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Evaluating genetic status and management tradeoffs for conservation of cutthroat trout (*oncorhynchus clarkii*)

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EVALUATING GENETIC STATUS AND MANAGEMENT TRADEOFFS FOR

CONSERVATION OF CUTTHROAT TROUT (*ONCORHYNCHUS CLARKII*)

By

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  Habitat fragmentation and invasive species are two of the primary threats to global biodiversity, yet biologists have tested few guidelines for protecting species under these conditions. These threats are particularly relevant to conservation of freshwater species like the Cutthroat Trout (*Oncorhynchus clarkii*). Hybridization with introduced Rainbow Trout (*O. mykiss*) has already caused extinction of one subspecies and threatens extant populations. Additionally, Cutthroat populations have lost genetic diversity across their range due to habitat destruction and fragmentation. These threats create a catch-22 for managers, wherein treating one problem (connecting populations) may lead to the other (interactions with invasive species). Furthermore, little is known about requirements for persistence of populations isolated to protection against invasive species.

  I assessed tradeoffs in conservation strategies for Westslope Cutthroat Trout (*O. c. lewisi*). In connected populations, steeper streams had smaller hybrid zones and less introgressive hybridization. I found that geomorphology (slope) limited hybridization between Rainbow and Cutthroat Trout and provided a natural refuge for native fish in connected systems.

  Isolated Cutthroat populations residing in under 5km of habitat above anthropogenic barriers (<80yrs) suffered loss of genetic diversity independent of habitat size, quality, and time since isolation. Geologically isolated populations in larger fragments (up to 18km) also experienced loss of genetic diversity, likely from stochastic events causing population bottlenecks. Significant loss of genetic diversity compared to connected populations occurred despite exceeding habitat size and population recommendations derived from genetic theory for maintaining diversity. Thus over the long-term, isolated populations may not retain genetic diversity even if they meet suggested conservation thresholds.

  In these anthropogenically isolated populations, population growth rate (lambda) was positively associated with water volume during summer base flow and declined with increasing land use in the watershed. Lambda was most sensitive to probability of maturity, and increased as size of maturity decreased. Populations with low adult survival had rapid somatic growth rates, thus reaching maturity sooner. This highlighted the potential for local adaption under isolation as populations adjust to shifting environmental conditions and life history tradeoffs. Although isolated population may have reduced genetic diversity, actions such as genetic rescue should be considered with caution.

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CHAPTER 1

INTRODUCTION AND OVERVIEW

For over three decades, biologists have been documenting an unprecedented loss of flora and fauna from their native habitats, and have called for global action to thwart what has become known as the "biodiversity crisis" (Soule 1986, Western 1992, Butchart et al. 2010). To protect wildlife species, recommendations based in population ecology often call for maintenance of large, high quality habitat that meets the needs of a species throughout all life stages (Caughley 1994). To achieve this same goal, recommendations based in conservation genetics typically call for maintenance high levels of genetic diversity to avoid inbreeding depression and maintain variation to adapt to future circumstances (Franklin 1980, Waples 1995).

In practice, these recommendations have helped protect many imperiled wildlife species. For example, the federal policies outlined in Northwest Forest Plan successfully protected millions of hectares of old growth forest from destruction by logging practices on federal lands, thereby securing critical habitat for Northern Spotted Owls (*Strix occidentalis caurina*) in the Pacific Northwest (Noon and Blakesley 2006). In another example, assisted immigration of bighorn sheep to a disconnected and severely bottlenecked population on the National Bison Range in western Montana resulted in greater than two-fold increase in annual reproductive success of offspring between residents and immigrants and reversing the declining trend in population growth rate (Hogg et al. 2006).

Despite these efforts, many wildlife species continue to decline because the existing recommendations do not address the most common threats to wildlife persistence. Invasive species and habitat loss and fragmentation are considered two of the largest threats to native species persistence (Sala et al. 2000). Although substantial amounts of critical habitat have been protected, the Northern Spotted Owl population continues to decline due to hybridization and competition with invasive Barred Owls (*S.varia;*(S. varia; Kelly et al. 2004, Dugger et al. 2011). The rapid decline and extirpation of native prairie fishes in North America has been attributed to construction of dams throughout waters ways of the southern Great Plains (Perkin and Gido 2012, Walters et al. 2013). Because these dams often provide valuable human resources, they are

unlikely to be removed to restore habitat connectivity. Consequently, conservation biologists are faced with problem of conserving populations threatened by interactions with invasive species and changing or novel communities, as well as populations existing in potentially small and isolated habitat fragments. To develop the most effective conservation strategies, we must gain a better understanding of the requirements for population persistence under isolation, as well as an understanding of when and how multispecies interactions can exacerbate or inhibit the impacts of invasive species on native populations and communities.

Theory based in population ecology does not currently provide clear answers for how to conserve wildlife threatened by fragmentation and invasive species. However, theory based in conservation genetics has provided biologists with some guidelines for maintaining genetic diversity in wildlife. For example, the 50/500 rule, presented first by Franklin (1980), recommends an effective population size of 50 to avoid the short term risks of inbreeding depression, and a minimum effective size of 500 to allow mutation to add functional diversity into the genome at the same rate it is removed by drift. The underlying basis for the 50/500 rule was calculated using maximum inbreeding loads observed by domestic animal breeders, and mutation rates observed in fruit flies, and assumes that these rates are applicable across taxa. Furthermore, accurate estimates of effective population size can be difficult to obtain for wildlife populations because many estimation methods have assumptions that oversimplify the dynamics of wildlife populations (Neel et al. 2013). In short, many guidelines and rules of thumb (such as the 50/500 rule) are based in theory and have unrealistic assumptions of a population's dynamics, making them unreliable for widespread conservation application. As a result, they must be empirically tested across a range of taxa and ecosystems to ensure that they are properly considered and applied to strategies for wildlife conservation.

These gaps in our understanding of requirements for protection of native species are particularly relevant to North America's freshwater ecosystems. Here faunal extinction rates are estimated to be five times greater than for terrestrial fauna (Ricciardi and Rasmussen 1999), and, over the next century, will be impacted most by spread of nonnative species and human activities that alter habitat (Sala et al. 2000). Species in streams and rivers are particularly vulnerable to fragmentation due to the dendritic nature of stream networks (Fagan 2002) and habitat

degradation is pervasive across aquatic systems (Malmqvist and Rundle 2002). Connected systems facilitate the spread of nonnative species (Korsu et al. 2010), which predate, parasitize, compete and hybridize with natives species, all of which can lead to alterations in available habitat as well as extirpation and extinction of native species (Behnke 2002, Dunham et al. 2004, Baxter et al. 2007, McDonald et al. 2008, Holitzki et al. 2013). To protect native stream dwelling species and their habitat, we must improve our understanding of how these impacts interact and affect genetic diversity and native species viability. This information will be critical as we prioritize conservation efforts and evaluate tradeoffs for current and future challenges associated with increasing human population and need for natural resources, climate change, and continued spread of nonnative species. Fortunately, substantial genetic work has been done evaluating theory and applying genetic information to conservation of freshwater species (Vrijenhoek 1998, Piorski et al. 2008) making these systems ideal for testing theory and beginning to explore these challenging questions.

My dissertation assesses tradeoffs in devising long-term management strategies for Cutthroat Trout (*Oncorhynchus clarkii*) native to Rocky Mountains and the Pacific Northwest. This species has a vast evolutionary history comprised of 14 subspecies. However two of these subspecies have already gone extinct from human related impacts (Behnke 2002). Across salmonids, fragmentation and subsequent isolation of Cutthroat populations has led to declines in genetic diversity, reduced dispersal capabilities, and loss of migratory life histories, leaving many populations at greater risk of local extinction (Rieman and McIntyre 1995, Morita et al. 2009, Cook et al. 2010, Sato and Gwo 2011). To increase habitat, rebuild metapopulation function, and expand migratory life histories for trout, many managers have chosen to restore and reconnect habitat (e.g., Vehanen et al. 2010, Pierce et al. 2013). However, highly connected landscapes have left many Cutthroat populations at a greater risk for competition with invasive Brook Trout (*Salvelinus confluentus*), hybridization with invasive Rainbow Trout (*O. mykiss*), and exposure to disease, threatening Cutthroat Trout across their range (Hess 1996, Dunham et al. 2002a, Peterson et al. 2004, Metcalf et al. 2008, Muhlfeld et al. 2009c, Rasmussen et al. 2010, Kovach et al. 2011). To reduce the impacts of these factors on native Cutthroat populations, managers are forced to decide between either connecting habitat for increased gene flow and diversity of life histories, or isolating populations for protection from nonnative species (Fausch et al. 2006,

Peterson et al. 2008b, Fausch et al. 2009). The major concerns influencing the tradeoff between isolation and connection of native populations focus on maintaining their social, ecological, and evolutionary values (Fausch et al. 2009). My dissertation seeks to help resolve this catch-22 for inland salmonids by studying factors influencing hybridization with nonnative species in connected landscapes and examining loss of genetic diversity and population viability in isolated populations of Cutthroat Trout. My research also informs the broader field of conservation genetics by testing theory and rules of thumb in an applied context.

Chapter 2 investigated whether interactions between nonnative species can alter the impact of a single species on native trout. Specifically, we explored how landscape variables and biotic interactions may mediate hybridization between Rainbow and Cutthroat in the Blackfoot River Basin of west central Montana. In addition to known hybridization gradients associated with habitat quality, temperature, elevation, stream slope, and distance from the source of Rainbow Trout alleles, we explored whether whirling disease, caused by the invasive parasite *Myxobolus cerebralis*, can alter hybridization patterns between these two species. Specifically, we hypothesized that higher vulnerability of Rainbow Trout to the parasite would result in lower levels of introgressive hybridization at a site scale (stream reach of 100-300m), and a smaller hybrid zone at the whole stream scale for streams where the disease was present. At a site scale, levels of introgression decreased with increasing elevation, stream slope, distance from source of Rainbow Trout alleles, and habitat quality- all of which corroborated previous studies on introgression between various Cutthroat subspecies and Rainbow Trout (Hitt et al. 2003, Weigel et al. 2003, Muhlfeld et al. 2009c, Rasmussen et al. 2010, Kovach et al. 2011, Buehrens et al. 2013). At the whole stream scale, we found that stream slope was the only variable with significant influence on the size of the hybrid zone. Here, the steepest streams had the smallest hybrid zones- regardless of whether whirling disease was present. These results suggest that the presence of whirling disease does not alter rates of hybridization between Rainbow and Cutthroat Trout, but rather, the most important factors preventing negative impacts from invasive species may actually be landscape features that are not predicted to change substantially in the foreseeable future.

Chapter 3 examined whether anthropogenically isolated populations of Cutthroat Trout in the Flathead River Basin of northwestern Montana have lost genetic diversity where barriers to fish passage are being maintained to protect Cutthroat from hybridization with nonnative Rainbow Trout. Overall, genetic diversity was between 25-70% lower in small streams (< 5km of occupied habitat) compared to nearby connected systems. This loss was not associated with time since isolation, length of occupied habitat, temperature, summer base flows, or habitat quality. Rules of thumb based in genetic theory predict that a minimum of 8km of stream habitat would be necessary for isolated Cutthroat Trout to avoid loss of genetic diversity. Our one large stream with 14 km of occupied habitat did maintain genetic diversity at levels similar to connected populations. To examine whether these inferences would be similar for isolated populations on longer time scales, we examined populations isolated for roughly 2500 generations by geologic barriers (e.g., waterfalls). In all of these naturally isolated populations, genetic diversity was roughly 50-75% lower than connected populations- even those isolated with up to 10 to 18 km of occupied habitat. Thus, isolation may be a viable short-term measure to protect against interactions with invasive fish species for populations isolated in large high quality habitat. However genetic drift, and population bottlenecks caused by environmental stochasticity will inevitably reduce genetic diversity in isolated populations, and significant loss of diversity can happen in as few as 12 generations in small fragmented populations.

Using the same anthropogenically isolated streams in Chapter 3, Chapter 4 used integral projection models (IPMs) to estimate population growth rates (lambda), and explored the relationship of these growth rates to habitat characteristics and genetic diversity. Estimates of lambda for most isolated populations were less than one. Genetic diversity showed no relationship to lambda, but was positively associated with population size. We did not find any relationships between population growth rate and most habitat variables (length of occupied habitat, summer base flows, stream temperature). Although the relationship was not statistically significant, we did observe a negative relationship between the percent of land in the watershed leased for agricultural practices and lambda. Additionally, when we considered populations residing in < 5km of stream habitat, summer base flow had a significant positive relationship with population growth rates.

Contrary to the rapid population decline expected with our estimates of lambda, isolated Cutthroat populations of the Lower Flathead River Basin have persisted for 10 to 20 generations. Across all populations, lambda was most sensitive to size of maturity, with smaller size of maturity causing an increase in lambda. Evidence from biologists sampling the system indicated that some of these populations may have smaller size of maturity than accounted for in our population viability models. Our results highlight the life history tradeoffs associated with somatic growth rate, adult survival, and size of maturity where populations in small, isolated systems may see a selective advantage for individuals with rapid growth in early life stages and smaller size of maturity, particularly in populations where adult survival is low.

Overall, this dissertation adds substantially to our understanding of conservation for inland trout and to other freshwater species in several ways. Firstly, this research highlights the broad and consequential role stream geomorphology and hydrology play in native fish species conservation. Chapter 2 demonstrates that streams with higher gradients may have an inherent resistance to certain invasive species, and Chapter 4 highlights that small population persistence may be particularly vulnerable to summertime water extraction, drought, and other changes in summer base flow.

While many researchers have estimated minimum thresholds and requirements for persistence from ecological and genetic theory, this dissertation quantitatively examined requirements for persistence and tested rules of thumb often used in management. Chapter 3 highlighted that untested guidelines based in theory make assumptions that can drastically alter their efficacy. Specifically, we found several populations of Cutthroat Trout isolated were not able to maintain genetic diversity despite residing in fragments exceeding minimum recommended habitat size. Chapter 4 utilized the first ever integral projection model applied to a salmonid species, and is the second instance that this technique has been used for any fish species (see Vindenes et al. 2013). The use of this modeling technique allowed for higher resolution in sensitivity analysis than the typical matrix model, allowing us to identify specific vital rates substantially influencing viability and life history tradeoffs faced by isolated trout populations. Furthermore, the lack of a relationship between genetic diversity and viability in populations examined in Chapters 3 and 4 highlight the fact that loss of genetic diversity does not necessarily confer population decline. In

conjunction with the potential for local adaption observed in these small isolated populations, managers should carefully consider demographic status while determining the need for genetic rescue and associated risk of outbreeding depression.

More broadly, this dissertation identified potential hazards in using rules of thumb in wildlife conservation. Rules of thumb are not a substitute for monitoring and understanding of genetic and demographic dynamics in conservation of wildlife populations. Low levels of genetic diversity may leave wildlife populations with less material to adapt to changes in their environment and biotic community, while reduced gene flow from habitat fragmentation may also foster local adaptation for persistence under isolation. Under such circumstances, anthropogenic movement of individuals between populations could cause outbreeding depression, and result in population decline instead of growth. Together, the results and discoveries from this dissertation highlight the interconnectedness of population ecology and genetic theory in successful conservation of wildlife species. Moving forward, conservation practitioners should consider and test theoretical guidelines within the specific context their population and ecosystem. Considering the specific needs and violation of assumptions in a particular population will maximize the outcomes of conservation efforts.

CHAPTER 2

A BATTLE OF INVADERS: CAN A NONNATIVE PARASITE ALTER HYBRIDIZATION
BETWEEN NATIVE AND INVASIVE TROUT?

INTRODUCTION

Freshwater ecosystems are highly imperiled, with the greatest number of threatened and
endangered species, as well as the highest rates of species extinction world-wide (Pimm et al.
1995, Ricciardi and Rasmussen 1999, Burkhead 2012). Anthropogenic degradation of habitat
has caused fragmentation of aquatic populations, loss of critical habitat, and subsequent loss of
biodiversity on a global scale (Dudgeon et al. 2006). In addition, both climate change and human
activities are facilitating the spread of nonnative species (including, but not limited to protozoa,
plants, and animals) across freshwater ecosystems at alarming rates (Walther et al. 2002, Strayer
and Dudgeon 2010). This spread of nonnative species creates novel species assemblages, where
the impacts of several species on one another may alter our expectations for viability of native
species across landscape. As conservation biologists, we need to consider how landscape factors
alter interactions between native and nonnatives species, and how this in turn influences our
conservation strategies (Lindenmayer et al. 2008, Hobbs et al. 2009).

As in many aquatic ecosystems across the world, biotic assemblages in lotic systems of the
Rocky Mountain region are a mix of native and nonnative species. Yet, the impact of invasive
species may vary across the landscape due to natural variation in abiotic conditions favoring
certain species over others (Buehrens et al. 2013). Furthermore, interactions between multiple
invasive species have varied and unpredictable consequences. For example, invaders may
negatively impact one another through competition or predation (Simberloff and Von Holle
1999, Braks et al. 2004), or they may have commensal or mutualistic interactions that increase
spread and intensity of their individual impacts (Ricciardi 2001). In some cases, the presence of
multiple invaders may amplify impacts on native species, despite negative interactions between
invaders (Ross et al. 2004, Johnson et al. 2009). Nonetheless, interactions between invasive
species are explored less frequently than the negative impacts of invasive species on the native
community (Simberloff and Von Holle 1999). Due to the complexity and unpredictable

outcomes of these interactions, we need to consider the effects of the "invasive community" when quantifying risks to native species persistence.

The persistence of native Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*, hereafter "cutthroat") is threatened by loss of habitat from human activities and hybridization with nonnative Rainbow Trout (*Oncorhynchus mykiss*, hereafter "rainbow"*;* Shepard et al. 2005). Studies have shown that the proportion of rainbow alleles present in a population sample (i.e., introgression) vary with distance from source of rainbow alleles and are altered by tributary characteristics (such as stream slope, flow regime, temperature) as well as human disturbances (Hitt et al. 2003, Weigel et al. 2003, Muhlfeld et al. 2009a, Heath et al. 2010, Rasmussen et al. 2010, Kovach et al. 2011). However, research has not explored whether additional nonnative species may alter these landscape level gradients associated with hybridization both within and among watersheds.

The unintentional spread of parasites has impacted wildlife populations globally, and differential vulnerability to disease between native and invasive species may be a mechanism influencing the spread of exotic species (Moyle and Light 1996, Peterson and Fausch 2003). For example, whirling disease, is hypothesized as a factor limiting the invasion of Rainbow Trout in the United Kingdom (Fausch 2007). The myxosporean parasite *Myxobolus cerebralis*, the causative agent of whirling disease (hereafter "WD"), is endemic to Eastern Europe. Human facilitated transport of infected fish after World War II spread the parasite across continents, and caused epizootics which have decimated wild fish populations across multiple continents (Bartholomew and Reno 2002). *M*. *cerebralis* utilizes two hosts to complete its lifecycle- oligochaete worms (*Tubifex spp*.) and salmonid fish (Hedrick and El-Matbouli 2002). Young fish which have substantial skeletal cartilage are most susceptible to infection (Ryce et al. 2005). Infection can lead to substantial cartilage destruction resulting in whirled swimming patterns, skeletal deformities, reduced growth rates and death (MacConnell and Vincent 2002).

Salmonids of the genus *Oncorhynchus* appear to be some of the most susceptible species to WD, but susceptibility between species in this genus vary. Vincent (2002) found that rainbow suffered higher infection rate and severity compared to various subspecies of Cutthroat Trout when

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exposed to whirling disease in a laboratory setting. In many field populations, rainbow may also be more vulnerable than cutthroat due to differences in preferred spawning habitat (Pierce et al. 2009). The infection rate of whirling disease decreases predictably in an upstream direction presumably due to the reduction in habitat (i.e., slow moving water with fine sediment) for the oligochaete hosts (De la Hoz and Budy 2004, Hallett and Bartholomew 2008) and cutthroat spawn higher in tributaries than rainbow (Muhlfeld et al. 2009b, Buehrens et al. 2013). Thus, in addition to lower susceptibility, cutthroat likely experience a lower level of exposure to *M. cerebralis* than rainbow.

While research has yet to explore the susceptibility of rainbow-cutthroat hybrids, evidence from other species suggests that hybridization between salmonid species of differential susceptibility alters the susceptibility of F1-hybrids. F1-hybrids of moderately susceptible Brook Trout (*Salvelinus fontinalis*) and mildly susceptible Lake Trout (*Salvelinus namaycush*) showed intermediate susceptibility compared to parental strains (Wagner et al. 2002). Therefore, rainbow-cutthroat hybrids may be more susceptible to WD than pure cutthroat due to their rainbow ancestry. And, like rainbow, hybrids may also be more vulnerable than cutthroat due to preferred spawning and rearing of hybrids in warmer, lower elevation areas (Muhlfeld et al. 2009b). If hybrid offspring of rainbow and cutthroat are more susceptible to WD, then we would expect the presence of WD to alter patterns of introgression between the two species both within and between streams.

Our research objective was to determine whether there was an impact of WD on introgressive hybridization between cutthroat and rainbow in the Blackfoot River Basin. We focused on the following questions:
1) Is introgressive hybridization between rainbow and cutthroat at the site associated with the same landscape characteristics and habitat quality variables identified by other studies? Given these characteristics, does the presence of whirling disease influence introgressive hybridization between the two species?
2) How do these variables (landscape characteristics, habitat quality, and WD) interact to predict spatial extent of introgression within a stream?

Overall, we expected that the same general landscape characteristics associated with other hybridization studies would be present in the Blackfoot River Basin. Specifically, that introgression will decline with increases in elevation, distance from river, and slope, and introgression will be lower in areas with higher habitat quality. If WD does influence hybridization, we expect that the presence of WD in a stream will interact with other variables such as landscape characteristics and habitat quality, increasing the strength of their expect impacts on levels of introgression and extent of hybridization.

STUDY AREA

The Blackfoot River, a free-flowing, fifth-order tributary of the upper Columbia River, drains a 5,998-km^2 watershed through 3,038 km of perennial streams. It lies in west-central Montana and flows west 212 river kilometers from the Continental Divide to its confluence with the Clark Fork River at Bonner, Montana. Our study focuses on 10 tributaries located in the lower half of the Blackfoot River Basin (Figure 2.1). Nonnative rainbow are present primarily in the lower half of the basin (Pierce et al. 2009) where they express both resident and fluvial life histories. Native cutthroat are present basin-wide, but most prevalent in streams of the mid-to-upper elevations such as upper reaches of tributaries to the mainstem, and throughout the upper basin of the Blackfoot River (Pierce et al. 2008). Despite intensive stocking throughout streams of the Blackfoot River watershed into the 1970's, hybridization between rainbow and cutthroat has been detected most commonly in lower watershed, and rarely detected in the upper basin (Pierce et al. 2005, Pierce et al. 2008). Other salmonid species present in the basin include native Bull Trout (*Salvelinus confluentus*) and Mountain Whitefish (*Prosopium williamsoni*), as well as nonnative Brook Trout (*S. fontinalis*) and Brown Trout (*Salmo trutta*). Whirling disease was present in the Blackfoot River Basin when it was first tested in 1998, just a few years after Montana's first documented outbreak in the Madison River in 1994.

METHODS

Stream Selection

For the last two decades, Montana Fish Wildlife and Parks (MFWP) conducted sentinel cage exposures with hatchery rainbow to monitor for presence and severity of whirling disease in streams throughout the Blackfoot River Basin following the methods of Pierce et al. (2009). We

selected streams with known hybridization in the lower reaches that had been monitored for whirling disease within 4.5 km of the confluence (median distance 0.7km) at least once between 2004-2008 (Table 2.A1; Pierce et al. 2001, Pierce and Podner 2006, Pierce et al. 2008).

In our study, we assumed that whirling disease was not present ("disease negative") in streams draining into the Blackfoot River if no infection was detected in sentinel cage fish for all tests conducted in that stream. We categorized streams as "disease positive" if sentinel cage exposures resulted in at least 70% of the individual caged fish with infections of $\geq$ 3 on the MacConnell-Baldwin rating scale, and a total mean grade infection for all exposed fish of $\geq$ 3. This level of disease severity is considered high enough to influence fish survival and have population level effects based on lab experiments and case studies (Vincent 2002, Granath et al. 2007). For example, multi-year study in the Rock Creek drainage of the Clark Fork River, MT found declines in wild trout associated with increasing infection severity (>2.5) of trout held in sentinel cages throughout the drainage (i.e., mean grade infection of fish in a cage was typically 2.7 or higher on the MacConnell-Baldwin rating scale; Granath et al. 2007). Six disease negative streams and four disease positive streams with known hybridization between *Oncorhynchus* species met these criteria for inclusion in the study.

Sampling

Within each stream, we sampled three to four locations between 2009 and 2011 to determine level of introgression between rainbow and cutthroat (Figure 2.1, Table 2.A1). Two sites were sampled again in 2013 to increase sample sizes. The lowest sampling site in each stream corresponds to the location of sentinel cage exposures for that stream. Sites were spaced roughly 1.3-16.2 km apart (median= 3 km) in order to define the longitudinal pattern of introgressive hybridization. The uppermost sampling site targeted areas where we expected to find little to no introgression between rainbow and cutthroat (i.e., <5% rainbow alleles). We used 5% introgression as a threshold for defining the end of the hybrid zone because this threshold allows for the occurrence of natural polymorphisms, which may otherwise alter the detection of non-hybridized populations in these systems (Allendorf et al. 2012).

At each site, we collected all *Oncorhynchus* species present using a backpack electrofishing unit until we obtained a sample size of 25 individuals or until sampling time exceeded 2.5 hours or 550 meters. For each fish, we measured total length (mm), obtained a fin clip, and stored it in 95% ethanol for genetic analysis. For sites that were sampled in multiple years, we examined the genetic results (allele calls) and length of individuals sampled to ensure that the same individual was not sampled more than once in our data set.

To assess habitat quality and other tributary characteristics known to influence hybridization, we recorded elevation, distance from the confluence (Stream_km), stream slope and temperature, as well as information on fine sediment deposition (i.e., embeddedness) and bank stability at each site. Elevation and Stream_km were measured in ArcMap (ESRI 2010). We calculated stream slope as the change in elevation from the confluence divided by distance from confluence for each site. To obtain information on temperatures throughout the main part of the growing season, we used HOBO temperatures loggers (Onset Computer Corporation, Pocasset, Massachusetts, ± 0.2°C of accuracy), and recorded the temperature at hourly intervals at each sampling site. Using this information, we calculated growing degree days (GDD) above 0°C between July 16th and August 31st, 2011. Sediment deposition was measured as embeddedness of the streambed by fine sediment at each site using the Platts/Bain visual assessment (Platts et al. 1983, Sylte and Fischenich 2002). We measured embeddedness of the streambed three times within each of three representative riffles for a total of nine readings per site. We then averaged the readings within each riffle, and then across all riffles to obtain a single estimate of deposition representative of the entire site. High scores of embeddedness using this technique indicate low levels of fine sediment deposition, which is associated with increased quality habitat for spawning and rearing or salmonids, and viability of invertebrate populations (Kemp et al. 2011). Thus, for clarity we refer to this measure of embeddedness as "streambed quality". To assess bank stability and impacts of animals we used the rating systems for vegetation cover, bank stabilization by rock, and animal damage outlined in Stevenson and Mills (1999) and summed the ratings across these three categories to obtain a single variable for bank stability at a site.

Genetic Analysis

To ensure that our data were representative of the spawning population at a given site, we sampled all fish between 70-230mm in total length. For all samples, DNA was extracted following the Gentra Isolation Kit protocol. All samples were amplified in 10ul reactions and analyzed using three different PCR profiles following instructions in the QIAGEN Mulitplex PCR Kit (QIAGEN, Valencia, CA).

To determine levels of introgression, we analyzed two panels consisting of a total of 11 diagnostic markers (denoted by an * below). The first panel consisted of five insertion/deletion loci and one microsatellite locus: *Occ34*, Occ35*, Occ36*, Occ37*, Occ38*, Occ42**, and *Om55** (Ostberg and Rodrigues 2004) and *Ssa408*(Cairney et al. 2000)*. The second panel consisted of *Omm1037-1, Omm1037-2, Omm1050** (Rexroad et al. 2002), *Omy0004* (Holm and Brusgaar 1999)*, *Omy1001** (Spies et al. 2005), and *Oki10* (Smith et al. 1998). 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). We quantified introgression at a given site as the proportion rainbow alleles in a sample using the following equation:

$$Pr(RBT) = (RBT\ alleles/2LN)$$

where RBT alleles is the number of rainbow alleles detected in a sample from a given site, and L is the number of loci examines, and N is the total number of fish analyzed from that site (see Bennett et al. 2010). With the 11 diagnostic markers listed above, a sample size of 25 fish gives us the 99.6% probability of detecting as little as 1% admixture with rainbow.

All genetic analyses were conducted at the University of Montana Conservation Genetics Lab, Missoula, USA.

Statistical Analyses

What variables are associated with introgression at a site?

To evaluate whether WD influences levels of introgression at a given site, we standardized variables and used a linear mixed regression model with a logit-link function. Temperature was

not included in this site-level analysis of introgression because we were unable to collect temperature data at all sites (see below).

Our full model regressed introgression on the following fixed effects: Stream_km, elevation (Elev), stream slope (Slope), bank stability (Bank) streambed quality (Bed) and presence of WD in the stream. The full model also contained an interaction of WD with all other variables because we hypothesized the presence of WD would alter the collective influence of these variables on introgression. Because there were multiple sites within a stream, stream was included in the model as a random effect. We analyzed all possible combinations of these variables and interaction terms. Model selection was based on Akaike information criteria (AIC; Burnham and Anderson 2002) and error around parameter estimates. The top model was the one with the lowest AIC that also had significant parameter estimates for all interaction terms as well as any base variables not included in interaction terms. We evaluated significance in parameter estimates at the level of $\alpha=0.05$.

To assess multicollinearity of variables, we looked at the variance inflation factor (VIF) of all variables in the full model. The variance inflation factor quantifies the degree to which the variance increased as a result of multicollinearity with other variable in an ordinary least squares regression model. For example a VIF=10 for a single variable would mean that the variance of the parameter estimate for that variable is ten times larger than it would be if that variable was completely uncorrelated with all others in the model (Montgomery et al. 2012). If VIF was high (>5) for variables in the top model, we removed highly correlated variables one at a time while keeping all other variables constant, and selected the resulting model with the lowest AIC value in which all parameter estimates were significantly different from zero.

What influences the spatial extent of introgression within a stream?
To examine what variables best predict the size of the hybrid zone (i.e., stream km where introgression = 5%), we first estimated the stream km where introgression would equal 5% by fitting a linear regression (introgression versus stream km) between the two sites where introgression was closest to 5%. When possible, we interpolated between two adjacent sampling sites that tested above and below this threshold (respectively). If we were unable to obtain a

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sample in a stream where introgression was below 5%, we extrapolated, and used the two adjacent sites with the introgression levels closest to 5%.

After estimating the upstream extent of hybridization within each stream, we obtained the elevation at that location using digital elevation layer on ArcMap (ESRI 2010). To obtain a measure of slope that was independent of the response variable, we used slope of the entire stream as a predictor variable in the second analysis. We calculated whole stream slope as change in elevation over distance from headwaters to confluence (as indicated on ArcMap).

To obtain a measure of temperature corresponding to the overall temperature profile of each stream (i.e., temperature spanning the elevational relief of each stream), we performed a linear regression of growing degree days on elevation for all sites where temperature was recorded. We then calculated the average residual value across all data points (i.e., temperature monitoring site) for a given stream. The result was a single value describing the temperature profile for each stream in this study (Temp).

To quantify bank stability and embeddedness within the hybrid zone, we averaged the scores for these variables across all sampling sites within the hybrid zone to obtain a single estimate of riparian quality (Bank_zone) and streambed quality (Bed_zone) within the hybrid zone.

To evaluate which variables best predict the size of the hybrid zone in a stream, we standardized variables and performed a multiple linear regression of our estimate of size of the hybrid zone on Elev, Slope, Temp, Bank_zone and Bed_zone. We compared every possible combination of these variables. The top model was the model with the lowest AIC value corrected for small sample size (AICc) in which parameter estimates for all variables was significantly different from zero at the level of $\alpha=0.05$. We checked for multicollinearity of variables using VIF as described above.

All statistical analyses were conducted in program R (R Developement Core Team 2012).

RESULTS

Quantification of Introgressive Hybridization

The length of stream sampled at each site varied between 73 – 574 m (median 178 m). At five sites in three different streams (West Twin, Monture, and Gold Creeks), we were unable to achieve a sample size of 25 fish due to low densities of cutthroat (0.03 to 0.09 fish/meter; Table 2.A1). In one case (site WT3), we were only able to obtain 13 unique samples over three sampling years. However, all fish captured at this site tested as non-hybridized with a 94% probability of detecting as little as 1% population admixture given the number of diagnostic markers and this sample size (Kanda et al. 2002). As a result, we are confident that the population at this site is non-hybridized. At the four other sites with samples size under 25, we detected levels of introgression between 0 -71% based on sample sizes ranging from 20-24 individuals.

In three of the ten streams, we did not obtain a genetic sample with population level introgression $\leq$ 5% (Figure 2.2). In Elk Creek we detected 7.8% admixture at the highest site (EK3), but cutthroat were not present at the next site upstream. The highest site sampled at Bear Creek had 7% admixture (BR3), but we only obtained two fish at the next site upstream of BR3. In Johnson Gulch, the uppermost site (JG2) had 5.7% admixture, and we were not able to access higher sites. To estimate the size of the hybrid zone, we interpolated for all streams except for Johnson Gulch, Bear and Elk Creeks (Table 1). For these three streams, we estimated the size of the hybrid zone by extrapolating between the two highest elevation sampling sites.

What site-scale variables are associated with introgression?

    Model results

We expected that introgression would decrease with increasing measures of habitat quality, elevation, stream slope, and distance from the Blackfoot River. We also expected the presence of whirling disease in a stream to interact with these landscape variables, increasing their impact on introgression at a site scale. Initially our full model included elevation, distance from river, slope, and bank stability and streambed quality with an interaction between all of these and WD. However, this model structure produced parameters estimates with associations between introgression and predictor variables that were not observed in the raw data, indicating that

multicollinearity between predictor variables may be affecting model results (Montgomery et al. 2012). Although VIFs were less than 3 for all variables in this model, a Welch's t-test reveled that slope was significantly more shallow in streams where WD was present (p-value <0.001). As a result, we created two different full models, including only one of these variables at a time. Our full models for analyzing predictors of introgression at a site scale were as follows:

Full Model A: Introgression ~ WD*(Stream_km + Elev + Bank + Bed)

Full Model B: Introgression ~Slope+ Stream_km + Elev + Bank + Bed

For A, the best model had the second lowest AIC and included an interaction between WD Stream_km and Elev, but not Bank or Bed. This model was chosen over the model with the lowest AIC because all parameter estimates for interactions terms as well as base variables not included in interaction terms were significantly different from zero (Table 2.2, Figure 2.A3). For B, the full model was chosen as the top model. Here, the full model produced the lowest AIC with all parameter estimates significantly different from zero (Table 2.2, Figure 2.A4). All variables in both the full model for A and B had a VIF $\leq$ 2.25.

As expected, results from Model A show that lower levels of introgression at a particular site were associated with increasing distance from the confluence, higher elevation and in higher quality habitat (as indicated by greater bank stability and higher streambed quality). The presence of whirling disease increases the impact of these variables on introgression except for the effect of distance from the confluence with the Blackfoot River. While the association between introgression and Stream_km was still negative in the presence of WD, the effect of Stream_km was attenuated.

Similar to Model A, results from Model B show a negative association between introgression and distance from the confluence, elevation and habitat quality. In addition, Model B demonstrated a highly significant negative relationship between introgression and slope. Overall the top model for both A and B agree in the overall relationships between abiotic landscape characteristics and introgression at a site scale, and, based on estimates of log-likelihood and confidence in parameter estimates, appear roughly equal in their ability to predict levels of introgression at a site scale.

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What influences the spatial extent of introgression within a stream?

   Temperature Data

Temperature data was successfully collected at a total of 34 sites across all streams in this study from July 16-August 31, 2011 (Figure 2.4). This included 26 of 33 sites sampled for introgression, as well as eight additional locations within the hybrid zone of five streams (two additional sites in Blanchard at 6km and 11.9km, Elk at 15lm and 19km, Monture at 7km and 19.3km; one additional site in Belmont at 12.9km and Gold at 8.4km).

   Model results

Our full model predicting size of the hybrid zone was as follows:

Zone Size ~ Elev + Slope + Temp + Bank_zone + Bed_zone

The top model for predicting the size of the hybrid zone in a stream contained only stream slope, (Table 2.4 and Table 2.5). The second ranked model fell within two AIC points of the top model and contained both stream slope and bank stability within the hybrid zone (Table 2.4), however, the parameter estimate for bank stability was not significantly different from zero (Table 2.5). In the full model, VIFs were high for elevation at the end of the hybrid zone (6.44), and bank stability (6.22) and moderate for whirling disease (3.99) and temperature (3.26).

DISCUSSION

In our study, WD positive streams tended to have more gradual stream slopes. For a given set of site scale characteristics (i.e., elevation, habitat quality), these shallow sloping, disease positive streams had a lower level of introgression than steeper, disease negative streams. Additionally, streams with steeper slopes typically had smaller hybrid zones, but the presence of WD did not play a significant role in determining size of the hybrid zone. Research by Eby et al. (In review) found that a landscape level estimate of valley slope in the Blackfoot River Basin was correlated with stream slope at a site scale, and was a good predictor of both fine sediment loads and WD severity at a site. Specifically, shallower sites had higher disease severity in sentinel cage studies, presumably due to the higher loads of fine sediment, which provide habitat for the disease's alternative host, *T. tubifex*. These data suggest that slope may generally serve as a better variable than whirling disease for predicting overall levels of ingression at a site because it likely

incorporates both the preferences of cutthroat and rainbow to varying geomorphic habitat characteristics, as well as any biotic impact of whirling disease on introgression.

Consistent with other studies, we found that introgression decreased with distance from the confluence of the Blackfoot River, the putative source of rainbow trout alleles (Hitt et al. 2003, Weigel et al. 2003, Muhlfeld et al. 2009c, Rasmussen et al. 2010, Kovach et al. 2011). But surprisingly, the effect of distance from source was dampened in the presence of whirling disease. This unexpected interaction between whirling disease and distance from source may be attributed to the fact that the slope was generally more shallow (Figure 2.3a) in disease positive versus negative streams. Given that slope was the strongest predictor of the upstream extent of hybridization on the whole stream scale, one may conclude that the more gradual slopes observed in disease positive streams could allow for further upstream extent of hybridization, and thus higher levels of introgression at a given distance from the confluence compared to disease negative streams. Eby et al. (In review), found that the low gradient, disease positive streams in this study registered some of highest instances of disease severity in sentinel cage studies conducted throughout the Blackfoot River Basin. In particular, Monture Creek had the lowest stream slope and the largest hybrid zone in our dataset. At our lowest elevation sampling site in Monture Creek, 2.9 km upstream from the confluence, we observed introgressive hybridization in excess of 70%. Over 90% of sentinel cage fish at this location had mean grade infections > 3 on the MacConnell- Baldwin rating scale in 2005, 2006, 2007 and 2009 (Eby et al. In review). If WD were truly impacting wild rainbow trout and hybrid populations in a manner that reduced introgressive hybridization with cutthroat, we would expect a stream like Monture Creek to have a much smaller hybrid zone, and lower levels of introgressive hybridization at sites known to induce high severity infection. This suggests that whirling disease actually has little effect on introgressive hybridization between rainbow and cutthroat populations in the Blackfoot River, and that WD is simply serving as a proxy for slope in our model analyzing introgression on a site scale.

Stream slope was the key predictor for size of the hybrid zone. This association speaks to differences in life history between rainbow and cutthroat trout. Multiple studies comparing habitat and occupancy of rainbow, cutthroat, and hybrids have found that rainbow and hybrids

occupy lower gradient sections of stream in areas where rainbow have been introduced as well as where the two species are naturally sympatric (Hitt et al. 2003, Weigel et al. 2003, Buehrens et al. 2013). Furthermore, cutthroat trout are headwater specialists, inhabiting some of the highest gradient streams of all salmonids and often occupying reaches where no other fish are present (Bozek and Hubert 1992, Paul and Post 2001, Quist and Hubert 2004, Rasmussen et al. 2010, D'Angelo and Muhlfeld 2013). Elevation generally displays a negative correlation with introgressive hybridization between our two focal species in this and other studies (Hitt et al. 2003, Bennett et al. 2010, Rasmussen et al. 2010, Yau and Taylor 2013). For example, in the Upper Oldman River of Alberta, Canada, Rasmussen et al. (2010) found that the proportion of rainbow alleles present in a population decreased exponentially with elevation of a site, and observed only one site with introgression > 5% at all sites at 1471 m or higher (16 sites, median introgression=1% and maximum elevation=1722 m). Hitt et al. (2003) found a similar transition to non-hybridized cutthroat in the Upper Flathead River Basin of northwestern Montana at roughly 1450 m. Out of a total of 12 sites above 1305m, we observed only one site with introgression > 5% (median introgression for sites above 1305m was 1% and maximum elevation of 1699). A subsequent study on physiological performance by Rasmussen et al. (2012) suggests that the metabolic needs of purebred and hybrid individuals with rainbow ancestry are not met in less productive, high elevation habitat, allowing cutthroat to dominate these areas. Our findings in conjunction with these studies indicate that stream conditions near 1300m in elevation in the Blackfoot River Basin may maintain certain climatic, biological and/or geomorphic conditions unsuitable for rainbow and hybrids.

In previous studies, temperature has emerged as one of the best predictors of, and is consistently negatively associated with both the occurrence of hybridization and the degree of introgression at a site scale (Muhlfeld et al. 2009c, Yau and Taylor 2013). Thus, we were surprised that temperature did not have an effect on size of the hybrid zone in this study. To categorize the temperature profile of each stream, we calculated the average residual temperature of logger sites within the hybrid zone compared to the mean for all sites where temperature was monitored in the basin. Our results suggest that even generalized summertime temperature metrics alone may not represent the key limiting climatic conditions affecting hybridization on a whole stream scale. For example, Fausch et al. (2001) found that success of rainbow invasions in Colorado, the

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Southern Appalachians, and Japan was strong influenced by flow regime. Bennett et al. (2010) found that tributaries to the Upper Kootenai River (British Columbia) located in warmer and dryer biogeoclimatic zones were associated with higher levels of introgression between cutthroat and introduced rainbow. These studies suggest that a broader climatic variable incorporating aspects of temperature, precipitation and flow regime may serve as better predictor of hybridization between rainbow and cutthroat than site-level estimates of summer temperature.

Similar to (Muhlfeld et al. 2009c), we found that sites with higher habitat quality generally had lower levels of introgression. In our study streams, introgression tended to increase with disturbances that erode stream banks and increase rates of sedimentation such as hoof sheering, lack of vigorous riparian vegetation and bank stabilization by rocks. A potential mechanism for this trend could be associated with development, where embryos of rainbow trout and hybrids may have a higher tolerance for fine sediment than cutthroat trout. Sowden and Power (1985) did not find a negative association between survival and fine sediments (under 2mm in diameter) for nonnative rainbow in a tributary to Lake Erie in Ontario, Canada. Conversely, Bonneville Cutthroat Trout hatch and emergence survival declined significantly with the proportion of fine sediment <4mm in redds (Budy et al. 2012). Furthermore, increases in smaller substrates may augment suitable spawning habitat for rainbow trout invaders. For Westslope Cutthroat Trout in the Blackfoot River Basin, the dominant substrates in redds are large gravels (16-32mm) and cobble (32-110mm), which are amongst the largest spawning substrate observed for cutthroat subspecies (Schmetterling 2000, Joyce and Hubert 2004, Budy et al. 2012). While size of spawning substrates for rainbows have not been documented in the Blackfoot River, their median substrate size in redds located in the Missouri River Drainage, MT was sand (12-15mm) with more than 10% of substrate finer than 0.85mm (Kondolf 2000). In short, habitat alterations resulting in an increased proportion of smaller substrate and fine sediment may promote spawning success of rainbow.

Studies predict that climate change will warm stream temperatures, reducing habitat for native trout and increasing habitat for nonnative trout throughout the Rocky Mountains (Williams et al. 2009, Wenger et al. 2011). Similarly, human activities and climate change will only continue to cause expansion of wildlife disease and alter host-pathogen interactions (Daszak et al. 2001,

Fuller et al. 2012, Gallana et al. 2013). In our study system, hybridization between a native cutthroat and invasive rainbow is primarily driven by stream slope (a landscape-level characteristic) and was not influenced by multispecies interactions with an introduced parasite. Instead, stream geomorphology may play a larger role in defining quality habitat for many nonnative trout species, such as rainbow and hybrids. Geomorphic characteristics, such as stream slope, are not expected to change in the next century and may limit species expansions in certain types of streams, such as high gradient, high elevation tributaries. Biologists should incorporate geomorphic variables in addition to variables such as temperature and precipitation when outlining expectations for community composition and conservation of native species in the coming decades. Additionally, as community assemblies continue to change, we must continually evaluate the effects of biotic interactions. Interactions between various nonnative species could serve as a bio-control mechanism, reducing the impacts of invasive on native species. But at worst, these novel community assemblages could compound threats to native species, and potentially speed rates of extirpation and/or extinction. Knowledge of how nonnative species interact with each other and with native species in the ecosystems they invade will help managers prioritize and strategize conservation action for long-term protection of native species in the wild.

CONCLUSIONS

Whirling disease does not appear to be a factor mediating introgressive hybridization between rainbow and cutthroat trout in the Blackfoot River Basin. Overall, stream slope appears to be the most influential factor determining the size of the hybrid zone in streams of this basin, regardless of whether WD is present. While our study did not find an effect of multispecies interactions on hybridization, biotic factors should not be overlooked, particularly as species assemblages are altered by climate change. Knowledge of how nonnative species interact with each other and with native species in the ecosystems they invade will help managers prioritize and strategize conservation action for long-term protection of native species.

Table 2.1. Estimated stream km, elevation, and change in elevation from confluence (Delta Elevation) marking the end of the hybrid zone in each stream. Slope refers to the whole stream slope from headwaters to confluence for each stream. Habitat quality variables include the average scores for bank stability and streambed quality (measured as embeddedness) averaged across all sites within the hybrid zone, as well as the average temperature residual for all temperature logger sites (Temp) within the hybrid zone for each stream.

Map ID	Stream	Stream Km	Elevation (m)	Delta Elevation (m)	Slope	Bank Stability	Streambed Quality	Temp
Disease Negative								
1	Johnson	2.89	1177	171	0.140	11.33	4.89	-115.70
2	WestTwin	5.22	1424	388	0.112	11.50	4.94	-90.27
3	EastTwin	6.58	1429	391	0.084	10.75	4.56	-68.33
4	Bear	5.4	1350	311	0.079	9.33	4.89	-72.72
5	Gold	14.7	1344	299	0.036	10.00	4.39	-5.19
6	Blanchard	8.7	1433	261	0.028	9.25	3.78	183.28
Disease Positive								
7	Belmont	7.18	1330	263	0.046	8.50	4.56	-66.35
8	Elk	11.63	1275	158	0.028	10.00	4.39	21.14
9	Chamberlain	5.44	1292	105	0.039	7.50	4.56	37.79
10	Monture	26.42	1341	140	0.023	9.00	4.39	19.01

Table 2.2. Top five models predicting the levels of introgression at a site. Models listed under A include whirling disease (WD) but exclude slope, while models listed under B include slope and exclude whirling disease. The number of parameters (k) includes the intercept and the random factor Stream. Uninformative Parameters refers to variables whose parameter estimates were not significantly different from zero at the level of $\alpha=0.05$. Top models are shown in bold.

	Model	ΔAIC	k	Log-Likelihood	Uninformative Parameters
A1	WD*(Elev + Stream_km + Bank)+ Bed + (1|Stream)	0.00	10	-194.8	WD*Bank, WD, Bed
A2	**WD*(Elev + Stream_km) + Bed + Bank + (1|Stream)**	**0.91**	**9**	**-196.3**	**WD**
A3	WD*(Elev +Stream_km+ Bed+ Bank) + (1|Stream)	1.73	11	-194.7	WD*Bank, WD*Bed, Bed, WD
A4	WD*(Elev + Stream_km + Bed) + Bank + (1|Stream)	2.69	10	-196.2	WD*Bed, WD
A5	WD*(Stream_km + Bed + Bank) + Elev + (1|Stream)	8.11	10	-198.9	WD*Bank, WD, Bed
B1	**Slope + Elev + Stream_km + Bed + Bank + (1|Stream)**	**0.00**	**7**	**-192.0**	**None**
B2	Slope + Elev + Stream_km + Bank + (1|Stream)	2.35	6	-194.2	None
B3	Elev + Stream_km + Bed + Bank + (1|Stream)	30.89	6	-208.5	None
B4	Elev + Stream_km + Bank + (1|Stream)	34.97	5	-211.5	None
B5	Slope + Elev + Stream_km + Bed + (1|Stream)	114.23	6	-250.1	None

Table 2.3. Details of top models predicting levels of introgression at a site, including parameter estimates, standard error (SE), and p-values for fixed effects variables, and variance estimate for the random effect in the top models. Model A refers to the model structure excluding slope. Model B refers to the model structure excluding whirling disease (WD), but including slope.

Fixed Effects	Model A			Model B		
	Estimate	SE	p-value	Estimate	SE	p-value
Intercept	-2.06	0.33	<0.001	-1.98	0.32	<0.001
Slope	--	--	--	-0.31	0.05	<0.001
WD	-0.07	0.34	0.84	--	--	--
Elev	-2.34	0.10	<0.001	-2.54	0.09	<0.001
Stream_km	-1.10	0.11	<0.001	-0.72	0.07	<0.001
Bed	-0.44	0.04	<0.01	-0.11	0.05	0.04
Bank	-0.16	0.05	<0.001	-0.46	0.04	<0.001
WD*Elev	-0.40	0.09	<0.001	--	--	--
WD*Stream_km	0.47	0.10	<0.001	--	--	--
Random Effects	**Variance**			**Variance**		
Stream	1.07			1.01		

Table 2.4. Top five models and the full model for predicting the size of the hybrid zone in a stream. Uninformative Parameters refers to variables whose parameter estimates were not significantly different from zero.

	Model	ΔAICc	k	Multiple R-sq	Log-Likelihood	Uninformative Parmaters
1	**Slope**	**0.00**	**2.00**	**0.40**	**-30.45**	**None**
2	Slope + Bank	1.52	3.00	0.54	-29.07	Bank
3	WD + Slope + Bank	2.42	4.00	0.72	-26.52	WD
4	WD	3.24	2.00	0.16	-32.07	WD
5	Slope + Temp	3.61	3.00	0.44	-30.11	Slope, Temp

Table 2.5. Parameters estimates, standard error and p-values for the top model and models within two AIC points of top model.

Parameters	Top Model			Second Ranked Model		
	Estimate	SE	p-value	Estimate	SE	p-value
Intercept	9.416	1.797	<0.001	9.42	1.67	<0.001
Slope	-4.336	1.894	p=0.05	-7.7	2.86	<0.05
Bank	--	--	--	4.27	2.86	0.18
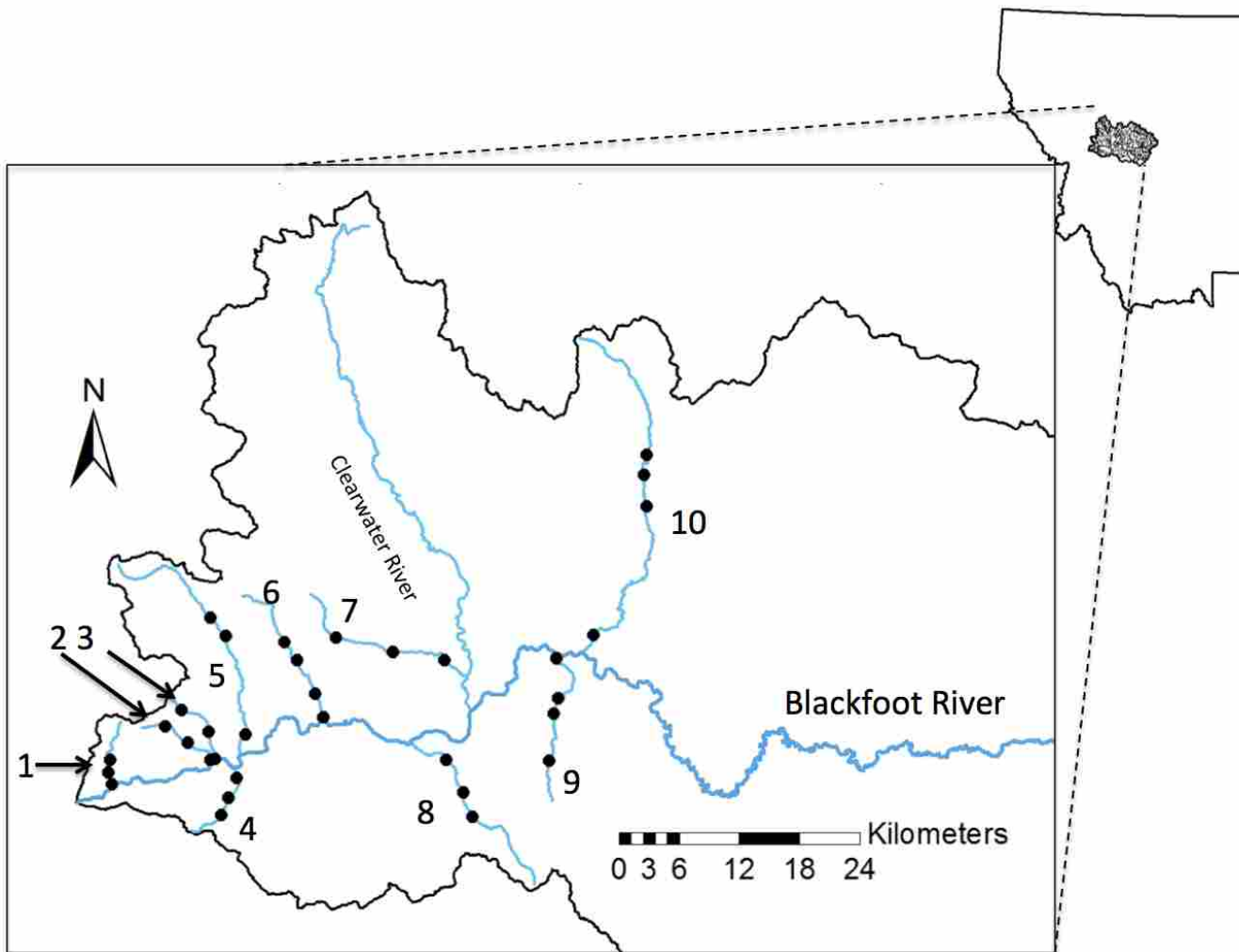
Figure 2.1. Points indicate sampling locations and numbers correspond to Stream ID in Table 1. The lowest site in each stream also corresponds to the sentinel cage exposure site for whirling

a)
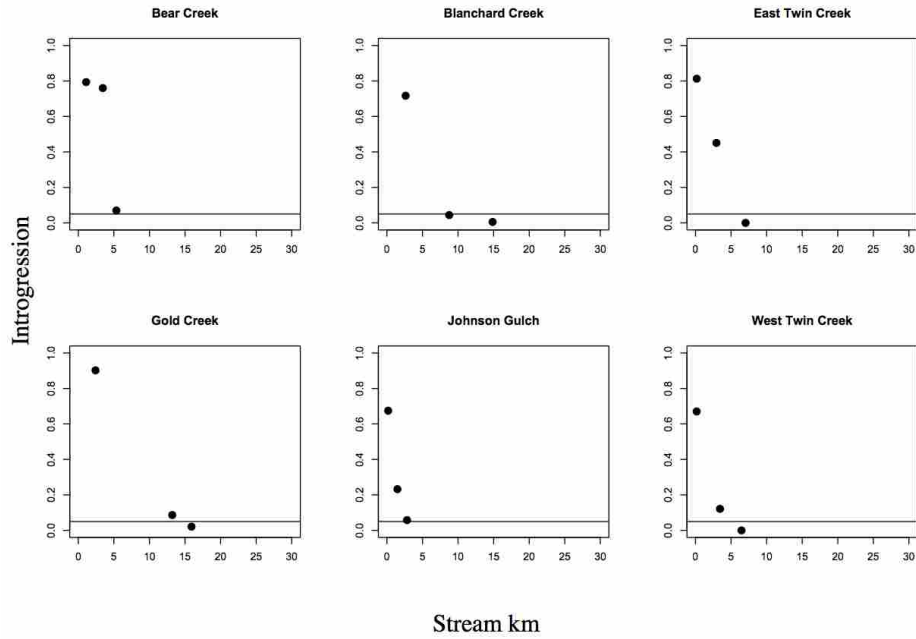
b)
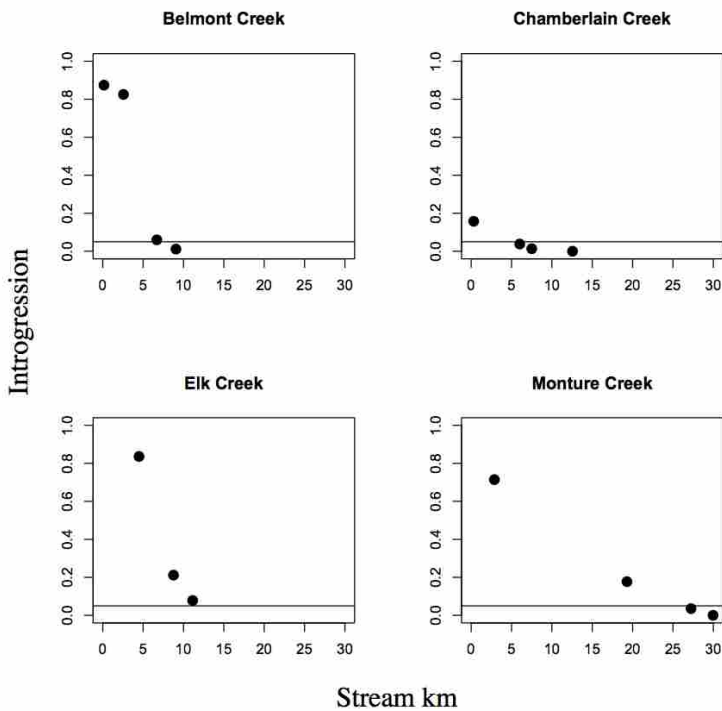
Figure 2.2. Level of introgression (proportion of rainbow alleles in a sample) versus distance upstream from confluence (Stream_km) for all sites sampled in disease negative (a) and disease positive streams (b). The horizontal line represents 5% introgression of Cutthroat with Rainbow Trout.

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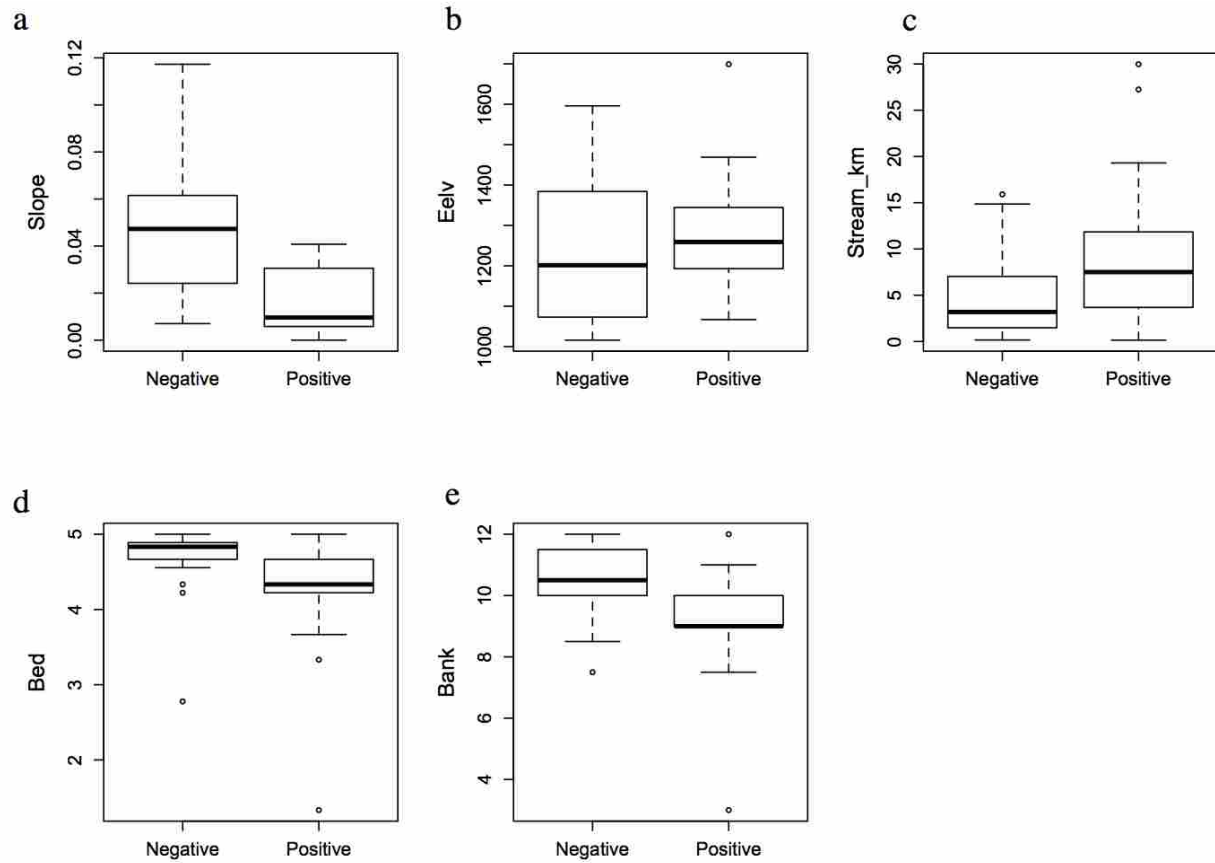
Figure 2.3. Box and whisker plots showing the range of standardized values between whirling disease negative and positive streams for five variables including slope, elevation (m), distance upstream from confluence (Stream_km), streambed quality ranking measured as embeddedness, and bank stability ranking across all sites. High values of Bed indicate low levels of fine sediment and thus high quality habitat. Whiskers represent 1.5 * the interquartile range. Note that plots d and e refer to the two habitat quality dimensions obtained from data reduction (via non-metric multidimensional scaling) that were used in the model identifying variables that influence introgression at the site scale.

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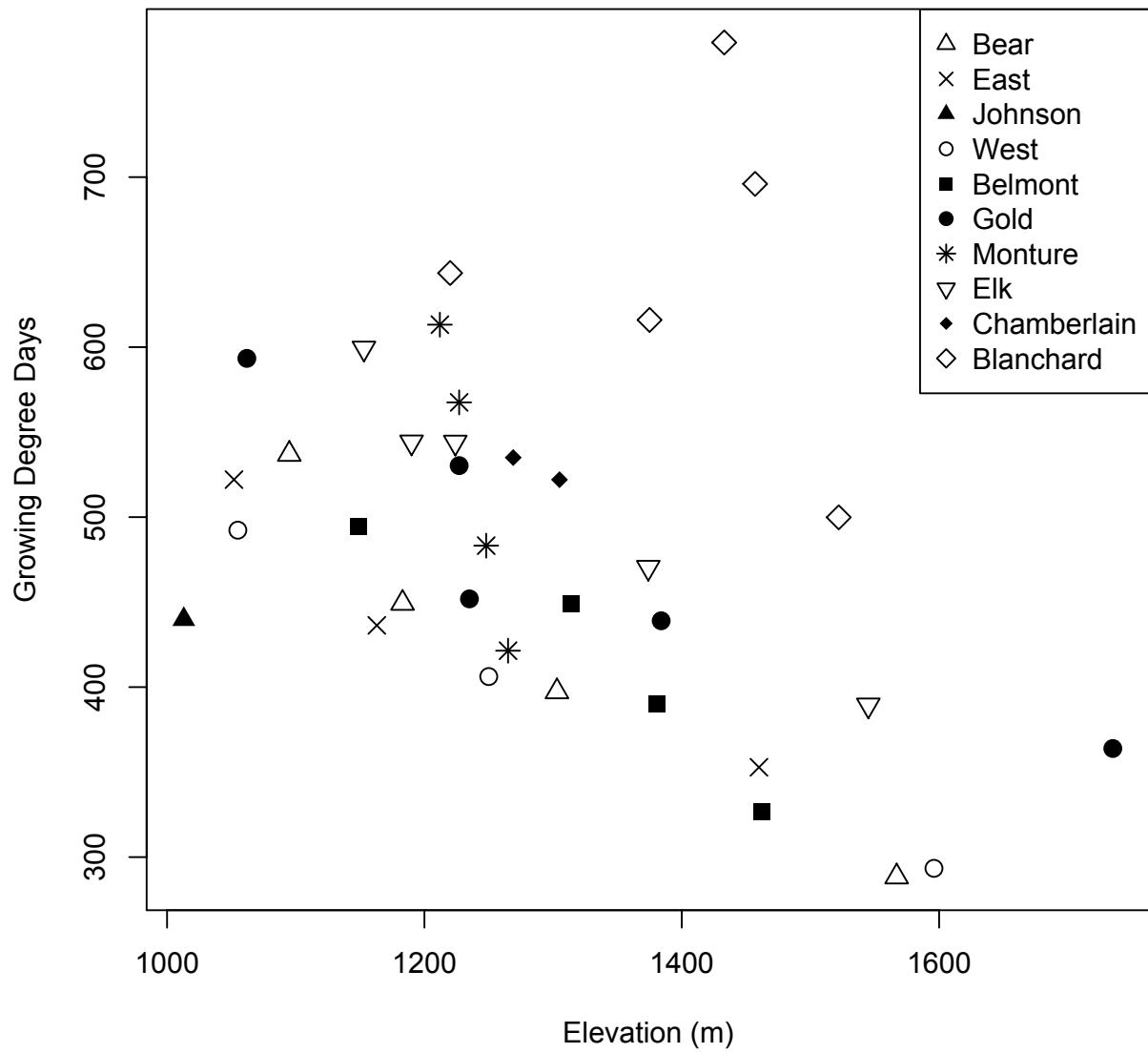
Figure 2.4. Growing degree days versus elevation for all sites where temperature was recorded.

Table 2.A1) Summary of data collected at each site in this study. Abbreviations of column names are as follows: Intro- proportion of RBT admixture; Stream_km – distance from confluence; Delta Elevation- change in elevation from confluence; Slope- stream slope; WD- presence of whirling disease (1=present, 0=not detected); Embed-average embeddedness score; Veg- vegetation rating; Rock- rock stabilization rating for the site; Animal damage observed at the site. Note that in the field rankings higher values of Embed, Veg, Rock, and Animal generally indicate higher quality habitat. The lowest elevation site in each stream corresponds to the location of whirling disease sentinel cage studies in that stream.

| Stream	Site	Latitute	Longitude	Years Sampled	Section Length (m)	Sample Size	Intro	Stream_km	Elevation (m)	Delta Elevaiton (m)	Slope	WD	Embed	Veg	Rock	Animal
Diesease Negative Streams																
Bear	BR1	46.898430	-113.680560	2009	114	41	0.794	1.10	1095	56	0.0511	0	4.89	2	3	3.5
Bear	BR2	46.880390	-113.690310	2009	162	29	0.760	3.45	1183	144	0.0418	0	4.89	2.5	2	3
Bear	BR3	46.864660	-113.699550	2011	151	26	0.070	5.35	1303	264	0.0494	0	4.89	4	4	4
Blanchard	BC1	47.009260	-113.413850	2011	243	26	0.717	2.63	1220	57	0.0217	0	4.78	1.5	3	4
Blanchard	BC3	47.014580	-113.482410	2011	73	28	0.044	8.75	1433	261	0.0298	0	2.78	3	3	4
Blanchard	BC5	47.025990	-113.557550	2011	209	27	0.005	14.86	1522	359	0.0242	0	4.78	3.5	3	4
EastTwin	ET1	46.914620	-113.710220	2009, 2011	142	26	0.813	0.17	1052	14	0.0819	0	4.78	3	3	4
EastTwin	ET2	46.938130	-113.719020	2011	178	25	0.451	2.94	1163	125	0.0425	0	4.33	3.5	4	4
EastTwin	ET3	46.957270	-113.755940	2009	93	29	0.000	7.03	1460	422	0.0600	0	4.78	3.5	3	3.5
Gold	GD1	46.937500	-113.671200	2009	235	26	0.902	2.42	1062	17	0.0070	0	4.56	3	4	3
Gold	GD3	47.024200	-113.700650	2009	218	30	0.086	13.20	1235	190	0.0144	0	4.22	3	4	3
Gold	GD4	47.040050	-113.722650	2011	258	24	0.021	15.91	1384	339	0.0213	0	4.67	3.5	3	4
Johnson	JG1	46.888800	-113.842690	2011	166	25	0.675	0.18	1016	10	0.0556	0	4.89	4	4	4
Johnson	JG1_5	46.899220	-113.848300	2011	410	27	0.232	1.48	1073	67	0.0452	0	4.89	4	3	4
Johnson	JG2	46.910050	-113.846760	2011	89	26	0.058	2.83	1180	174	0.0615	0	4.89	4	3	4
WestTwin	WT1	46.913030	-113.715500	2009	134	28	0.670	0.16	1055	19	0.1173	0	5.00	3	4	4
WestTwin	WT2	46.927650	-113.746290	2011	247	27	0.121	3.44	1250	214	0.0622	0	4.89	4	4	4
WestTwin	WT3	46.941670	-113.776440	2009, 2011, 2013	200	13	0.000	6.47	1596	560	0.0866	0	4.89	4	3.5	4
Diesease Negative Streams																
Belmont	BL1	46.954940	-113.570290	2009	97	35	0.874	0.15	1067	0	0.0000	1	4.78	2.5	3	3.5
Belmont	BL2	46.975470	-113.582050	2009	90	33	0.825	2.56	1149	82	0.0320	1	4.67	2.5	1	4
Belmont	BL3	47.004760	-113.606900	2011	244	25	0.060	6.70	1314	247	0.0369	1	4.22	4	1	4
Belmont	BL4	47.020540	-113.624310	2011	188	28	0.011	9.07	1381	314	0.0346	1	4.22	4	2	4
Chamberlain	CH1	47.014110	-113.268490	2009, 2011	91	30	0.158	0.31	1196	9	0.0291	1	4.56	2	2	3.5
Chamberlain	CH3	46.977940	-113.263580	2009	101	36	0.038	6.02	1305	118	0.0196	1	4.33	3.5	2.5	4
Chamberlain	CH4	46.964150	-113.268960	2011	127	31	0.013	7.50	1335	148	0.0197	1	4.33	4	4	4
Chamberlain	CH5	46.922120	-113.273790	2011	80	30	0.000	12.56	1699	512	0.0408	1	5.00	3	2.5	4
Elk	EK1	46.920360	-113.407990	2009	402	28	0.836	4.49	1153	26	0.0058	1	1.33	1	1	1
Elk	EK2	46.891330	-113.384120	2009, 2011	147	29	0.212	8.76	1190	73	0.0083	1	3.67	4	2	3
Elk	EK3	46.870440	-113.372120	2011	350	25	0.078	11.13	1224	107	0.0096	1	3.33	3	3	4
Monture	MO1	47.035580	-113.220500	2009, 2011	316	21	0.714	2.88	1212	11	0.0038	1	4.22	2	3	4
Monture	MO4	47.118700	-113.146800	2009, 2013	550	20	0.177	19.30	1259	58	0.0030	1	4.56	3	2	4
Monture	MO6	47.179730	-113.159480	2011	283	27	0.035	27.24	1354	159	0.0058	1	4.67	2	4	4
Monture	MO7	47.197630	-113.156720	2011	574	24	0.000	29.97	1469	274	0.0091	1	4.78	3	4	4
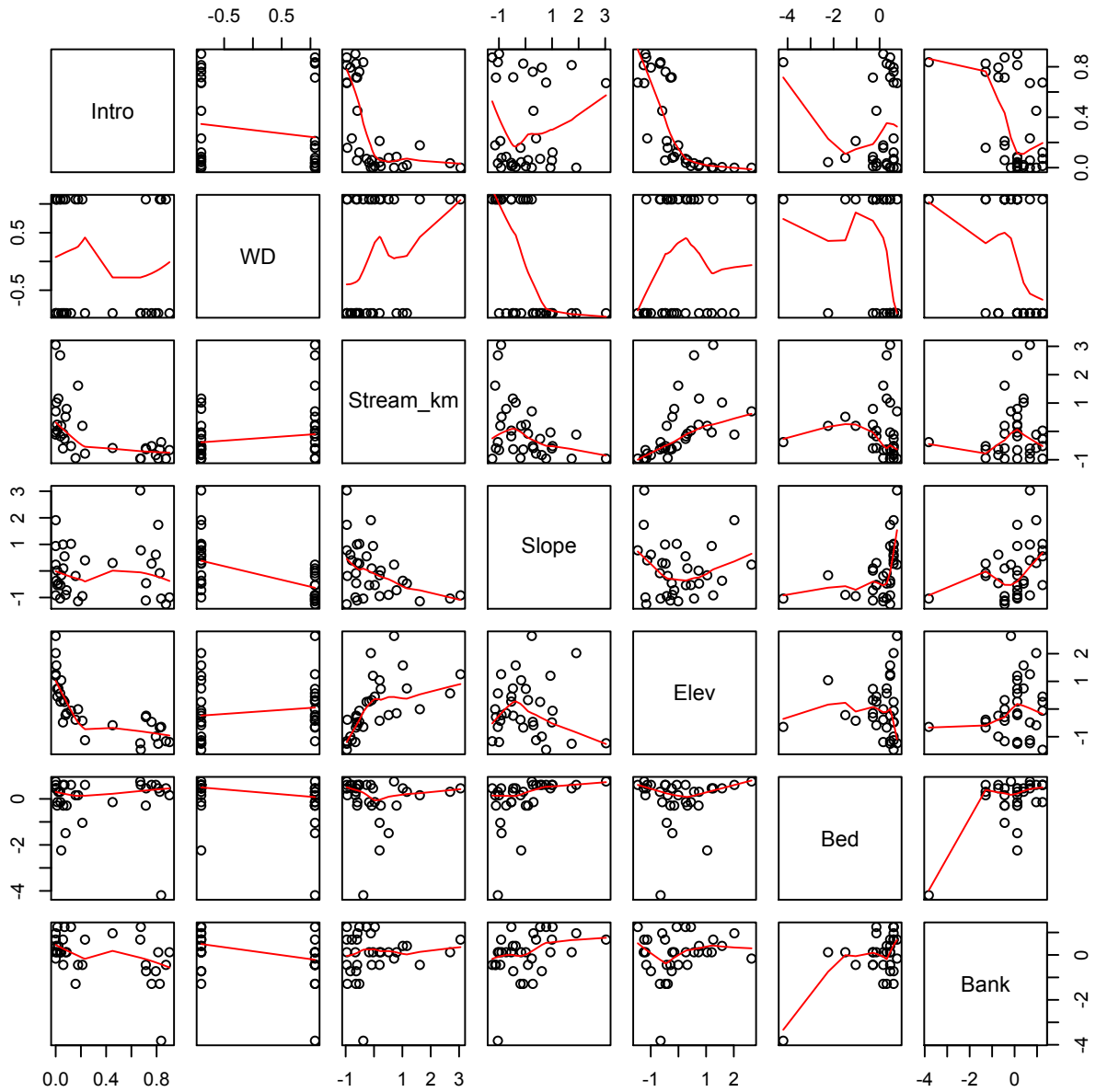
Figure 2.A2. Matrix scatter plot of levels of introgression at a site (Intro; the dependent variable) and all scaled independent variables measured in this study used in the two full models predicting levels introgressive hybridization at a site scale. Variables include presence of whirling disease (WD), stream slope (Slope), and elevation at a site (Elev), streambed quality determined by levels of embeddedness (Bed) and bank stability (Bank).

a)
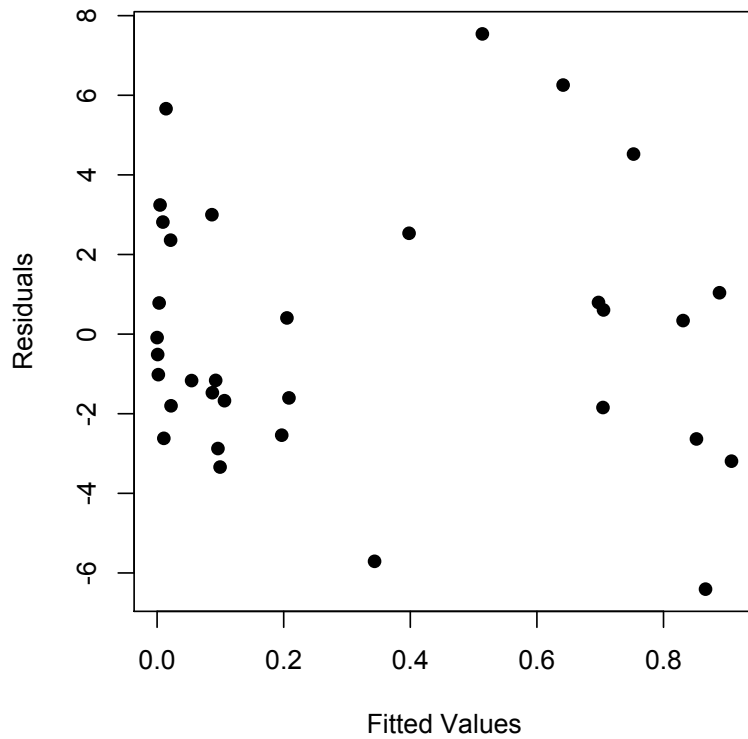
b)
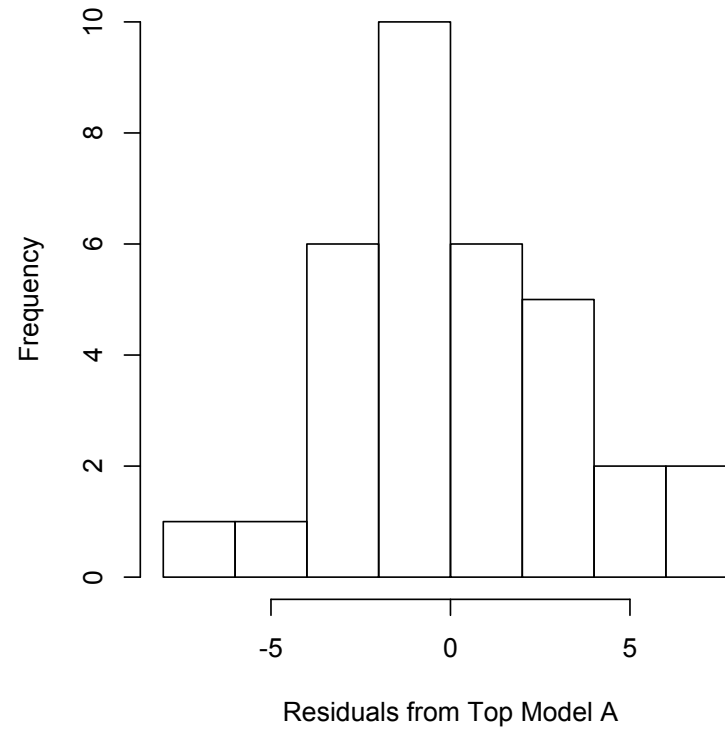
Figure 2.A3. Residuals versus fitted values (a) from the top model from model structure A and histogram of residuals from this same model (b). The top model from structure A predicted levels of introgression at a site using the following model:

$$\sim WD*(Stream\_km + Elev) + Bank + Bed$$

where WD is presence of whirling disease, Stream_km is distance from confluence, Elev is elevation, and Bank is bank stability and Bed is streambed quality as determined by levels of embeddedness.

a)
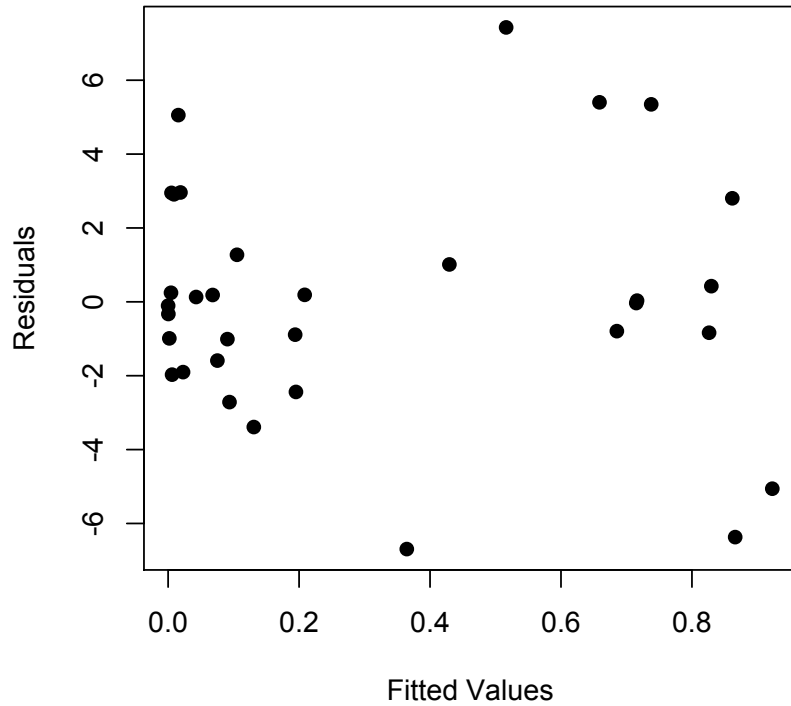
b)
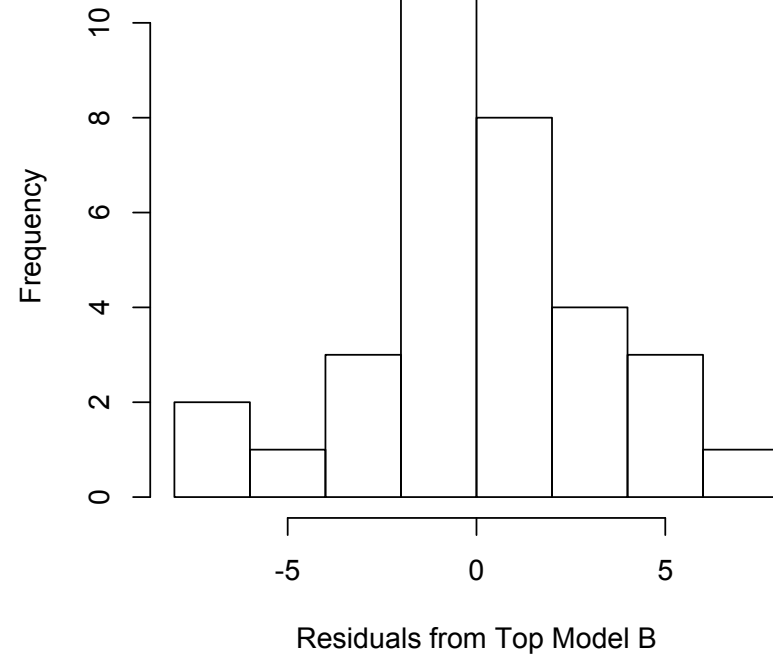
Figure 2.A4. Residuals versus fitted values (a) from the top model from model structure B and histogram of residuals from this same model (b). The top model from structure B predicted levels of introgression at a site using the following model:

$$\sim Slope + Stream\_km + Elev + Bank + Bed$$

where Slope is the slope of the stream at a site, Stream_km is distance from confluence, Elev is elevation, and Bank is bank stability and Bed is streambed quality as determined by levels of embeddedness.

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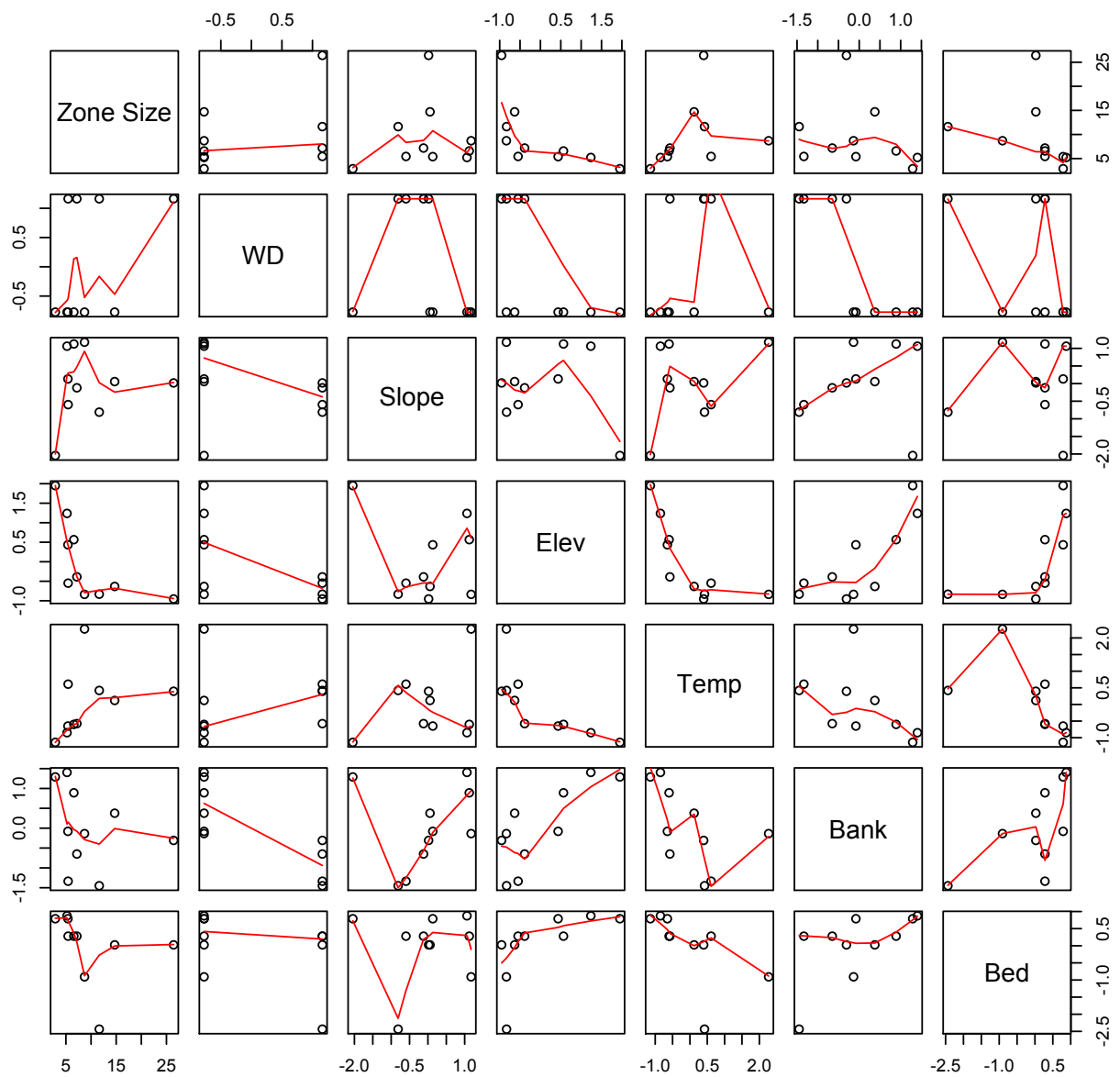
Figure 2.A5. Matrix scatter plot of size of hybrid zone (Zone Size, dependent variable) and all scaled independent variables assessed for estimating the extent of hybridization in a stream. Variables include presence of whirling disease (WD), stream slope (Slope), elevation at the upper end of the hybrid zone (Elev), stream temperature (Temp), streambed quality determined by levels of embeddedness (Bed) and bank stability (Bank).
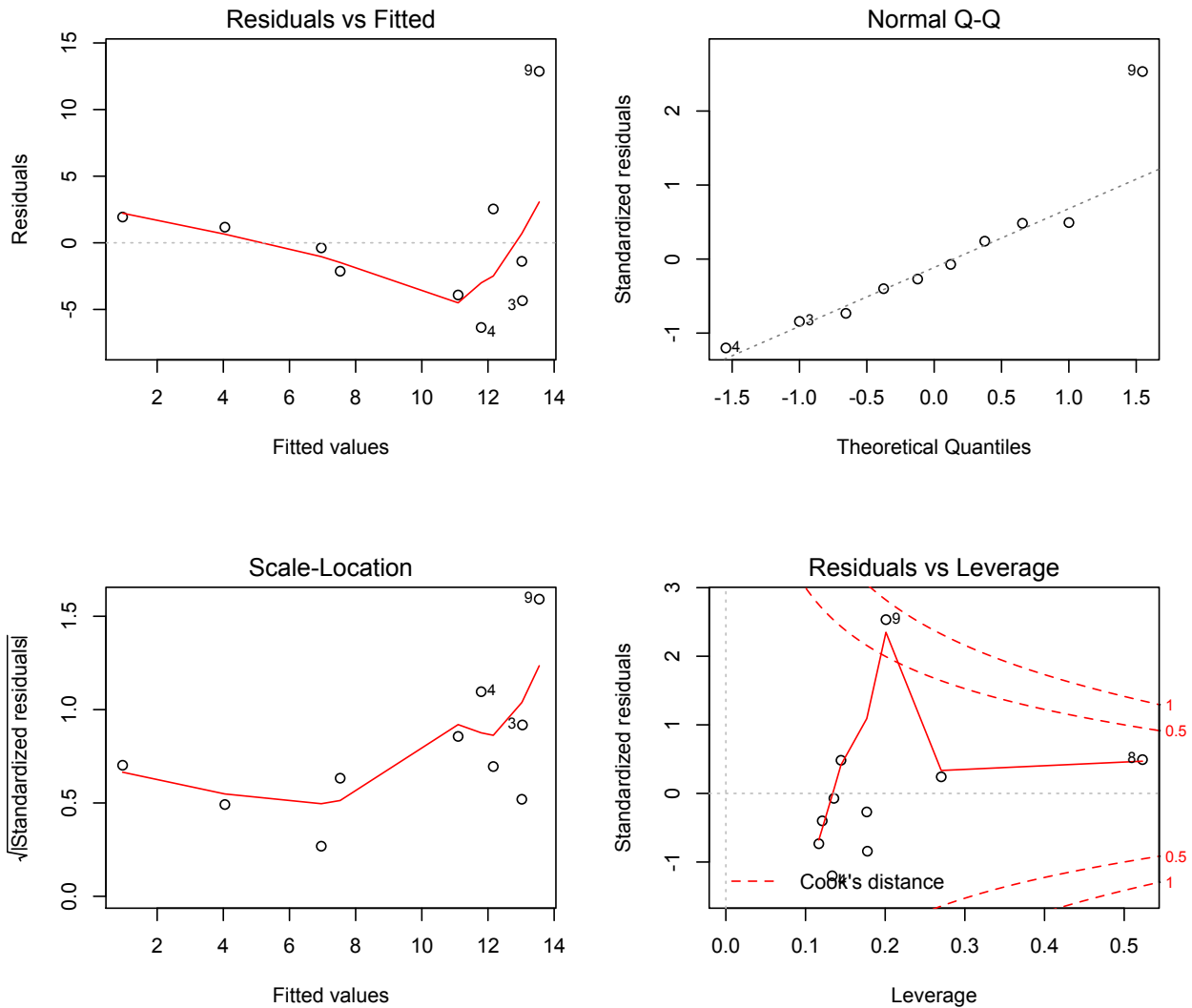
Figure 2.A6. Residual plots for the top model regressing size of the hybrid zone on whole stream slope. Plots show a) residuals versus fitted values for the model, b) normal Q-Q plot showing the deviation of residuals from a normal distribution, c) square root of standardized residuals versus fitted values, and d) standardized residuals versus leverage showing cooks distances. In d, point 9 outside the dashed lines is substantially influencing the results of the model. When removed, from the linear regression analysis, the p-value for the parameters estimate for slope decreases from p=0.5 to p=0.03.

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CHAPTER 3

INEVITABLE LOSS OF GENETIC DIVERSITY IN ISOLATED POPULATIONS- WHERE
DO RULES OF THUMB FALL SHORT IN WILDLIFE CONSERVATION?

INTRODUCTION

Over the last several decades, conservation biologists have promoted the use of genetic
information when prioritizing wildlife populations for conservation efforts. In response, both the
U.S. Endangered Species Act (16 U.S.C. §§ 1532[16] Section 3(15)) and the United Nations
Convention on Biodiversity (www.cbd.int) has recognized the importance of genetic
considerations in the protection of endangered species and maintenance of biodiversity. The
inclusion of genetic considerations in conservation management stems from genetic theory and
mounting empirical evidence demonstrating the links between the loss of genetic diversity and
reduced fitness and survival, higher incidence of disease, and ultimately demographic decline
across taxa (Madsen et al. 2004, McCallum 2008, Wagenius et al. 2010, Dunn et al. 2011, Heber
et al. 2013).

While the reasons for loss of genetic diversity vary, fragmentation of habitat and populations is
considered a primary factor leading to loss of genetic diversity across taxa (Dixon et al. 2007,
Clark et al. 2010, Alexander et al. 2011, Vranckx et al. 2012). Due to the dendritic nature of
stream networks, stream-dwelling organisms (such as salmonid fish) are particularly susceptible
to fragmentation (Fagan 2002). Various human activities, such as dam construction, building of
roads, water diversions and agricultural practices have degraded habitat and caused population
isolation, loss of migratory life histories and reduced genetic diversity in salmonid populations
on a global scale (Dunham et al. 1997, Aarts et al. 2004, Wofford et al. 2005, Morita et al. 2009,
Sato and Gwo 2011). Subsequently, these populations are left at increased risk of extirpation
with little hope for natural recolonization or restored genetic integrity. Despite these negative
consequences associated with isolation, construction and/or maintenance of barriers to fish
movement are becoming more common as connected populations of native fish are at risk from
increasing interactions with invasive species through hybridization, competition, and predation
(Fausch et al. 2009, Rahel 2013).

Due to the varied threats to native fish, conservation managers are left with a catch-22 between constructing and maintaining barriers to protect native fish from invaders, versus restoring connectivity to promote gene flow and associated metapopulation dynamics (Fausch et al. 2006, Fausch et al. 2009). Cutthroat Trout (*Oncorhynchus clarkii*) is one species for which these tradeoffs are of increasing concern. In connected riverscapes of western North America, Cutthroat Trout are threatened by hybridization and with expanding populations of introduced Rainbow Trout (*O. mykiss*) and competition with similarly expanding Brook Trout (*Salvelinus fontinalis*). Using isolation management, managers often choose to maintain and construct barriers to fish passage to protect the remaining purebred populations of Cutthroat Trout from invasive species. Intentional and unintentional isolation of cutthroat populations is increasingly common on a landscape scale (Dunham et al. 1997, Kruse et al. 2001, Young and Harig 2001, Shepard et al. 2005, Young et al. 2005) and understanding these tradeoffs is critical for effective long term conservation of the species.

Despite the strong influence of habitat connectivity on subpopulation persistence, many salmonid populations, including those of the Cutthroat Trout subspecies, have persisted above natural barriers such as waterfalls (Taylor et al. 2003, Shepard et al. 2005, Wofford et al. 2005). This suggests isolated populations may be somewhat buffered against extinction risk if habitat fragments are relatively large and contain suitable environmental conditions. To minimize the risks associated with intentional isolation, substantial research over the last decade has focused on quantifying specific habitat and population requirements for maintenance of genetic diversity and long-term population persistence of trout under isolation (Harig and Fausch 2002, Morita and Yokota 2002b, Novinger and Rahel 2003, Peterson et al. 2008b, Fausch et al. 2009, Muhlfeld et al. 2012). Generally speaking, occurrence of inland trout is associated with larger and higher quality habitat (Dunham et al. 2002b, Peterson et al. 2013). Whiteley et al. (2013) found a positive relationship between patch size and levels of genetic diversity in populations of isolated Brook Trout in their native range. Neville et al. (2006) found higher levels of genetic diversity in Lahontan Cutthroat Trout (*O. c. henshawi*) populations with migratory lift histories, as well as populations residing in relatively higher quality habitat characterized by cooler temperatures and more consistent (perennial) stream flows.

Genetic theory states that isolated populations will lose genetic diversity through drift at a rate of one over two times the effective population size (N_e) per generation, indicating that smaller populations will lose genetic diversity more quickly than larger populations. The most common rule of thumb for preservation of genetic diversity is the "50/500" rule (Franklin 1980). This rule of thumb estimates that N_e of 50 is desirable to reduce the short-term likelihood of extinction due to the harmful effects of inbreeding depression on population demography. Franklin (1980) also estimates that, based on mutation rates in fruit flies, N_e of 500 is required for mutation to add genetic diversity back into a population at the same rate that it is removed by drift, thereby maintaining long-term genetic diversity in a population. Estimates for wild Pacific salmon stocks equate a N_e of 500 to a census size of roughly 2,500 (Allendorf et al. 1997). With these guidelines, Hilderbrand and Kershner (2000) estimated that at densities of 0.3 fish/m, a minimum of 8km of high quality stream habitat would be necessary to sustain an inland trout population at this census size, and that larger habitat fragments would be necessary in low quality areas that cannot support fish at these densities. Considering that population size and genetic diversity are usually positively associated with habitat quality and characteristics of habitat volume such as number of pools and stream width (Harig et al. 2000, Harig and Fausch 2002, Morita and Yokota 2002a), Young et al. (2005) estimated that the length of stream necessary to preserve both population viability and evolutionary potential across Cutthroat species is a minimum of 8.8 km for densities of 0.2 fish/m. These theoretically derived rules of thumb are being regularly considered for management but there is very little empirical work evaluating these recommendations for maintaining the genetic diversity and population viability of isolated salmonid populations. To assess these rules of thumb, we studied isolated populations of Westslope Cutthroat Trout (*O. c. lewisi*) in western Montana to address the following research questions:

1) How do time since isolation and length of occupied stream habitat affect loss of genetic diversity in anthropogenically isolated populations? We hypothesized that isolated populations residing in large habitat patches (>8km) would maintain levels of genetic diversity similar to connected populations, regardless of time since isolation. For populations isolated in smaller fragments (i.e., <8 km of occupied habitat), we hypothesized that genetic diversity would decline with decreasing fragment size and habitat quality habitat, as well as increasing time since

isolation.

2) Do stream characteristics that influence population abundance, such as habitat quality, temperature, and flow influence levels of genetic diversity in anthropogenically isolated streams? We hypothesized that populations in streams with fewer human impacts, cooler temperatures and higher stream flows would have higher levels of genetic diversity.

3) How does loss of genetic diversity compare between streams that have been isolated on short time scales (anthropogenic isolation, 10s of generations) and those that have been isolated on very long time scales (geologic isolation, 1000s of generations)? Based on the rules of thumb outlined above, we hypothesized that populations residing in large habitat fragments would have levels of genetic diversity similar to connected populations.

STUDY AREA

The Flathead River watershed drains over 22,780 km^2 of land, and encompasses the headwaters of the Columbia River Basin. Stream flows in the basin are dominated by snowmelt runoff, with majority of the annual discharge occurring during spring and early summer. The hydrograph typically declines to base flows by late July to early August. Fishes of the watershed include native Westslope Cutthroat (hereafter "cutthroat") and Bull Trout (*S. confluentus*), as well as introduced Brown Trout (*Salmo trutta*), Brook Trout and Rainbow Trout (hereafter "rainbow"). Hybridization between native cutthroat and invasive rainbow readily occurs in connected tributaries where populations are sympatric, and is pervasive throughout the larger river network (Boyer et al. 2008, Corsi 2011). Many of the remaining pure cutthroat populations in this watershed are found above anthropogenic barriers, such as perched culverts and irrigation canals installed over the last century or in first or second order streams above natural barriers, such as waterfalls formed by isostatic rebound of land after the last glacial maximum (Pardee 1950).

Populations included in this study reside in streams located in a range of habitat types, from high gradient mountain environments to low gradient grassland environments. The majority of the Lower Flathead River Basin (tributaries to Flathead Lake and waters downstream) drains through tribally owned lands of the Flathead Indian Reservation. Here, human impacts on streams are common and associated with agricultural and ranching practices, including stream dewatering

and cattle grazing (Figure 3.1). Conversely, the Upper Flathead River Basin (upstream of Flathead Lake) is relatively unimpacted by human activities, with a majority of watershed draining through several national wilderness areas, Glacial National Park, and areas of Flathead National Forest with little history of resource extraction.

METHODS

Sample Collection and Habitat Assessment in Anthropogenically Isolated Populations

To compare genetic diversity between connected and anthropogenically isolated populations of cutthroat, we studied two connected and 12 isolated genetically pure populations located in headwater streams of the Lower Flathead River Basin. For these isolated streams, we used records from the Montana Department of Transportation and the Bureau of Indian Affairs Flathead Indian Irrigation Project to date all isolating barriers. If a perched culvert was the isolating barrier, we collected information on culvert dimensions, material and construction (e.g., corrugations), and we surveyed longitudinal profiles that extended through the culvert. We analyzed these data in FishXing program to ensure that culverts were impassible by *Oncorhynchus* species (FishXing 3; http://www.stream.fs.fed.us/fishxing/). For all cutthroat populations isolated by a perched culvert, rainbow and cutthroat-rainbow hybrids were present immediately below the barrier. Lack of introgression with rainbow in these isolated populations further confirmed that upstream fish passage into these streams was not possible. For two populations isolated by irrigation canals, there was no immediate barrier, such as a fish screens, preventing individuals from moving in and out of the canal. However, fish were not observed in the canal, and there were no nearby streams supporting *Oncorhynchus* species that drained into the canal that could provide a source for gene flow.

Length of occupied habitat for cutthroat in streams of the Lower Flathead River was determined by electrofishing upstream from the confluence (in connected streams), or the isolating barrier (in isolated streams) until cutthroat were no longer captured. At this location, a GPS point was taken and we used Arc GIS (ESRI ArcMap 9.3) and stream data layers created by the Confederated Salish and Kootenai Tribes (unpublished data) to measure the length of occupied habitat in each stream between the isolating barrier and upper extent of the cutthroat distribution.

Between late June and early September of 2010-2013, we estimated fish densities in each stream using standard mark-recapture or depletion methods at sites that ranged from 120-155m in length. For depletion estimates, we repeated collection passes until we captured less than 20% of the number of fish caught in the first depletion pass. Recapture runs at mark-recapture estimate sites were conducted between six to nine days after the marking run. Typically, two sites were sampled in each stream with one site located in the upper and lower half of the occupied habitat. We averaged densities for each stream. In three streams (Teepee, Talking Water and Yellow Bay Creeks) density estimates were performed at only one site due to short total habitat lengths ($\leq$ 1.4km).

To obtain a representative sample of the each population's genetic diversity, we collected tissue samples at all density estimate sites, and an additional one to three locations throughout the length of occupied habitat in every stream. The average distance between sampling locations in a given stream was 0.74 km, with a maximum of 2.11 km in Revais Creek. All tissue samples were collected between late June and early September of 2009-2012.

To assess habitat quality in all streams we asked two fisheries biologists and a hydrologist that have worked in these streams for 10-20 years to complete an expert opinion survey on habitat quality as outlined in Peterson et al. (2013; Appendix 3B). Briefly, the survey asked experts to rate the portion of the stream above the isolating barrier as high (1), moderate (2), or low (3) quality habitat based on anthropogenic and natural disturbances including road densities, logging and grazing activities, wildfire, floods and debris flow. We averaged the results from our three experts to obtain a single value of habitat quality for each stream.

We also measured temperature and summer base flows for all streams. We used temperatures loggers (HOBO and Tidbit V2 models, Onset Computer Corporation, Pocasset, Massachusetts, ± 0.2°C of accuracy) to record temperature at one-hour intervals at from July 1 through September 8, 2013 (70 days). Temperature was recorded at one easily accessible location per stream targeting the middle of the cutthroat distribution. We calculated relative growing season as growing degree days (GDD) above 0°C for the 70 day period that temperature was measured in each stream. Base flows were recorded as cubic feet per second (CFS) between August 6th-8th,

2013 in the lower half of the cutthroat distribution in each stream (Table 3.A1) using handheld Acoustic Doppler Velocimeter (FlowTracker).

Sample Collection in Geologically Isolated Populations

To quantify genetic diversity in geologically isolated populations of cutthroat, we analyzed samples from four connected and eight isolated populations located above waterfalls at least two meters in height in the Upper Flathead River. The formation of these waterfalls, and subsequent isolation of cutthroat populations occurred as a result of isotactic rebound after the last glacial retreat roughly 10,000 years ago (Pardee 1950). For these streams, extent of occupied habitat was determined from Montana Fish Wildlife and Parks past fish monitoring records and expert opinion of local fisheries biologists (Matt Boyer, personal communication). We measured occupied habitat from either the confluence or isolating waterfall to the upper extent of fish bearing habitat. We obtained genetic samples previously collected which followed methods outlined in Boyer et al. (2008). Fish were captured by electrofishing or angling in stream reaches ranging from 250 m to 1 km to minimize sampling of related individuals, and to obtain a representative sample of the genetic diversity in the entire stream. All samples were collected between late July and early September of 2003 and 2004.

For all fish sampled in both the Lower and Upper Flathead basins, total length was recorded and a small portion of fin tissue was excised and stored individually in 95% ethanol until genetic analysis could occur. Based on the lengths and the time of year at which they were collected (i.e., post-spawn), all fish sampled in connected streams were either resident life history forms or juvenile progeny, and thus native to their stream of capture.

Based on the length frequency distributions of fish in these populations, as well as another study in the Flathead River Basin (Fraley and Shepard 2005), we estimated the average age of reproductive maturity to be 4 years. To obtain the number of generations each population was isolated, we determined the number of years between the date of isolation and the first year of sampling, and divided this number by four. For geologically isolated populations, we assumed 2,500 generations of isolation, based on the estimated time of the last glacial retreat. Because

population level changes in genetic structure are detected on a time scale of generations, all sampling efforts were conducted within the span of a single generation.

Genetic Analysis

For all samples, DNA was extracted following the Gentra Isolation Kit protocol. All samples were amplified in 10 ul reactions analyzed using three different PCR profiles following instructions in the QIAGEN Mulitplex PCR Kit (QIAGEN, Valencia, CA). Multiplex 1 consisted of Ogo8 (Olsen et al. 1998), Omm1019, Omm1050, Omm1060 (Rexroad et al. 2002) and Omy 0004 (Holm and Brusgaar 1999). Multiplex 2 consisted of Omy1001 (Spies et al. 2005), Ogo4, Ssa456, and Sfo8 (Small et al. 1998). Multiplex 3 consisted of Ogo3 (Olsen et al. 1998), Oki10 (Smith et al. 1998), Ots 107 (Nelson and Beacham 1999), Ssa408, and Ssa407 (Cairney et al. 2000). Eight of these markers (Ogo8, Omm1019, Omm1050, Omm1060, Omy004, Ogo4, Sfo8, and Ssa408) are diagnostic for cutthroat and rainbow trout for most watersheds tested in Montana (Sally Painter, Montana Conservation Genetics Laboratory, personal communication) and were used to ensure that each population contained only pure Westslope Cutthroat Trout. 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). All genetic analyses were conducted at the University of Montana Conservation Genetics Lab, Missoula, USA.

Statistical Analyses

We used Arlequin v 3.5 (Excoffier and Lischer 2010) to calculate expected heterozygosity and test for linkage disequilibrium and deviations form Hardy-Weinberg expectations. To quantify genetic diversity, we used FSTAT (Goudet 1995) to calculate the allelic richness (R_s) for each population because this program uses rarefaction to estimate the number of alleles per population scaled to the population with the smallest sample size (Petit et al. 1998), which in this study was N=25.

Genetic Diversity in Anthropogenically Isolated Populations

We assumed that levels of genetic diversity in connected populations were not altered by genetic drift due to the potential for dispersal from neighboring subpopulations (as in Whiteley et al. 2010). Thus, the levels of genetic diversity in connected populations represent the highest level that an isolated population could possibly maintain. We used a Welch's two-sample t-test to compare average allelic richness between open and isolated systems within Lower Flathead River. To determine the individual roles of time since isolation and amount of habitat on maintenance of genetic diversity, we also performed linear regressions comparing the number of generations since isolation and amount of occupied habitat to allelic richness in anthropogenically isolated populations.

To further explore the relationships between habitat variables and genetic diversity in anthropogenically isolated populations in small fragments, we performed a multiple linear regression of allelic richness on length of occupied habitat, generations isolated, habitat quality, growing degree days, and base flow. We compared every possible subset of these variables. We assessed multicollinearity by calculating the variance inflation factor (VIF) for all five independent variables in the full model. The variance inflation factor quantifies the degree to which the variance is increased as a result of multicollinearity with other variables in an ordinary least squares regression model. For example a VIF=10 for a single variable would mean that the variance of the parameter estimate for that variable is ten times larger than it would be if that variable was completely uncorrelated with all others in the model. If VIF was high (>5) for variables in the top model, we identified highly correlated variables using Pearson's correlation tests and did not consider any models that contained highly correlated variables (Montgomery et al. 2012) . The model with the lowest Akaike information criteria corrected for small sample size (AICc; Burnham and Anderson 2002) whose parameters estimates were all significantly different from zero was considered the top model.

Comparison between Anthropogenically and Geologically Isolated Populations

We used a Welch's two-sample t-test to compare average allelic richness between 1)

geologically isolated populations and connected populations in the Upper Flathead River Basin; and 2) between populations isolated on anthropogenic (short) versus geologic (long) time scales.

All statistical analysis was conducted in R Statistical Software (R Developement Core Team 2012), and p-values were assessed at the level of $\alpha=0.05$.

RESULTS

Between the Upper and Lower Flathead watershed, we analyzed a total of 25 streams and 994 individuals. The number of individuals analyzed for genetic diversity varied by stream (Table 3.1). We performed 210 tests for departure from Hardy-Weinberg equilibrium, and found 22 tests (10%) with significant departures at the level of $\alpha=0.05$, where 10 were expected by chance. After Bonferroni correction, no tests were significant for departure from Hardy-Weinberg equilibrium (corrected p= 0.00024, Table 3.1). We performed 2275 independent tests for linkage disequilibrium across the whole dataset, and found that 161 (7%) were significant at the level of $\alpha=0.05$, where 114 were expected by chance. After Bonferroni correction, only four tests were significant (corrected p = 0.00002). For all populations sampled, heterozygosity and allelic richness were highly correlated with $R^2=0.96$ (p<0.001).

Genetic Diversity in Anthropogenically Isolated Populations

For anthropogenically isolated populations, median length of occupied habitat above the isolating barrier was 3.2 km with a range of 0.4 to 14 km, compared to 5.3 and 10.4 km for the two connected systems in the same watershed. Isolated populations had an average of $R_s=2.84$, which was significantly lower than that of connected populations in the same region (R_s in connected populations = 5.21, p<0.001). Revais Creek, the only anthropogenically isolated stream over 8 km in our dataset, maintained $R_s=5.25$, similar to that of the connected populations in the same watershed (Figure 3.2, Table 3.1). All other anthropogenically isolated populations examined were found in less than 5 km of occupied habitat and had lower allelic richness (average R_s = 2.62 +/- 0.33S.E.). Across all anthropogenically isolated populations, length of occupied habitat was a significant predictor of allelic richness (p<0.01, $R^2=0.576$), but time since isolation was not (p=0.791, $R^2=0.007$). However, when analyzing populations in habitat less than 5km, the effect of habitat length on allelic richness was substantially reduced (p=0.103, $R^2=0.268$), and time since isolation still had no effect (p=0.625, $R^2=0.028$).

Scores for habitat quality across the 14 streams in our data set ranged from 1 (high quality) to 3 (low quality) with a median of 1.9. Due to failure of the temperature logger in Centipede Creek, this population was not included in the multiple regression analysis examining variables influencing genetic diversity. Across all streams, growing degree days and base flow ranged from 630-1134 GDD (median=828) and 0.12-8.28 CFS (median= 1) respectively (Table 3.A1).

None of the multiple regression models relating habitat characteristics, length of occupied habitat, and time since isolation to genetic diversity met our criteria for selection as the top model (Table 3.2, Figures 3.A1 and 3.A2). Specifically, the multiple regression analysis did not produce any models in which all parameters estimates were significantly different from zero. Variance inflation factors for all variables in the full model were less than five, indicating that multicollinearity was not substantially influencing the parameters estimates in this analysis.

Comparison Between Anthropogenically and Geologically Isolated Populations
Geologically isolated streams had a median of 6.7 km (range 2.1 to 18.6 km) of occupied habitat, versus a median of 15.1 km (range 13.2 km to 16 km) for the connected populations in the same watershed. Geologically isolated populations had an average R_s=1.79, which was significantly lower than that of the connected populations in the same basin (p<0.001, Figure 3.3). While several of the geologically isolated streams had occupied habitat of 10km or more (Table 3.1), none of the geologically isolated systems, demonstrated maintenance of genetic diversity compared to connected populations in the same basin. Although geologically isolated populations were found in substantially larger habitat fragments compared to anthropogenically isolated populations (p>0.05), geologically isolated streams had significantly lower genetic diversity (p=0.01).

DISCUSSION

Based on populations on this study, loss of genetic diversity appears to be inevitable in isolated populations of cutthroat. Genetic diversity was not maintained in our geologically isolated populations, regardless of habitat size. These results suggest levels of genetic diversity in isolated cutthroat populations are more strongly affected by genetic drift and stochastic events resulting in population bottlenecks than by habitat size. Furthermore, in the anthropogenically isolated populations residing in less than 8 km of habitat, no combination of environmental

variables in this study (indicative of habitat quality and demographic persistence) explained genetic diversity. In two populations, we observed loss of genetic diversity in less than a dozen generations of isolation. These results suggest that genetic diversity could be lost rapidly, despite efforts to maintain or increase habitat quality in isolated stream fragments. Thus the potential for temporary isolation lasting longer than several generations to be an effective management tool is limited, particularly in the context of more pervasive conservation challenges such as climate change and invasive species not limited by the same barriers.

The fact that all geologically isolated populations in our study demonstrated a loss of genetic diversity compared to populations in connected systems may be attributed to two potential explanations. First, rules of thumb for maintaining genetic diversity in isolated population are based in genetic theory, which assumes an ideal Fisher-Wright population- something which is rarely, if ever observed in the wild. Suggesting that a N_e=500 is appropriate for maintenance of diversity in cutthroat populations assumes 1) the estimated rate of functional mutations per generation equal to 0.001 for fruit flies is similar for inland trout (see Franklin 1980); and 2) the N_e:N ratio is similar between inland trout and Pacific salmon (see Allendorf et al. 1997). While functional mutation rates have not been explored thoroughly across salmonids, Steinberg et al. (2002) estimated neutral mutation rates in pink salmon ranging from 0-0.0085 per generation. However, there is no estimate of how these rates may relate to frequency of functional mutations, and the wide variation in neutral mutation rates observed does not necessarily support similarity in mutation rates between fruit flies and salmonid species. Furthermore, N_e:N ratios vary between species, and even populations due to various factors including (but not limited to) population size, historical population bottlenecks, variation in life history strategy, and whether variance or inbreeding N_e was estimated (Hedrick et al. 2000, Palstra and Ruzzante 2008, Hare et al. 2011, Gomez-Uchida et al. 2013). Given that mutation rates vary across species and isolated populations face different life history tradeoffs than connected population, the minimum effective population size for maintenance of genetic diversity is likely to be specific at both the species and population level.

The second potential explanation for the loss of genetic diversity in geologically isolated populations is that environmental stochasticity was not incorporated into rules of thumb for maintaining genetic diversity in isolated trout populations. On a landscape scale, trout

populations persist as a group of subpopulations that interact through dispersal and are regularly impacted by natural disturbance (Dunham and Rieman 1999, Rieman and Dunham 2000). Natural disturbances such as floods, droughts, fire and debris and ice flows are common in stream ecosystems (Resh et al. 1988, Lake 2000, Miller et al. 2003). When severe, they can cause population bottlenecks and subsequent loss of genetic diversity (Hakala and Hartman 2004, Pujolar et al. 2011), particularly in isolated populations that lack potential for gene flow. And populations in connected habitat are expected to rebound from disturbances through dispersal (Roghair et al. 2002, Neville et al. 2006, Pierce et al. 2013), which will restore both population size and genetic diversity in a given habitat patch. Populations isolated in larger habitat fragments are expected to be less susceptible to bottlenecks and loss of genetic diversity because larger habitat will support larger populations and provide more refugia under adverse conditions (Dunham and Rieman 1999, Neville et al. 2009). However, populations in larger fragments are not immune to bottlenecks. Salmonid species observed above geological barriers commonly show lower levels of genetic diversity compared to connected populations in the same region (Costello et al. 2003, Neville et al. 2006, Guy et al. 2008, Whiteley et al. 2010). Over the course of several thousand generations, it would be surprising if the populations in our study were able to evade all events capable of such an impact.

The low levels of genetic diversity we observed in geologically isolated populations suggests that genetic diversity may not be as important to population persistence as theory predicts. However, this conclusion should be regarded with caution. While persistence of cutthroat above geological barriers is not uncommon, studies have shown that salmonids occur less frequently in streams and stream networks above barriers, even when ample high quality habitat is present (Dunham et al. 1997, Hastings 2005). Because researchers have not closely observed and documented the extirpation of cutthroat under these circumstances, it is difficult to discern whether population extinction was a result of demographic factors, genetic factors, or some combination of the two.

The consistently observed reduction of genetic diversity in, anthropogenically isolated salmonid populations both here and in other studies (Yamamoto et al. 2004, Morita et al. 2009, Horreo et al. 2011, Sato and Gwo 2011, Kitanishi et al. 2012, Whiteley et al. 2013) indicates that isolation is not a reliable stopgap measure if managers wish to avoid the risk of inbreeding depression.

Under these circumstances, managers may choose to perform assisted migration to restore and maintain genetic diversity. However, managers should closely analyze demographic parameters to determine if genetic rescue through assisted migration would actually benefit the population (See Chapter 4 which outlines demographic persistence in the anthropogenically isolated stream of this study). Across salmonid species, including cutthroat, mounting evidence suggests that adaption to local habitat characteristics is common, and can occur rapidly- in as few as six generations (Fraser et al. 2011, Drinan et al. 2012, Narum et al. 2013). Local adaptation may be more common in small isolated populations because advantageous alleles can be quickly driven to high frequencies by natural selection if selection pressure is sufficiently high (Allendorf and Luikart 2008) and isolation will limit gene flow that could reduce the frequency of the most advantageous alleles. Thus, for small isolated populations of trout, the introduction of fish that are ill-adapted to the local environment could result in outbreeding depression, causing population decline. This is particularly relevant to populations in our study where time since isolation for the all populations exceeds ten generations.

The one anthropogenically isolated population of cutthroat residing in a large habitat fragment (Revais Creek with 14 km of occupied habitat) maintained levels of genetic diversity similar to populations in connected systems, suggesting that the 8 km rule of thumb isolation may be a useful short-term solution to prevent interactions with invasive species isolated in large, high quality habitat supporting sufficiently high trout densities. However, the combined minimum habitat size and maximum isolation time under which populations will be able to maintain genetic isolation could vary from one population to the next based on populations size, habitat quality and refugia, and occurrence of stochastic environmental events. Climate change is predicted to alter flow regimes and warm streams temperatures (Wenger et al. 2011). Over the next several decades these effects are predicted to increase the frequency of stochastic events, such as ice flows, debris flows, and winter flooding whose impacts target young age classes (Goode et al. 2013). Furthermore, warmer stream temperatures will reduce viable habitat for trout with lower thermal tolerances such as Brook Trout and cutthroat (Williams et al. 2009, Wenger et al. 2011). As a result, isolated populations that may be maintaining genetic diversity and viability under current conditions will likely struggle to do so in the foreseeable future.

Table 3.1. Map identification, stream name, number of individuals sampled (N), type of isolating barrier, number of significant tests for linkage disequilibrium after Bonferroni correction (LD), average heterozygosity (H_e) across all loci, average allelic richness (R_s) across all loci, length of occupied habitat (Length, km), and the estimated number of generations isolated for each population. Average density of fish was only estimated for populations in the Lower Flathead River Basin. We found no significant departures from Hardy-Weinberg after Bonferroni corrections. Asterisks (*) denotes connected populations.

Map ID	Stream	N	Barrier Type	LD	He	Rs	Length (km)	Generations Isolated	Average Density (fish/m)
Lower Flathead River Basin (Anthropogenic Isolation)									
1	Camas	53	Rerouted/dispersed into agricultural field	0	0.120	1.69	3.5	27.5	0.44
2	Centipede	55	Irrigation Diversion or Canal	0	0.193	1.74	2.7	22.75	0.5
3	Cold	62	Irrigation Diversion or Canal	0	0.419	3.75	4.9	21.25	0.71
4	Frog	44	Perched Culvert	0	0.420	3.74	3.7	11.5	0.37
5	Magpie*	55	NA	0	0.526	5.23	5.3	18	0.57
6	Magpie Spring	54	Perched Culvert	0	0.435	3.29	2.9	NA	0.18
7	Revais	42	Irrigation Diversion or Canal	0	0.489	5.25	14.0	23.75	0.31
8	Schley	46	Perched Culvert	0	0.418	3.47	1.7	11.5	0.73
9	Seepay*	68	NA	2	0.501	5.18	10.4	NA	1.11
10	Talking Waters	40	Perched Culvert	0	0.187	1.95	0.6	15.5	0.28
11	Teepee	36	Perched Culvert	0	0.176	1.50	0.4	15.5	0.2
12	Thorne	51	Irrigation Diversion or Canal	1	0.416	3.48	3.5	24.5	0.35
13	West Magpie	44	Irrigation Diversion or Canal	1	0.233	2.30	4.6	12	0.21
14	YellowBay	57	Perched Culvert	0	0.189	1.93	1.4	15.5	0.61
Upper Flathead River Basin (Geological Isolation)									
15	Addition	26	Waterfall	0	0.149	1.84	6.7	~2,500	
16	Bunker	28	Waterfall	0	0.132	1.54	15.6	~2,500	
17	Colts*	25	NA	0	0.431	4.19	5.4	NA	
18	Emery*	27	NA	0	0.439	4.33	8.0	NA	
19	Goldie	25	Waterfall	0	0.090	1.43	2.1	~2,500	
20	Hungry Horse*	24	NA	0	0.439	4.14	9.0	NA	
21	Kneiff	25	Waterfall	0	0.060	1.14	4.4	~2,500	
22	Middlepass*	25	NA	0	0.420	4.11	10.9	NA	
23	Quintonkin	27	Waterfall	0	0.245	2.19	10.0	~2,500	
24	South	27	Waterfall	0	0.285	2.13	2.8	~2,500	
25	Upper Twin	28	Waterfall	0	0.281	2.26	18.6	~2,500	

Table 3.2. Top five models with the lowest AIC$_c$ values predicting allelic diversity across ten populations with <8 km of occupied habitat (Centipede Creek was excluded from this analysis due to lack of temperature data). Parameters present in the full model include length of occupied habitat (Length), generations isolated (Iso), summer base flows in cubic feet per second (CFS), growing degree days (GDD), and average expert opinion rating of habitat quality. All parameters estimates for all models shown here were not significantly different from zero.

	Model	k	ΔAICc	Multiple R-sq	Log-Likelihood
1)	~Length	2	0.00	0.29	-10.99
2)	~Length+GDD	3	1.84	0.43	-9.95
3)	~Length+Iso	3	3.09	0.35	-10.57
4)	~CFS	2	3.33	0.02	-12.66
5)	~GDD	2	3.38	0.01	-12.69
Full)	~Length+Iso+CFS+GDD+Quality	6	22.61	0.57	-8.55

Figure 3.1. Map of Flathead River Basin where all study streams are located (a). Figure 3.1b highlights the location of streams in the Lower Flathead River Basin, and Figure 3.1c includes study streams in the Upper Flathead Watershed. Numbers correspond to "Map ID" in Table 3.1. For d-f, bold sections represent stream section occupied by Cutthroat Trout, triangles represent density estimate locations, and black circles show location of temperature loggers in each stream.

a)
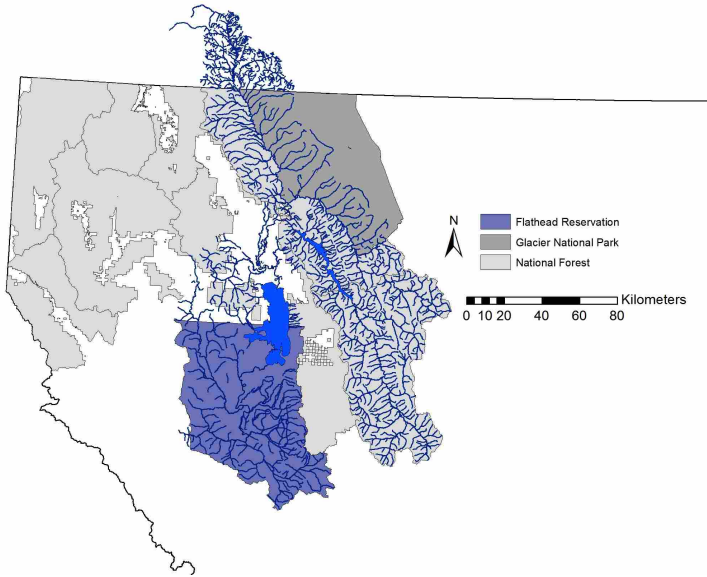
b)
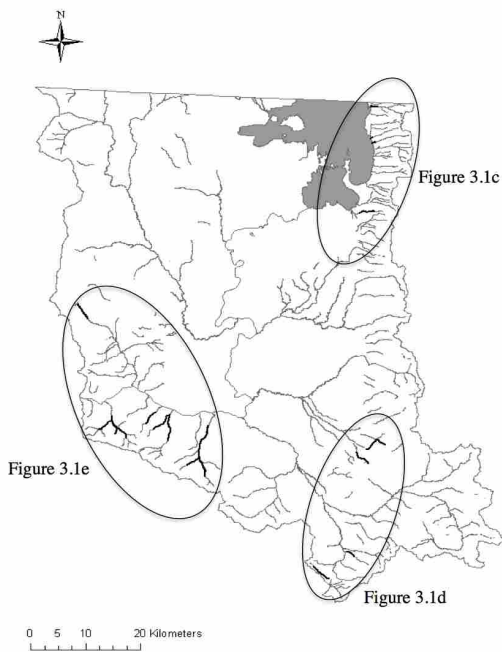
c)
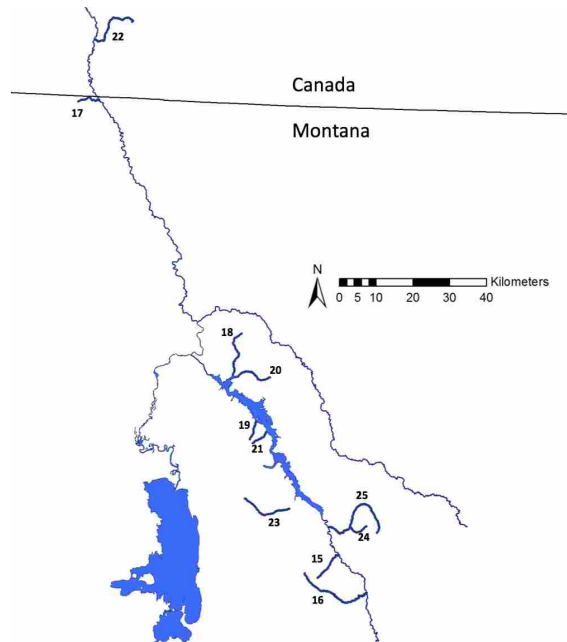
d)
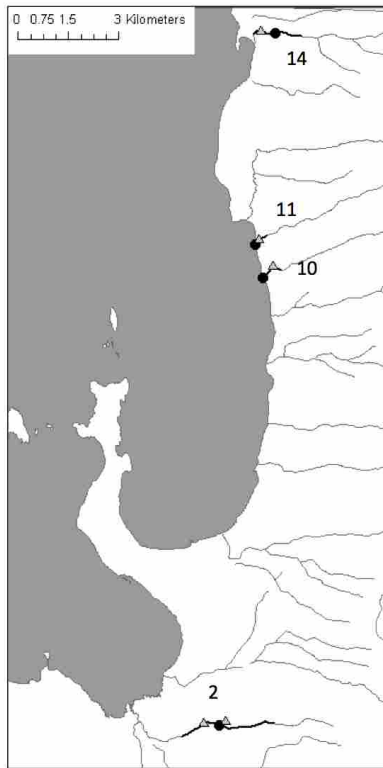
e)
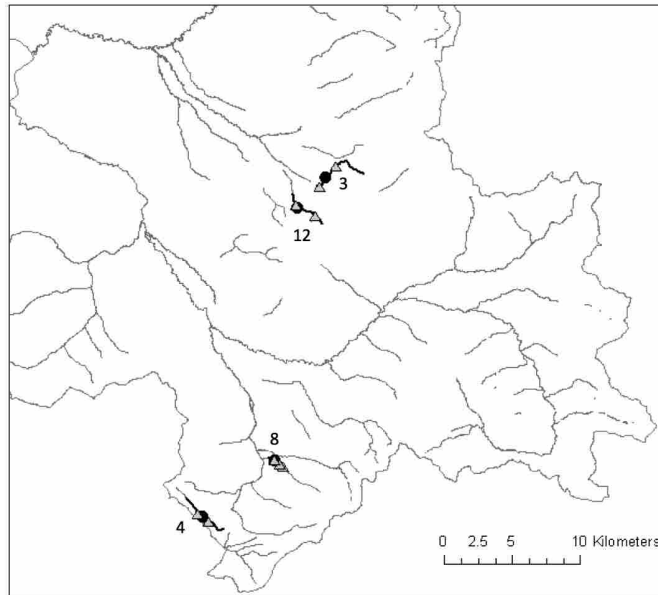
f)
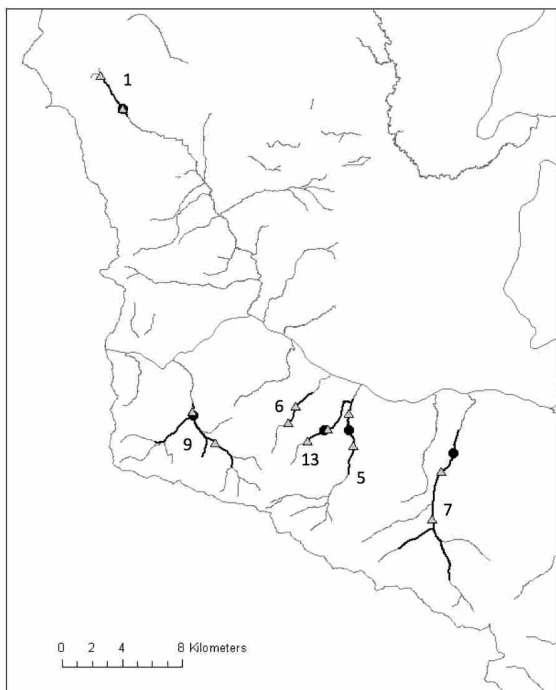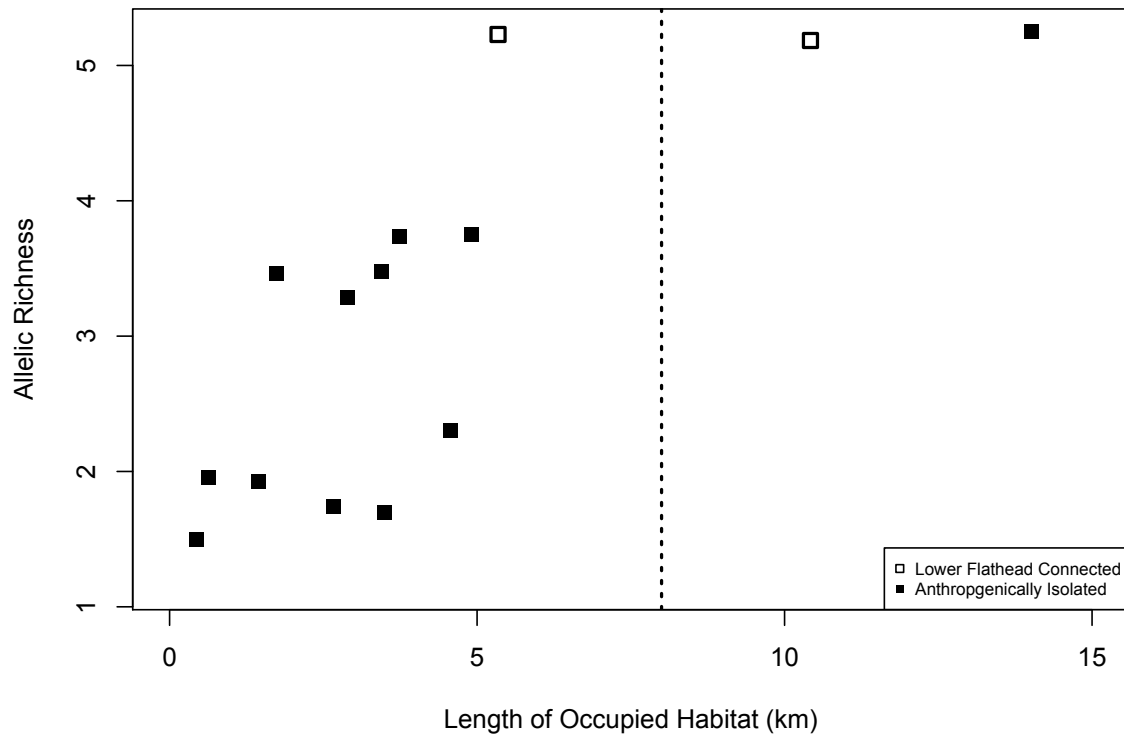
Figure 3.2. Allelic richness versus length of occupied habitat in streams of the Lower Flathead River Basin. The dashed line marks 8 km of occupied stream habitat. For the purposes of this study, a stream fragment with less than 8 km of occupied habitat is considered "small", while one with more than 8 km is considered "large".
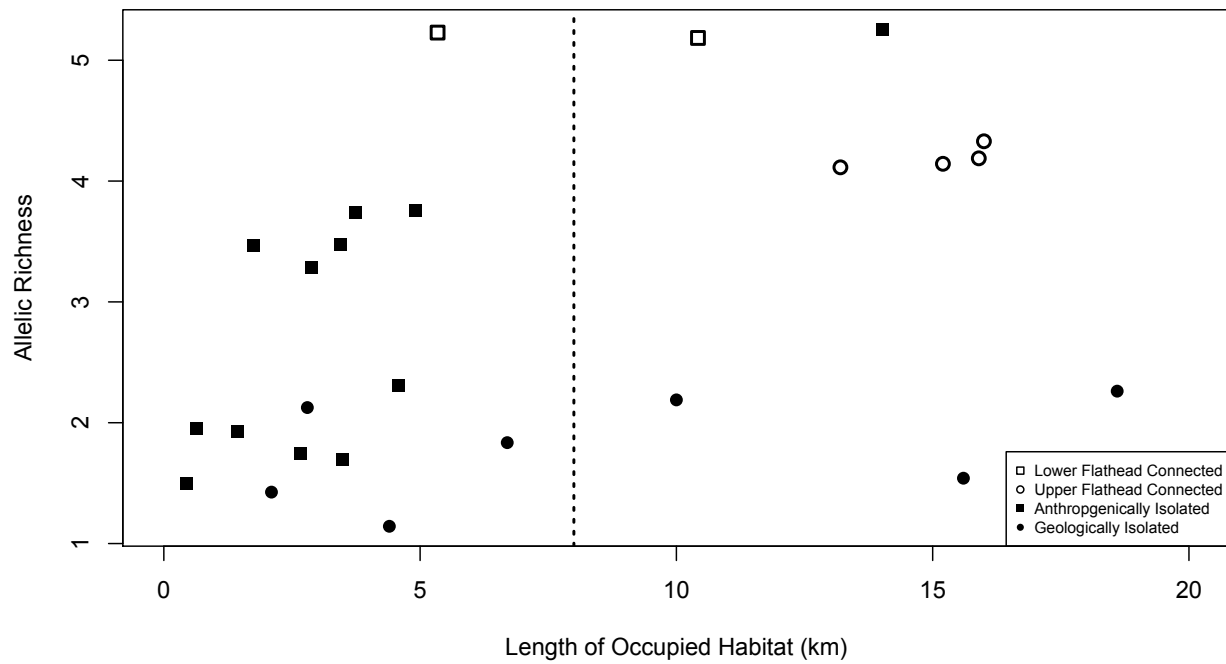
Figure 3.3. Allelic richness versus length of occupied habitat for all twenty-five streams included in this study. The dashed line marks 8 km of occupied stream habitat. For the purposes of this study, a stream fragment with less than 8 km of occupied habitat is considered "small", while one with more than 8 km is considered "large".

APPENDIX 3.A

Table 3.A1. Habitat quality metrics for streams in this study, including road density (km of road per km^2) and percent of watershed area leased for grazing above the isolating barrier in each stream, growing degree-days (GDD), summer base flows in cubic feet per second (CFS) and location of CFS measurement as distance from the confluence in km.

Stream	GDD	CFS	CFS Location
Camas	1134	0.12	1.06
Centipede	--	0.81	1.29
Cold	785	2.04	0.96
Frog	912	0.18	2.09
MagpieSpring	780	0.49	0.39
Revais	970	8.28	3.5
Schley	689	1.30	0.57
TalkingWaters	735	1.00	0.01
Teepee	838	0.90	0.01
Thorne	919	0.30	1.16
WestMagpie	828	0.46	2.7
YellowBay	630	3.53	0.73
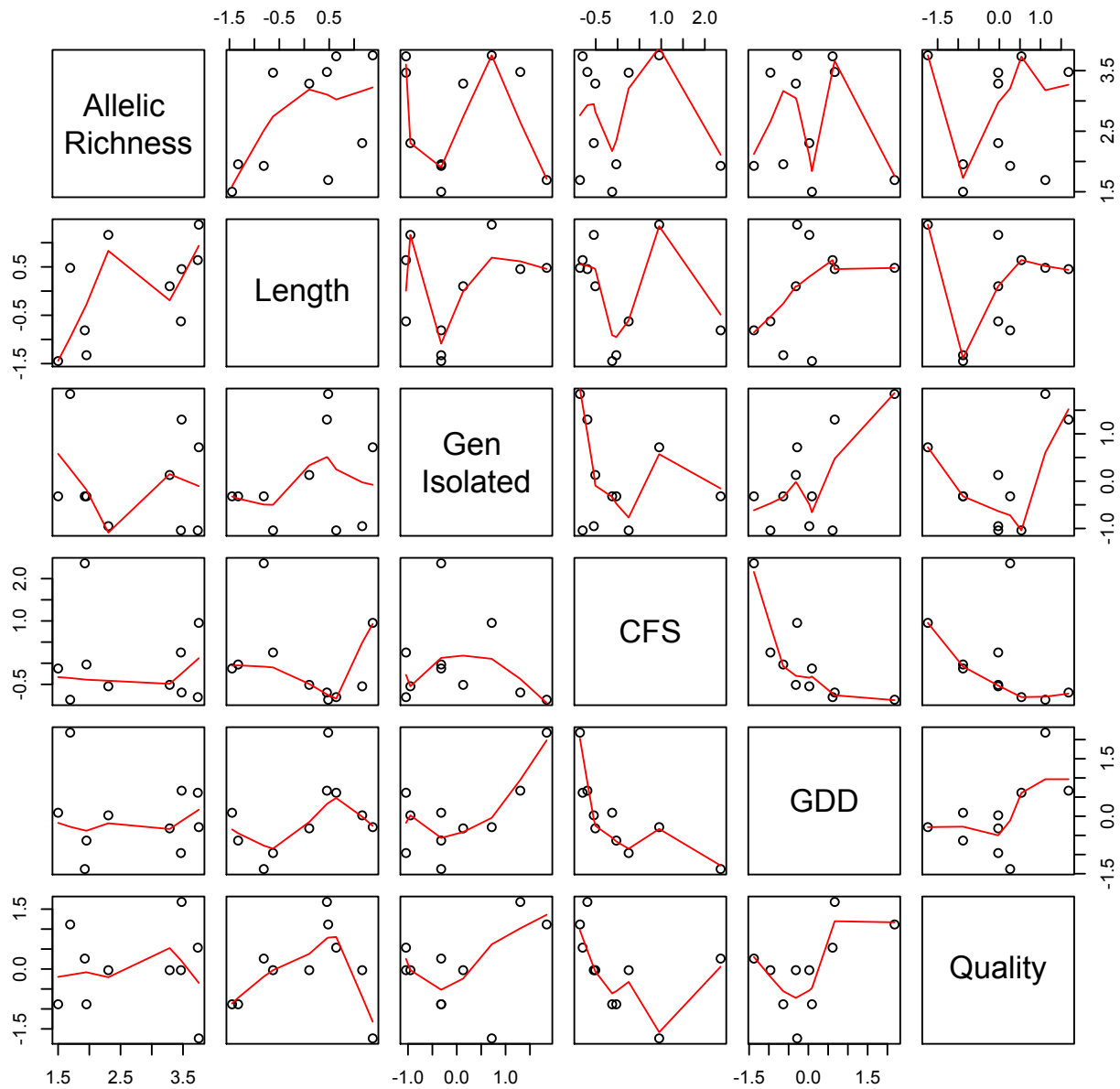
Figure 3.A1. Matrix scatter plot of allelic richness and all scaled independent variables used in the full model predicting levels of genetic diversity in population of Cutthroat Trout isolated in under 8 km of habitat. Variables include length of occupied habitat (Length), generations isolated (Gen Isolated), cubic feet or water per second at base flow (CFS), growing degree days (GDD) as well as expert opinion rating of habitat quality for each population. The multiple linear regression analysis and this plot did not include the population in Revais Creek, which is isolated with 14 km of occupied habitat, or Centipede Creek, for which we did not obtain temperature information.
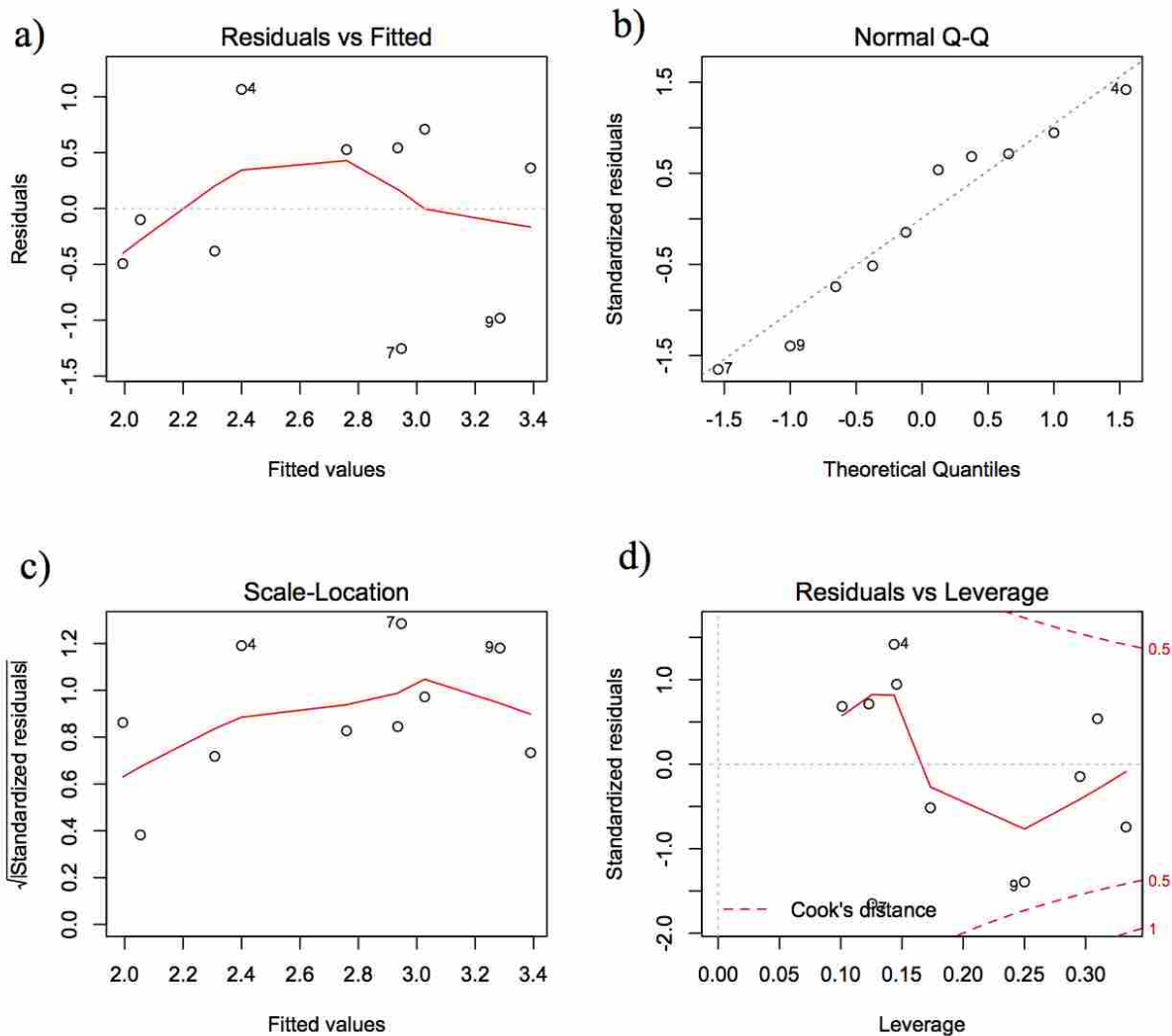
Figure 3.A2. Residual plots for the model regressing allelic richness on length of occupied habitat for Cutthroat Trout populations isolated in less than 8km of occupied habitat. Overall, this model has the lowest AIC value of all possible models examined, however, none of the models examined met our criteria for selection as the top model with all parameter estimates significantly different from zero. Plots show a) residuals versus fitted values for the model, b) normal Q-Q plot showing the deviation of residuals from a normal distribution, c) square root of standardized residuals versus fitted values, and d) standardized residuals versus leverage showing cooks distances.

APPENDIX 3B

Habitat Quality Survey modified from Peterson et al. (2013). Scores: 1= high quality; 2= moderate quality; 3= low quality.

Habitat quality should be considered high (1) if the watershed above the barrier has not been extensively disrupted by management activities including grazing, roading, logging or has not been extensively disturbed by severe fire, floods or debris flows since the barrier was installed. Roads, if present, exist at densities less than 1 km/km2, do not directly constrain or impact the stream channels, and are not believed to have an important influence on hydrologic or geomorphic processes (i.e., the stream is connected with its floodplain). The riparian community is intact and functioning as expected under natural conditions. Habitat quality should be considered moderate (2) if it is not high and not low.Habitat quality should be considered low (3) if the watershed above the barrier has clearly been extensively disrupted by management activities including grazing, roading, logging or a severe wildfire that occurred since the barrier was installed. Instream habitat conditions can be shown to be consistently and significantly degraded from expected natural or reference conditions because processes influencing the hydrologic regime, sediment regime (or other geomorphic processes), or linkages and function of the riparian community and flood plain have been obviously constrained or degraded by past management activities, existing roads, or severe fire that burned a majority of the watershed and or the riparian corridor.

CHAPTER 4

POPULATION VIABILITY AND THE POTENTIAL FOR LOCAL ADAPTATION IN

ISOLATED TROUT POPULATIONS

INTRODUCTION

Human activities leading to habitat destruction and fragmentation are the primary cause of the

global biodiversity decline. As a result, biologists have been increasingly concerned with the

effects of habitat fragmentation on species persistence (Hanski 1998, Fahrig 2002, Fahrig 2003).

Across wildlife taxa, habitat loss and fragmentation can alter life history patterns, limit migration

and dispersal patterns, and disrupt gene flow, all of which can lead to population decline (Bolger

et al. 2008, Morita et al. 2009, Haag et al. 2010, Pavlacky et al. 2012, Ruell et al. 2012).

Stream dwelling organisms are particularly susceptible to fragmentation due to the dendritic

nature of stream networks (Fagan 2002). For many freshwater aquatic species, genetic,

phenotypic, and life history diversity, as well as population viability relies on habitat

connectivity that allows movement within and dispersal among subpopulations (Green 2003,

Noël et al. 2007, Morita et al. 2009, Watanabe et al. 2010). Inland salmonid species are one

group for which the potential effects of fragmentation have been documented. Trout and char

whose habitat is fragmented suffer from loss of migratory life histories, reduced genetic

diversity, and are at increased risk of extirpation (Dunham et al. 1997, Morita et al. 2009,

Whiteley et al. 2010, Sato and Gwo 2011, Whiteley et al. 2013).

Despite the strong influence of habitat connectivity on subpopulation persistence, many

populations of Cutthroat Trout (*Oncorhynchus clarkii*) have persisted since the last glacial period

behind natural barriers (e.g., waterfalls) that prevent immigration from other subpopulations

(Taylor et al. 2003, Shepard et al. 2005, Wofford et al. 2005, Whiteley et al. 2010). This

suggests that the probability of extinction for isolated populations may vary depending on habitat

characteristics, environmental conditions, and population size. For example, Hilderbrand (2003)

estimates that the probability of extinction for isolated populations of Cutthroat experiencing

little environmental variability (i.e., stochasticity) is less than 5% at carrying capacities above

2000 individuals. Populations may also be able to persist at even smaller population sizes if they

can adapt to the limitations of an isolated environment (Morita and Yokota 2002a, Letcher et al. 2007, Morita and Fukuwaka 2007, Morita et al. 2009). For example, Morita et al. (2009) report smaller size and younger age at maturity, higher growth rates, and increased expression of resident life history forms in populations of White Spotted Char (*Salvelinus leucomaenis*) that have been able to persist for several decades above anthropogenic barriers. Similarly, persistence of isolated Brook Trout populations (*Salvelinus fontinalis*) relies on different demographic characteristics for population viability than connected populations including younger age and smaller size of reproductive maturity, as well as higher survival for early life stages (Letcher et al. 2007).

The shifts in traits observed in isolated populations may be attributed to tradeoffs in life history to maximize lifetime fitness. Life history theory suggests that for fish like trout, low adult survival will be associated with increase juvenile growth rates favoring high reproductive effort and earlier age/size of reproduction in individuals to ensure at least one spawning opportunity before death. Once individuals reach maturity, somatic growth becomes marginal because more energy is allocated to production of gametes and not towards growth, and so individuals in these circumstances may achieve relatively smaller adult sizes. Empirical data support the theory behind these tradeoffs (Hutchings 1993, Haugen 2000), and data demonstrate an underlying genetic component associated with the traits involved, such as growth and adult body size (Nilsson 1994, Letcher et al. 2011, Hu et al. 2013). In isolated populations where adult survival is low, we expect a selective advantage would be given to individuals who grow fast and mature early in order to maximize their reproductive potential.

Despite the potential to adapt to the conditions of isolation, it is possible that the ability of salmonids to make the necessary shifts for persistence under isolation may occur too slowly for some populations and may not be ubiquitous across populations (Morita et al. 2009). Furthermore, genetic diversity may be lost rapidly in isolated populations due to genetic drift and lack of gene flow between subpopulations, leaving isolated populations less able to adapt to changes in environment and at higher risk of inbreeding depression. Several authors have suggested that a minimum of eight to nine km of high quality stream habitat is necessary support a population large enough to maintain genetic diversity on evolutionary time scales (Hilderbrand

and Kershner 2000, Young et al. 2005). Currently, a majority of core conservation populations of Cutthroat Trout persist in isolated stream fragments less than ten km in length (Dunham et al. 1997, Shepard et al. 2005, Young et al. 2005), meaning that many core populations are not meeting estimated minimum requirements for persistence.

While some of these populations are naturally isolated above geologic barriers, most have been isolated by anthropogenic disturbances such as road crossings, dams and dewatering of streams at lower reaches (e.g., Dunham et al. 1997, Morita et al. 2009, Cook et al. 2010, Nislow et al. 2011, Kitanishi et al. 2012). The potential for reconnecting habitat for these isolated populations comes with tradeoffs as anthropogenic isolation has protected Cutthroat Trout from negative impacts associated with the spread of non-native species such as competition, predation, and introgressive hybridization. Managers often choose to maintain these barriers to avoid extirpation of threatened Cutthroat Trout populations (Fausch et al. 2006, Peterson et al. 2008b, Fausch et al. 2009). In order to effectively manage these high-risk populations into the future, we must evaluate the ability of isolation strategies to maintain self-sustaining native populations, and preserve the evolutionary and ecological values of threatened trout species (Fausch et al. 2009, Rahel 2013).

To evaluate the habitat and population characteristics that may influence persistence of isolated trout, we explored population viability and genetic diversity in Westslope Cutthroat Trout ($O. c.$ lewisi) populations isolated for varying lengths of time and in different habitat sizes. We asked the following research questions:

1) Are population growth rates lower in streams with smaller habitat size and lower habitat quality?

2) What demographic rates have the most influence on population viability, and how do population growth rates vary with estimated abundance?

3) How does genetic diversity relate to population growth rate and demographic parameters, including adult survival, somatic growth rate, and population size?

STUDY AREA

This study was conducted on Westslope Cutthroat Trout (hereafter "cutthroat") populations in first and second order streams in the Lower Flathead River watershed, located on the Flathead Indian Reservation of western Montana (Figure 4.1). The Flathead River watershed drains approximately 22,780 km^2 of land, encompassing the headwaters of the Columbia River Basin. The basin is primarily fed by precipitation with highest annual flows associated with spring runoff. The hydrograph typically declines to base flows by early August. Streams in the basin are located in a range of habitat types, from high gradient, mountain environments, to arid grassland environments. The Lower Flathead River Basin drains through private lands of the Flathead Indian Reservation. Here, human impacts on streams are common, and generally associated with agricultural and ranching practices, including stream dewatering and cattle grazing.

Fish of the watershed include native cutthroat and Bull Trout (*Salvelinus confluentus*), as well as introduced Brown Trout (*Salmo trutta*), Brook Trout and Rainbow Trout (*Oncorhynchus mykiss*). In connected systems, cutthroat display both resident and migratory life histories. Here, populations of cutthroat and Rainbow Trout are sympatric and hybridization between the two species is common. Cutthroat that are not hybridized are primarily found in small isolated tributaries above anthropogenic barriers.

METHODS

Data Collection

We examined cutthroat populations in 12 streams that have been isolated for 12 to 28 generations (assuming four years/generation) by anthropogenic structures, such as perched culverts at road crossing or irrigation canals. We used records from the Montana Department of Transportation and the Bureau of Indian Affairs Flathead Indian Irrigation Project to date isolating barriers for each population in this study. If the barrier was a perched culvert, we collected information on culvert dimensions, material and construction (e.g., corrugations), and surveyed longitudinal profiles that extended through the culvert. We analyzed these data in FishXing program to ensure that culverts were impassible by *Oncorhynchus* species (FishXing 3; http://www.stream.fs.fed.us/fishxing/). For all cutthroat populations isolated by a perched

culvert, Rainbow Trout or hybridized populations were present immediately below the barrier. Lack of introgression in these isolated populations (Chapter 3) further confirmed that upstream fish passage was not possible. Nonnative Brook Trout are the only salmonid other than cutthroat present above barriers, and were observed in two streams in this study (Revais and Centipede Creek).

The upper extent of cutthroat distribution in each stream was identified by sampling upstream until no additional cutthroat trout were observed. At this location, a GPS point was taken and we used Arc GIS (ESRI ArcMap 9.3) and stream data layers created by the Confederated Salish and Kootenai Tribes (unpublished data) to measure the length of occupied habitat in each stream between the isolating barrier and upper extent of the cutthroat distribution.

We estimated population density and size between 2010 and 2013 by sampling fish with a backpack electrofishing unit during base flow. We identified, counted and measured total length (mm) for every fish encountered and estimated population density for cutthroat >70mm using standard mark recapture or depletion methods (Guy and Brown 2007) at sampling sites that ranged from 120-155m in length. For depletion estimates, we repeated collection passes (typically 2-3) until we captured $\leq$20% of the number of fish captured in the first pass. Recaptures runs at mark-recapture estimate sites were conducted between six to nine days after the marking run. Typically we sampled two sites per stream with one located in the upper and one in lower half of the occupied habitat. But in three streams (Teepee, Talking Waters and Yellow Bay) density estimates were performed at only one site due to short total habitat lengths ($\leq$ 1.4km). To calculate population size, we averaged all density estimates and multiplied the average fish density by the total length of occupied habitat in a given stream.

In each stream, we sampled additional reaches ranging from 20 to 300m in length to increase the sample size for the length frequency distributions necessary for the catch curves used in survival estimates. In total, catch data comprised between two to five sampling sites (including density estimate sites) per stream over two to three sampling years (Table 4.A1). When sampling spanned two or more years, we combined catch data from multiple years throughout the study

period to reduce error associated with annual variation in recruitment and survival (Guy and Brown 2007).

Habitat Measurements

To assess habitat quality in each stream, we collected information on temperature, summer base flows, road density and land use in the watershed upstream of the isolating barrier. During the summer of 2013, we used temperatures loggers (HOBO and Tidbit V2 models, Onset Computer Corporation, Pocasset, Massachusetts, ± 0.2°C of accuracy) to record temperature at one-hour intervals from July 1 through September 8 (70 days). Temperature was recorded at one location per stream at an accessible site targeting the middle of the known cutthroat trout distribution (Figure 4.1). We calculated the number of growing degree days (GDD) above 0°C for the 70 day period in each stream. Base flows were recorded as cubic feet per second (CFS) between August 6th -8th, 2013 in the lower half of the cutthroat distribution (Table 4.1) in each stream using handheld Acoustic Doppler Velocimeter (FlowTracker). We calculated road density as total kilometers of road over total watershed area above the barrier for a given stream in Arc GIS using data layers generated by the Confederated Salish and Kootenai Tribes (unpublished data). Similarly, we calculated land use as the total number of square kilometers used for grazing or agriculture upstream of the barrier, and then used this number to calculate percentage of the watershed leased for these human activities.

Somatic Growth and Survival Estimates

Populations were analyzed individually (by stream) when estimating somatic growth and survival with the exception of Teepee and Talking Waters Creeks. Due to small population sizes in these creeks, we took caution not to impact these populations more than absolutely necessary. These creeks are in very close proximity and have similar habitat lengths and characteristics. As a result, we combined the information collected in these two creeks to estimate somatic growth and construct age-length keys (described below).

To determine somatic growth rates and survival of fish in each stream, we collected sagittal otoliths from 6-26 individuals in each population. Otoliths were clarified and analyzed for length at age following methods of (Corsi et al. 2013). Briefly, we took digital photographs of the distal surface of each otolith at 32 to 50X magnification in a dissecting microscope under reflected

light using SPOT Advanced version 4.7 (Diagnostic Instruments Inc., Sterling, MI). At the same magnification and focus as each photo, we also took a digital photograph of a micrometer to convert pixel length of the structure to mm. Using these images, at least two independent readers aged each otolith and a consensus age was determined for any individual for which there was disagreement. 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 90° angle from the anterior-posterior axis. This measurement axis provided the most consistent readability and measurements of inter-annual growth across all otoliths. We marked annuli 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 (Kruse et al. 1997, Klumb et al. 2001).

To determine somatic growth rates for each population we used the back-calculated lengths from otoliths in a given populations to calculate the increase in body length in the next growing season (length at t+1) given current body length (length at t). Consider, for example, an individual with back-calculated length of 60mm at age-1, 90mm at age-2. With a length of 60mm at time t, the delta length at t+1 would be 90-60=30mm.

Next, we performed a linear regression of delta length at t+1 versus length at time t. We used the resulting trend line to calculate the length an average individual would gain in one growing season given their current length. The slope and intercept of the resulting trend line was used in the integral projection model for each stream (below) to determine the size of an individual at the next time step, given its current size. Note that the slope of the somatic growth equation for all populations indicates the relative rate at which fish achieved their maximum size, and is negative because somatic growth declines as fish grow larger (Figure 4.2). The x-intercept of the growth equation is the estimated length at which increases in body size from one growing season to the next is marginal, and is similar to the parameter "L-infinity" or asymptotic growth in a von Bertalanffy growth curve.

To estimate annual adult survival for each population, we created age-length keys using back-calculated length-at-age information obtained from otoliths. Age-length keys began at 70mm and

assigned probability of a given age based on 10mm length categories. The maximum size range included in the age length key varied between populations, from 165-215mm, depending on the size of the largest fish captured for otolith analysis. Most streams had at least one size interval that lacked aging information because no fish sampled in that population had a back-calculated length-at-age within that particular size range. To fill in these gaps, we estimated probability of ages in uninformed length intervals based on information in the surrounding intervals. For example, consider age length key that is uninformed for the 130-140mm size category. If fish between 120-130mm had a 0.5 probability of being either age-2 or age-3, and all fish between 140-150 mm were categorized as age 3, we estimated that fish between 130-140mm would have a 0.25 and 0.75 probability of being ages 2 and 3 respectively.

To calculate adult survival for each population, we applied the age-length key to the catch data for a given population to examine the age-frequency distribution for each population. We then estimated annual adult survival using the Robson-Chapman method of survival estimation (Chapman and Robson 1960). To standardize datasets and survival estimates across populations, we included only the first three age classes on the descending limb of the catch curve in the Robson-Chapman estimation of adult survival.

For several streams the sample for the age-length key did not encompass the entire population size structure required for the survival estimates (Camas, Thorne, Teepee, Talking Waters, and Yellow Bay Creeks). To remedy this in Camas, Thorne, Yellow Bay Creeks, we applied the somatic growth rate of the two oldest age categories measured in the age length key, and projected the length at age for the next three years (i.e., ages) of growth. We then incorporated this predicted length at age information into the existing age-length key to obtain length at age information for additional age categories (Appendix 4.B).

Due to the small population size, our lethal samples for otolith analysis for Teepee and Talking Waters Creeks were low. These creeks are close in proximity, and similar in habitat quality, size, slope, stream aspect and population abundance, so we combined age and length data from both creeks into one back-calculated length at age analysis. While Teepee and Talking Waters Creeks had the same age-length key, the catch obtained from sampling each creek was not

combined. As a result, we were able to obtain unique survival estimates for Teepee and Talking Waters. To assess potential error resulting from combining age data for Teepee and Talking Waters, we calculated the sum of square errors (SSE) of predicted and actual age for a combined age-length key versus considering the age-length key from each population alone. In Teepee, the SSE did not change when information from Talking Waters was incorporated into the age-length key (SSE=0 without Talking Waters; SSE=0.04 with Talking Waters). For Talking Waters, the SSE was much lower for the combined aged length key than for the key created from Talking Waters alone (SSE=9.4 versus SSE=16.4 respectively),

Integral Project Models

We used integral projection models (IPMs) to determine the population growth rate of cutthroat in isolated streams. Our IPMs are individual, length-based models, which use the size of an individual to estimate vital rates. Our models were female based and density-independent with a pre-breeding census, and were adapted from Vindenes et al. (2013).

The model for each population was built using the following information: size distribution of age-1 fish entering the model; sex ratio, annual survival for juveniles (ages 1-2), sub-adults and adults; size of transition from juvenile to sub-adult; somatic growth rates; size based probability of maturity and fecundity; and survival of eggs to age-1. We estimated size distribution of age-1 fish, somatic growth and survival of sub-adults and adults as outlined above. All other information was obtained from previous studies (Table 4.2; Downs et al. 1997, Peterson et al. 2004). In all populations, a majority of fish at Age 3 were $\leq$ 110 mm in length. Therefore we applied adult survival rates to all fish $\leq$ 110 mm in length.

To obtain population growth rates, we entered information for a given population and ran the model until the size structure stabilized, and we obtained the population growth rate, lambda. We incorporated error of our survival and somatic growth estimates in the model for each population by randomly generating a distribution of possible survival and somatic growth parameters using the standard error in our estimates of survival as well as the slope and intercept of the growth equation. We then ran the model 500 times, each time randomly selecting estimates of survival

and parameters for somatic growth from these distributions. From the resulting distribution of 500 lambdas we calculated a 95% confidence to represent uncertainty in our estimate of lambda.

Genetic Samples and Analysis

Methods outlining sample collection, DNA extraction and amplification, genotyping, and analysis of genetic data are described in Chapter 3. Briefly, for each population we collected and analyzed between 36-54 samples across 14 polymorphic loci. To quantify levels of genetic diversity in each population, we calculated allelic richness for each population using rarefaction to account for unequal sample sizes.

Data Analyses

To determine the influence of habitat length and quality on population growth rates, we standardized the independent variables of length of occupied habitat, GDD, CFS, road density, and land use. We assess multicollinearity by calculating the variance inflation factor for all five independent variables, and found a high degree of collinearity between CFS and habitat length (Figure 4.A1). As a result, our analysis included two independent "full" models as follows:

Full Model 1) Lambda ~ Habitat Length+ Road Density +Percent Leased+ GDD
Full Model 2) Lambda ~ CFS+ Road Density +Percent Leased+ GDD

We compared every possible subset of these two models. The model with the lowest Akaike information criteria corrected for small sample size (AICc; Burnham and Anderson 2002) that contained only informative variables (i.e., parameter estimates significantly different from zero at the 95% confidence level) was considered the top model.

To determine the most influential demographic parameters on population growth rate, we performed sensitivity analysis by quantifying the change in population growth rate by manually perturbing one vital rate at a time by roughly 5% of its input value. The three parameters with the highest sensitivity values were ranked, and compared across all populations. We also used linear regression to compare lambda to estimated population abundance.

We used linear regression to examine the relationship between genetic diversity and lambda. We also performed linear regressions of allelic richness on the following demographic parameters that were directly estimated from each population: adult survival, somatic growth rate (i.e., slope of the growth equation) and population abundance.

All analyses were conducted in program R. For these analyses we used several R packages specific to analysis of fisheries data including the "FSA" (Ogle 2012)and "fishmethods" (Nelson 2012).

RESULTS

Are population growth rates lower in streams with smaller habitat size and lower quality?

Lambda varied across the 12 populations in this dataset (Figure 4.3). Incorporating error in our estimates of somatic growth and adult survival generally produced a normal distribution of lambdas after 500 simulations, with the exception of Camas Creek. Due to high variance in survival and growth estimates in Camas Creek, the distribution of possible lambdas was positively skewed, indicating the majority of simulations produced a population growth estimate greater than the point estimate.

Temperature information was not successfully collected in Centipede Creek therefore this population was removed from the dataset when performing the multiple linear regression examining the influence of environmental variables on population growth rate. Percent of the drainage leased was the only variable present in the model with the lowest AICc value (Table 4.3). The model with the second lowest AICc contained only GDD and fell within two AICc points of the top model. Population growth rate decreased as both of these variables increased (Figure 4.4c and d), although parameter estimates in both models were not significantly different from zero (Model 1: Leased=-0.069, p=0.06; Model 2: GDD=-0.035, p=0.38, Figures 4.A2 and 4.A3). Population growth rate was not related to CFS in our full dataset (Figure 4.4a). However, when considering populations in small habitat ($\leq$ 5km) the relationship between CFS and population growth rate was significant (<5km) (R^2=0.50; p=0.02).

What demographic rates and parameters have the most influence on population growth rate?

Across all streams, the slope of probability of maturity equation consistently had the highest sensitivity, sometimes up to three times greater than the second ranked parameter (Figure 4.5). The next two highest ranked parameters were typically the slope of the somatic growth equation (i.e., somatic growth rate) and adult survival. The only exception to this pattern was observed in Yellow Bay where the parameter with the third highest sensitivity value was egg to age-1 survival with a sensitivity value of 1.6. Here, adult survival had the fourth highest sensitivity value at 1.592. Population growth rate did not have any relationship to estimated abundance (R^2=0.17, p=0.19).

How does genetic diversity relate to lambda and demographic parameters?

Allelic richness was not associated with lambda (R^2=0.17, p=0.19; Figure 4.6a) or adult survival (R^2=0.04, p=0.53; Figure 4.6b). Allelic richness was positively correlated with somatic growth rate (R^2=0.38, p=0.03; Figure 4.6c) and population size (R^2=0.46, p=0.01; Figure 4.6d).

DISCUSSION

In our study, population viability models indicated that most isolated cutthroat populations were in decline, with no relationship between population growth rate and habitat size, quality, or genetic diversity. We found that increasing the probability of maturity for a given size fish (analogous to decreasing size of maturity) would have the largest positive effect on population growth rate for all populations, with sensitivity values often two to three times that of the vital rate with the second highest ranked sensitivity (Figure 4.5). While genetic diversity did not display any relationship with overall population growth rate, we did observe higher levels of genetic diversity in relatively larger populations and populations with more gradual declines in somatic growth.

The cutthroat population in Revais Creek had the largest estimated abundance at roughly 4400 adults occupying 14km of habitat. However, this stream is suffering from an aggressive Book Trout invasion, which may explain this population's decline. Throughout the Intermountain West, invasive Brook Trout have displaced native cutthroat populations, (Dunham et al. 2002a), creating substantial concern for protection cutthroat and highlighting the benefits of isolation that successfully prevents spread of invasive species (Dunham et al. 2004, Peterson et al. 2004,

Peterson et al. 2008b). The situation in Revais Creek highlights that even robust isolated populations may not be able to tolerate non-native species that are anthropogenically introduced or not deterred by the existing barrier. Under these circumstances, ongoing conservation efforts are needed to maintain viability of isolated populations (Peterson et al. 2008a).

While results from multiple regression analysis exploring the best predictors of populations growth, lambda, did not produce a model that met our selection criteria for a top model, the model with the lowest AICc value did show a negative relationship between population growth rate and percent of drainage area leased for agricultural practices. These results generally support other studies showing decline and extirpation of cutthroat trout in association with common dewatering and cattle grazing practices (Thurow et al. 1997, Peterson et al. 2010, Pierce et al. 2013). In our study, the p-value of this relationship was only slighter over the threshold of 0.05. A larger dataset with populations residing in streams with 20-80% of the drainage area leased may elucidate a clearer relationship. Additionally, we were surprised to find that length of occupied habitat was not a significant predictor of population growth rates. These results contradict multiple studies finding that length of habitat is positively correlated with population viability. For example, population viability and occurrence has been positively correlated with habitat size and connectivity in Bull Trout, White-spotted Char, and Masu Salmon (Rieman and McIntyre 1995, Morita et al. 2009, Tsuboi et al. 2013).

Even though we have a relatively small range of habitat lengths, isolated cutthroat populations have been observed in remarkably small habitat fragments under one km (Cook et al. 2010, also this study) and have not always displayed a consistent association between persistence and habitat size (Peacock and Dochtermann 2012). These data suggest that habitat characteristics beyond size alone are important for persistence of isolated cutthroat trout. Despite the fact that length of occupied habitat and base flows were highly correlated, base flow but not length of occupied habitat, emerged as an important variable for population growth rates in populations isolated in < 5km of occupied habitat. This suggests that information on stream flows may incorporate not only information on habitat size or volume of habitat, but also structural quality. For example Harig and Fausch (2002) found that mean pool width at bank full and number pools with residual depth $\geq$ 30 cm were significant predictors of successful translocations of

Greenback and Rio Grande cutthroat trout above isolating barriers, presumably because large pools provide overwintering habitat and refuge from high spring flows and drought (Bisson et al. 1982, Behnke 1992). Additionally, streams with lower base flows will have higher rates of sedimentation which can reduce available spawning habitat and embryo survival for cutthroat (Magee et al. 1996). Changes in anthropogenic water use will directly impact these habitat characteristics, and thus CFS at base flow may better represent anthropogenic activities such as dewatering of streams for agricultural practices than length of occupied habitat alone. Due to climate change and human resource use, current summer base flows have decreased 20% in the upper Columbia River Basin compared to the average for the 1980's, and are projected to decline an additional 10% by 2080 (Wu et al. 2012). The negative associations we observed between population growth rates and both base flow CFS and land use suggest that efforts to find more efficient and less impactful use of natural resources will play a critical role in viability of cutthroat populations into the future.

Generally, population growth rates were lower than expected given that these populations have persisted for 10-20+ generations under isolation. Even after accounting for error in our estimates of adult survival and somatic growth (i.e., 95% confidence interval) the distribution of our estimates of lambda indicated that our estimates in most streams were likely <1. Intermittent monitoring of isolated cutthroat populations over the last several decades indicates that populations in this study are not declining as rapidly as IPM models suggest. For example, the population in Schley Creek has an estimated abundance of 1271 individuals, at roughly 0.73 fish/m. With a population growth rate of 0.681, we would expect the population size to decline to 273 in only four years, and less than two individuals in only 17 years. However, sampling a designated monitoring site in Schley showed little difference in catch per unit effort for fish age-3 and older between 2009 and 2013 (catch $\leq$ age-3 = 15.25 in 2009 and 17.75 in 2013). There were no substantial changes to habitat quality throughout the stream between 2009 and 2013, so we are confident that these numbers reflect the population as a whole. If the population in Schley Creek is in decline, it is certainly not declining as rapidly as our model suggests.

Probability of maturity was one of our most sensitive parameters and was estimated at 11% for females 140 mm in length (see Downs et al. 1997). However, based on sampling records during

spawning between 2007 and 2013 in Camas, Centipede, Magpie Spring, Schley, Yellow Bay, and West Magpie Creeks, we regularly encountered gravid females under 140mm, with two as small as 119mm. This evidence suggests that populations in our study are maturing at smaller sizes than accounted for in our models. If we increase probability of maturity for a 120 mm female from 1% to 5% (equivalent to increasing the slope parameter for probability of maturity from 0.13 to 0.169; Table 4.2), the estimate of lambda in Magpie Spring Creek increases from 0.874 to 1.02. In short, the probability of maturity for small cutthroat is likely higher in our small, isolated systems than the published values from similar systems used in our models.

Through modeling populations dynamics of Brook Trout in western Massachusetts, Letcher et al. (2007) found that persistence of isolated trout populations was associated with higher survival for smaller size classes and earlier age (and thus smaller size) of maturity compared to connected populations. As a result, populations that persisted under isolation in their simulations tended to have a size structure that was skewed towards smaller individuals compared to connected systems. Our data also suggest that individuals in isolated populations may be under similar selection pressures. For example, our shortest streams Teepee and Talking Waters (<0.6km stream length) had the lowest estimated adult survival and abundance, but had the fastest growing individual fish. Given that these are small, steep streams with moderate temperatures regimes compared to other streams in this study, there is no evidence that the observed high somatic growth rates were a result of environmental conditions. Instead, low adult survival in these streams may have caused selection for individuals that mature sooner to maximize lifetime fitness.

Larger populations tended to have higher levels of genetic diversity. This was not surprising, given that smaller populations are expected to lose genetic diversity through drift more quickly than larger populations, and may be more likely to suffer from population bottlenecks when faced with stochastic environmental events. In our isolated populations of cutthroat, genetic diversity was also higher in populations whose decline in somatic growth from one year to the next was more gradual. For example, fish in Teepee and Talking Waters Creeks were characterized by the fastest growth rates for small fish and they rapidly approached their estimated maximum adult size. These two populations also had some of the lowest observed

levels of allelic richness in this dataset. What can this association tell us about life history tradeoffs faced by isolated populations? Low levels of allelic richness observed in these populations do not directly indicate local adaptation because we analyzed neutral markers. However, if our markers are linked to genes that code for traits such as somatic growth and size-based probability of maturity, it is possible that the low levels of allelic richness observed in these populations could be a result of natural selection. However, more information on the genome wide location of loci coding for these traits, as well as analysis of diversity at these loci would be needed to confirm this hypothesis.

Loss of genetic diversity has led to demographic decline across wildlife taxa. In some cases generalized loss of diversity and inbreeding are associated with lower survival and fitness, and increased susceptibility to disease (Slate et al. 2000, Höglund et al. 2002, Isomursu et al. 2012, Ruiz-Lopez et al. 2012, Mattey et al. 2013) which can cause population decline (McCallum 2008, Johnson et al. 2010). Other times, the association is more subtle. In some cases, loss of particular alleles or diversity at specific loci, but not loss of overall genetic diversity, can increase susceptibility to disease (Spielman et al. 2004, Meyer-Lucht et al. 2010, Kerstes and Wegner 2011). In contrast, Peacock and Dochterman (2012) found no relationship between genetic diversity at neutral makers and extinction risk across three connected and ten isolated populations of Lahonton Cutthroat Trout (*O. c. henshawi*). Similarly, we did not detect a relationship between loss of generalized genetic diversity at neutral markers and overall population growth rate, suggesting that assisted migration and genetic rescue would not benefit our populations at this time. Furthermore, if local adaptation is substantially contributing to viability in these populations, assisted migration could lead to outbreeding depression (and potential extirpation) if the introduced individuals are not appropriately suited to the local environment.

While levels of genetic diversity at neutral markers is not currently associated with changes in population growth rates, more information on how diversity at genes coding for demographic parameters such as somatic growth and probability of maturity will enhance our understanding of local adaption in these systems. Furthermore, lower levels of genetic diversity in these populations (Chapter 3) leave them with less material to adapt to expected changes in their

environment associated with climate change (Williams et al. 2009, Wenger et al. 2011). While isolation may be a short-term solution for preventing interactions with many invasive aquatic species, we cannot effectively prevent interactions with organisms that are not limited by the same barriers to movement across the landscape. As a result, isolated populations maintained for conservation purposes should be closely monitored for declines associated with inbreeding depression and outside factors affecting vital rates. If genetic rescue does become a necessary step, we caution managers to carefully consider which populations they use as donors to avoid outbreeding depression in populations that may have high levels of local adaptation.

Table 4.1. Growing degree-days (GDD), summer base flows in cubic feet per second (CFS) and location of base flow readings (CFS Location) listed as km upstream from the isolating barrier for the12 anthropogenically isolated streams.

ID	Stream	GDD	CFS	CFS Location
1	Camas	1134	0.12	1.06
2	Centipede	--	0.81	1.29
3	Cold	785	2.04	0.96
4	Frog	912	0.18	2.09
5	MagpieSpring	780	0.49	0.39
6	Revais	970	8.28	3.5
7	Schley	689	1.30	0.57
8	TalkingWaters	735	1.00	0.01
9	Teepee	838	0.90	0.01
10	Thorne	919	0.30	1.16
11	WestMagpie	828	0.46	2.7
12	YellowBay	630	3.53	0.73

Table 4.2. Vital rate information and sources used in integral population models.

Parameter	Estimate or Equation	Notes	Source
Egg to Age-0 Survival	0.4		Peterson et al. (2004)
Age-0 to Age-1	0.318		Peterson et al. (2004)
Juvenile Survival ($\leq$ Age 2)	0.394	Average of age-1 and age-2 survival estimates	Peterson et al. (2004)
Adult Survival	Directly measured from each population	Robson-Chapman method	This study
Length Distribution of Age-1 Fish	Directly measured from each population	Lengths of age-1 fish back-calculated from otoliths	This study
Somatic Growth	Directly measured from each population	Estimated from otoliths	This study
Fecundity	4.4*Length - 494.4		Downs et al. 1997
Probability of Maturity	$\dfrac{e^{(-20.28+0.13*Length)}}{1+e^{(-20.28+0.13*Length)}}$		Downs et al. 1997
Sex Ratio (F:M)	1:2.3		Downs et al. 1997

Table 4.3. Results from the top five models quantifying the influence habitat characteristics on population growth (lambda), as well the two full models. Full Model 1 includes occupied habitat length, road density, percent of drainage leased for agriculture, and growing degree days. Full model 2 includes the same parameters but replaces length of occupied habitat with base flow CFS. K is the number of parameters including the intercept. A listing of "None" under the informative parameters column indicates that no parameter estimates in the model were significantly different from zero at the 95% confidence level.

	Model	K	ΔAICc	Multiple R-sq	Informative Parameters
1)	~% Leased	2	0.00	0.33	None
2)	~GDD	2	1.99	0.09	None
3)	~ %Leased + Road Density	3	2.54	0.41	Leased (p=0.046)
4)	~%Leased + CFS	3	3.79	0.34	None
5)	~ %Leased + Length	3	3.79	0.34	None
Full 1)	~Length + Road Density+ %Leased+GDD	5	13.11	0.51	None
Full 2)	~CFS + Road Density + %Leased + GDD	5	13.50	0.49	None

Figure 4.1. Map of the study area. Numbers on c-e refer to Stream ID in Table 1.

a)

b)

c)

d)

e)

81

a)

b)
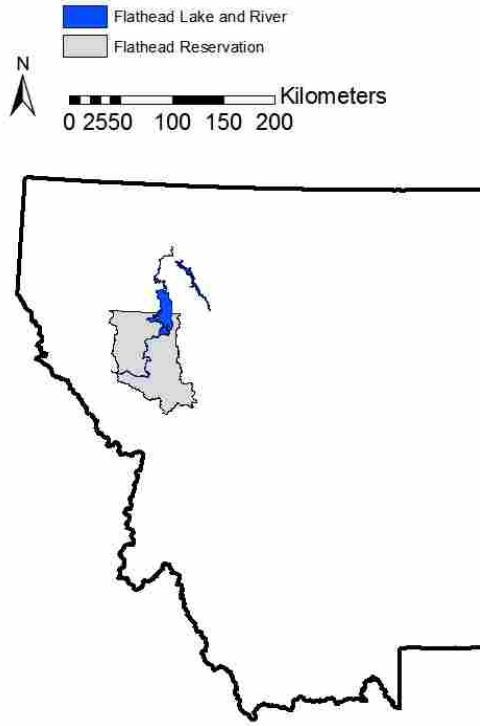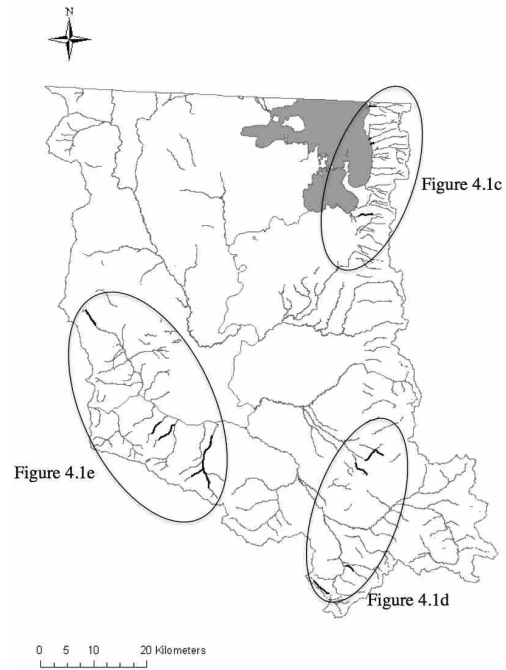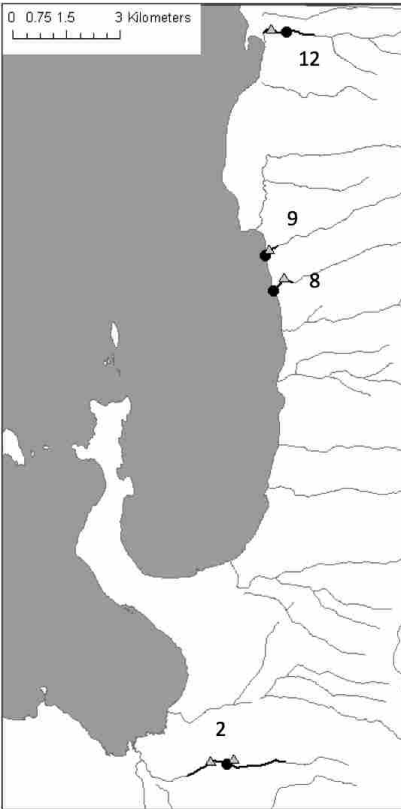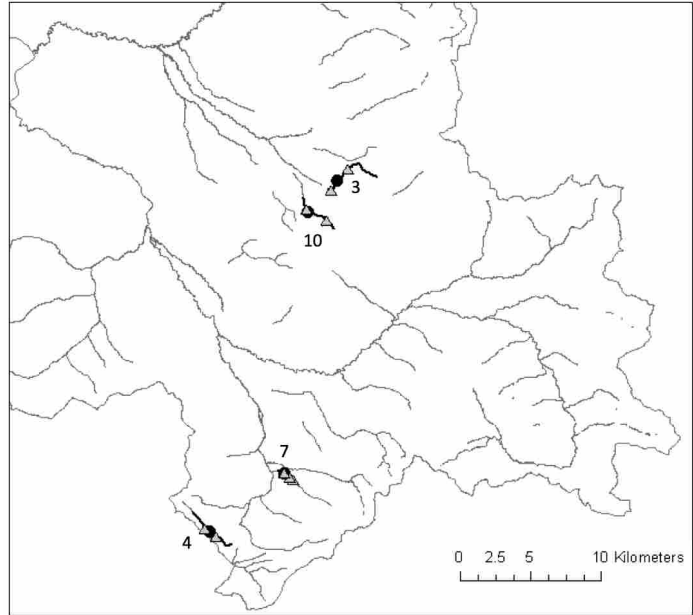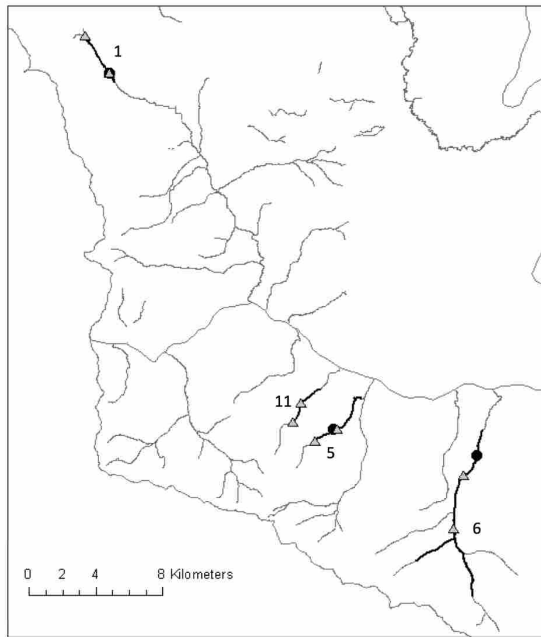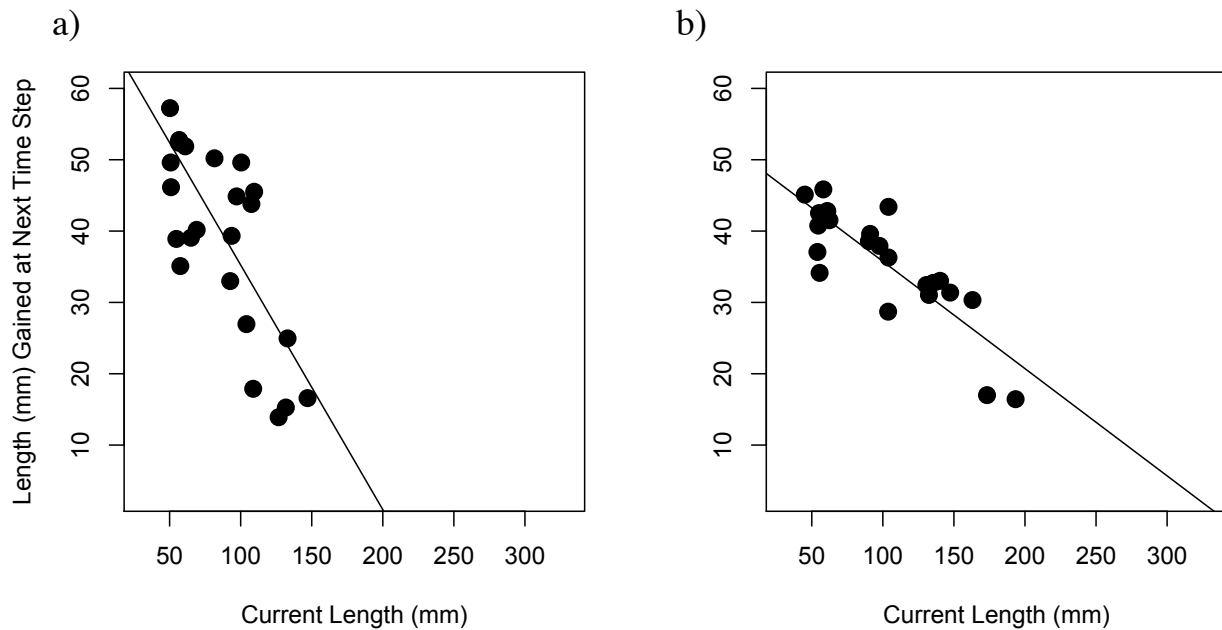
Figure 4.2. Length gained in next time step (in mm) based on current total length for Teepee and Talking Waters Creeks combined (a) and Cold Creek (b). Data for these figures was obtained from back-calculated length data from otoliths in each population (with information from Teepee and Talking Waters combined), and represents the growth equation used in population viability modeling. The x-intercept is roughly 201mm total length for Teepee and Talking Waters (a) and 314mm total length in Cold Creek (b). This point is the estimated maximum adult body size, beyond which annual increases in fish length are marginal. The growth equation for Teepee and Talking Waters (a) had a more rapidly declining slope than that observed in Cold Creek (b) indicating that individuals in Teepee and Talking Waters grow faster in early life stages. Conversely, fish in Cold Creek approach their point of asymptotic growth more slowly, but reach a bigger size compared to fish in Teepee and Talking Waters Creeks.
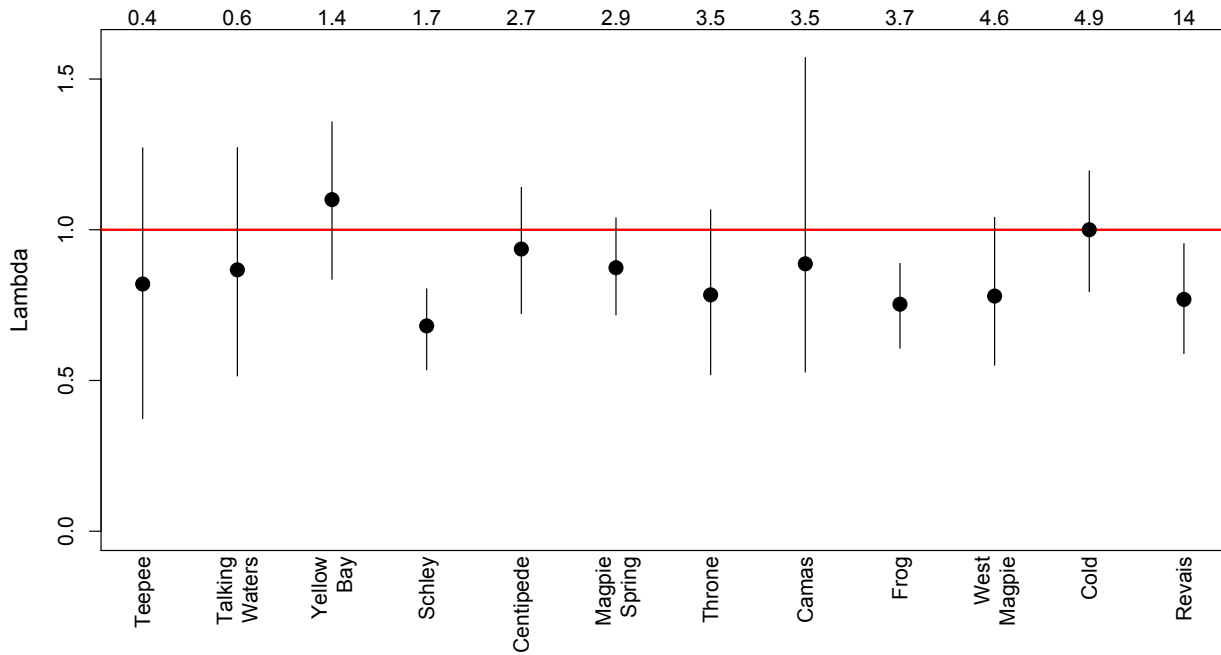
Figure 4.3. Estimates of population growth rate (lambda) for each population, ordered by length of habitat. Bars represent the 95% confidence interval after incorporating error in somatic growth and adult survival estimates. Numbers along the top of the figure show length of habitat in km.
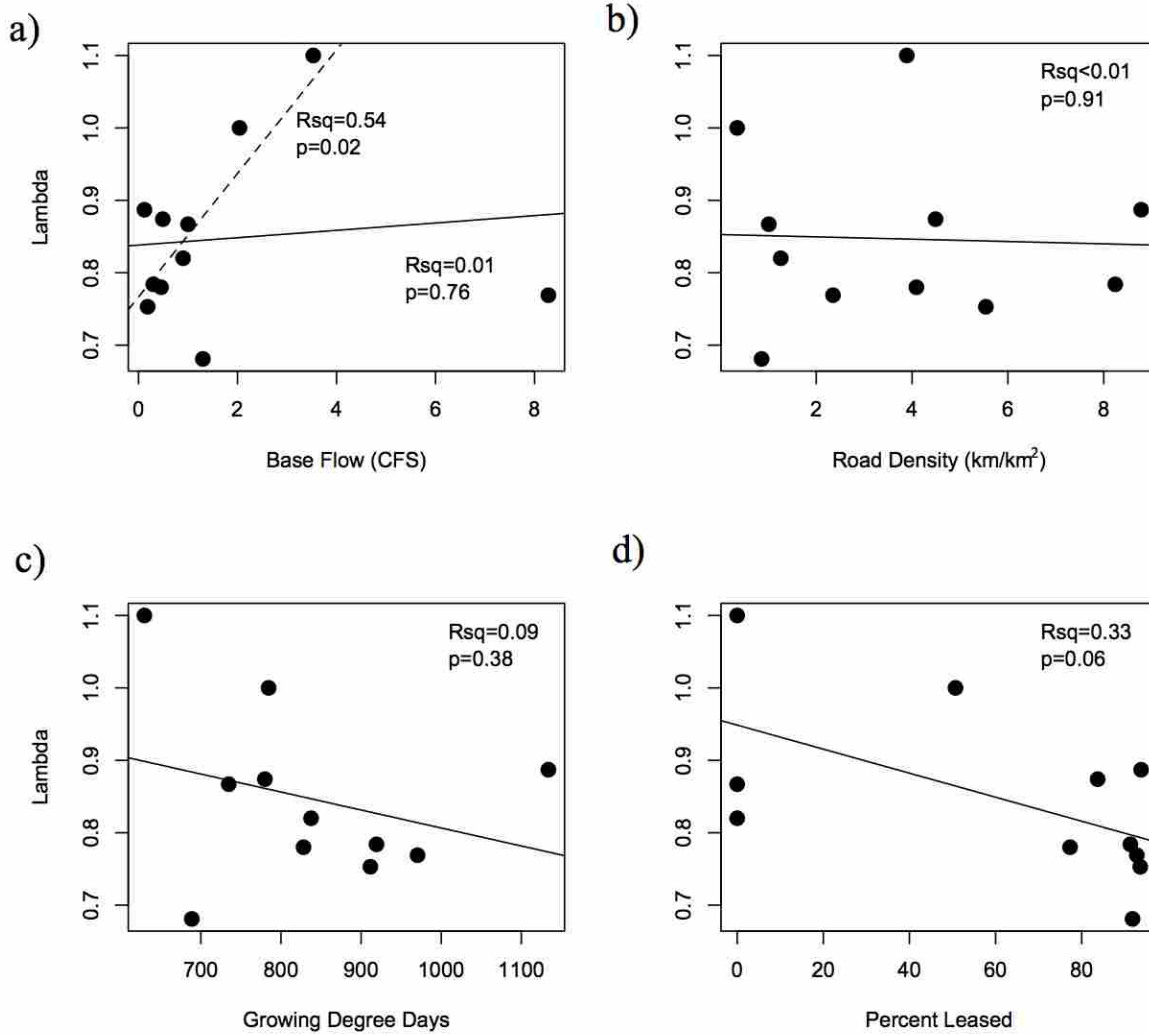
Figure 4.4. Population growth rate (Lambda) versus habitat quality metrics, base flow (CFS), road density (as km of road per km^2), growing degree-days, and percent of the watershed leased for grazing or agricultural production. In a, the dashed line represents the trend line when Revais Creek, at 8 CFS, is removed from the dataset. Centipede Creek is absent from all figures because we were not able to obtain temperature information for this stream.
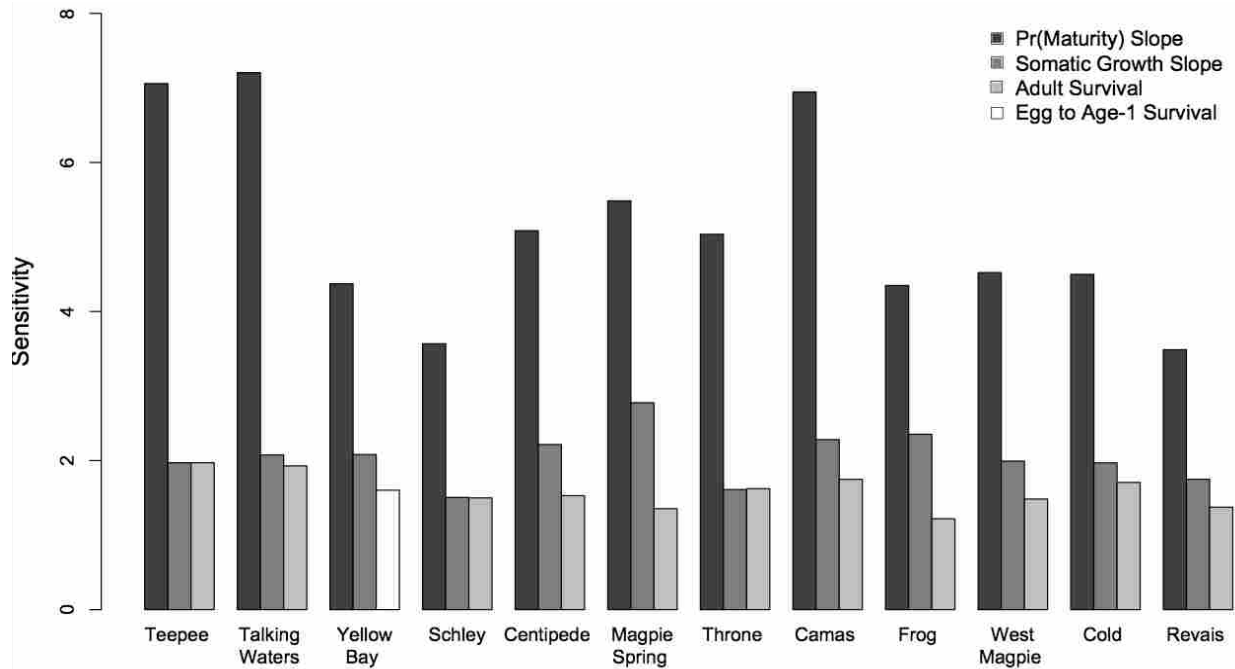
Figure 4.5. Ranking of the top three demographic parameters with highest sensitivity values for each population. We have displayed the absolute value of sensitivity values to highlight their relative ranking. The true sensitivity value for somatic growth rate is negative because the value of the parameter in the model is negative.
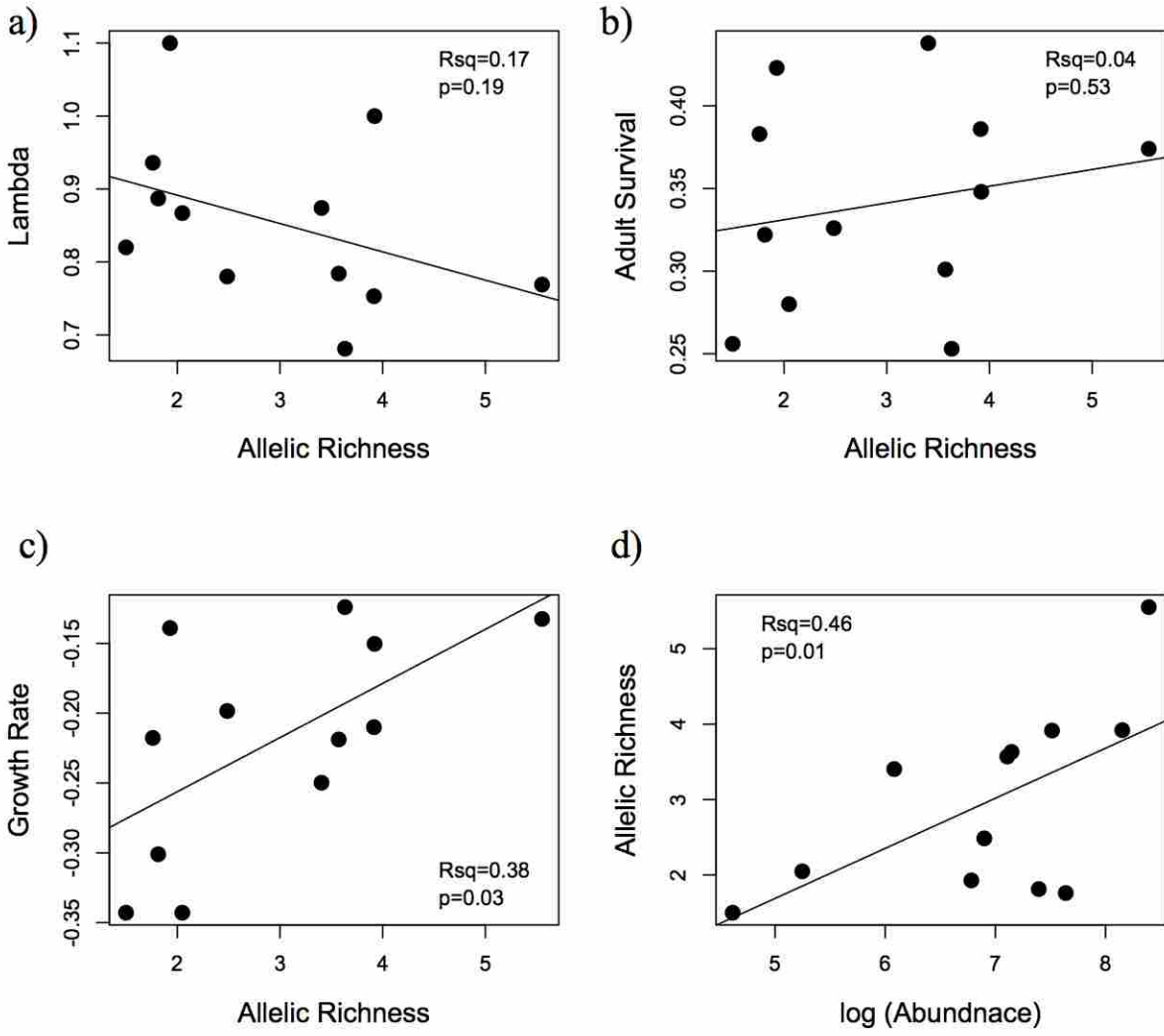
Figure 4.6. Allelic richness versus lambda (a), adult survival (b), slope of the somatic growth equation used in the population viability modeling (c). Panel d demonstrates the relationships between abundance and allelic richness (d).

APPENDIX 4.A

Table 4A.1. Information on habitat length, number of sites for fish collection (for length frequency distribution, average density, and lethal otolith collection), and year/s the fish collections took place. Barrier type indicates the structure creating the passage barrier and the year it was installed.

Stream	Habitat Length (km)	# Sampling Sites	Average Density (fish/m)	Years Sampled	Barrier Type (Date Installed)
Camas	3.5	4	0.44	2010, 2011, 2013	Irrigation diversion (1900)
Centipede	2.7	4	0.5	2011, 2013	Irrigation diversion (1920)
Cold	4.9	3	0.71	2011	Irrigation canal (1925)
Frog	3.7	3	0.37	2010, 2011	Perched culvert (1964)
Magpie Spring	2.9	5	0.18	2010, 2011, 2013	Percehed culvert (1938)
Revais	14	4	0.31	2010, 2011, 2012	Irrigation diversion (1915)
Schley	1.7	3	0.61	2010, 2013	Perched culvert (1964)
Talking Waters	0.6	4	0.28	2010, 2011, 2013	Perched culvert (1948)
Teepee	0.4	2	0.20	2010, 2011, 2013	Perched culvert (1948)
Thorne	3.5	3	0.35	2011, 2012	Irrigation diversion (1912)
Yellow Bay	1.4	3	0.61	2011	Perched culvert (1948)
West Magpie	4.6	4	0.21	2011, 2013	Irrigation diversion (1963)
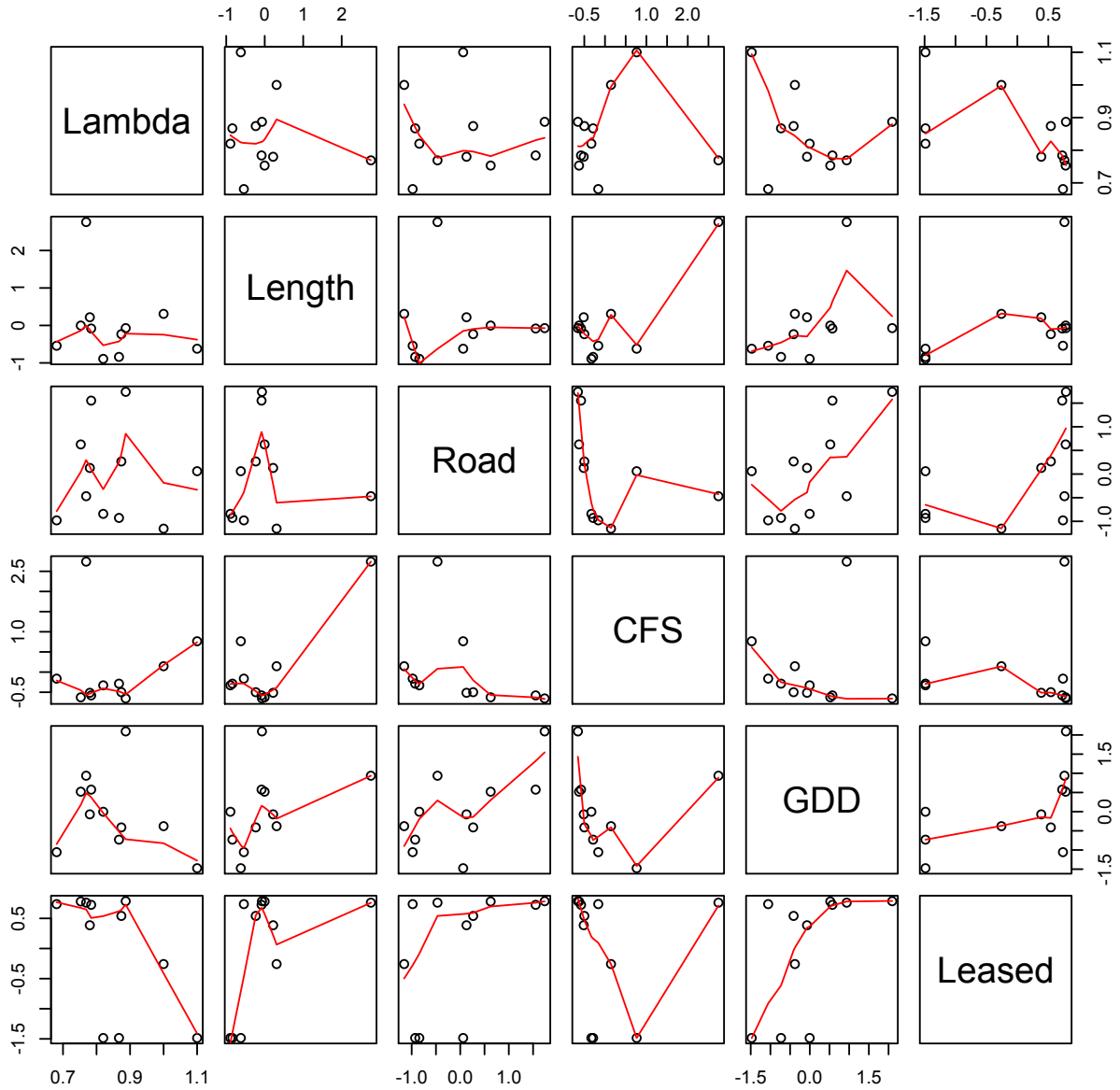
Figure 4.A1. Matrix scatter plot of the dependent variable (Lambda) and all scaled independent variables (length of occupied habitat; road density; base flow CFS; growing degree days; percent of drainage leased for agriculture) in the two full models exploring the relationship between habitat characteristics and population growth rate Information from Centipede Creek is not included in these plots because it was not included in the multiple linear regression analysis.
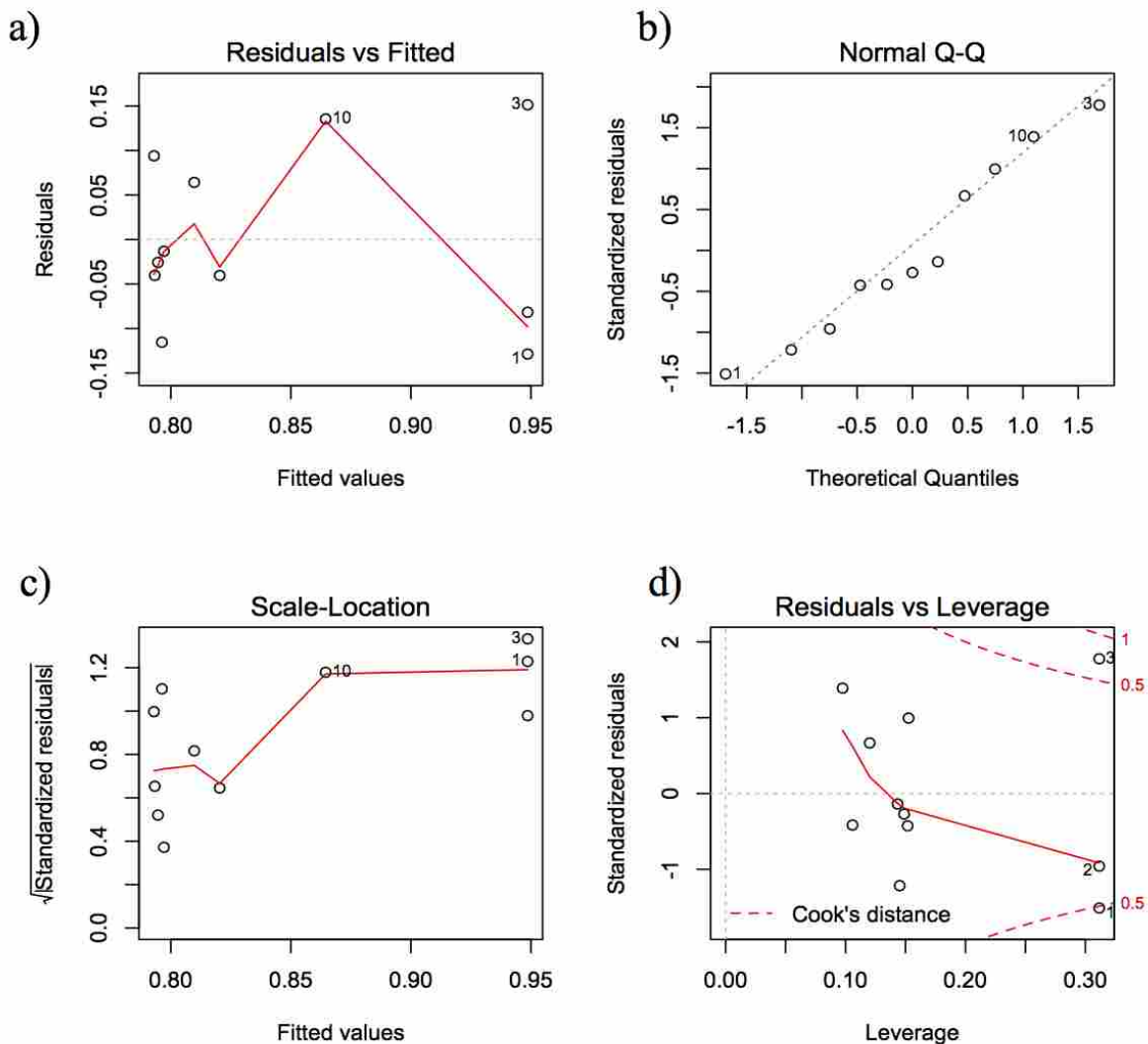
Figure 4. A2. Residual plots for the model regressing population growth rate lambda on percent of drainage area leased. Overall, this model has the lowest AIC value of all possible models examined, however, none of the models examined met our criteria for selection as the top model with all parameter estimates significantly different from zero. Plots show a) residuals versus fitted values for the model, b) normal Q-Q plot showing the deviation of residuals from a normal distribution, c) square root of standardized residuals versus fitted values, and d) standardized residuals versus leverage showing cooks distances. In d, points 1, 2, and 3 (Teepee, Talking Waters, and Yellow Bay Creeks respectively) fall outside the dashed lines are substantially influencing the results of the model and may be outliers in the full model. We believe that conditions experience by these three populations have selected for smaller size of maturity than is reflected in the viability model, producing lower than expected population growth rates.

APPENDIX 4.B
Age-length keys for each population are below. The number in each cell indicates the proportion of the population in that age and length class.

Camas

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5
55	1				
65	1				
75	0.667	0.333			
85	1				
95		1			
105		0.667	0.333		
115		1			
125		0.5		0.5	
135		0.333	0.667		
145			1		
155			0.333	0.667	
165				1	
175					1

Centipede

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5
55	1				
65	1				
75	0.5	0.5			
85		1			
95		0.8	0.2		
105		1			
115		0.5	0.25	0.25	
125			0.833		0.167
135			1		
145			0.167	0.833	
155			0.25	0.5	0.25
165				0.667	0.333
175				0.5	0.5
185				0.5	0.5

Cold

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6
55	1					
65	1					
75	1					
85	0.5	0.5				
95		1				
105		1				
115		0.667	0.333			
125		0.333	0.667			
135			1			
145			1			
155			1			
165				1		
175				1		
185				1		
195					1	
205					0.5	0.5
215						1

Frog

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7	Age-8
55	1							
65	1							
75		1						
85		1						
95		1						
105			1					
115			1					
125			0.333	0.667				
135				0.5	0.5			
145					0.5	0.5		
155				0.333		0.667		
165					0.333		0.667	
175						0.5		0.5

Magpie Spring

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6
55	1					
65	1					
75	1					
85		1				
95		1				
105		0.667	0.333			
115			1			
125			1			
135				1		
145				0.667	0.333	
155					1	
165					1	
175						1

Revais

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7
55	1						
65	0.333	0.667					
75		1					
85		1					
95		0.455	0.545				
105		0.5	0.5				
115			0.2	0.8			
125			0.571	0.286	0.143		
135			0.5	0.333	0.167		
145			0.167	0.333	0.333	0.167	
155				1			
165				0.6	0.2	0.2	
175					0.8	0.2	
185					1		
195					0.5	0.5	
205						1	
215							1

Schley

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7
55	1						
65	0.5	0.5					
75		1					
85		1					
95		0.75	0.25				
105			1				
115			1				
125			1				
135			0.333	0.667			
145				1			
155				1			
165					1		
175					0.5	0.5	
185						1	
195							1

Thorne

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7
55	1						
65	1						
75	1						
85		1					
95		1					
105		1					
115		0.5	0.5				
125			1				
135			0.75	0.25			
145				1			
155				1			
165				0.333	0.667		
175					0.6	0.4	
185						0.667	0.333
195						0.5	0.5

Teepee & Talking Waters

Length (mm)	Age-1	Age-2	Age-3	Age-4
55	1			
65	1			
75	1			
85	1			
95		1		
105		1		
115		1		
125		0.5	0.5	
135		0.2	0.8	
145		0.333	0.333	0.333
155			1	
165				1

Yellow Bay

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7
55	1						
65	1						
75	0.5	0.5					
85		1					
95		1					
105		1					
115			1				
125		1					
135			1				
145			0.5	0.5			
155			0.667	0.333			
165				1			
175				0.333	0.667		
185				0.5	0.5		
195					0.667	0.333	
205						1	
215						0.667	0.333

West Magpie

Length (mm)	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7	Age-8	Age-9
55	1								
65	1								
75		1							
85		1							
95		1							
105		0.5	0.5						
115			1						
125			0.333	0.667					
135			1						
145				1					
155				0.667	0.333				
165					0.5	0.5			
175					0.6	0.2	0.2		
185								1	
195									1

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