conditions (Einum et al. 2004). Both egg size and number have been shown to be strongly related to fitness, and often represent an important life history tradeoff, as females must allocate limited resources, and it is energetically expensive to produce many eggs and large eggs. There was evidence of egg size – fecundity tradeoffs between zero and high hybrids, as the zero category fish have higher fecundities at a given length (Figure 3.4, Table 3.2), but high category fish have larger egg sizes at a given length (Table 3.3). Along with larger eggs at a given length, an earlier age at maturity could also mitigate for reduced fecundity in high category hybrids. While we did not observe significant differences among categories for age at migration, high category hybrids did have a younger median age at migration (4 years old) relative to zero category fish (5 year old), and substantially more high category hybrids were classified as age 3 (14%) relative to zero category fish (4%).

Migration Timing and Reproductive Separation

The primary difference we observed among groups in migration timing was that high hybrids appeared to have a consistent and abrupt end to their migration with the first major jump in discharge caused by spring runoff (Figure 3.5). Fish in the zero and low hybrid categories in this study often arrived in the K-Canal trap during the rising limb of the hydrograph. This is concordant with Schmetterling (2001) in the Blackfoot River drainage, MT, where WCT migrated to the mouths of spawning tributaries on the rising limb of the hydrograph before spawning in those tributaries on the descending limb. Muhlfeld et al. (2009b) found RBT and RBT backcrossed hybrids tended to migrate earlier at lower discharges and spawn on the rising limb of the hydrograph, and WCT and WCT backcrossed hybrids tended to spawn on the descending limb of the hydrograph. These authors observed substantial overlap in spawning time between the hybrid categories and the parental categories, surmising that hybrids are likely facilitating further introgression between parental types. Spatial and temporal separation in spawning is likely to be a primary factor limiting introgression (DeRito et al. 2010), but if hybrids express intermediate phenotypes and continue to introgress, reproductive isolation between the species may continue to erode.

Hybrid Policy and Levels of RBT Introgression in WCT Populations

Critics of the current USFWS policy to include hybridized populations with < 20% RBT introgression have contended that this genetic invasion has the potential to disrupt local adaptations, leading to lower fitness of hybridized populations (Allendorf et al. 2004; Allendorf et al. 2001). While we did not measure the relative fitness of the hybrid categories in this study, differences observed in growth, fecundity, and egg size are likely related to the survival and reproductive success of WCT and high hybrids in their respective habitats (Kinnison et al. 2001; Schaffer 2004). Depending on the links between migration timing, spawn timing, and emergence timing relative to flood pulses, even life history differences related to migration time could influence the reproductive success of individuals and, consequently, population success (Fausch et al. 2001). The scale that the traits addressed in this study are locally adapted is unknown, but given the strong selection on factors related to fecundity and egg size, local and environmentally-dependent adaptations are likely (Hendry and Day 2003; Lobon-Cervia et al. 1997). For example, clutches comprised of larger egg sizes exhibited by the high hybrids may have lower total metabolic demand than a larger clutch of smaller eggs, thus creating an offspring survival advantage in lower elevation habitats where fine sediments and temperatures are elevated, leading to lower dissolved oxygen content (Hendry and Day 2003). Continued introgression of high hybrids into habitats dominated by WCT phenotypes may result in intermediate phenotypes or may otherwise erode local adaptations.

Despite these important differences between the high and zero categories, the low and zero categories were similar in all tests in this study. While this study had many limitations inhibiting our ability to detect differences in these groups (see above), this study does provide empirical evidence in support of the USFWS policy that low category hybrids are morphologically and behaviorally indistinguishable from WCT. However, other studies have provided evidence to the contrary. Muhlfeld et al. (2009a) described a 25% reduction in reproductive success (as measured by number of out-migrating juveniles per parent) with 10% RBT admixture. It is unclear from this study what proportion of these measured differences is a result of true vital rate reductions versus alternative life history strategies (such as higher survival from larger egg sizes, increased growth, or increased rates of residency in hybrids). Other studies have demonstrated important differences in growth (Allendorf and Leary 1988; Seiler and Keeley 2009), developmental stability (Leary et al. 1985), morphology (Hawkins and Quinn 1996), and survival (Leary et al. 1995) as a result of F_1 crosses of RBT and cutthroat trout, but none of these studies addressed differences between WCT-backcrosses and WCT in wild populations. The linear relationship of the residuals (versus a step-function) and high variance in this study implies that a biologically-derived threshold from field studies is not promising.

Given the small number of studies and degree of uncertainty regarding whether there are appreciable morphological, behavioral, life history, and, ultimately, fitness differences between WCT and hybrids with low RBT ancestry, further study of these issues is important. Furthermore, we have no knowledge of the role of low levels of hybridization in disease resistance, nor whether the increased genetic diversity caused by hybridization leads to increased evolutionary potential in rapidly changing habitats (see Ellstrand and Schierenbeck 2000). A diversity of studies addressing life history, fitness-related metrics, population viability, and ecosystem dynamics in a broader diversity of habitats is required before we can better understand the ecological and evolutionary consequences of introgressive hybridization in cutthroat trout populations.

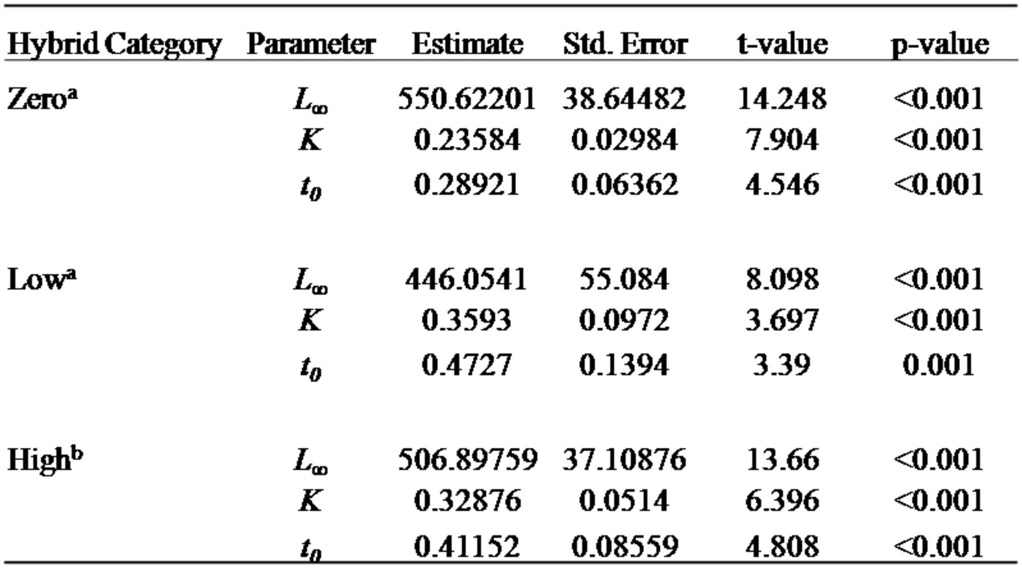
Study Limitations and Suggestions for Future Research

The primary limitations of this study – the inability to control for early life stage growth environment, and issues related to the limited sample sizes of low category hybrids – have been addressed above. However, there are some additional issues that limit this study and provide important avenues for future research. First, it is important to remind the reader that this study was conducted only on comparisons among migratory fish. Life history differences between WCT and hybrids may also occur for resident populations, and this may be a very important future research topic. We have no reason to think that resident fish would show differences from their migratory counterparts analyzed in this study, but a study that examined these life history characteristics in an array of habitats with an array of pRBT levels would be a highly beneficial contribution as it may be able to explicitly deal with some of these confounding issues.

Another major limitation is the fact that we had no ability to directly link growth and age of maturity. There was no evidence from our analysis of the otoliths that we could document the age of previous spawning events in an older, potentially repeat spawning fish. Certainly, many or most of the fish captured during spawning migrations may have reached their first year of maturity, especially since the majority of fish were aged 3-5. If increased growth rate observed in high category hybrid trout relative to low and zero category trout could lead to earlier average age at maturity, thus reducing the generation time, it could give high category hybrids a substantial mediation for the reduced fecundity rates we observed. A better understanding of the growth maturity relationship would be an important contribution to our understanding of the consequences of hybridization.

Another important limitation of this study is the inability to directly link migration timing to spawn timing. Since overlap in space and timing of spawning is what leads to hybridization between RBT, hybrids, and WCT, it would be very helpful for research and management to understand the conditions of spawn timing, rather than migration timing. Muhlfeld et al. (2009b) indirectly measured spawn timing using radio telemetry, but this may be the best kind of data available for spring spawning salmonids, where we usually cannot observe the timing of redd construction. We should point out that while enumerating eggs for this study, we noticed that females with more RBT characteristics tended to also have loser eggs indicative of ripeness and imminence of spawning. While we have no quantitative measure of ripeness, this anecdotal evidence indicates there may be even greater evidence of separation for spawn timing that we observed for migration timing.

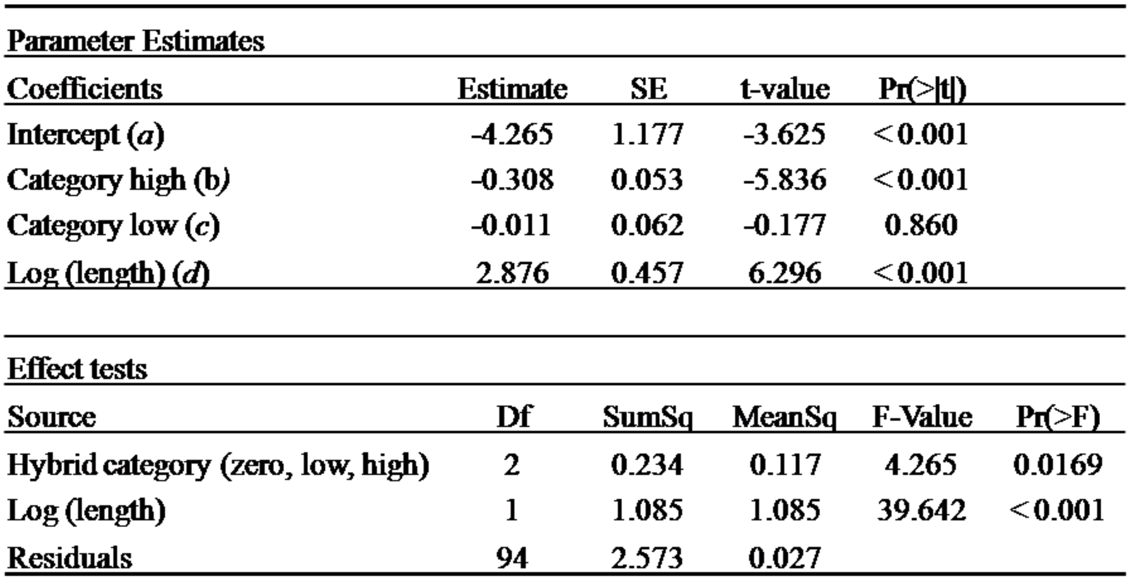
Table 3.1. Parameter estimates of the von Bertalanffy curves fit to back-calculated size at age data for each hybrid category.



Hybrid Category	Parameter	Estimate	Std. Error	t-value	p-value
Zero[a]	L_∞	550.62201	38.64482	14.248	<0.001
	K	0.23584	0.02984	7.904	<0.001
	t_0	0.28921	0.06362	4.546	<0.001
Low[a]	L_∞	446.0541	55.084	8.098	<0.001
	K	0.3593	0.0972	3.697	<0.001
	t_0	0.4727	0.1394	3.39	0.001
High[b]	L_∞	506.89759	37.10876	13.66	<0.001
	K	0.32876	0.0514	6.396	<0.001
	t_0	0.41152	0.08559	4.808	<0.001

[a,b] Categories with different letter superscripts were significantly different in pairwise likelihood ratio tests for differences in all parameters.

Table 3.2. Parameter estimates and ANOVA results for ANCOVA testing for different intercepts of each hybrid category for the length – fecundity relationship. The model used was: $\log(F) = a + b(\text{high}) + c(\text{low}) + d(\log(\text{length}))$.



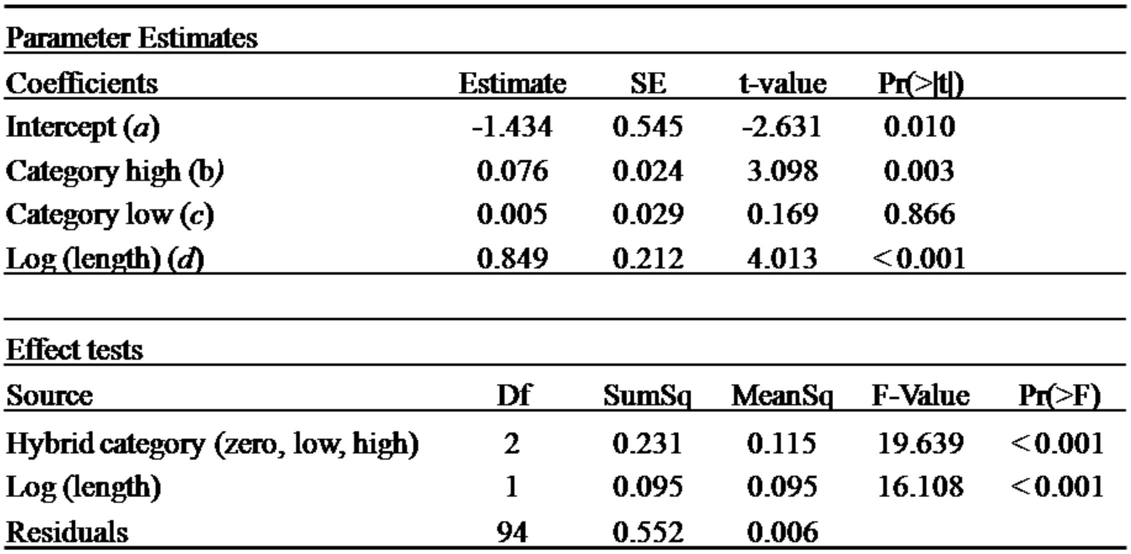
Parameter Estimates

Coefficients	Estimate	SE	t-value	Pr(>|t|)
Intercept (*a*)	-4.265	1.177	-3.625	<0.001
Category high (b)	-0.308	0.053	-5.836	<0.001
Category low (*c*)	-0.011	0.062	-0.177	0.860
Log (length) (*d*)	2.876	0.457	6.296	<0.001

Effect tests

Source	Df	SumSq	MeanSq	F-Value	Pr(>F)
Hybrid category (zero, low, high)	2	0.234	0.117	4.265	0.0169
Log (length)	1	1.085	1.085	39.642	<0.001
Residuals	94	2.573	0.027		

Table 3.3. Parameter estimates and ANOVA results for ANCOVA testing for different intercepts of each hybrid category for the length – E_{100} relationship (E_{100} is the volume of 100 eggs). The model used was: $\log(E_{100}) = a + b(\text{high}) + c(\text{low}) + d(\log(\text{length}))$



Parameter Estimates

Coefficients	Estimate	SE	t-value	Pr(>|t|)
Intercept (*a*)	-1.434	0.545	-2.631	0.010
Category high (b)	0.076	0.024	3.098	0.003
Category low (*c*)	0.005	0.029	0.169	0.866
Log (length) (*d*)	0.849	0.212	4.013	<0.001

Effect tests

Source	Df	SumSq	MeanSq	F-Value	Pr(>F)
Hybrid category (zero, low, high)	2	0.231	0.115	19.639	<0.001
Log (length)	1	0.095	0.095	16.108	<0.001
Residuals	94	0.552	0.006		

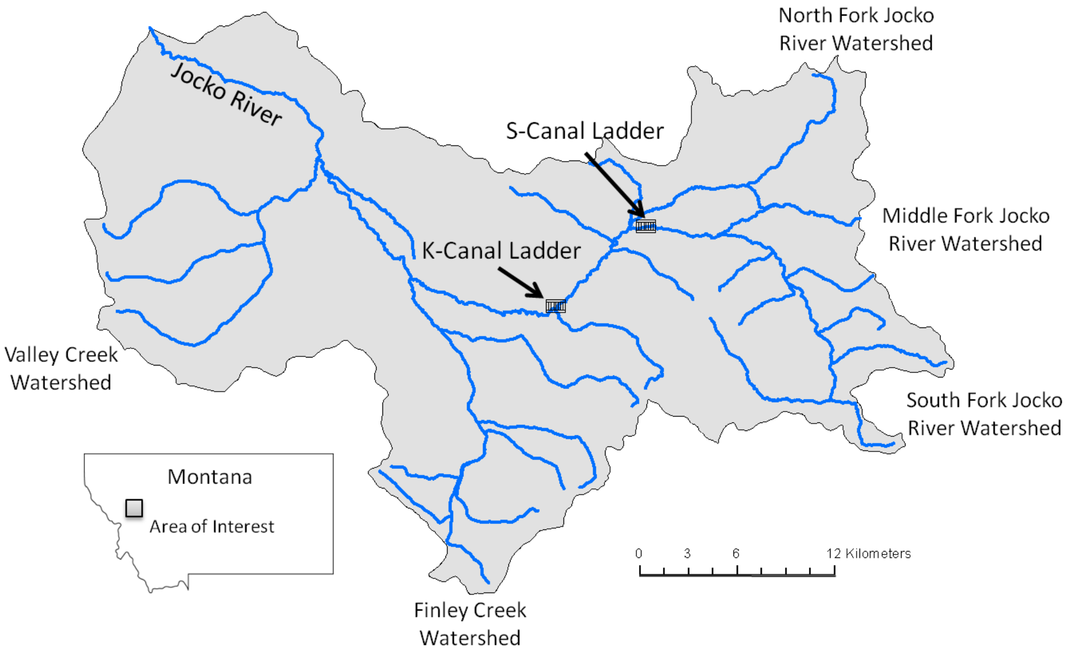


Figure 3.1. Map of study watershed. K-Canal and S-Canal are irrigation diversion structures on the main-stem Jocko River, MT. These structures have fish ladders and migration traps and are complete barriers to upstream movement at most flows.

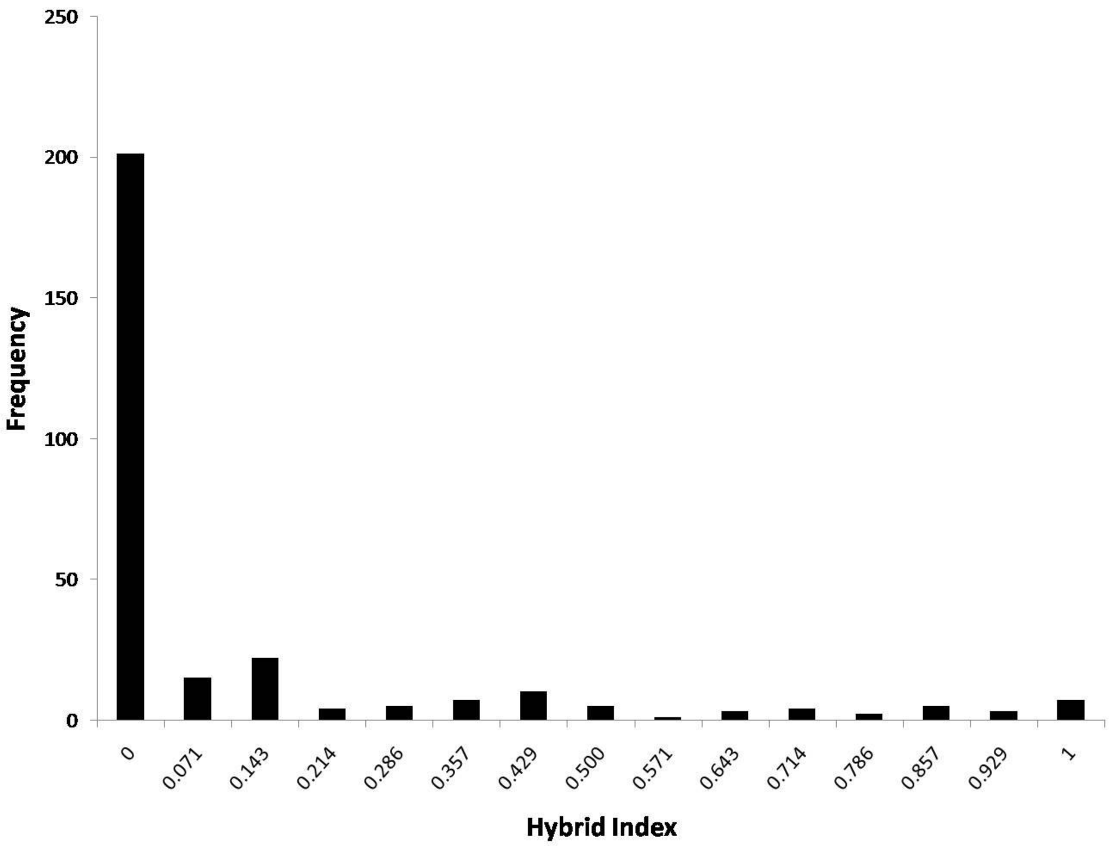


Figure 3.2. Frequency distribution of hybrid index scores of individuals collected at K and S canal ladders that were used to address life history questions in this study. Each analysis used a subset of these individuals, depending on study question. The bin labels here are the HI thresholds we evaluated using 7 diagnostic microsatellites (14 alleles). It should be noted that not every individual HI exactly matches one of the bin labels in this figure, because not all individuals amplified at all loci.

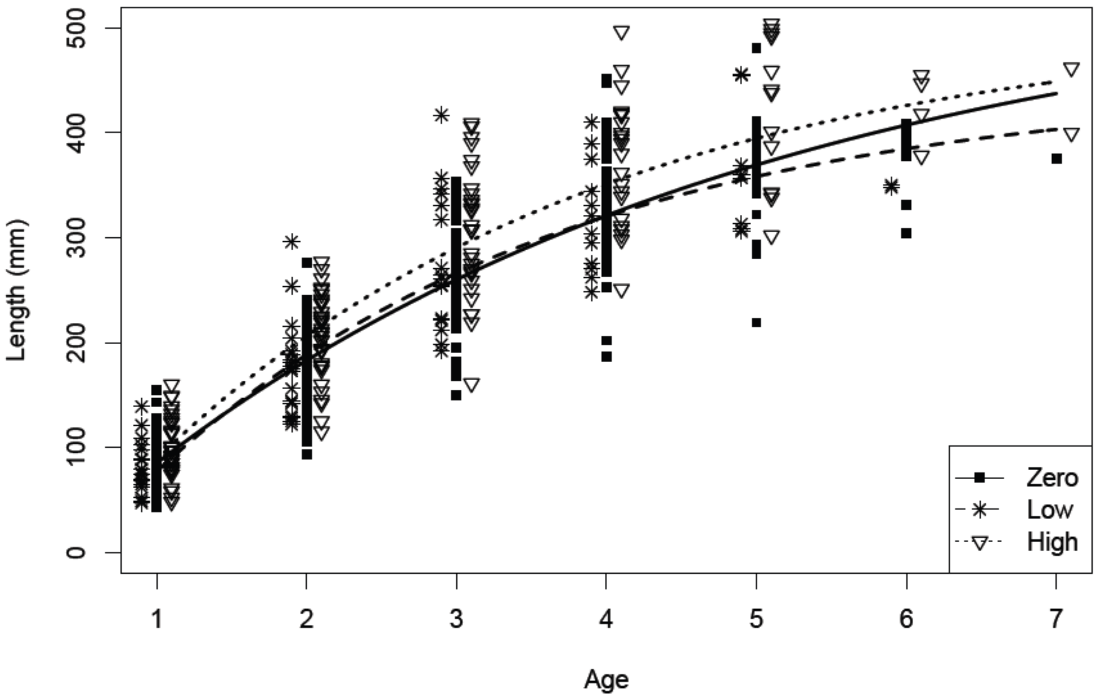


Figure 3.3. Von Bertalanffy (VB) curves fit to back-calculated size at age data for fish in each of the hybrid categories. Data points for hybrid categories are offset to highlight differences of length distributions of each category at each age.

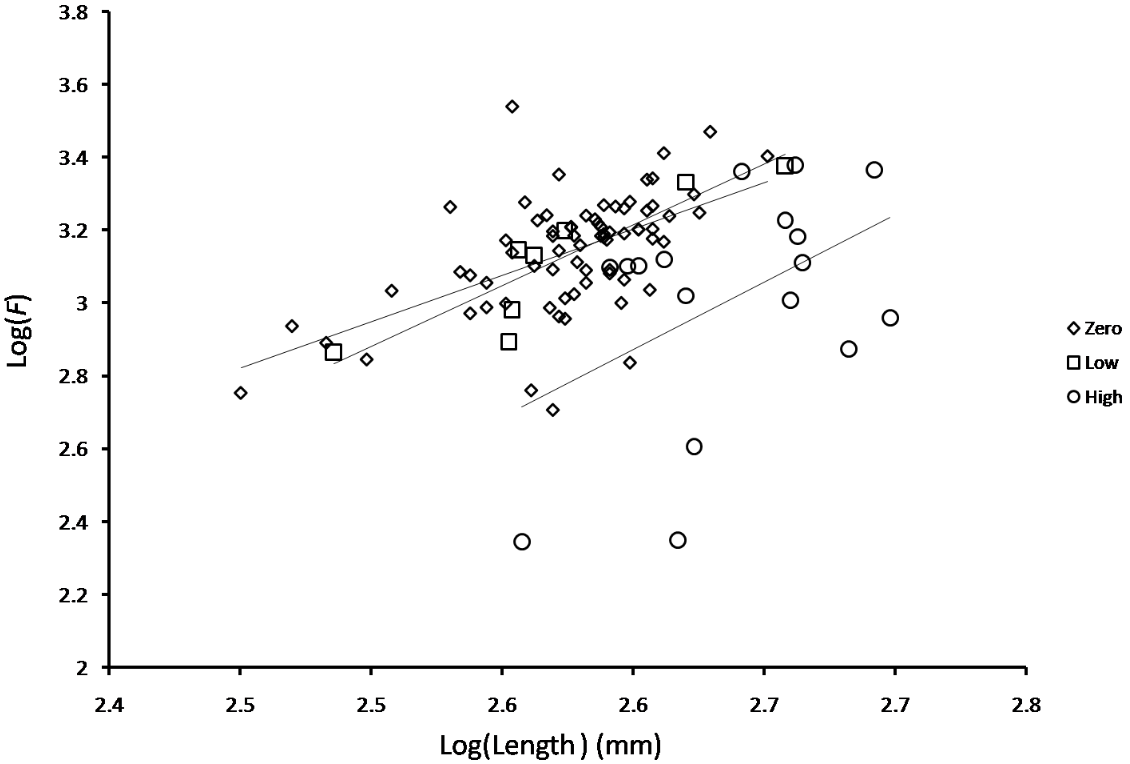


Figure 3.4. Relationship of log transformed fecundity (*F*) to log transformed total length (L,mm). Separate lines are fit to the data for each of the hybrid categories: zero (R^2 = 0.31), low (R^2 = 0.80), and high (R^2 = 0.21).

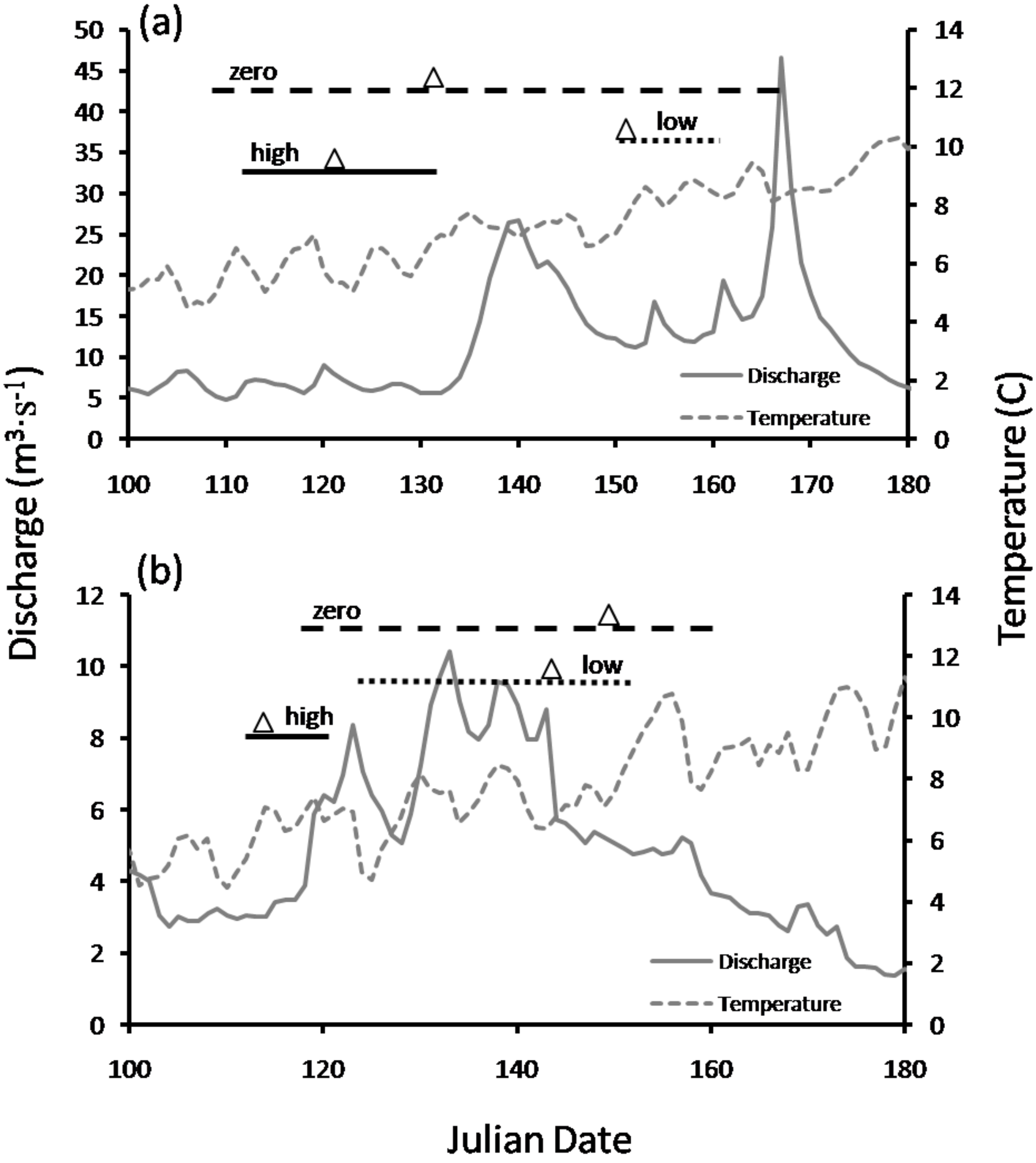


Figure 3.5 pt 1. Distributions of arrivals in K-Canal trap for each of the hybrid categories across Julian date, discharge, and temperature for 2006 (a), 2007 (b), 2008 (c), and 2009 (d). Triangles represent median arrival date, and the lines represent the entire range of days of arrival in the trap.

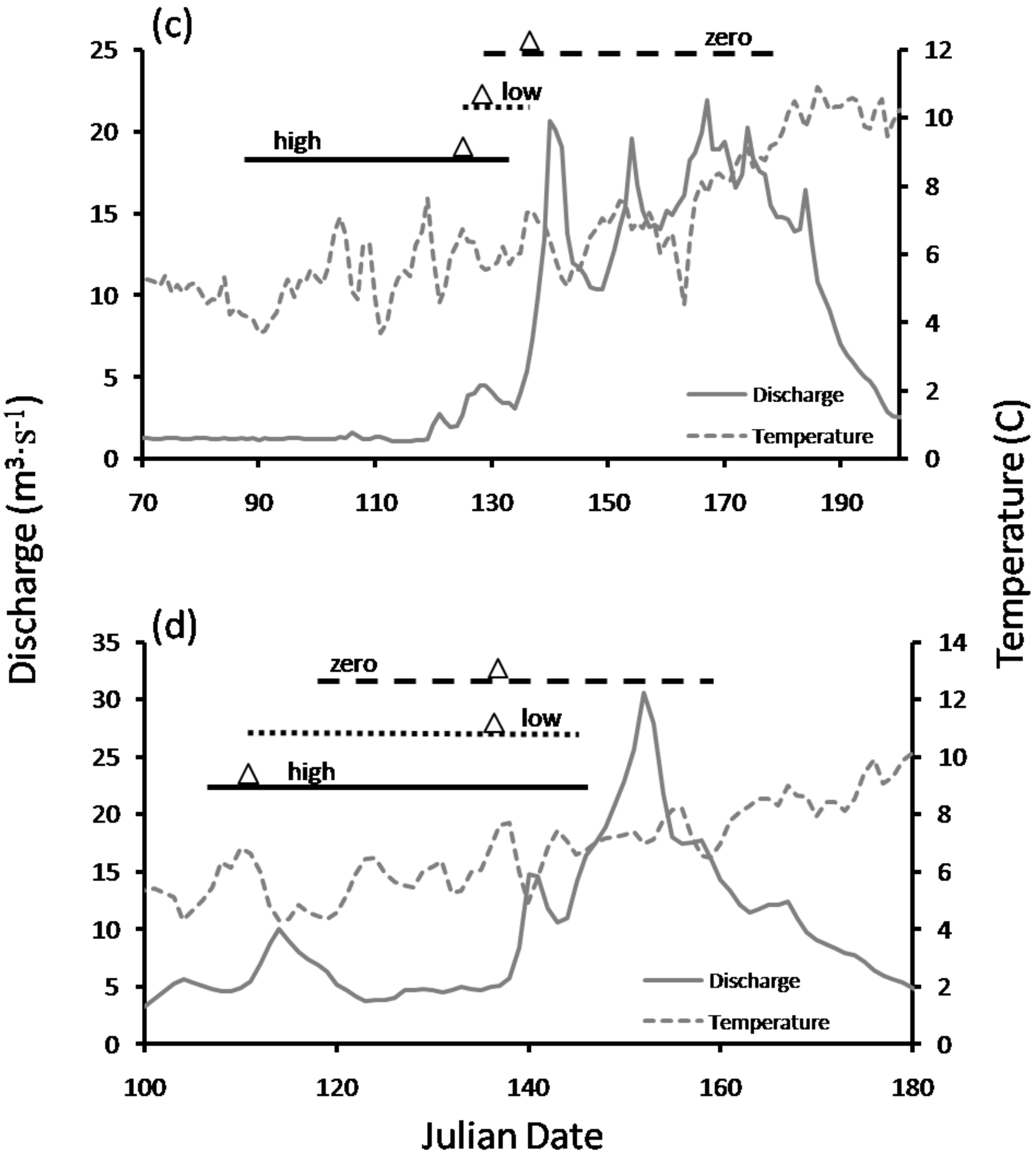


Figure 3.5 pt. 2.

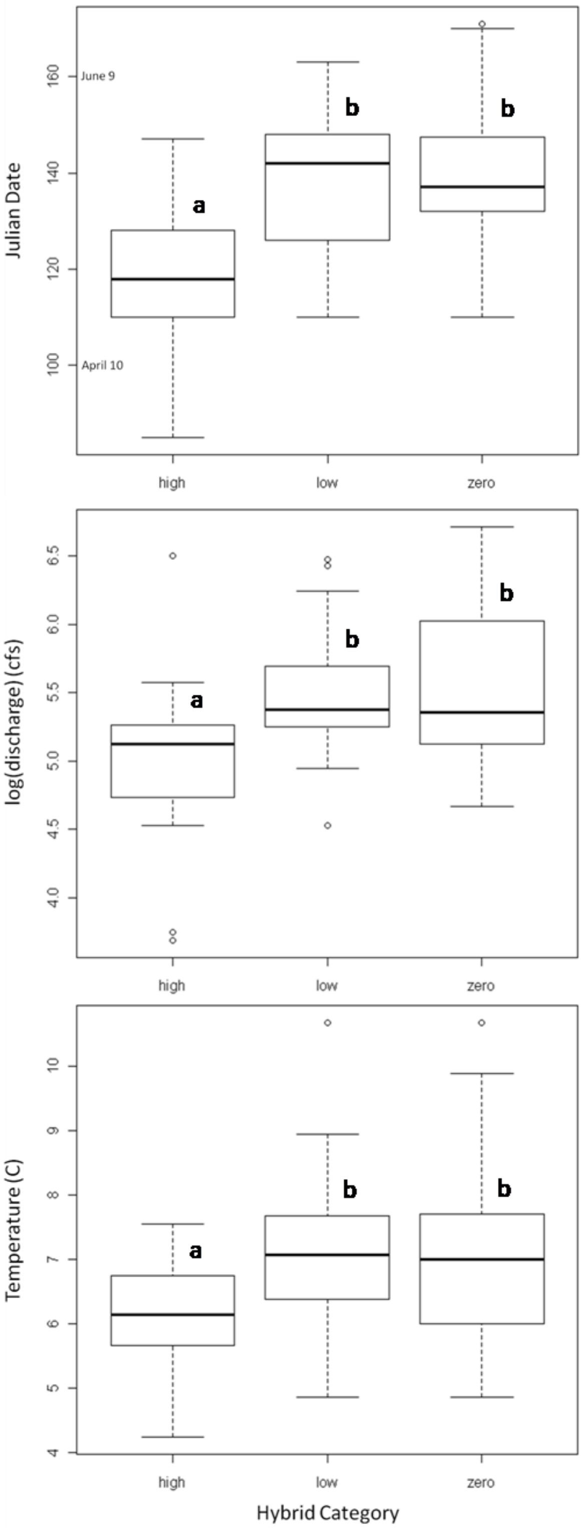


Figure 3.6. Box plots of the distributions of conditions (Julian day, discharge, and temperature) at which fish migrated into K-Canal ladder 2006-2009. Letters above the distributions indicate significant differences (Tukey HSD, $P < 0.05$).

CHAPTER 4

RESTORATION OF A MIGRATORY LIFE HISTORY TO A CUTTHROAT TROUT POPULATION: A POPULATION VIABILITY ANALYSIS TO ASSESS TRADEOFFS IN BARRIER MANAGMENT

Abstract

Cutthroat trout are declining throughout their range due to habitat degradation, isolation, and invasive species. Negative consequences of invasion, such as hybridization, often force fishery managers to make difficult decisions about the use of barriers as a management tool. Barriers can be effective for limiting the spread of invasive species, but they have negative side-effects of restricting success of migratory life histories. We used a multiple life history, stochastic, stage-structured population model to assess the relative viability of a cutthroat trout population in the Jocko River, MT, with and without a migratory life history. In the population model, young of year survival, the transition of subadults to adult1 stage, and reproductive contribution of the adult1 stage had the highest elasticities by an order of magnitude over all other matrix elements. Restoration of passage above barriers had strongly positive effects on the long-term viability of the population. Allowing limited escapement of migratory fish decreased the cumulative proportion of populations extinct at the end of 200 years from 0.83 to 0.54. Reducing young-of-year survival and fecundity rates to reflect the influence of hybridization estimated in recent studies led to dramatic declines in viability where 100% of population trajectories went extinct before 125 years. Results of a population

genetic model indicate that restoration of a migratory life history could result in substantial introgression in above barrier populations after 4-10 generations of passage. Selective passage could effectively mitigate that threat. This study illustrates the benefits of restoring migratory life histories, but elucidates the risk for increased introgression. Finally, analysis of model elasticity values highlights the need to better address vital rate variation due to hybridization of poorly understood early life stages from young of year through subadult.

Introduction

The introduction of non-native species is a well-established cause in the imperilment of native freshwater fish populations (Rahel 2000; Ricciardi and Rasmussen 1999). Declines in native salmonids due to interactions with non-native fishes occur for a variety of reasons, including predation (Stapp and Hayward 2002), competition (Peterson et al. 2004), and hybridization (Leary et al. 1995). One of the primary factors restricting invasion of non-native fishes is the presence of barriers to movements (Fausch et al. 2009). Unfortunately, fragmentation of habitats and populations is another important factor leading to the decline of native salmonids (Harig and Fausch 2002; Morita and Yokota 2002; Letcher et al. 2007). This conundrum presents an important challenge for salmonid conservation efforts (Peterson et al. 2008; Fausch et al. 2009) that will only be exacerbated by climate change and other anthropogenic alterations to salmonid habitats (Williams et al. 2009).

Rainbow trout (RBT, *Oncorhychus mykiss*) have been introduced in nearly every suitable habitat worldwide (Lowe et al. 2000; Fausch et al. 2001), and they interbreed and produce fertile hybrids with cutthroat trout (*O. clarkii spp.*) in areas where they are naturally and anthropogenically sympatric (Leary et al. 1995; Behnke 2002; Kozfkay et al. 2007; Metcalf et al. 2007). Westslope cutthroat trout (WCT, *O. c. lewisi*) are listed by states as a species of concern, and they have been considered for listing as threatened under the Endangered Species Act (USFWS 2003). Hybridization with RBT is generally considered to be a primary conservation threat to native populations of WCT (Rubidge et al. 2001; Allendorf et al. 2004; Muhlfeld et al. 2009a). Even though existing and future barriers may protect WCT population from hybridization, they may decrease the conservation value of these populations through limiting the diversity of life history types (UDWR 2000; Peacock and Kirchoff 2004; Shepard et al. 2005; Williams et al. 2007).

Many salmonids exhibit partial migration, where a component of the population carries out their entire life cycle in or near natal habitats, and another component of the population leaves natal habitats for more productive rearing habitats (Trotter 1989; Behnke 2002). Relative to the resident life history strategy, migratory life histories generally gain a reproductive benefit through increased fecundity due to larger body size (Hendry et al. 2004). Migratory life histories may increase population viability through increased reproductive potential, mobility (to seek refugia during times of stress), and expression of phenotypic plasticity (Hutchings 2004). Habitat connectivity and migratory life histories are crucial for rapid recolonization of habitats following catastrophic events, such as wildfire and debris flows (Dunham et al. 2003; Gresswell 1999). Migratory fish also help maintain genetic and demographic connectivity between populations (Neville et al. 2006; Shepard et al. 2005). The presence of nonnative species often forces fishery managers to make difficult decisions regarding the tradeoffs of managing for river connectivity to support migratory life histories versus maintaining or placing barriers to limit the upstream movement of invasive species (Fausch et al. 2009).

There are approximately 75,000 dams at least 2m high in the contiguous United States and innumerable barriers to fish movement resulting from small dams, road culverts, irrigation diversions, and other hydrologic alterations (Graf 1999; Warren and Pardew 1998). Given the ubiquity of non-native fish invasions (Rahel 2000), managers will have to contend with the tradeoffs of fragmented populations versus increased interactions with non-native species in nearly all fishery management jurisdictions. Where feasible, selective fish passage at barrier structures can promote connectivity of native fish populations to habitats for a full expression of life history. For example, selective fish passage is currently used as a tool to return migratory bull trout and Yellowstone cutthroat trout (*O.c. bouveri*) to spawning habitats above major dams and permanent weirs (Henderson et al. 2000, USFWS 2002). Where selective passage of native fishes at barriers is not feasible, managers will need to weigh the relative merits of removal versus maintenance of the barrier (Peterson et al. 2008; Fausch et al. 2009).

An understanding of both the demographic benefits of restoration of migratory life histories and the genetic risks to upstream conservation populations need to be weighed as managers make decisions regarding barriers. Previous work has indicated that habitat diversity and volume, carrying capacity, dispersal among populations, and synchrony all play important roles in population viability (Harig and Fausch 2002; Hilderbrand 2003; Morita and Yokota 2002; Novinger and Rahel 2003; Young et al. 2005), but there is limited quantification of the importance of migratory life history types on viability for inland trout (but see Letcher et al. 2007; Peterson et al. 2008). Demographic population models in concert with population genetic simulation models are useful tools to evaluate relative hybridization risks of complete or selective passage of migratory individuals at a barrier. Because both demographic and genetic viability are time dependent (e.g. Epifanio and Philipp 2000), conservation decision making could benefit from linking the dynamics of genetic introgression and population persistence to evaluate these tradeoffs directly.

The goal of this project is to assess demographic and genetic tradeoffs of restoration of migratory life history versus isolation in a WCT conservation population. We used two simulation frameworks to make this assessment. First, we used a stochastic matrix population model to address the relative benefits of different passage scenarios for migratory WCT and to test demographic consequences of the potential observed reductions in juvenile survival (Muhlfeld et al. 2009a) and fecundity (Chapter 3) due to hybridization. Second, we used an individual-based population genetic simulation model to address the hybridization consequences of allowing potentially hybridized individuals to return to an unhybridized population. Using these models, we evaluated no passage, selective passage (using phenotypic characteristics – visual calls), and complete passage. We applied this approach to a case study in the Jocko River basin where two large irrigation diversion structures restrict fish passage, both fluvial migratory WCT and non-native RBT and hybrids, to populations above the barriers.

Study Area

The Jocko River system is a 979 km^2 tributary basin of the Flathead River in northwestern Montana (Figure 4.1). The basin lies within the boundaries of the Flathead Indian Reservation, and the basin's fisheries are managed by the Confederated Salish and Kootenai Tribes (CSKT). We detected evidence of RBT introgression throughout the Jocko River system, but frequencies of RBT alleles remain below 0.05 in nearly all regions of the upper main-stem Jocko River and the North, Middle, and South Forks of the Jocko River (Chapter 2). The two large irrigation diversions, the K-Canal and S-

Canal ladders on the upper Jocko River, have limited the upstream spread of nonnative salmonids including RBT and brown trout *Salmo trutta*. Brook trout *Salvelinus fontinalis* are present in many locations throughout the system. Both diversions are barriers to movement and migration, although fish can occasionally pass the K-Canal diversion when the Jocko River is at bank-full or higher flows and irrigation managers uncheck the diversion (Craig Barfoot, CSKT, personal communication). These barriers have been modified with fish ladders leading into traps so that biologists could selectively pass native species if appropriate. Currently, only bull trout *Salvelinus confluentus* are passed.

The *Oncorhynchus sp.* samples collected in the K-Canal and S-Canal ladders are dominated by individuals with WCT genotypes, but RBT and hybrids are collected in the migration traps at K-Canal and S-Canal ladders during spring spawning migrations. From 2006-2009 we obtained genotypes for individuals captured in the migration traps at 7 microsatellite loci diagnostic for RBT and WCT ancestry (see Chapter 2 for methods). To quantify their ancestry, each individual was assigned a hybrid index score (HI) based on the number of RBT alleles amplified from seven diagnostic microsatellite loci divided by the number of total alleles amplified per individual. Individuals with HIs of zero have a WCT genotype, and individuals with HIs of 1 have a RBT genotype. Individuals with HIs of zero dominate the migratory component (comprising 74% of all individuals in the migration traps; Figure 4.2). Individuals with relatively high RBT ancestry ($HI > 0.2$) comprise 20% of the individuals captured in the trap (Figure 4.2).

Methods

Demographic Model

We designed a multiple life history, stochastic, stage structured population model using the Matlab program VitalSim (Morris and Doak 2002). The deterministic projection model was a stage-based model, with young of year in the first stage and three length-based stages that represented either resident or migratory individuals (Figure 4.3). Length-based stages are appropriate for use in a salmonid population model because survival, maturity, and fecundity are strongly dependent on length (Downs et al. 1997; Einum et al. 2004; Hilderbrand 2003). The transition to either migratory or resident life histories occurred as a transition in the subadult stage as Muhlfeld et al. (2009a) found out-migrating juvenile trout had a median age of 2 and rarely out-migrated as fry (<75mm).

Survivorship and transition values came from the literature and were corroborated with data from the Jocko River system when possible. Survivorship for the resident life history was representative of moderate to high survivorship reported by Shepard et al. (1997) and similar to Hildebrand (2003). Survivorship for the migratory life history was based on ranges in Shepard et al. (1997) (see Appendix C for all vital rate values and variances). We assumed a 50% transition probability for each stage to be consistent with empirically derived growth rates from this system (Chapter 2), as well as previously reported length frequency and size at maturity data reported by Downs et al. (1997) for residents and Thurow et al. (1988) for migratory Yellowstone cutthroat trout.

Fecundity values are based on the values reported by Downs (1997) for resident WCT and Shepard et al (1997) and this system (Chapter 2) for migratory fish. Migratory fish are usually alternate year spawners, but about 15% of migratory Yellowstone cutthroat trout spawn annually (Thurow et al. 1988). Propensity to breed was therefore calculated as 1.0 for resident fish and 0.65 (0.5+0.15) for migratory fish. This model assumed a 1:1 sex ratio, and only females were modeled.

We calculated elasticity values for the matrix elements following Morris and Doak (2002) to evaluate the relative influences of each element on population growth. Some elements are made up of multiple vital rates (e.g., fertility and young of year survival which includes incubation success and fry survival). We also calculated the elasticity of individual vital rates. All elasticities were calculated using the MATLAB program Limitsens (Morris and Doak 2002).

We modeled environmental stochasticity by randomly varying every vital rate around a predetermined mean value. Vital rates were drawn from a beta distribution (survivorship and breeding probabilities) or stretched beta values (fecundity values). To estimate the standard deviation required to compute a beta distribution in VitalSim, we obtained ranges of values for vital rates reported in the literature, then divided the reported range by four to have an estimate of standard deviation. The shape of the distribution was visually examined for each vital rate by randomly simulating 500 values from the distribution in the Matlab program BetaDemo (Morris and Doak 2002). We simulated 1000 population trajectories over 200 years where random projection matrices were calculated for each population trajectory at each time step. We set a quasi-extinction threshold of 25 adult female individuals of all age classes (equal to 50 total adults). Starting population sizes were 40,000 individuals at stable age distribution, which approximates densities of subadult and adult WCT in 3 to 6 km of habitat in the upper Jocko River system (CSKT, unpublished data).

Passage Scenarios

The goal was to establish the demographic effect of passing migratory native WCT above a migration barrier. The base scenario represents current management in the Jocko River where no fish of the genus *Oncorhynchus* are passed above the irrigation diversion dams to avoid further introgression in the upper Jocko River. To model a population dominated by resident life history where migratory individuals are lost to lack of connectivity, we made the migratory sub-adult survival approximately zero (0.001). We simulated two additional scenarios (Table 4.1): (1) where there is selective passage so migrants have moderate total subadult survival (0.32) and (2) complete passage where migrants have high total subadult survival (0.50), effectively recruiting more migratory individuals into adulthood.

For all scenarios, we estimated population viability as the cumulative proportion of population trajectories that had reached quasi-extinction by the end of the 200-year scenarios (i.e. probability of extinction). If 100% of the population trajectories were extinct after 200 years, we reported the year by which all trajectories had gone extinct. Population growth rate across all trajectories within a scenario was recorded as the mean of log of stochastic lambda ($\log\lambda_s$), which is the arithmetic mean of the log ratios of population sizes in adjacent years (Morris and Doak 2002). The log of the stochastic lambda is analogous to $\log(\lambda)$ recorded for a single deterministic matrix. We recorded the mean values of $\log\lambda_s$ for each population trajectory in order to examine the distribution of $\log\lambda_s$ across all trajectories for each scenario.

Consequences of Hybridization

We examined demographic consequences due to hybridization by decreasing mean vital rates in the stochastic matrix model described above in the complete passage scenario according to reduced reproductive output of hybrids compared to WCT described in recent studies. Muhlfeld et al. (2009a) described an approximate 50% lower reproductive output of individuals with HIs of 0.20 and an approximate 25% lower reproductive output of individuals with HIs of 0.10 relative to unhybridized fish. It is unclear from that study whether differences in reproductive output were due to life history differences, lower fecundity, lower mating success, lower egg through young of year survival, or some other factor. Results of relative survival studies for F1 WCT X RBT hybrids versus parental types reported in Leary et al. (1995) suggest reduced survival in hybrids occurs primarily post-hatching. Therefore, we modeled the reduced reproductive output of hybrids described in Muhlfeld et al. (2009a) as a reduction in young of year survival. In addition, our research suggested that average size dependent fecundity of hybrids with HI > 0.20 was approximately 50% of that for individuals with HIs of 0 – 0.20 (Chapter 2). We ran three additional scenarios in the stochastic matrix model where (1) we reduced fecundity of all adult age classes by 50%, (2) we reduced young of year survival by 25%, and (3) we reduced young of year survival by 50%. We analyzed the output of these scenarios as described above for migration scenarios.

Introgression Model

We modeled the potential for introgression for the selective and complete passage scenarios using PEDAGOG (Coombs et al. 2010). PEDAGOG is an individual-based population genetics simulation framework where each individual has a multi-locus genotype, in this case, for the 7 diagnostic microsatellite markers used to genotype individuals in Chapters 1 and 2. Under selective passage management, it is important to acknowledge that passage of hybrid individuals can still occur because phenotypic characteristics are a notoriously inaccurate basis for identification of backcrosses to WCT (Weigel et al. 2002). Under the complete passage scenario, individuals of all possible HIs passed the K-Canal and S-Canal diversions into the upstream population. We modeled two populations, Trap and upper Jocko River. We generated allele frequencies for each locus and population using raw individual genotypes entered for individuals collected in the K-Canal and S-Canal traps (Trap, below barrier), as well as upstream population genetic samples collected in the upper Jocko River and the lower South and Middle Forks of the Jocko River (JR, above barrier). While population samples from the South Fork and Middle Fork Jocko River used to create the JR simulated population were mildly introgressed ($pRBT < 0.02$), we removed individuals with RBT alleles in order to test the direct influence of the hybridized trap population on the total change in pRBT in the above-barrier JR population as if it were unhybridized. This also effectively modeled another generalized scenario, where managers must make a decision to maintain or remove barriers below an isolated, unaltered cutthroat population.

We modeled individual WCT during their subadult and adult life stages (and used identical survival rates to those in the matrix model). Individual growth was modeled using modifications of the von Bertalanffy growth model developed for individuals with WCT genotypes in Chapter 3, where the migratory Trap population had a maximum length (L_∞) of 550mm and the resident JR population had L_∞ of 380mm. Individual fecundity was modeled using the length-fecundity relationship estimated for individuals with WCT genotypes in Chapter 3. Maturity was modeled to be age based (individual must be at least two years old), as well as size based (individual must be at least 150mm). Movement of individuals from the trap to the upstream population required an individual to be >300mm (and mature). At each reproductive time step, parents were randomly chosen from the mature individuals, and matings were carried out randomly until annual cohorts of 10,000 1-year-olds were created. Cohorts were allowed to overlap (e.g. a mature 3 year-old could mate with a mature 5 year-old). To model sneaker male mating, the number of potential mates for females was set at 2, and the number of potential mates for males was set at 3. See Appendix C for a complete list of specific parameters.

Each scenario was run for 6 generations to approach a stable age distribution. Frequencies of RBT alleles in the trap population were similar before and after the initial period, but no individuals with HI > 0.3 remained after (Figure 4.4). We modeled movement of individuals from a hybrid swarm (trap population) into the upstream JR population. Five output generations were modeled to examine changes in introgression in JR over a reasonable management time-frame (10-20 years), with movement from trap to JR allowed after the first generation (4 generations with movement). PEDAGOG sampled the population at each time step. We estimated the pRBT in each sample as the number of RBT alleles an individual had divided by 14 (total alleles possible per individual for 7 diagnostic microsatellites).

Passage Scenarios

A review by Allendorf et al (2004) suggests populations with pRBT < 0.20 are morphologically indistinguishable from WCT populations based on meristics (from Leary et al. 1984), and populations had to have pRBT > 0.50 to be reliably identified from morphological characteristics (from Weigel et al. 2002). To establish a range for identification errors, we downscaled these population-level admixture cutoffs to individual admixtures (i.e. an individual with HI = 0.20 should often be morphologically distinguishable from WCT, and an individual with HI = 0.50 should be reliably distinguishable from WCT). We simulated selective passage with two different levels of identification error and then a complete passage (barrier removal) scenario. In the first scenario (Selective/HI ≤ 0.2), individuals collected in the migration traps with HI ≤ 0.20 were used as starting genotypes for the simulated Trap population. In the second scenario (Selective/HI ≤ 0.5), individuals collected in the migration traps with HI ≤ 0.50 were used as starting genotypes for the simulated trap population. As a final scenario (barrier removed) for the trap population, we established a starting population for Trap that included all individuals collected in migration trap samples with hybrid and RBT genotypes to mimic a management strategy where all migratory fish were passed, or the barriers were removed completely (see Figure 4.4 for frequency distributions of HI before and after burn-in).

Results

Passage and Population Viability

The λ of the deterministic matrix for the base (no passage scenario) model was 1.0. Young of year survival, the transition of subadults to adult1 stage, and reproductive contribution of the adult1 stage had the highest elasticities by an order of magnitude over all other matrix elements (Table 4.2). The elasticities of all vital rates for the selective passage scenario were low relative to those of the base scenario. The resident subadult to migratory subadult transition and migratory subadults to migratory adult 1 transition had equal elasticities (0.02) that were the highest of migratory vital rates.

Despite low elasticity values for migratory life history vital rates (Table 4.2, also see Appendix C, Table C.3.), population viability was substantially increased by allowing migration, regardless of whether passage was selective (moderate migrant survival) or complete (high migrant survival). In the stable age distribution used to start all simulations, there were 0 adult migrants in the scenario with no passage, 12 in the scenario with selective passage, and 16 in the scenario with complete passage. The no passage scenario had a logλ_s of -0.027 with a SD of 0.0138 (Figure 4.6). There was an increase in mean logλ_s of 0.013 (48%) under the scenario with selective passage (mean logλ_s = -0.008, S.D. = 0.0136). Further increasing the migrant survival under the complete passage scenario resulted in an increase mean logλ_s of 0.027, a nearly 100% increase relative to the no passage scenario (mean logλ_s = -0.0004, S.D. = 0.0131). At the end of the 200-year time period, the cumulative proportion extinction was 0.35 with complete passage, 0.83 for no passage, and 0.54 for selective passage.

Consequences of Hybridization

After 200 years, 100% of simulated population trajectories were extinct for the scenarios with a 50% reduction in fecundity (40 years to 100% extinction) and a 50% reduction in young of year survival (38 years to 100% extinction) (Figure 4.7). Both of these scenarios had substantially negative $\log\lambda_s$ (-0.173 and -0.174, respectively), indicative of rapidly declining populations (deterministic $\lambda \sim 0.86$) (Figure 4.8). Time to 100% extinction (124 years) and $\log\lambda_s$ (-0.074) were substantially higher for the scenario with a 25% decrease in young of year survival, but all scenarios had dramatically lower viability than the base scenario with high migrant survival (Figure 4.7).

Passage and Introgression

At the end of 6 burn-in generations, pRBT in the Trap population averaged 0.013 (S.D. = 0.0023) for the selective/HI ≤ 0.2 scenario, 0.04 (S.D. = 0.0048) for the selective/HI ≤ 0.5 scenario, and 0.10 (S.D. = 0.01) for the barrier removed scenario (also see Figure 4.4). Introgression was detectable in the upstream JR population in the first sample following the start of movement from the Trap population. The trajectory of pRBT was asymptotic in both selective passage scenarios after 4 generations of migration approaching the starting pRBT of the Trap population (Figure 4.9). After 4 generations, pRBT was 0.007 (S.D. = 0.002), 0.023 (S.D. = 0.004), and 0.058 (S.D. = 0.01) for the selective/HI ≤ 0.2, selective/HI ≤ 0.5 scenario, and barrier removed scenarios, respectively. After 10 generations, the JR population pRBT (0.086) had still not increased to the Trap population pRBT (0.108) in the high admixture scenario (also see

Figure 4.9). Migratory adults from Trap averaged 7% of the total mature adults in the JR population, which was slightly higher than the percentage of adults that were migratory at stable age distribution in the demographic models (3-4%).

Discussion

Westslope cutthroat populations in the Jocko River are threatened both by hybridization and barriers to movement that limit hybridization and migratory life histories. The demographic model results indicate that restoration of even a few migratory individuals in an otherwise resident WCT population could substantially increase population growth rate and viability. If reestablishing a migratory life history, however, also increases hybridization and decreases juvenile recruitment through reductions in fecundity (Chapter 3) or early survival (e.g., Muhlfeld et al. 2009a), the demographic costs associated with hybridization could outweigh the benefits of reconnection. Selective passage may offer the best alternative to this apparent conundrum. Results of the genetic introgression model indicate that an upstream population would be sufficiently hybridized to reduce viability only with a full passage from a moderate or highly hybridized downstream population. Selective passage could essentially limit the migrants to pRBT less than 0.03 and negligible declines in fitness associated with further hybridization.

Passage and Population Viability

This study quantifies the potential role of migratory multiple life histories in population viability of WCT. The improved viability in the scenarios with selective and complete passage relative to the viability of the scenario with no passage suggest that even very few surviving migrants with high individual fecundity rates impart much improved population growth rates. We observed this improvement in viability, despite the fact that migrants have lower survival to adulthood than resident forms (Shepard et al. 1997).

We assumed no density dependence in our model, which could have altered the results. We ignored density dependence because habitats above the K-Canal and S-Canal diversions support low trout densities despite the presence of widely suitable habitat (Craig Barfoot, CSKT, unpublished data). Whether or not increasing density would constrain viability with restoration of migratory life histories would depend on the carrying capacity, the strength of density dependence, and the life stages it influences. Hilderbrand (2003) explored density dependence in cutthroat trout populations and demonstrated that carrying capacity can be positively correlated with population viability. Strong density dependence acting on young of year and juvenile fish could limit the viability gains if highly fecund migratory adults flood early life history rearing habitats with offspring. However, because partial migration in salmonid populations is a conditional strategy that may be influenced by density-related factors (Jonsson and Jonsson 1993), it is reasonable to assume increased juvenile densities are likely to result in compensating shifts in proportions of migratory type fish in the population. Rather than decreased population viability through exceeded carrying capacity, the system would likely settle in to a new equilibrium, but with the compensatory reserve to grow if survival declined for other reasons. While the magnitude of the increase of population viability is likely to change with some limits to population size, we would generally expect an increase in viability with passage.

In addition to their benefits to population viability, migratory life histories in WCT are highly valued by anglers and managers. Aside from their value as sportfish because of larger body size, migratory trout fill important ecological roles (e.g. Koel et al. 2005), are crucial for dispersal and recolonization following catastrophic events (Dunham et al. 2003; Gresswell 1999), and are considered crucial for maintaining genetic and demographic connectivity between populations (Neville et al. 2006; Shepard et al. 2005). Efficacy of restoration of migratory life histories into any river system may depend on several factors. Barrier removal or selective passage projects should be assessed from a cost-benefit standpoint. Projects should be feasible from the perspective of human and financial capital, and there should be adequate habitat and a high likelihood of achieving population goals (Kemp and O'Hanley 2010).

The degree to which migratory versus resident life history is expressed seems to be due to interplay of genetic and environmental effects, and the relative importance of those effects is highly context-specific in trout and charr (Northcote 2010). Resident populations can evolve, or re-express migratory life histories in relatively short time frames (e.g. Pascual et al. 2001). Bohlin et al. (2001) provided evidence that the density of migratory populations of anadromous brown trout *Salmo trutta* increased with decreases in migration cost due to elevation, suggesting restoration of migratory life histories may be most successful where migratory costs are otherwise low. Likewise, Morinville and Rasmussen (2003) demonstrated that migratory forms of brook trout *Salvelinus fontinalus* have higher metabolic costs and higher consumption rates than resident forms. These studies indicate the magnitude to which migratory life history forms flourish may depend on the biotic constraints and distance to suitable habitats. In the case of the Jocko River, low-elevation, main-stem habitats are still suitable for growth of migratory fish and are close proximity from spawning habitats, making this a good candidate system for restoring connectivity before migratory components of basin populations are lost.

Introgression and Restoration of Migratory Life Histories

While it has been demonstrated that introgressive hybridization can rapidly lead to the formation of a hybrid swarm, even when there is strong selection against hybrids (Epifanio and Philipp 2000), we are not aware of any research demonstrating large-scale declines in population sizes of WCT with the onset of RBT hybridization that are implicated by the simulations in this study. We found declines in the numbers of juveniles produced could have dramatic consequences for population viability in highly hybridized populations (Figure 4.7). This is consistent with the high elasticities for young of year survival and fertility of resident adults. Modeling population viability consequences of reduced individual fecundity in isolation of other factors may be overly simplistic. While highly hybridized individuals had lower fecundities, they also had significantly higher egg size (Chapter 3), which may improve embryo or subsequent juvenile survival (Einum et al. 2004; Hendry and Day 2003). Similarly, high RBT ancestry individuals also demonstrated significantly higher growth rates and earlier (but not significant) median age at spawning migrations (Chapter 3). However, increases in survival of young of year (or later life stages) attributable to hybrid ancestry are unknown and Leary et al. (1985) described developmental instability and high fluctuating asymmetry in F1 hybrids. Thus, evaluating the differences in how fish of different ancestry express these key life history tradeoffs is needed to better understand the demographic consequences of hybridization.

The demographic simulations support the importance of limiting further introgression and the value of migratory life histories. Even though there is still uncertainty about the full consequences of introgression on persistence, caution is important. The results of the introgression simulations suggest that we might solve both problems with selective passage that could be used to limit upstream introgression. Even in a scenario where hybrids with HI of 0.5 were misidentified as WCT, the pRBT of the JR population was only 0.023 after 4 generations (10-15 years) of passage. Although introgression is detectable at most sites (Actual estimates of pRBT in the Jocko River above S-Canal range from 0 to 0.07, Chapter 2), selective passage of migratory fish at the K-Canal and S-Canal Ladders would not likely result in meaningful increases in measured pRBT. It is also important to point out that this model assumes random mating and a complete overlap in spawn timing and location, thus the modeled scenarios are essentially worst-case. We know that high HI hybrids have different migration and spawning patterns than WCT (Chapter 3, Muhlfeld et al. 2009b). Additionally, in natural systems, such as the Jocko River, where above barrier populations have very low rates of RBT hybridization, most migratory individuals produced by these populations would have WCT genotypes (HI = 0, Figure 4.2), and most individuals with HIs > 0.20 would likely be potential strays from populations well downstream of the traps. Finally rates of misidentification from field morphology, and thus increased introgression risk, could be drastically reduced with implementation of rapid genotyping to select migrants for passage.

Limitations and Utility of Models

There are important limitations to the models described in this study. First and foremost, the vital rates used to parameterize these models were not derived from demographic studies conducted on Jocko River WCT populations. Rather, we used mean vital rate estimates from the literature and demographic model structure in a similar fashion to Hilderbrand (2002) and Hilderbrand (2003). Several authors (e.g. Beissinger and Westphal 1998; Reed et al. 2002) have described the pitfalls of parameter estimates based on closely related species or populations, expert opinion, or range-wide approximations in predictions about specific populations. Our approach of making relative comparisons, to consider the nature of the tradeoffs is generally considered a robust application of viability analyses (Reed et al. 2002). While we place little certainty on the accuracy of the point estimates, the magnitude of the differences among scenarios has utility for evaluating the potential implications for management (Beissinger and Westphal 1998).

Another common criticism of viability analysis is the omission of factors in population models that have important influences on real populations (density dependence, inbreeding depression, catastrophes, correlation of vital rates, etc., Morris and Doak 2002). In structuring these models we made the decision not to incorporate density dependence, because we had little or no information to develop parameters. This is a common problem, however, and while density dependence could undoubtedly be important, growth rates at small population sizes critical to persistence probably are not strongly density dependent (Boyce 1992). Although we may not capture the full dynamics of the range of population sizes possible in the system, we believe our simulations are useful for considering the most pertinent comparisons involving passage and introgression consequences.

Perhaps one of the biggest uncertainties was the implication of the growth and egg size differences between high-RBT hybrids and WCT observed in Chapter 3. To model the effects of these differences on viability, we would need to know the link between growth and survival and between egg size and subsequent embryo or emergent fry survival, but these are links for which information was not readily available. Given the high elasticity of young of year survival in the demographic model, increases in survival at this life stage due to increased growth or increased egg size certainly have the potential to balance the apparent reductions in viability due to lower fecundity rates described in this study. This illustrates the importance of focusing future research efforts on measuring vital rates across the spectrums of life stages, admixture, and habitats.

The primary limitation of the PEDAGOG model was that below barrier populations were required to randomly mate during the initial simulations to achieve stable age distribution. Random mating created a hybrid swarm in the below-barrier population and eliminated any individuals with exceptional HIs. This leads to a somewhat artificial modeling scenario relative to what occurs in the Jocko River system, where the below-barrier mixture is made up primarily of individuals with no RBT ancestry and a few individuals with very high HIs. We predict reduction in the variation of below-barrier HIs likely leads to reduction in the variation of pRBTs in the above-barrier population following the onset of movement above the barrier. However, the general conclusions about the relative changes in pRBT in the above-barrier population should be robust to this limitation.

Even with the limitations, this study makes three important steps for cutthroat trout management and research: (1) the development of a multiple life history cutthroat trout matrix model to consider tradeoffs among demographic connectivity scenarios, (2) an elasticity analysis of that matrix, which allows us to identify priorities for viability research in multiple life-history trout populations and (3) the application of a population genetic model to consider hybrid identification errors and tradeoffs between no passage, selective passage, and complete passage scenarios. In short, despite its limitations, this study was effective in answering the primary research goal: to assess the demographic and genetic tradeoffs of restoration of connectivity for fish with migratory life histories.

Management and Research Recommendations

Barrier Management and Selective Passage

The demographic benefits of restoration of migratory life history forms are clear and can be substantial. Improved production of large migratory fish have the associated benefits of fulfilling important ecological roles as prey for larger vertebrates (Koel et al. 2005) and important socio-economic roles as sportfishes. Use of barriers as a management tool has the undesired effect of isolating populations and fragmenting habitats, which can, in turn decrease the viability of local populations (Harig and Fausch 2002; Morita and

Yamamoto 2002; Hilderbrand 2003). On the other hand, depending on the environmental context and the threat of non-native fishes in addition to hybridizing species (such as brook trout), removing barriers can also lead to reductions in viability (Peterson et al. 2008). Based on the results of this study, the decision whether or not to restore a migratory life history, or restore connectivity to an artificially isolated fish population, should be largely dependent on the magnitude of the hybridization threat downstream and the magnitude of previous introgression upstream. The potential for invasion of other detrimental non-native species, the degree of population isolation, and whether or not there is sufficient habitat volume above the barrier are also important factors (Harig and Fausch 2002, Fausch et al. 2009).

Selective passage to restore migratory life histories, where feasible, offers an attractive alternative to complete barrier removal that could allow a multitude of invaders into upstream habitats. If non-native introgression is already present in populations upstream of barriers, and introgression occurs at acceptable rates below barriers, or selective passage can be conducted with acceptable error rates, the results of this study suggest there is considerable opportunity to improve system connectivity and long-term population viability while maintaining conservation populations under current management policy (UDWR 2000). If there is no detectable introgression above a barrier, managers may be prudent to maintain isolation for that population to conserve an unaltered genome, but they must realize that if habitat is limited, the population will likely need to be occasionally supplemented by other core conservation stocks to maintain long-term viability.

Critical Areas for Future Research

The declines in modeled viability demonstrated in this study when young of year survival and fecundity are reduced suggest knowledge of vital rates in hybridized populations is important. The approach taken to model reductions in vital rates was simplistic because data are limited and mechanisms are still unclear. We modeled vital rate reductions under the assumption that they would affect migratory and resident life history types equally, even though both Muhlfeld et al. (2009a) and Chapter 3 were studies conducted exclusively on migratory life histories. We also did not account for any influence of egg size on young of year survival given the lack of data.

Elasticity values of matrix elements (Table 4.2) and individual vital rates (Appendix C, Table C.3.) suggest that the vital rates that have the greatest influence on population growth rate are total young of year survival, the transition probability of resident subadults to adulthood, and the fertility of small adult residents. We recommend increased research effort be placed on these previously poorly understood life stages. Unfortunately young of year survival is notoriously difficult to measure in wild populations, but it would be possible to estimate growth, fecundity, and survival rates of individuals with various RBT ancestries in a variety of habitats at juvenile through adult life stages.

Understanding the links between habitat variation and hybrid fitness may be one of the most crucial avenues for future research. The results of Chapter 2 suggest landscape resistance plays an important role in structuring of WCT x RBT hybrid zones. Measuring fitness surrogates in resident populations with various times since the start of RBT introgressions may help us better understand the interplay of natural selection and introgression over time (Taylor 2004). The results of Chapter 3 suggest there are genetic and/or environmental influences on differential growth rates between WCT and high RBT ancestry hybrids. Increased growth observed in high RBT ancestry hybrids may reduce the age at maturity, or increase juvenile survival, both of which may counteract potential reductions in young of year survival or fecundity (Hutchings 1993). As described above, earlier age at maturity, in particular, can dramatically increase viability of salmonid populations (Letcher et al. 2007; Morita et al. 2009). Further research addressing the influences of environment versus hybrid ancestry on variation in vital rates across hybrid zones would make a fundamental contribution to our understanding of the conservation needs of cutthroat trout. In summary, while the results of the reduced vital rate scenarios in this study suggest there may be some high costs to removing barriers and allowing introgression, we have much to learn about the contexts in which such actions would be so costly.

Table 4.1. Demographic scenarios to demonstrate the improvement in population viability from allowing escapement of adult migratory fish. Base scenario with migrant subadult survival near zero allows no escapement of migratory fish (no passage). selective and complete passage scenarios model various levels of escapement of adult migratory fish back to the resident (above trap) population. Total stage survival can be obtained by adding survival and transition probabilities within a stage. For example, total migratory subadult survival is 0.25 + 0.25 = 0.50 under the complete passage scenario.

Survival Parameter	No Passage	Selective Passage	Complete Passage
Resident Subadult Survive/ Stay Subadult	0.14	0.14	0.14
Resident Subadult Survive/ Become Migrant Subadult	0.02	0.02	0.02
Resident Subadult Survive/ Become Resident Adult 1	0.14	0.14	0.14
Migrant Subadult Survive/ Stay Subadult	0.001	0.16	0.25
Migrant Subadult Survive/ Become Migrant Adult 1	0.001	0.16	0.25

Table 4.2. Elasticities of matrix elements for matrix models with base (no passage; “Res”) selective passage (“Mig”) scenarios.. (For elasticities of individual vital rates see Appendix C, Table C.3.). Elasticities are estimated using mean vital rates under the selective passage scenario.

	YOY	Res SA	Mig SA	Res Ad1	Mig Ad1	Res Ad2	Mig Ad2
YOY	0	0	0	0.182	0.016	0.054	0.005
Res SA	0.256	0.041	0	0	0	0	0
Mig SA	0	0.021	0.007	0	0	0	0
Res Ad1	0	0.236	0	0.068	0	0	0
Mig Ad1	0	0	0.021	0	0.006	0	0
Res Ad2	0	0	0	0.054	0	0.028	0
Mig Ad2	0	0	0	0	0.005	0	0.002

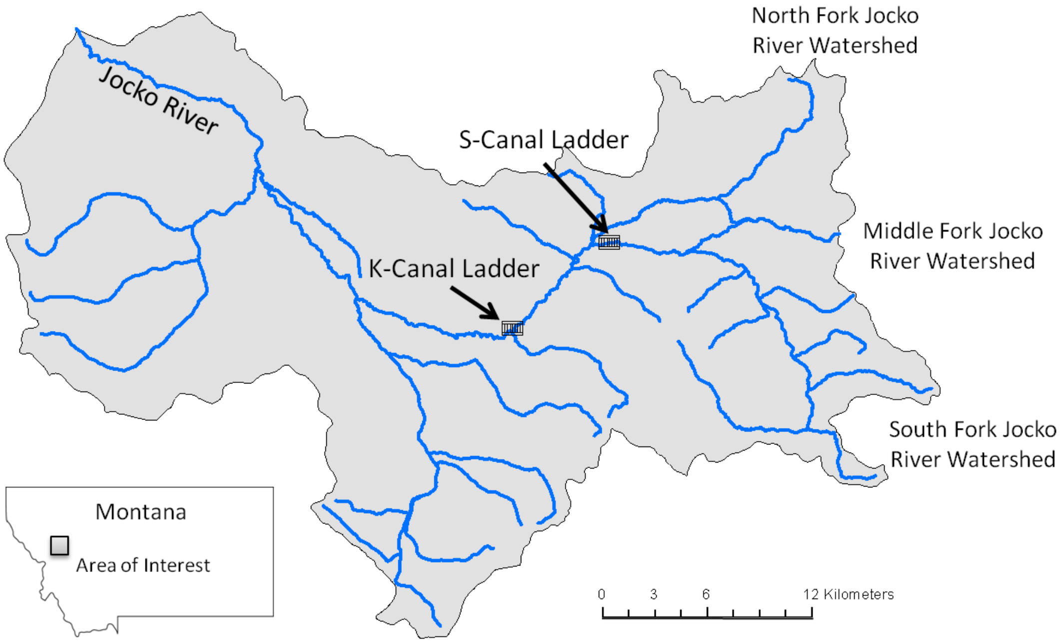


Figure 4.1. Map of the Jocko River drainage, Montana. The selective passage management actions tested with this population viability analysis would be implemented at the K- and S-Canal ladders.

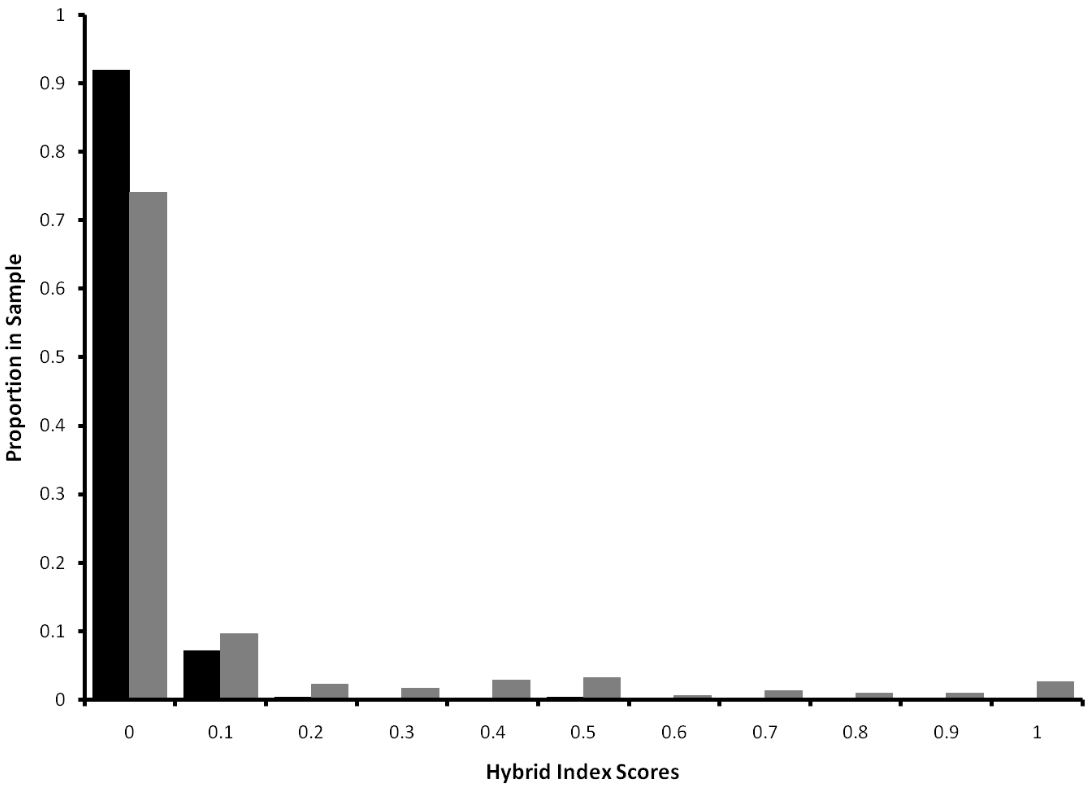


Figure 4.2. Distribution of hybrid index scores for individuals collected in the Jocko River above S-Canal (black bars) and for all individuals captured at the K-Canal and S-Canal ladder traps (trap population, grey bars). Histogram bin labels are the upper boundary of that bin.

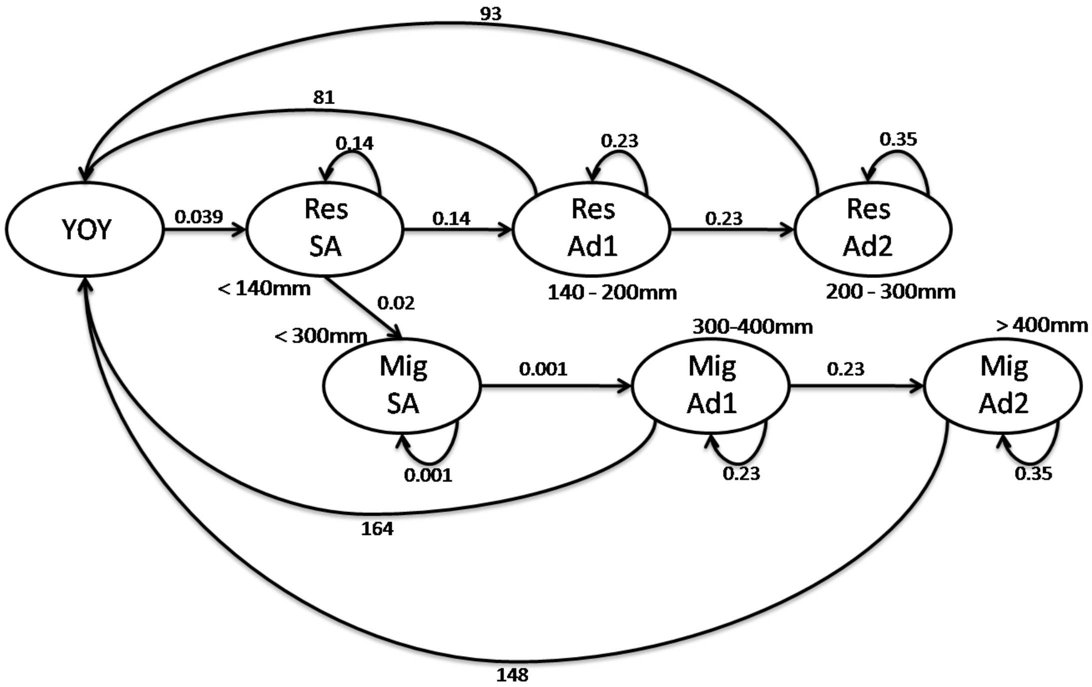


Figure 4.3. A stage-structured population model including stream resident (Res) life history types and fluvial, or migratory (Mig), life history types. The values associated with the arrows are the mean vital rates used to develop the base model for this study. The two life history types are subject to the same young of year (YOY) survival, which includes estimates of fry survival and incubation success from Shepard (1997). Migrant individuals must pass through an additional subadult (SA) life stage before reaching adulthood (Ad) to reflect later ages at maturity and decreased survival of migrants relative to residents. Life stages are length based, except young of year.

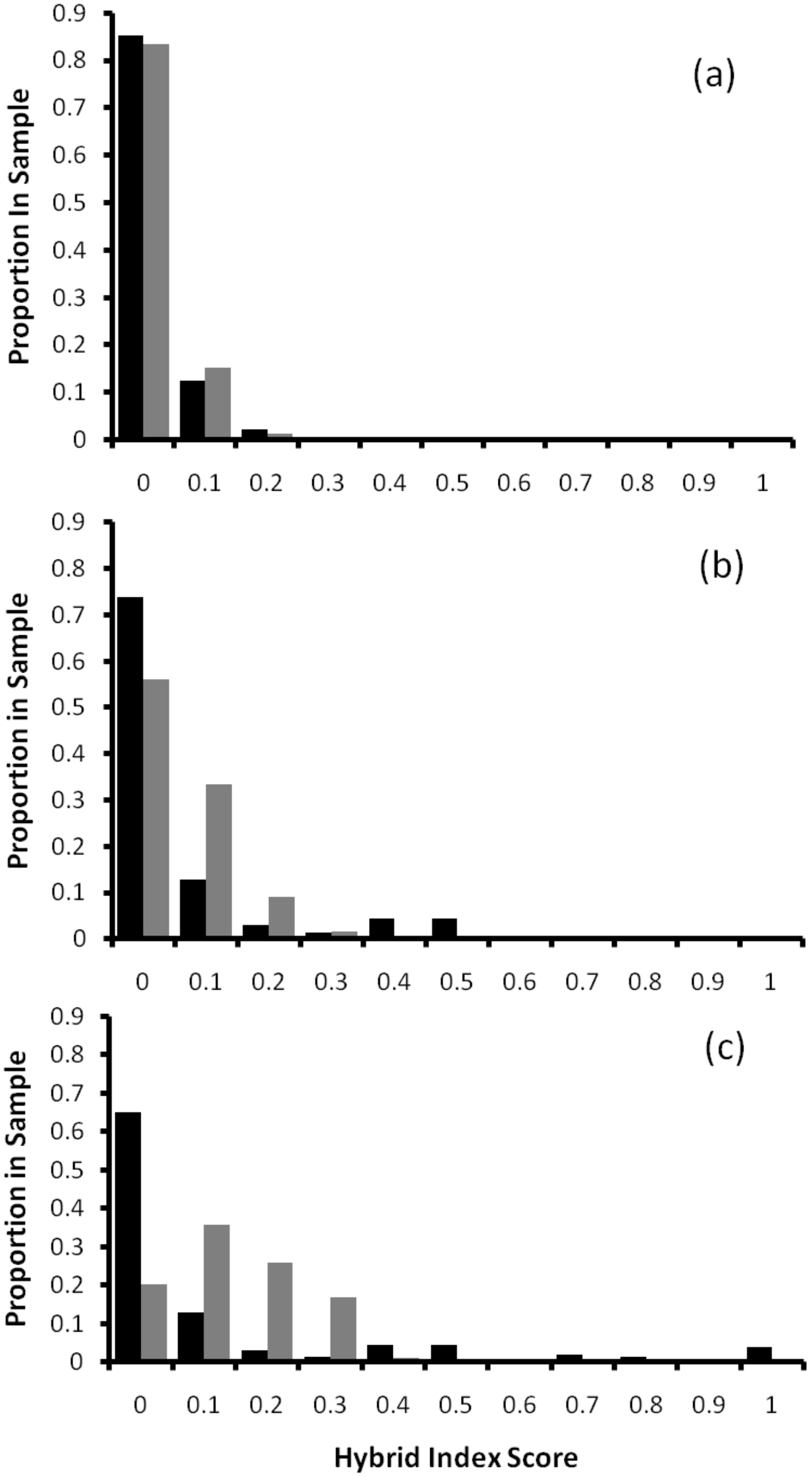


Figure 4.4. Distribution of hybrid index scores for initial trap population (black bars) and for the trap population (grey bars) following a 6 generation burn-in of random mating for (a) selective/HI ≤ 0.2, (b) selective/HI ≤ 0.5, and (c) complete passage PEDAGOG scenarios.

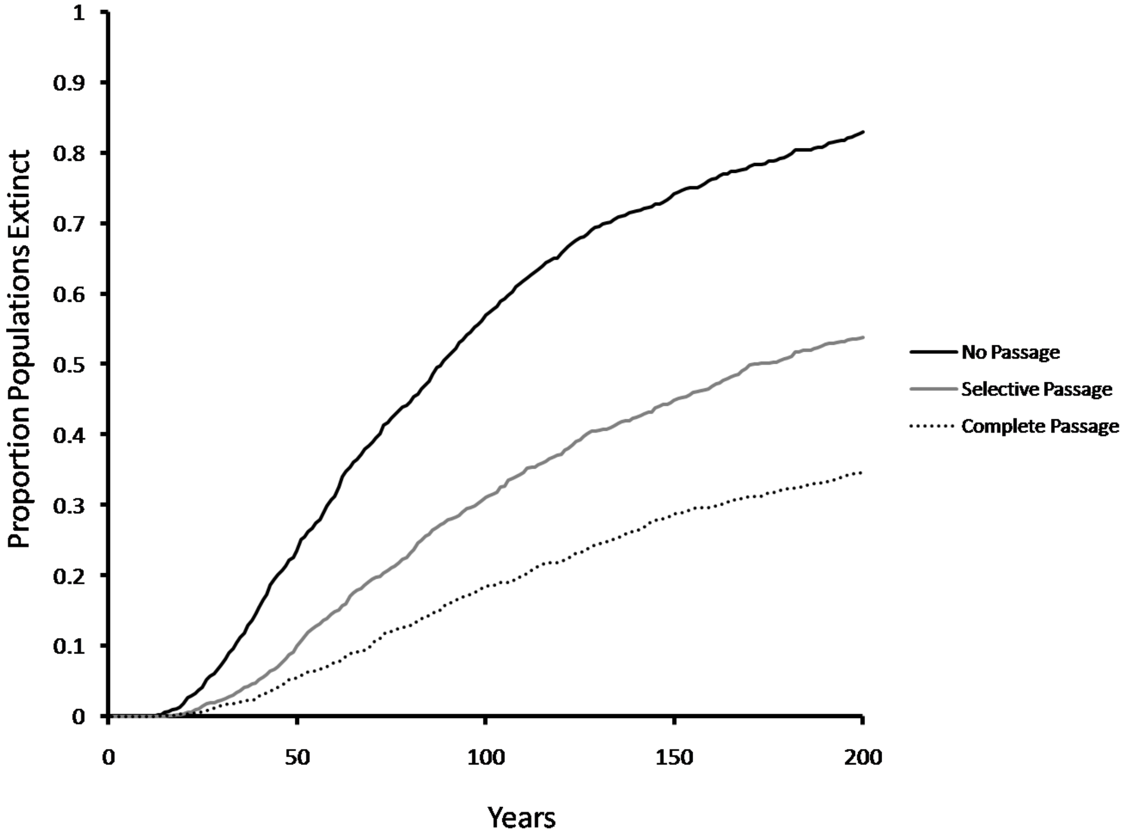


Figure 4.5. Cumulative probability of quasi-extinction of the 1000 random population trajectories.

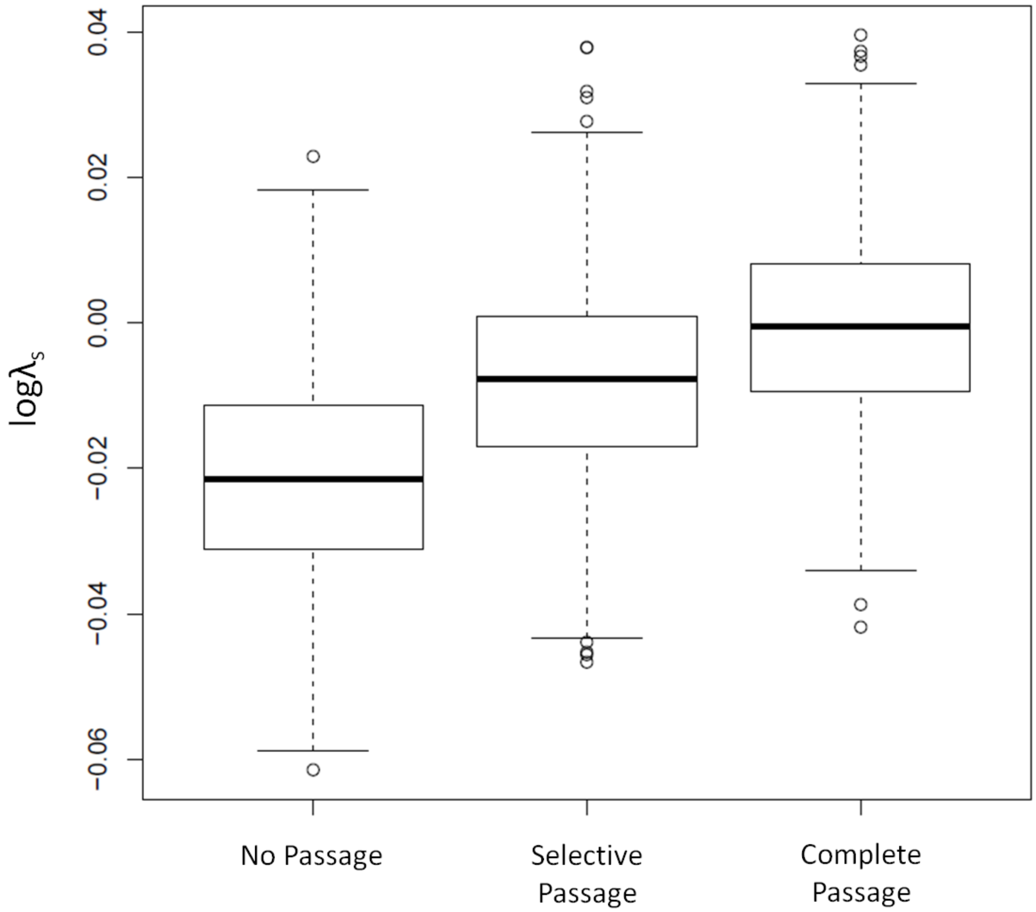


Figure 4.6. Mean stochastic log λ ($\log\lambda_s$) values for the migrant passage scenarios used in this study. No passage is the base model, representing current, pre-management population growth. Under the selective passage scenario, total migrant subadult survival was increased to 0.32. Under the complete passage scenario, total migrant subadult survival was increased to 0.50. Negative $\log\lambda_s$ are indicative of a declining population, positive of a growing population, and $\log\lambda_s$ of zero are indicative of a stable population.

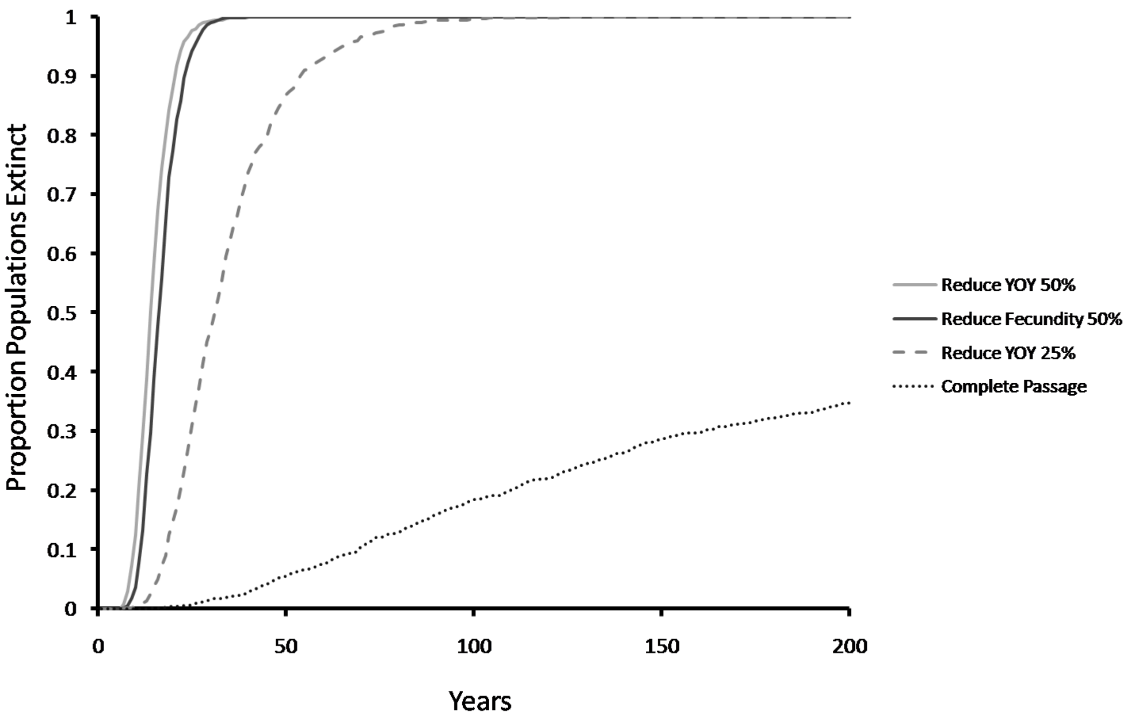


Figure 4.7. Cumulative probabilities of quasi-extinction for simulations where young of year (YOY) survival and fecundity were reduced to model the potential demographic impacts to populations with pRBT of zero (Complete Passage), pRBT = 0.10 (Reduce YOY 25%), and pRBT = 0.20 (Reduce YOY 50% and Reduce Fecundity 50%) in concordance with results from Muhlfeld et al. (2009a) and fecundity results from Chapter 3.

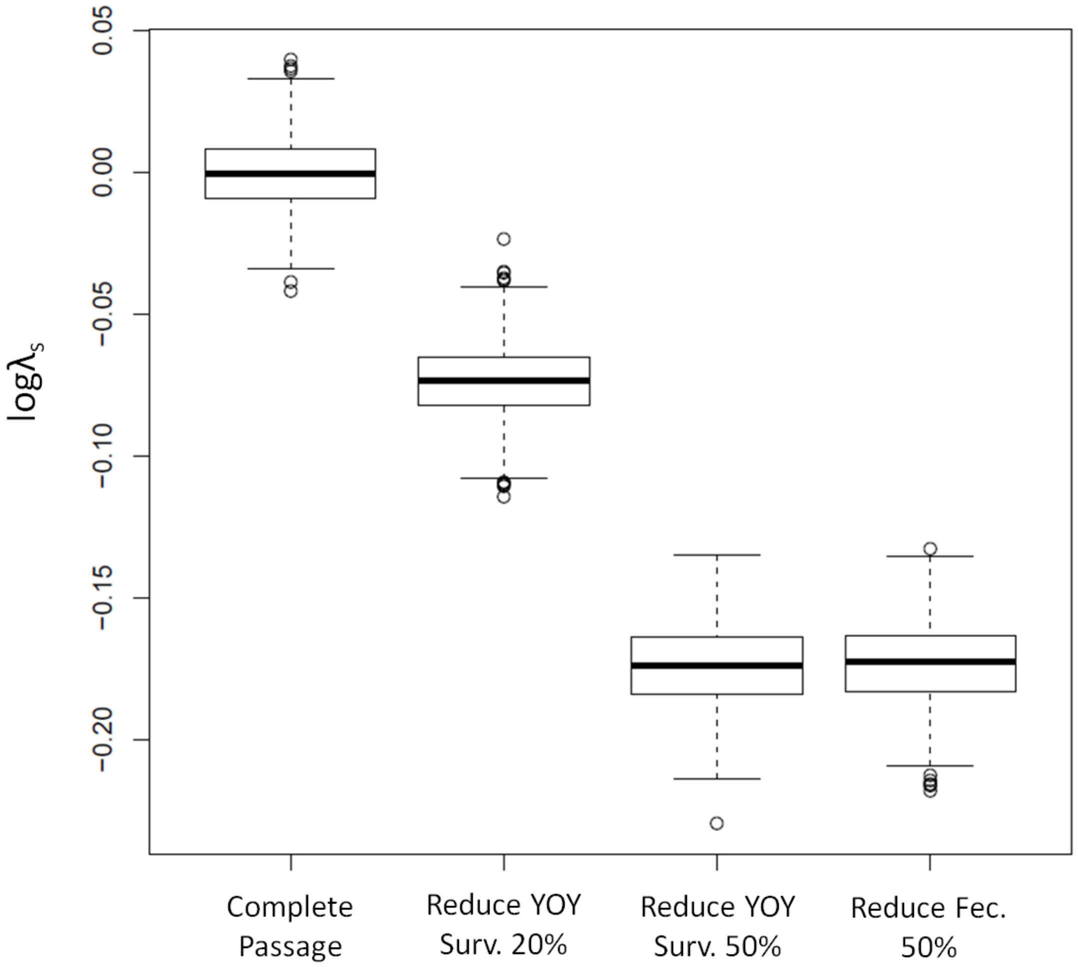


Figure 4.8. Mean stochastic log λ (logλ_s) value distributions for the reduced vital rate scenarios. For these scenarios, the complete passage scenario is the base model. Results are for scenarios with pRBT of zero (complete passage), pRBT = 0.10 (Reduce YOY 25%), and pRBT = 0.20 (Reduce YOY 50% and Reduce Fecundity 50%) in concordance with results from Muhlfeld et al. (2009a) and fecundity results from Chapter 3.

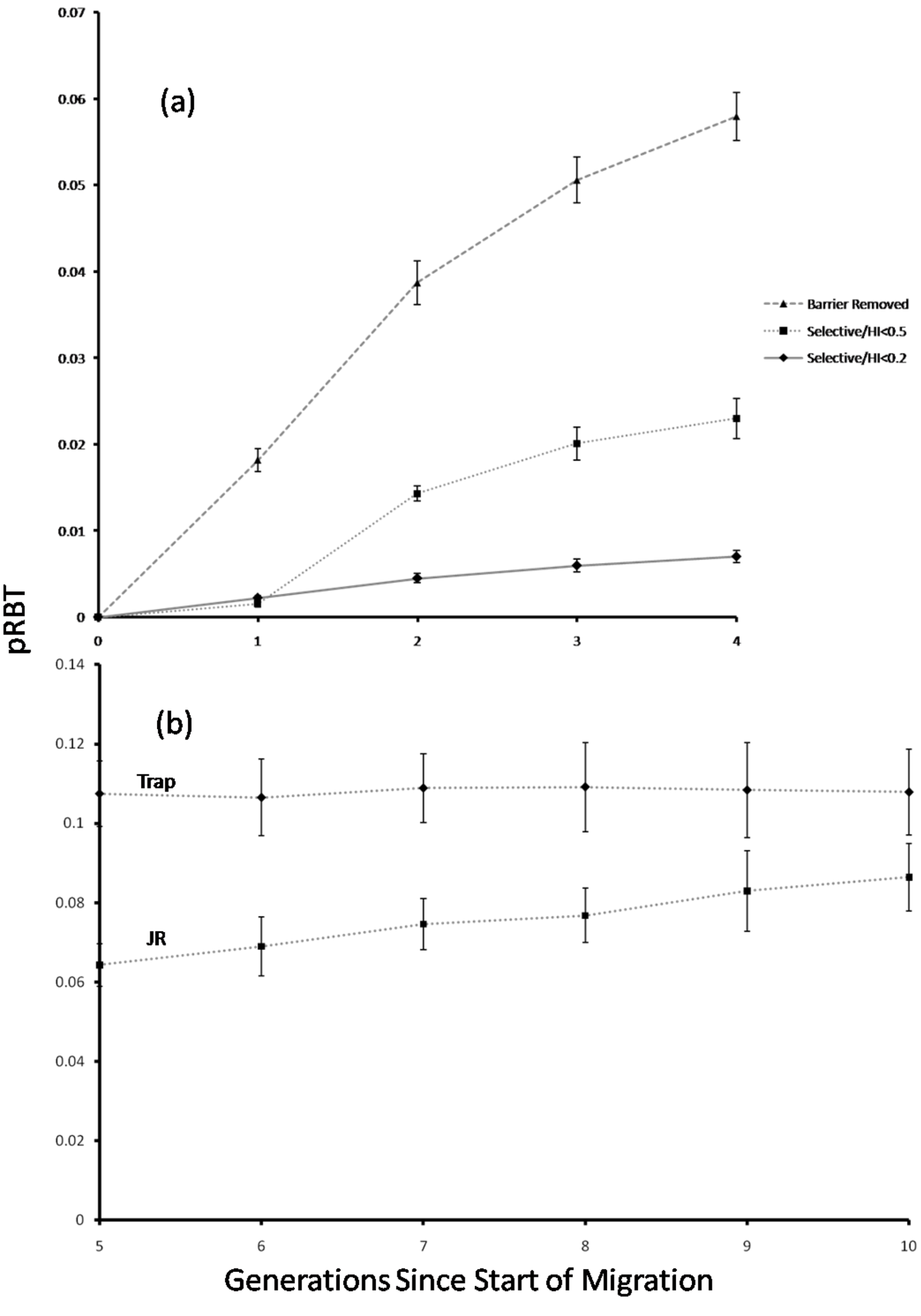


Figure 4.9. Results of PEDAGOG simulations of increased RBT admixture (pRBT) following the start of migration from Trap to Jocko River (JR). The top panel (a) demonstrates the increase in mean pRBT for 10 replications in the JR population for each of the passage scenarios. The bottom panel (b) is the mean pRBT for 10 replications for the Trap population and the JR population under the barrier removed scenario from 5 to 10 generations following start of migration. In both panels error bars are 95% confidence intervals estimated from 10 replications of each scenario.

GLOSSARY OF HYBRIDIZATION TERMS

Admixture: Development of novel genetic combinations due to introgressive hybridization between genetically distinct taxa (Allendorf et al. 2001).

Genomic Extinction: A situation where parental genomes no longer exist due to admixture and the production of hybrid swarms (Allendorf and Luikart 2007).

Hybrid Index (HI): An estimate of individual levels of hybridization. HI is the proportion of the total number of RBT alleles observed divided by the total number of alleles amplified for each individual across all diagnostic loci (Muhlfeld et al. 2010a).

Hybridization: Interbreeding between individuals from genetically distinct populations (Allendorf et al. 2001)

Hybrid Swarm: A population where all individuals are hybrids that have varying levels of parental ancestry due introgressive hybridization (Allendorf et al. 2001)

Hybrid Zone: A geographic region where two taxa are sympatric and hybridize (Allendorf et al. 2001)

Introgression: Gene flow between hybridizing taxa (Allendorf et al. 2001)

Introgressive Hybridization: Hybridization between genetically distinct taxa that results in fertile hybrid offspring that subsequently mate among themselves and with parental types.

pRBT: Proportion of rainbow trout alleles in a sample of individuals. It is calculated by dividing the number of rainbow trout alleles detected across all loci by the total number of alleles amplified (UDWR 2000).

BIBLIOGRAPHY

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., and coauthors. 2005. Cutthroat trout hybridization and the U.S. Endangered Species Act: one species, two policies. Conservation Biology 19(4):1326-1328.

Allendorf, F. W., and coauthors. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conservation Biology 18(5):1203-1213.

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.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford University Press, New York.

Bart, J. 1995. Acceptance criteria for using individual-based models to make management decisions. Ecological Applications 5(2):411-420.

Bear, E. A., T. E. McMahon, and A. V. Zale. 2007. Comparitive thermal requirements of westslope cutthroat trout and rainbow trout: implications for species interactions and development of thermal protection standards. Transactions of the American Fisheries Society 136:1113-1121.

Behnke, R. J. 2002. Trout and salmon of North America, First edition. The Free Press, New York.

Beissinger, S. R. and M.I. Westphal. 1998. On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management 62(3): 821-841.

Bettles, C. M., M. F. Docker, B. Dufour, and D. D. Heath. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. Journal of Evolutionary Biology 18(5):1220-1233.

Bohlin, T., J. Pettersson, and E. Degerman. 2001. Population density of migratory and resident brown trout (*Salmo trutta*) in relation to altitude: evidence for a migration cost. Journal of Animal Ecology 70(1):112-121.

Boyce, M.S. 1992. Population Viability Analysis. Annual Review of Ecology and Systematics. 23(1): 481-506

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.

Burnham, K. P., and D. R. Anderson. 1998. Model selection and multimodel inference: a practical information - theoretic approach, 2nd edition. Springer-Verlag, New York.

Campton, D. E., and L. R. Kaeding. 2005. Westslope cutthroat trout, hybridization, and the U.S. Endangered Species Act. Conservation Biology 19(4):1323-1325.

Cerrato, R. M. 1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy Equation. Canadian Journal of Fisheries and Aquatic Sciences 47:1416-1426.

Clarkson, R. W., P. C. Marsh, S. E. Stefferud, and J. A. Stefferud. 2005. Conflicts between native fish and nonnative sport fish management in the Southwestern United States. Fisheries 30(9):20 - 27.

Coombs, J. A., B. H. Letcher, and K. H. Nislow. 2010. PEDAGOG: software for simulating eco-evolutionary population dynamics. Molecular Ecology Resources 10(3):558-563.

DeRito, J. N., A. V. Zale, and B. B. Shepard. 2010. Temporal reproductive separation of fluvial Yellowstone cutthroat trout from rainbow trout and hybrids in the Yellowstone River. North American Journal of Fisheries Management 30(4):866-886.

Devries, D. R., and R. V. Frie. 1996. Determination of age and growth. Pages 483-512 *in* B. R. Murphy, and D. W. Willis, editors. Fisheries Techniques, Second edition. American Fisheries Society, Bethesda, Maryland.

Downs, C. C., R. G. White, and B. B. Shepard. 1997. Age at sexual maturity, sex ratio, fecundity, and longevity of isolated headwater populations of westslope cutthroat trout. North American Journal of Fisheries Management 17:85-92.

Dunham, J. B., M. K. Young, R. E. Gresswell, and B. E. Rieman. 2003. Effects of fire on fish populations: landscape perspectives on persistence of native fishes and nonnative fish invasions. Forest Ecology and Management 178(1-2):183-196.

Eby, L. A., W. J. Roach, L. B. Crowder, and J. A. Stanford. 2006. Effects of stocking-up freshwater food webs. Trends in Ecology & Evolution 21(10):576-584.

Einum, S., M. T. Kinnison, and A. P. Hendry. 2004. Evolution of egg size and number. Pages 126-153 *in* A. P. Hendry, and S. C. Stearns, editors. Evolution Illuminated. Oxford University Press, New York.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants. Proceedings of the National Academy of Sciences of the United States of America 97(13):7043-7050.

Epifanio, J., and J. Nielsen. 2000. The role of hybridization in the distribution, conservation and management of aquatic species. Reviews in Fish Biology and Fisheries 10(3):245-251.

Epifanio, J., and D. Philipp. 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):339-354.

Fausch, K. D., B. E. Rieman, J. B. Dunham, M. K. Young, and D. P. Peterson. 2009. Invasion versus isolation: trade-offs in managing native salmonids with barriers to Upstream Movement. Conservation Biology 23(4):859-870.

Fausch, K. D., B. E. Rieman, M. K. Young, and J. B. Dunham. 2006. Strategies for conserving native salmonid populations at risk from nonnative fish invasions: tradeoffs in using barriers to upstream movement. USDA Forest Service, Rocky Mountain Research Station. RMRS-GTR-174.

Fausch, K. D., Y. Taniguchi, S. Nakano, G. D. Grossman, and C. R. Townsend. 2001. Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. Ecological Applications 11(5):1438-1455.

Graf, W. L. 1999. Dam nation: a geographic census of American dams and their large-scale hydrologic impacts. Water resources research 35:1305-1311.

Gresswell, R. E. 1999. Fire and aquatic ecosystems in forested biomes of North America. Transactions of the American Fisheries Society 128(2):193 - 221.

Gross, K., B. A. Craig, and W. D. Hutchison. 2002. Bayesian estimation of a demographic matrix model from stage-frequency data. Ecology 83(12):3285-3298.

Gunnell, K., M. Tada, F. Hawthorne, E. Keeley, and M. Ptacek. 2007. Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and introduced rainbow trout (*O. mykiss*) in the South Fork of the Snake River watershed, Idaho. Conservation Genetics.

Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. Ecological Applications 12(2):535-551.

Hawkins, D. K., and T. P. Quinn. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 53(7):1487-1496.

Henderson, R., J. L. Kershner, and C. A. Toline. 2000. Timing and location of spawning by nonnative wild rainbow trout and native cutthroat trout in the South Fork Snake River, Idaho, with implictions for hybridization. North American Journal of Fisheries Management 20:584-596.

Hendry, A. P., T. Bohlin, B. Jonsson, and O. K. Berg. 2004. To sea or not to sea? Anadromy versus non-anadromy in salmonids. A. P. Hendry, and S. C. Stearns, editors. Evolution illuminated: Salmon and their relatives, volume 1. Oxford University Press, New York.

Hendry, A. P., and T. Day. 2003. Revisiting the positive correlation between female size and egg size. Evolutionary Ecology Research 5:421-429.

Hendry, A. P., and S. C. Stearns. 2004. The salmonid contribution to key issues in evolution. Pages 3-19 *in* A. P. Hendry, and S. C. Stearns, editors. Evolution Illuminated. Oxford University Press, New York.

Hilderbrand, R. H. 2002. Simulating supplementation strategies for restoring and maintaining stream resident cutthroat trout populations. North American Journal of Fisheries Management 22(3):879-887.

Hilderbrand, R. H. 2003. The roles of carrying capacity, immigration, and population synchrony on persistence of stream-resident cutthroat trout. Biological Conservation 110(2):257-266.

Hitt, N. P., C. A. Frissell, C. C. Muhlfeld, and F. W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences 60:1140-1451.

Holm, L. E., & Brusgaard, K. 1998 Two polymorphic dinucleotide repeats in rainbow trout (*Oncorhynchus mykiss*) Animal Genetics 30:162-163.

Hutchings, J.A. 1993. Adaptive life histories effected by age-specific survival and growth rate. Ecology 74:673-684.

Hutchings, J. A. 2004. Norms of reaction and phenotypic plasticity in salmonid life histories. Pages 232-263 *in* A. P. Hendry, and S. C. Stearns, editors. Evolution Illuminated. Oxford University Press, New York.

Isely, J. J., and T. B. Grabowski. 2007. Age and growth. Pages 187-228 *in* C. S. Guy, and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in Ecology and Evolution 15(6):250-255.

Jonsson, B. 1985. Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Transactions of the American Fisheries Society 114(2):182 - 194.

Jonsson, B., and N. Jonsson. 1993. Partial migration: niche shift versus sexual maturation in fishes. Reviews in Fish Biology and Fisheries 3(4):348-365.

Jonsson, N., B. Jonsson, and I. A. Fleming. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic Salmon? Functional Ecology 10(1):89-96.

Kalinowski, S.T., Manlove, K.R., Taper, M.L. 2007. ONCOR: a computer program for genetic stock identification. Accessed online at http://www.montana.edu/kalinowski/Software/ONCOR.htm on 5/06/2011.

Kanda, N., R. F. Leary, P. Spruell, and F. W. Allendorf. 2002. Molecular genetic markers identifying hybridization between the Colorado River-greenback cutthroat trout complex and yellowstone cutthroat trout or rainbow trout. Transactions of the American Fisheries Society 131(2):312-319.

Kemp, P. S., and J. R. O'Hanley. 2010. Procedures for evaluating and prioritising the removal of fish passage barriers: a synthesis. Fisheries Management and Ecology 17(4):297-322.

Kennedy, E. J. 1983. Computations of continuous records of streamflow. . Pages 53 *in* USGS Survey Techniques of Water-Resources Investications, Book 3. U.S. Geological Survey

Kimura, D. K. 1980. Likelihood methods for the von Bertalanffy growth curve. Fishery Bulletin 77:765-776.

Kinnison, M. T., M. J. Unwin, A. P. Hendry, and T. P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55:1656-1667.

Klumb, R. A., M. A. Bozek, and R. V. Frie. 2001. Validation of three back-calculation models by using muliple oxytetracycline marks formed in the otoliths and scales of bluegill x green sunfish hybrids. Canadian Journal of Fisheries and Aquatic Sciences 58:352-364.

Koel, T. M., P. E. Bigelow, P. D. Doepke, B. D. Ertel, and D. L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30(11):10-19.

Kolar, C. S., and D. M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science 298:1233-1236.

Kozfkay, C. C., M. R. Campbell, S. P. Yundt, M. P. Peterson, and M. S. Powell. 2007. Incidence of hybridization between naturally sympatric westslope cutthroat trout and rainbow trout in the Middle Fork Salmon River drainage, Idaho. Transactions of the American Fisheries Society 136(3):624-638.

Kruse, C. G., W. A. Hubert, and F. J. Rahel. 1997. Using otoliths and scales to describe age and growth of Yellowstone cutthroat trout in a high elevation stream system, Wyoming. Northwest Science 71:30-38.

Leary, R. E., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native fish. American Fisheries Society Symposium 15:91-101.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1985. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39(6):1318-1326.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1984. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. Proceedings of the Montana Academy of Sciences 15:91-101.

Letcher, B.H., K.H. Nislow, J.A. Coombs, M.J. O'Donnell, and T.L. Dubreuil. 2007. Population response to habitat fragmentation in a stream-dwelling brook trout population. PLoS ONE 2: e1139. doi:10.1371/journal.pone.0001139

Lobon-Cervia, J., C. Utrilla, P. Rincon, and F. Amezcua. 1997. Environmentally induced spatio-temporal variations in the fecundity of brown trout Salmo trutta L.: trade-offs between egg size and number. Freshwater Biology 38(2):277-288.

Lowe, S. M., S. Browne, S. Boudjelas, and M. DePoorter. 2000. 100 of the World's worst invasive alien species. Pages 12 *in*. The Invasive Species Specialist Group, IUCN, A selection from the Global Invasive Species Database.

Metcalf, J. L., and coauthors. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology 16(21):4445-4454.

Meyer, K. A., D. J. Schill, J. A. Lamansky, M. R. Campbell, and C. C. Kozfkay. 2006. Status of Yellowstone cutthroat trout in Idaho. Transactions of the American Fisheries Society 135(5):1329-1347.

Morinville, G. R., and J. B. Rasmussen. 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries & Aquatic Sciences 60(4):401.

Morita, K., S. H. Morita, and S. Yamamoto. 2009. Effects of habitat fragmentation by damming on salmonid fishes: lessons from white-spotted charr in Japan. Ecological Research 24(4):711-722.

Morita, K., and S. Yamamoto. 2002. Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. Conservation Biology 16(5):1318-1323.

Morita, K., S. Yamamoto, and N. Hoshino. 2000. Extreme life history change of white-spotted char (*Salvelinus leucomaenis*) after damming. Canadian Journal of Fisheries & Aquatic Sciences 57:1300-1306.

Morita, K., and A. Yokota. 2002. Population viability of stream-resident salmonids after habitat fragmentation: a case study with white-spotted charr (*Salvelinus leucomaenis*) by an individual based model. Ecological Modelling 155(1):85-94.

Morris, W. F., and D. F. Doak. 2002. Quantitative conservation biology: Theory and practice of population viability analysis. Sinauer Associates, Inc., Sunderland.

MTFWP. 2007. Memorandum of understanding and conservation agreement for westslope cutthroat trout and Yellowstone cutthroat trout in Montana. Montana Fish, Wildlife, and Parks, Helena, MT.

Muhlfeld, C. C., and coauthors. 2009a. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters 5(3):328-331.

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.

Muhlfeld, C. C., T. E. McMahon, M. C. Boyer, and R. E. Gresswell. 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.

Nelson, G. A. 2010. fishmethods: fisheries methods and models in R.

Neville, H., J. Dunham, A. Rosenberger, J. Umek, and B. Nelson. 2009. Influences of wildfire, jabitat size, and connectivity on trout in headwater streams revealed by patterns of genetic diversity. Transactions of the American Fisheries Society 138(6):1314 - 1327.

Neville, H. M., J. B. Dunham, and M. M. Peacock. 2006. Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. Landscape Ecology 21(6):901-916.

Nielsen, J. L., and G. K. Sage. 2002. Population genetic structure in lahontan cutthroat trout. Transactions of the American Fisheries Society 131(3):376-388.

Nolan, M., and R. Shields. 2000. Measurment of stream discharge by wading. U. S. Geological Survey.

Northcote, T. G. 2010. Controls for trout and char migratory/resident behaviour mainly in stream systems above and below waterfalls/barriers: a multidecadal and broad geographical review. Ecology of Freshwater Fish 19(4):487-509.

Novinger, D. C., and F. J. Rahel. 2003. Isolation management with artificial barriers as a conservation strategy for cutthroat trout in headwater streams. Conservation Biology 17(3):772-781.

Olsen, J. B., Bentzen, P., & Seeb, J. E. 1998 Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1083-1090

Ostberg, C. O., and R. J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. Molecular Ecology Notes 4(1):26-29.

Ostberg, C. O., and R. J. Rodriguez. 2006. Hybridization and cytonuclear associations among native westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River drainage, North Cascades National Park. Transactions of the American Fisheries Society 135(4):924-942.

Ostberg, C. O., S. L. Slatton, and R. J. Rodriguez. 2004. Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), costal cutthroat trout (*O. clarki clarki*) and interspecific hybrids. Molecular Ecology 13(9):2773-2788.

Pascual, M., and coauthors. 2001. First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. Transactions of the American Fisheries Society 130(1):53 - 67.

Paul, A. J., and J. R. Post. 2001. Spatial distribution of native and nonnative salmonids in streams of the eastern slopes of the Canadian Rocky Mountains. Transactions of the American Fisheries Society 130(3):417-430.

Peacock, M. M., and V. Kirchoff. 2004a. Assessing the conservation value of hybridized cutthroat trout populations in the Quinn River Drainage, Nevada. Transactions of the American Fisheries Society 133:309-325.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6, 288-295.

Perry, W. L., D. M. Lodge, and J. L. Feder. 2002. Importance of hybridization between indigenous and nonindigenous freshwater species: an overlooked threat to North American biodiversity. Systematic Biology 51(2):255-275.

Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: effects of brook trout on native cutthroat trout. Ecological Applications 14(3):754-772.

Peterson, D. P., B. E. Rieman, J. B. Dunham, K. D. Fausch, and M. K. Young. 2008. Analysis of trade-offs between threats of invasion by nonnative brook trout (*Salvelinus fontinalis*) and intentional isolaton for native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Canadian Journal of Fisheries & Aquatic Sciences 65:557-573.

Piccolo, J. J. 2011. Challenges in the conservation, rehabilitation and recovery of native stream salmonid populations: beyond the 2010 Luarca symposium. Ecology of Freshwater Fish: 20: no. doi: 10.1111/j.1600-0633.2010.00463.x

Pister, E. P. 2010. California golden trout: perspectives on restoration and management. Fisheries 35:550 - 553.

R Core Development Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahel, F. J. 2000. Homogenization of fish faunas across the United States. Science 288(5467):854-856.

Railsback, S. F., and K. A. Rose. 1999. Bioenergetics modeling of stream trout growth: temperature and food consumption effects. Transactions of the American Fisheries Society 128:241 - 256.

Rantz, S. E., and others. 1982. Computation of discharge. U.S. Geological Survey

Rasmussen, J. B., M. D. Robinson, and D. D. Heath. 2010. Ecological consequences of hybridization between native westslope cutthroat (*Oncorhynchus clarkii lewisi*) and introduced rainbow (*Oncorhynchus mykiss*) trout: effects on life history and habitat use. Canadian Journal of Fisheries and Aquatic Sciences 67:357-370.

Raymond M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity, 86:248-249

Reed, M.J., L.S. Mills, J.B. Dunning Jr., E.S. Menges, K.S. McKelvey, R. Frye, S.R. Beissinger, M.C. Anstett, and P. Miller. Emerging issues in population viability analysis. Conservation Biology. 16 (1): 7-9.

Revenga, C., I. Campbell, R. Abell, P. d. Villiers, and M. Bryer. 2005. Prospects for monitoring freshwater ecosystems towards the 2010 targets. Philosophical Transactions: Biological Sciences 360(1454):397-413.

Rexroad, E., Coleman, R. L., Gustafson, A. L., Hershberger, W. K., & Killefer, J. 2002 Development of rainbow trout microsatellite markers from repeat enriched libraries. Marine Biotechnology 3:12-16.

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

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13(5):1220-1222.

Rubidge, E., P. Corbett, and E. B. Taylor. 2001. A molecular analysis of hybridization between native westslope cutthroat trout and introduced rainbow trout in southeastern British Columbia, Canada. Journal of Fish Biology 59:42-54.

Rubidge, E. M., and E. B. Taylor. 2004. Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhychus clarki lewisi*) and introduced tainbow trout (*O. mykiss*). Molecular Ecology 13(12):3735-3749.

Rubidge, E. M., and E. B. Taylor. 2005. An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. Conservation Genetics 6(3):369-384.

Schaffer, W. M. 2004. Life histories, evolution, and salmonids. Pages 20-51 *in* A. P. Hendry, and S. C. Stearns, editors. Evolution Illuminated. Oxford University Press, New York.

Schill, D., E. Mamer, and G. LaBar. 2010. Validation of scales and otoliths for estimating age of redband trout in high desert streams of Idaho. Environmental Biology of Fishes 89(3):319-332.

Schindler, D. E., and coauthors. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465(7298):609-612.

Schmetterling, D. A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River Drainage, Montana. North American Journal of Fisheries Management 21(3):507-520.

Schmetterling, D. A. 2003. Reconnecting a fragmented river: movements of westslope cutthroat trout and bull trout after transport upstream of Milltown Dam, Montana. North American Journal of Fisheries Management 23(3):721-731.

Seiler, S. M., and E. R. Keeley. 2007. Morphological and swimming stamina differences between Yellowstone cutthroat trout (Oncorhynchus clarkii bouvieri), rainbow trout (Oncorhynchus mykiss), and their hybrids. Canadian Journal of Fisheries & Aquatic Sciences 64(1):127-135.

Seiler, S. M., and E. R. Keeley. 2009. Competition between native and introduced salmonid fishes: cutthroat trout have lower growth rate in the presence of cutthroat-rainbow trout hybrids. Canadian Journal of Fisheries and Aquatic Sciences 66:133-141.

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.

Shepard, B. B., B. Sanborn, L. Ulmer, and D. C. Lee. 1997. Status and risk of extinction for westslope cutthroat trout in the upper Missouri River basin, Montana. North American Journal of Fisheries Management 17(4):1158-1172.

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.

Small, M. P., Beacham, T. D., Withler, R. E., & Nelson, R. J. 1998 Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. Molecular Ecology 7:141-155.

Spies, B., Brasier, D. J., O'Reilly, P. T. L., Seamons T. R., & Bentzen, P. 2005 Development and characterization of novel tetra-, tri- and dinucleotide markers in rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Notes 5:278-281.

Stapp, P., and G. D. Hayward. 2002. Effects of an Introduced Piscivore on Native Trout: Insights from a Demographic Model. Biological Invasions 4(3):299-316.

Taylor, E. B. 2004. Evolution in mixed company: evolutionary inferences from studies of natural hybridization in salmonidae. Pages 232-263 *in* A. P. Hendry, and S. C. Stearns, editors. Evolution Illuminated. Oxford University Press, New York.

Taylor, E. B., M. D. Stamford, and J. S. Baxter. 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. Molecular Ecology 12(10):2609-2622.

Thurow, R. F., C. E. Corsi, and V. K. Moore. 1988. Status, ecology, and management of Yellowstone cutthroat trout in the upper Snake River drainage, ID. R. E. Gresswell, editor. Status and management of interior stocks of cutthroat trout, 4th edition. American Fisheries Society, Bethesda.

Trotter, P. C. 1989. Coastal cutthroat trout: a life history compendium. Transactions of the American Fisheries Society 118(5):463-473.

UDWR. 2000. Cutthroat trout management: a position paper, genetic considerations associated with cutthroat trout management. Utah Division of Wildlife Resources, Publication Number 00-26, Salt Lake City, Utah.

USFWS. 2002. Chapter 3 Clark Fork River recovery unit, Montana, Idaho, and Washington bull trout (*Salvelinus confluentus*) draft recovery plan. 285p. U. S. Fish and Wildlife Service, editor.

USFWS. 2003. Endangered and threatened wildlife and plants: reconsidered finding for an amended petition to list the westslope cutthroat trout as threatened throughout its range. Pages 46989-47009 *in* Federal Register.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of earth's ecosystems. Science 277(5325):494-499.

Warren, M. L., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society 127(4):637 - 644.

Weigel, D. E., J. T. Peterson, and P. Spruell. 2002. A model using phenotypic characteristics to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. Transactions of the American Fisheries Society 131:2002.

Weigel, D. E., J. T. Peterson, and P. Spruell. 2003. Introgressive hybridization between native cutthroat trout and introduced rainbow trout. Ecological Applications 13(1):38-50.

Williams, J. E., A. L. Haak, N. G. Gillespie, and W. T. Coyler. 2007. The conservation success index: synthesizing and communicating salmonid condition and management needs. Fisheries 30(10):477-492.

Williams, J. E., A. L. Haak, H. M. Neville, and W. T. Colyer. 2009. Potential consequences of climate change to persistence of cutthroat trout populations. North American Journal of Fisheries Management 29(3):533-548.

Wofford, J. E. B., R. E. Gresswell, and M. A. Banks. 2005. Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecological Applications 15(2):628-637.

Young, M. K., P. M. Guenther-Gloss, and A. D. Ficke. 2005. Predicting cutthroat trout (*Oncorhynchus clarkii*) abundance in high-elevation streams: revisiting a model of translocation success. Canadian Journal of Fisheries & Aquatic Sciences 62:2399-2408.

Young, S. F., J. G. McLellan, and J. B. Shaklee. 2004. Genetic integrity and microgeographic population structure of westslope cutthroat trout, Oncorhynchus clarki lewisi, in the Pend Oreille Basin in Washington. Environmental Biology of Fishes 69(1-4):127-142.

APPENDIX A

CHAPTER 2 SUMMARY INFORMATION

Table A.1. Results of Hardy-Weinberg (HW) tests for equilibrium (HWE), binomial (Binom) tests for hybrid swarms, and summary information of the samples collected.

Sample #	Stream Name	HW Exact Test Probability	HWE? (α=0.05)	Swarm (Binom)	# Amplified Ind. (N)	Prop. Missing Alleles	Size Range	Hyb Sizes	# Ind. w/ RBT Alleles	Max HIS	pRBT
1	Jocko River	0.000	No	No	83	0.02	73-508	73-508	80	1.00	0.92
2	Spring Creek	0.000	No	No	23	0.01	77-374	77-374	23	1.00	0.97
3	Valley Creek	0.005	No	Yes	28	0.03	126-327	126-327	28	1.00	0.98
4	Valley Creek	0.000	No	No	13	0.09	63-270	63-270	13	1.00	0.86
5	Valley Creek	0.000	No	No	30	0.01	50-289	52-257	20	1.00	0.25
6	North Fork Valley Creek	0.266	Yes	Yes	23	0.01	96-180	147	1	0.07	0.003
7	Hewolf Creek	0.009	No	Yes	18	0.05	57-200	126	1	0.07	0.004
8	Hewolf Creek	0.986	Yes	No	7	0.02	64-122	N/A	0	0.00	0.00
9	Hewolf Creek	0.043	Yes	Yes	26	0.05	61-191	97-107	2	0.08	0.01
10	South Fork Valley Creek	0.093	Yes	Yes	27	0.01	70-245	245	1	0.07	0.003
11	Finley Creek	0.000	No	No	35	0.03	59-229	59-229	35	1.00	0.85
12	Finley Creek	0.000	No	No	29	0.07	140-388	140-388	14	0.93	0.13
13	Finley Creek	0.996	Yes	Yes	25	0.00	121-441	142-441	9	0.50	0.07
14	Finley Creek	0.295	Yes	Yes	30	0.21	87-253	87-115	3	0.17	0.01
15	Finley Creek	0.959	Yes	Yes	21	0.10	64-146	64-146	12	0.36	0.08
16	Finley Creek	0.652	Yes	Yes	17	0.07	108-194	111-155	3	0.10	0.01
17	Finley Creek	0.779	Yes	No	30	0.08	56-128	N/A	0	0.00	0.00
18	Agency Creek	0.448	Yes	No	29	0.04	63-233	229	1	0.21	0.01
19	Agency Creek	0.139	Yes	No	30	0.01	73-262	N/A	0	0.00	0.00
20	East Fork Finley Creek	0.007	No	Yes	22	0.21	66-210	148-210	2	0.10	0.01
21	Schley Creek	0.268	Yes	No	30	0.02	40-188	N/A	0	0.00	0.00
22	Kitty Girl Creek	0.577	Yes	Yes	18	0.05	91-196	108-184	3	0.07	0.01
23	Unnamed Finley Cr. Trib #6	0.000	No	Yes	25	0.00	86-186	130	1	0.07	0.003
24	Frog Creek	0.991	Yes	Yes	28	0.09	63-150	130	1	0.07	0.003
25	Big Knife Creek	0.388	Yes	No	29	0.02	76-202	120-187	10	0.36	0.06
26	Cold Creek	0.988	Yes	Yes	14	0.20	51-180	115-135	2	0.25	0.01
27	Pistol Creek	0.999	Yes	Yes	5	0.11	***	***	5	0.60	0.42
28	Jocko River	0.000	No	No	30	0.03	36-214	46-214	7	1.00	0.14
29	Jocko River	0.441	Yes	Yes	30	0.02	54-206	146	1	0.07	0.002
30	Jocko River	0.001	No	No	29	0.11	84-189	N/A	0	0.00	0.00
31	Kelly's Creek	0.158	Yes	No	14	0.04	80-166	N/A	0	0.00	0.00
32	North Fork Jocko River	0.000	No	No	6	0.16	82-158	110-158	3	1.00	0.32
33	North Fork Jocko River	0.000	No	No	29	0.03	72-286	110-271	4	0.86	0.06
34	North Fork Jocko River	0.875	Yes	Yes	28	0.07	70-316	79-89	3	0.07	0.01
35	North Fork Jocko River	0.000	No	No	30	0.29	75-167	88-118	6	1.00	0.13

***No size data available for Pistol Creek

Table A.1 pt. 2

Sample #	Stream Name	HW Exact Test Probability	HWE? (α=0.05)	Swarm (Binom)	# Amplified Ind. (N)	Prop. Missing Alleles	Size Range	Hyb Sizes	# Ind. w/ RBT Alleles	Max HIS	pRBT
36	North Fork Jocko River	0.126	Yes	No	26	0.03	67-270	N/A	0	0.00	0.00
37	North Fork Jocko River	0.977	Yes	Yes	28	0.02	67-255	74-242	4	0.14	0.01
38	North Fork Jocko River	0.646	Yes	Yes	31	0.04	67-245	129-173	3	0.14	0.01
39	North Fork Jocko River	0.000	No	No	29	0.03	73-229	N/A	0	0.00	0.00
40	Middle Fork Jocko River	0.368	Yes	Yes	29	0.05	51-194	125-176	7	0.08	0.02
41	Middle Fork Jocko River	0.396	Yes	Yes	34	0.10	98-253	112-197	3	0.07	0.01
42	Middle Fork Jocko River	0.118	Yes	Yes	30	0.02	54-301	93-150	3	0.14	0.01
43	Middle Fork Jocko River	0.446	Yes	Yes	20	0.09	107-254	141-208	6	0.07	0.02
44	Middle Fork Jocko River	0.015	Yes	Yes	38	0.01	55-313	64-144	2	0.14	0.01
45	South Fork Jocko River	0.212	Yes	Yes	33	0.00	80-214	125-170	3	0.07	0.01
46	South Fork Jocko River	0.030	No	Yes	29	0.03	69-225	136-211	2	0.07	0.005
47	South Fork Jocko River	0.440	Yes	No	28	0.05	88-300	174-199	2	0.42	0.02
48	South Fork Jocko River	0.677	Yes	Yes	30	0.01	76-226	76-226	4	0.07	0.01
49	South Fork Jocko River	0.000	No	No	30	0.00	95-215	123-215	8	0.86	0.07
50	South Fork Jocko River	0.256	Yes	Yes	30	0.04	107-220	189	1	0.07	0.002
51	South Fork Jocko River	0.000	No	No	22	0.18	107-226	132-211	3	0.36	0.02
52	South Fork Jocko River	0.995	Yes	No	29	0.02	101-235	139	1	0.36	0.01
53	South Fork Jocko River	0.104	Yes	Yes	29	0.07	121-218	139	1	0.07	0.003
54	South Fork Jocko River	0.072	Yes	No	24	0.18	75-206	134-206	2	0.25	0.01
55	South Fork Jocko River	0.529	Yes	No	30	0.02	88-194	N/A	0	0.00	0.00
56	Belmore Sloughs Creek	0.861	Yes	No	22	0.13	78-177	N/A	0	0.00	0.00
57	Unnamed SFJR Trib #1	0.462	Yes	Yes	29	0.05	56-167	111-158	2	0.07	0.01
58	Unnamed SFJR Trib #3	0.000	No	No	29	0.04	61-156	95-144	5	0.21	0.04
59	Unnamed SFJR Trib #2	0.120	Yes	Yes	29	0.09	55-153	97-134	5	0.25	0.02
60	Liberty Creek	0.000	No	No	26	0.06	70-251	70-251	25	1.00	0.88
61	Boles Creek	0.450	Yes	Yes	31	0.00	68-169	103-117	3	0.07	0.01

APPENDIX B

RESULTS OF ASSIGNMENT TESTS TO CONTROL FOR POPULATION OF ORIGIN OF MIGRATORY FISH

Methods

In an attempt to control for natal growth habitat and population of origin for fish analyzed as part of Chapter 3 life history investigations, I completed assignment tests using the program ONCOR (Kalinowski et al. 2007). ONCOR is specifically designed to handle population assignment of individuals collected in a mixed stock fishery (such as what are collected at K- and S-Canal ladders). In addition to the seven diagnostic microsatellite loci used in Chapters 2 and 3, I used four additional variable microsatellites, which were a subset of those used in Muhlfeld et al. (2009a) (Table B.1). Using GenAlEx (Peakall and Smouse 2006), I computed pairwise linearized Fst values for each sample site from Chapter 2. To create reporting groups for use in ONCOR, I grouped geographically proximate samples with relatively low pairwise Fst values. I used the PCA function in GenAlEx (which completes a PCA using distances in a triangular Fst matrix) as a means to plot groups of sample sites with low Fst values and determine which geographically proximate samples should be included in the same reporting group. I used the Leave-One-Out Test (Kalinowski et al. 2007) to determine how well individuals collected at the K- and S-Canal traps would assign to the baseline. This test sequentially removes each individual in the baseline and assigns it back to a sample site in the baseline. ONCOR records the proportion of individuals successfully assigned to both the correct sample site and the correct reporting group. When using reporting groups, ONCOR assigns individuals to sample sites (the genetic baseline), and sample sites are combined into reporting groups of genetically similar sites.

Results and Discussion

The results of the PCA of linearized Fst values suggest that, overall, populations grouped primarily by similarity of pRBT (see Chapter 2). This finding was not surprising, as most of the diagnostic markers were nearly fixed in populations that had primarily WCT genotypes. Reporting groups had low within-group pairwise Fsts. For example, pairwise Fsts for the lower South Fork Jocko River reporting group were 0.002-0.01. Occasionally higher pairwise Fsts were observed within reporting groups (up to 0.08 in the MFJ), but it was important to group these sample sites into reporting groups due to similarity of environment.

Results of the Leave-One-Out test indicated there were acceptable rates of assignment, but only for some of the populations likely to produce migratory fish, especially those with high pRBT. Baseline individuals assigned correctly to their sample sites at rates greater than 90% for sites with high pRBT. Baseline individuals correctly assigned to their sample sites at rates greater than 90% for only a fraction of populations with low pRBT likely to produce migratory trout. Individuals from upper mainstem Jocko River sites (those most likely to produce migratory fish) assigned very well to one population (94% correct), but very poorly to adjacent sites downstream (11% correct, 41%). Poor assignments were likely an artifact of upper mainstem sites consisting of a mixture of populations when sampling was feasible, but spawning does occur near those locations. Individuals from baselines with low pRBT often assigned into populations in other major drainages (e.g. fish from upper Jocko River assign to upper Finley sites above barriers or to South Fork Valley Creek) making it impossible to assign to an ecologically relevant scale.

I attempted to assign fish collected in S- and K-Canal traps used in growth and migration analyses (Chapter 3) to sample sites in the baseline. We used an *a priori* determined assignment probability cutoff of 0.90 to establish acceptable assignments. Only 37% of these samples assigned to a population or reporting group above the 0.90 probability cutoff, and given the results the Leave-One-Out tests, it is reasonably likely they were assigning to populations unlikely to have produced migratory fish (high elevation, above barriers).

Table B.1. Loci used to complete assignment analysis. Loci marked with asterisks were diagnostic markers used to detect hybridization in Chapters 2 and 3. Table is adapted from Muhlfeld 2009a.

Locus	Primer Dilution (uM)	Initial annealing temperature (°C)	Reference
Multiplex 1			
Ogo8*	0.12	58	(Olsen et al. 1998)
Omm1019*	0.20	58	(Rexroad et al. 2002)
Omm1050*	0.20	58	(Rexroad et al. 2002)
Omm1060*	0.12	58	(Rexroad et al. 2002)
Omm1037-1	0.20	58	(Rexroad et al. 2002)
Omm1037-2	0.20	58	(Rexroad et al. 2002)
Omy0004*	0.20	58	(Holm et al. 1998)
Multiplex 2			
Omy1001*	0.10	59	(Spies et al. 2005)
Ogo4	0.10	59	(Olsen et al. 1998)
Ssa456	0.10	59	(Angers et al. 1995)
Sfo8*	0.30	59	(Small et al. 1998)

APPENDIX C

FUNCTIONS AND PARAMETERS USED IN VITALSIM AND PEDAGOG MODELS

Table C.1. Parameter and variance estimates used in VitalsSim (Chapter 4).

Parameter	Estimate	Stretched Beta Range	Notes/Citations
Incubation Success	0.4 (SD =0.1125)		Shepard et al. 1997
Fry Survival	0.35 (SD =0.075)		Shepard et al. 1997
YOY surv	0.28 (SD =0.09)		Hilderbrand 2003, Shepard et al. 1997
Resident SubAd surv stay subadult	0.12 (SD =0.045)		Hilderbrand 2003, Shepard et al. 1997
Resident SubAd to migrant	0.02 (SD =0.045)		Modeled to reflect resident pop with remnant migrant LH
Resident SubAd surv become Adult1	0.12 (SD =0.045)		Hilderbrand 2003, Shepard et al. 1997
Migrant SubAd surv stay subadult	0.001 (SD =0.045)		Adjusted within ranges from Shepard et al. 1997
Migrant SubAd surv become Adult1	0.001 (SD =0.045)		Adjusted within ranges from Shepard et al. 1997
Resident Adult1 surv stay Adult1	0.23 (SD =0.075)		Hilderbrand 2003, Shepard et al. 1997
Resident Adult1 surv become Adult2	0.23 (SD =0.075)		Hilderbrand 2003, Shepard et al. 1997
Migrant Adult1 surv Stay Adult1	0.23 (SD =0.075)		Shepard et al. 1997
Migrant Adult1 surv become Adult2	0.23 (SD =0.075)		Shepard et al. 1997
Resident Adult2 surv	0.35 (SD =0.125)		Hilderbrand 2003, Shepard et al. 1997
Migrant Adult2 surv	0.35 (SD =0.125)		Shepard et al. 1997
Eggs per Resident Adult1	173 (SD =83.75)	99-265	Adjusted within ranges from Downs et al. 1997, Shepard et al. 1997, Chapter 3
Eggs per Resident Adult2	230 (SD =105)	112-322	Adjusted within ranges from Downs et al. 1997, Shepard et al. 1997, Chapter 3
Eggs per Migrant Adult1	540 (SD =50)	250-900	Adjusted within ranges from Downs et al. 1997, Shepard et al. 1997, Chapter 3
Eggs per Migrant Adult2	650 (SD =100)	500-1500	Adjusted withing ranges from Downs et al. 1997, Shepard et al. 1997, Chapter 3
Probability of Migrant Adult1 Breeding	0.65 (SD =0.008)		Thurow et al. 1988
Prob of Migrant Adult2 Breeding	0.65 (SD =0.008)		Thurow et al. 1988

Table C.2. Parameter estimates or functions used in PEDAGOG (Chapter 4)

Parameter	Estimate or Function	Vary (V) or Constant (C)	Notes/Citations
(Population Parameters)			
Population Pools	2000	N/A	
Burn in Generations	6	N/A	
Cohort Size	10000	C	
Density Dependence	No	N/A	
(Maturity, Survival, Movement)			
Maturity (Trap)	=IF(AGE>0.5,IF(LENGTH>150,1,0),0)	C	Downs et al. 1997; Corsi, Jocko River, Unpublished Data.
Maturity (JR)	=IF(AGE>0.5,IF(LENGTH>125,1,0),0)	C	Downs et al. 1997; Corsi, Jocko River, Unpublished Data.
Survival	=IF(LENGTH<150,NORMAL(0.3,0.045),NORMAL(0.46,0.075))	V	Shepard et al. 1997
Sample Capture (Trap)	=IF(LENGTH>200,NORMAL(0.9,0.1),IF(LENGTH>100,NORMAL (0.2,0.1),NORMAL(0.1,0.05)))	V	Shepard et al. 1997
Sample Capture (JR)	=IF(LENGTH>150,NORMAL(0.9,0.1),IF(LENGTH>100,NORMAL (0.5,0.1),NORMAL(0.1,0.05)))	V	
Movement (Trap only)	=IF(SAMPLE>1,IF(LENGTH>300,NORMAL(0.7,0.05),0),0)	V	
(Mating)			
Female Mates	2 (SD = 0.1)	V	Multiple mates to account for sneak/satellite matings.
Male Mates	3 (SD = 0.1)	V	Multiple mates to account for sneak/satellite matings.
Sire Contributions	Length Proportional	N/A	
Generation Overlap	2.0 (SD=0.1)	V	
Fecundity*YOY Survival	=(0.000373*LENGTH^2.5505)*0.039 (SD = 20.5)	V	Chapter 3
(Growth)			
von Bertalanffy (Trap)	Length At Age = 550*(1-exp(-0.3*(-0.7)))	V	All parameters vary according to estimates in Chapter 3. Parameters adjusted to account for only modeling adult, subadult survival.
von Bertalanffy (JR)	Length At Age =380*(1-exp(-0.24*(-1.0)))	V	All parameters vary according to estimates in Chapter 3. Parameters adjusted to account for only modeling adult, subadult survival.

Table C.3. Elasticity metrics calculated using LimitSens (Morris and Doak 2002) for individual vital rates used in VitalSim models used in Chapter 3. Max. λ is the maximum λ that can be achieved if all other vital rates are held constant.

	Max. λ	Max. prop. change in λ	Corr. (r^2) λ and vital rate	Elasticity From Mean Vital Rates
Resident Subadult Survival	1.01	0.04	0.00	0.04
Resident Subadult to Migrant	1.09	0.12	0.03	0.01
Resident SubAd to Adult1	1.17	0.21	0.08	0.24
Migrant SubAd Survival	1.08	0.11	0.06	0.00
Migrant SubAd to Adult1	0.98	0.01	0.03	0.01
Resident Adult1 Survival	1.08	0.12	0.01	0.15
Resident Adult1 to Adult2	1.07	0.10	0.03	0.16
Migrant Adult1 Survival	0.98	0.01	0.00	0.01
Migrant Adult1 to Adult2	0.98	0.01	0.01	0.01
Resident Adult2 Survival	1.14	0.17	0.03	0.09
Migrant Adult2 Survival	0.99	0.02	0.02	0.01
Incubation Success	1.03	0.06	0.24	0.26
Fry Survival	1.00	0.04	0.08	0.26
YOY Survival	1.06	0.10	0.16	0.26
Eggs per Resident Adult1	1.01	0.04	0.00	0.08
Eggs per Resident Adult2	1.03	0.06	0.03	0.16
Eggs per Migrant Adult1	0.97	0.00	0.00	0.00
Eggs per Migrant Adult2	0.98	0.01	0.01	0.01
Prob. Breed Migrant Adult1	0.97	0.00	0.00	0.00
Prob. Breed Migrant Adult2	0.97	0.00	0.00	0.01

APPENDIX D

GROW BIG OR STAY HOME: MODELING LIFE HISTORY AS A ROLL OF THE DICE

Introduction

Some of the fondest memories of many children involve an outing to their local waters for an afternoon of fishing with their best buddy or a day out in a boat with a grandparent. When kids venture outside, they are exposed to, and even acutely aware of, the life cycles of organisms in their surroundings. On a day at a pond, a child may pick a flower that has just bloomed, catch a tadpole with developing frog legs, and squeeze a puffball mushroom to make it poof out its spores. A child may have even noticed the difference of size in trout that she caught in a small mountain stream versus the trophy she caught in a lake in the valley below the mountains. In each of these activities, a child would be observing stages in the life histories of these organisms. Life histories are the series of behavioral changes and life stages that define the life of an organism. Scientists and science textbooks often depict the life history of an organism with a life cycle diagram (example: fig. 1). While these illustrations often give students understanding of the life history of a species, much of what happens to an individual organism depicted in a life cycle diagram is by luck of the draw. The fortunes of whole populations of individuals are what determine the successes of the species depicted in a life cycle diagram. To explore life histories, we developed a game of chance as an inquiry driven exercise and implemented it in a fifth grade classroom designed to investigate the question “why do some trout migrate to large rivers or lakes and some stay in their home streams?”

This game, *Grow Big or Stay Home*, is an ideal way to link mathematics, probability, and science. As a biologists and Ecologists in Residence at a local elementary school, we look for ways to foster student knowledge of local organisms and ecosystems. The game is a model designed to teach all of these concepts at the same time as students explore changes in populations and the ecology of wild organisms.

Cutting edge ecology often involves creating models based on scientific and mathematical principles that are appropriate for upper elementary students. *Grow Big or Stay Home* allows students to explore the advantages and disadvantages of two life cycle strategies of trout mathematically with rolls of the dice. Random events often influence when an organism dies, how many successful offspring it has, and whether it migrates to feeding grounds or stays at home. Through this investigation, students develop an understanding of the role of random events in shaping the structure of wild populations. This game also provided an outstanding way for students to test hypotheses about the ecology of popular local fishes, such as trout.

Why are the life cycles of trout like rolling the dice?

Every organism is faced with “tradeoffs” that determine how likely they are to survive to a certain age and how many offspring they are likely to have. For example, most elementary students have learned about the life cycle of salmon that migrate to the ocean, and then return to the stream where they were born to reproduce. This is only one life history strategy that salmonids (trout and salmon family) exhibit. Students may not know that some trout actually stay in the stream where they were born for their entire lives. One result of staying in their home stream is they are safer there from predators and fisherman and are more likely to survive to adulthood when they can breed and pass on their genetic lineage (genes). For the other trout, that leave their home stream and migrate to a large river, lake, or ocean, there are great rewards in the form of abundant food and these migrant fish can grow quite large. Usually, a trout that migrates can grow to a much larger size than a trout that stays in the home stream.

How do these life history "choices" affect how many trout there are?

Very large trout are capable of producing many more eggs (offspring) than small trout. On the other hand, it is more likely that a migrant trout will die before it makes the long migration home to reproduce. Thus, there is a "tradeoff" between migrating and staying in a home stream. Both strategies have their advantages and disadvantages, and there may be periods of time when one strategy is better than another. For example, imagine a dam of logs and debris that forms after a landslide and blocks the trout migration pathway for a few years. None of the migratory fish will be able to return home to complete their life cycle. In contrast, imagine what happens if a stream freezes solid two winters out of every three and all of the resident fish die. Clearly fishes born in that stream would have a huge advantage if they migrated before winter. Because there is natural variation, and a fair amount of luck, involved with how well a trout can compete for resources and survive to a breeding age, scientists create mathematical models where random chance (probability) plays a role to better understand complexities of nature.

What about assumptions?

It is important to remember that models are not exact replicates of nature. Instead, they are close representations, so scientists make several assumptions to simplify reality in these models. In *Grow Big or Stay Home*, we simplify nature in several ways to make a mathematical representation of natural reality. For example, we assume a migrant fish is as likely to survive to its next birthday as any other migrant fish, regardless of how old each of the fish are. As fisheries and wildlife biologists, we know this is rarely true, but making this assumption keeps the model (game) simple and easier to use. There is another reason for exploring the assumptions of *Grow Big or Stay Home*: having students explore the assumptions of a model and how violations of those assumptions could change their results will help them better understand the ecological system they are modeling. Students can be challenged to look for assumptions of the model by comparing the life cycle of a trout to the game instructions.

Playing Grow Big or Stay Home

Grow Big or Stay Home begins by presenting the importance of modeling to students. It is very difficult for scientists to study wild organisms for long periods of time. In order to overcome this difficulty, scientists develop models that try to closely represent what we see in nature. There are many types of models. Some students may have made a model train, volcano, or played with model dinosaurs. Those models are called physical models. Scientists often make physical models, but they also build numerical models. Models are easier to control, study, and are simpler than populations in nature. For example, to better understand why an organism such as a trout would have different life history types, we can develop a mathematical model to represent what is actually occurring in nature. As scientists we can use dice to decide whether an imaginary fish will live or die and when it will stay in its home stream or migrate to a large river or lake. Some percentage of fish (a random number or probability) will migrate and some percentage will stay in the stream in which they were born. As a fish matures, it has a certain probability of dying of predation, a flood, or a drought. A fish also has a certain probability that it will survive to breed once, twice, three times, or even more. In the game, dice are used to make all decisions about life or death of a fish at random, without controlling what happens on our own. In other words, we can get a glimpse of how a population will change in nature by playing a game with dice in the classroom.

How it went in a Fifth Grade Class

Before getting into the game, students learned about the idea of tradeoffs. Some trout leave their stream to grow large and have greater success when they reproduce, but they must take a gamble of lower survival. Other fish will stay at home, and enjoy high survival, but the number of eggs they lay if they survived will be much lower.

To begin the game, students formed small groups and pretended to be trout. Students formed hypotheses about which life history type, migrants or residents, was likely to have the greatest offspring production at the end of the game, therefore contributing the most baby fish to the next generation.   Then each student rolled the dice to determine if they were going to be a migrant trout (odd numbers) or stay at home as a resident trout (even numbers). Then students rolled the dice and matched the number rolled to their instruction sheet to their fate in the first year (Table B.1). For example, resident fish were killed by a flood with a roll of eleven and eaten by a predator with a roll of twelve. If the fish successfully survived three full years, it then breeds and produces offspring. The number of offspring produced was also determined by a roll of the dice. If a fish was a resident, the student rolled the dice and added the value of the two die to determine the number of offspring that fish contributed to the next generation. If a fish was a migrant, the values of the two die were multiplied by each other to determine the contribution to the next generation. The game ends, either when all fish die, or when all surviving fish have successfully bred in their fifth year.

Students playing *Grow Big or Stay Home* were definitely caught up in the competition of who survived and who produced more offspring. They were surprised by how fast good fortune can change and how the “slow and steady” resident fish can often produce the most offspring over their life span. The game stimulated lively discussion about other natural and human-caused factors that could change the survival probabilities of both resident and migrant fish. Students also asked many questions about how migrant fish and resident fish interacted with their environments differently. By the end of the game, students were thinking critically about aquatic ecosystems and talking about the nuances of what made each strategy viable in wild populations.

Our fifth grade class was surprised by their results. This was particularly true when we compared the results of all students in the class. Each group posted the number of offspring for resident and migratory fish on the chalkboard at the end of the game.

Because everything that happened to each fish at each step of the game was subject to random chance, results often varied widely between groups. To explore this, students were asked to explain in writing why their results varied so much. As an extension, students also wrote about why they thought trout have two different life history strategies. With some leading questions, they were able to determine that the reason for two strategies was that, in some years, one strategy does better than the other – just by chance, so both strategies are stable, depending on the environmental conditions for that year.

Conclusions

In *Grow Big or Stay Home*, mathematics and probability are integrated with science. In fact, mathematics is a critical tool for scientists. While playing *Grow Big or Stay Home,* students forgot they were getting a math lesson during the heat of the game. As the students began to use the math to explore ecology, they raised excellent questions about trout life history and causes of trout mortality. Application of the math component also lends itself well to extensions involving computer applications such as Excel, which can be programmed with random number generating functions, then easily manipulated so students can test hypotheses about the relative success of each life history strategy if survival or breeding parameters are altered. An Excel based model is available upon request from the author.

Students that played *Grow Big or Stay Home* will have a greater appreciation for life histories of trout, but hopefully they will also start thinking about the gambles and tradeoffs that any organism makes during different stages of its life. Perhaps the next time a student chases a firefly, she will think about the probability that the firefly will escape the mason jar, find a mate, and contribute its genes to the next generation.

National Science Education Content Standards Addressed with this Investigation

Grades 5-8
Unifying Concepts and Processes: Evidence, Models, and Explanation
Life Science: Reproduction and Heredity
Life Science: Regulation and Behavior
Life Science: Populations and Ecosystems
Life Science: Diversity and Adaptations of Organisms

Resources:

National Research Council (NRC). 1996. National science education standards. Washington DC: National Academy Press.

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Table D.1. *Grow Big or Stay Home* instruction sheet.

Grow Big or Stay Home?
Simulation Instruction Sheet

1) Roll dice to determine which life history the fish will have: Odd numbers are migrant life history; even numbers are resident life history.

2) Roll the dice each "year" to determine whether the fish survives to the next year or not:

Dice Value	**Resident**	**Migrant**
1	Survive	Survive
2	Survive	Survive
3	Survive	Survive
4	Survive	Survive
5	Survive	Survive
6	Survive	Survive
7	Survive	Survive
8	Survive	Survive
9	Survive	Eaten by Predator
10	Survive	Killed by Flood
11	Eaten by Predator	Drought - You are Dried Up!
12	Killed by Flood	Caught and Eaten by Fisherman

Whenever a fish dies, draw a line through the box.

3) If a fish survives to breeding age, roll the dice to determine the number of offspring they will produce during that year. Resident fish roll both dice and **add** the two numbers, while migrant fish roll two dice then **multiply** the two numbers. Write the number of offspring in the box for that year.

4) After the fish breeds, roll again to see if it survives to the next year.

5) Add the number of offspring for each fish and put the total in the offspring column.

6) The simulation ends when the table is full for one life history type.

7) Discuss and compare the outcome of the simulation for each life history strategy.

Table D.2. Results of *Grow Big or Stay Home* for 16 fish. Data presented here correspond to figure B.2.

Grow Big or Stay Home Score Sheet

Fish	**Migrant or Resident?**	Year 1 (Survive?)	Year 2 (Survive?)	Year 3 (# Offspring)	Year 4 (# Offspring)	Year 5 (# Offspring)	**Total Offspring**
1	**Migrant**	No	No	-	-	-	0
2	**Resident**	Yes	Yes	6	5	10	21
3	**Resident**	Yes	Yes	3	4	9	16
4	**Resident**	Yes	Yes	6	9	10	25
5	**Resident**	Yes	Yes	-	-	-	0
6	**Resident**	Yes	Yes	-	-	-	0
7	**Resident**	No	No	-	-	-	0
8	**Resident**	Yes	Yes	6	12	10	28
9	**Migrant**	Yes	No	-	-	-	0
10	**Migrant**	Yes	Yes	4	2	-	6
11	**Migrant**	No	No	-	-	-	0
12	**Migrant**	No	No	-	-	-	0
13	**Resident**	Yes	Yes	-	-	-	0
14	**Migrant**	Yes	Yes	24	8	36	68
15	**Resident**	Yes	Yes	8	2	3	13
16	**Migrant**	Yes	Yes	4	20	36	60

Migrant Total:	134
Resident Total:	103

Side Bar: Vocabulary

Fitness – The ability of an organism to survive and reproduce offspring. For example, a trout that survives for three breeding seasons and produces 10 surviving offspring has a higher fitness than a trout that only survives for one breeding season and can only produce 2 surviving offspring.

Life History – The series of behavioral shifts, maturation processes, and life stages that shape and define the life of an organism.

Mathematical Model – A close representation of nature that uses mathematics to describe the behavior of a system.

Migrant – For the purposes of this inquiry, a trout that leaves its natal stream to go to a habitat (large river, lake, or ocean) where it can grow larger than if it stayed.

Model – A simplified representation of nature.

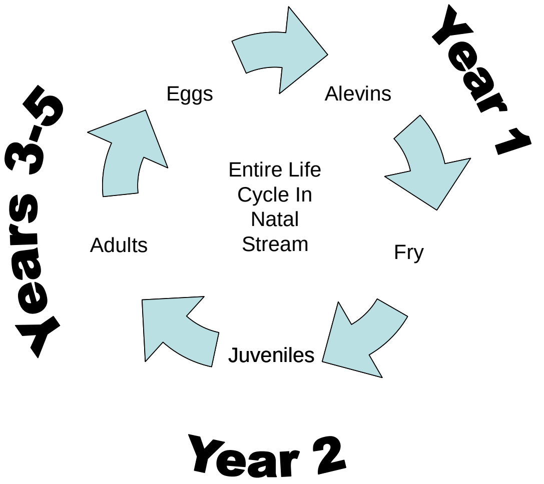
Mortality – For the purposes of this inquiry, the probability that an organism will die during any given year.

Physical Model – A physical representation of an object, organism, or system.

Resident – For the purposes of this inquiry, a trout that lives in its natal (home) stream its entire life.

Survival – The probability that an organism will survive a given length of time.

Figure D.1. Life cycle diagrams of resident trout (a) and migrant trout (b).
a)



b)

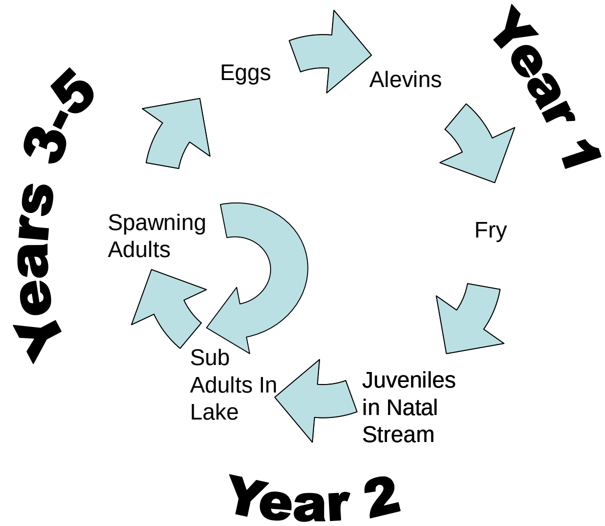
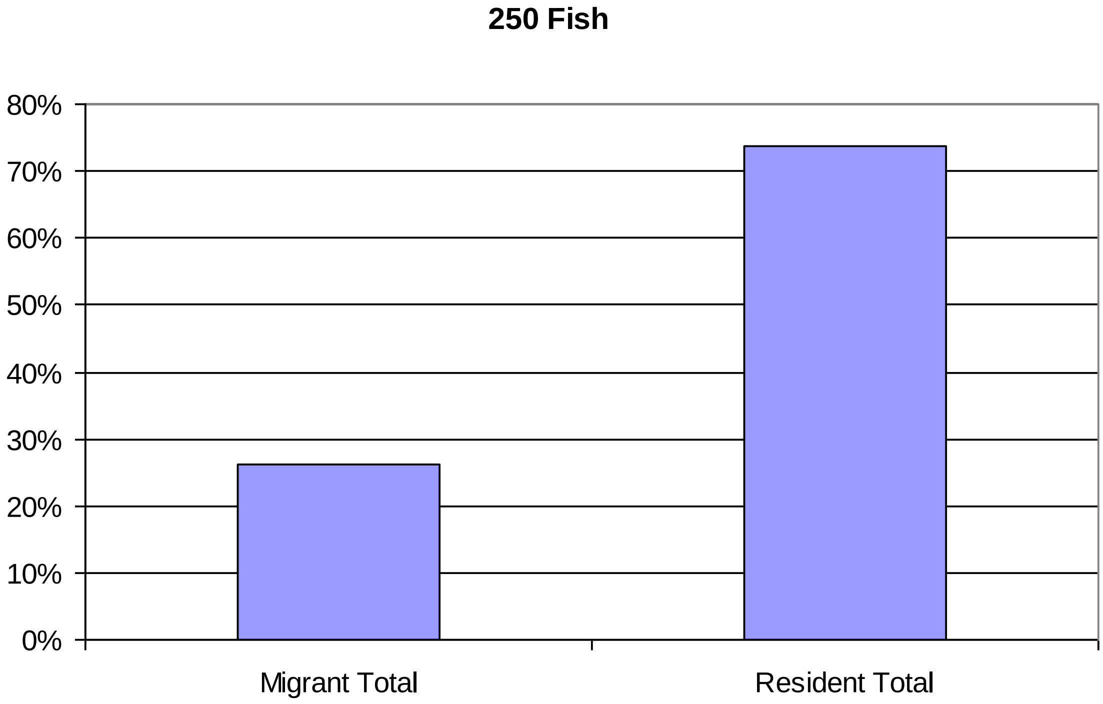
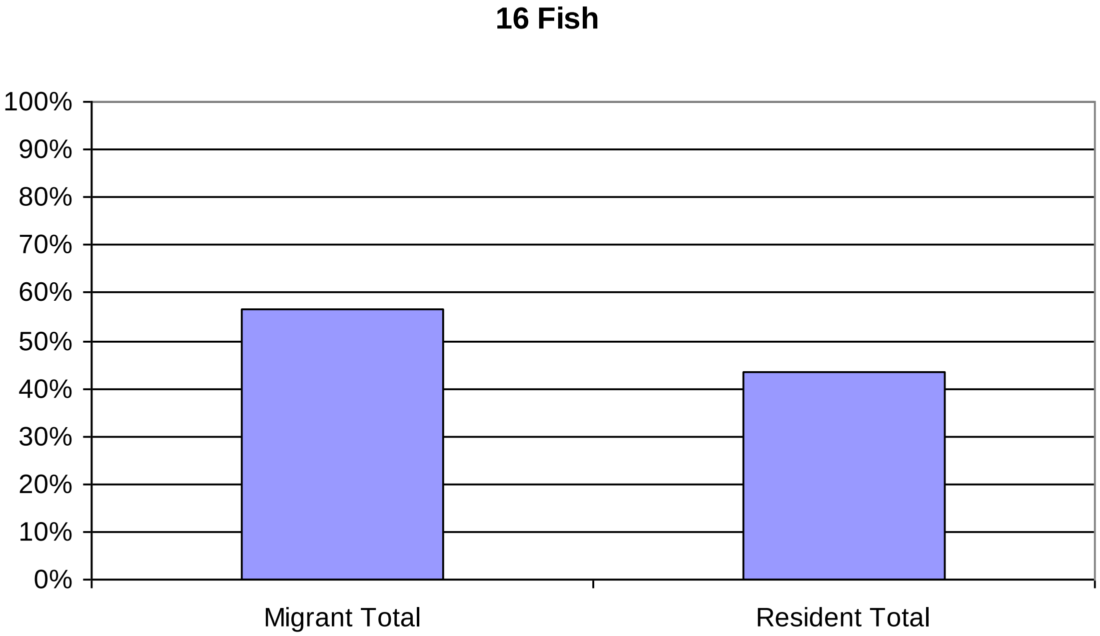


Figure D.2. Demonstration of the influence of using a small number of fish for a simulation. This example is one simulation with 250 fish. Figure B.2a is the results of all 250 fish in the simulation depicted as the proportion of individuals contributed to the next generation by each life history type. Figure B.2b is a similar bar chart showing the results of 16 fish that were simply the first 16 fish of the simulation.

a)



b)


16 Fish
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Migrant Total
Resident Total