Colonization of steelhead (*Oncorhynchus mykiss*) After Barrier Removal in a Tributary to the Methow River, Washington

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AUTHORIZATION TO SUBMIT DISSERTATION

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Abstract

Connectivity is important to the long-term persistence of populations allowing individuals to access essential habitats, and provide demographic support and genetic exchange among local populations. This exchange of individuals among populations increases genetic variation and the evolutionary potential of the species. Barriers to migration create fragmentation and isolation which interrupts these processes. This study explores the effects of small irrigation diversion dams on the migration of steelhead (*Oncorhynchus mykiss*) in tributaries to the Methow River, and the subsequent colonization of the anadromous life history after re-designing these diversions to allow passage. Passive integrated transponder tags were used with microsatellite markers to identify life history, source of colonizers and successful reproduction. Migratory *O. mykiss* successfully colonized Beaver Creek and offspring from the first two brood years successfully returned to the stream as adults. Inter-breeding between the fluvial and anadromous life history types was common and offspring from the fluvial parents returned to the basin as adult steelhead. Hatchery *O. mykiss* did not contribute to the first two brood years during this early colonization process despite high abundances in adult returns. Population genetic diversity and the percent hatchery admixture were significantly different at the lowest two monitoring sites in the stream after barrier treatment. Colonization was still progressing upstream one generation after barrier treatment (4-5 years). Migration estimates prior to treatment of the diversion dams indicated that there was no migration for at least a generation in Beaver Creek.

Comparisons with migration to no migration sites in reference streams (Libby and Gold creeks) found significant differences in distance, number of obstructions, obstruction height to depth ratio and stream gradient. However, when examining Beaver Creek in comparison to sites with migration in the reference streams, only the number of obstructions was significantly different. Diversion dams on Beaver Creek were preventing migration and the treatment of these barriers resulted in the re-colonization of the migratory life histories. The fluvial life history was important in the colonization process and acts as a genetic reserve for the wild genotypes.

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Dedication

To Linda Blackwelder Pall, J. D., Ph. D, scientist, attorney, philosopher, mother, community organizer, politician, philanthropist, professor – a woman with a heart as big as her personality

For any goal to be realized, a person's willpower must be greater than the resistance. Dr. Pall has defied medical predictions three-fold. Willpower and a strong sense of purpose carry her through each day. Dr. Pall's dedication is an inspiration to the pursuit of a life in service of others.

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Overview

Stream restoration actions are being conducted throughout the United States to conserve native species and habitats. Connectivity of habitats is a focus of restoration efforts, as migratory movements to support growth, survival and reproduction are critical to the persistence of species. Barriers may create isolation of habitats that can prevent exchange of individuals for demographic support and genetic diversity and result in lost rearing and spawning habitat. Barriers are generally considered undesirable due to the consequences of isolation and fragmentation; however, they can have benefits such as reduced competition and introgression from non-natives and/or hatchery stocks, reduced introduction of disease and/or invasive species, and preservation of native genotypes.

Stream restoration actions are being conducted on the landscape where many other factors simultaneously influence population abundance and genetic diversity. Many streams support hatchery and harvest programs for recreational and commercial fishing opportunities. Monitoring of restoration actions is needed to document the effects of these restoration actions and improve scientific understanding of the inter-dependence of numerous effects in aquatic habitats. Additionally, monitoring can identify when and how management actions may be warranted to improve the likelihood of achieving the desired result from the restoration actions. Sometimes, the identification of barriers is obvious, such as when the barrier exceeds the jumping or swimming ability of the subject species. However, some barriers may be smaller, incomplete or temporary and it is much less clear when such an obstruction may result in blocking fish passage. This dissertation

studies the effects of smaller irrigation diversion dams where the long-term passage effects were unknown prior to treatment.

This study is part of an intensive effectiveness monitoring research project on the removal of several small irrigation diversion dams. Effectiveness monitoring evaluates whether management activities achieve the desired effect or goal. The goal of intensive effectiveness monitoring research is to understand the mechanisms underlying environmental and survival changes in the target species. It demonstrates causal relationships based on testable hypotheses with detailed ecological and ecosystem experiments.

In this dissertation, I used the before-after-control-impact (BACI) design recommended for effectiveness monitoring. This design collects data simultaneously at treatment and control sites before and after treatment. I used data collected from similar nearby tributaries, Libby and Gold creeks as references to Beaver Creek where the barrier removal treatments were performed. I recognize that the selection of reference or control streams is difficult for numerous reasons, such as uncontrolled anthropogenic or natural processes on a landscape that may not be evenly applied (such as timber harvest or fire), the difficulty finding relatively unimpacted streams in many locations, and the difficulty finding reasonably matched watershed attributes (no two watersheds are identical). The data presented in Chapter 4 provide an understanding of the initial conditions prior to treatment, whereas the data presented in Chapters 2 and 3 test causal relationships and examine mechanistic responses between the treatments, management actions and the population survival and growth.

Colonization of stream habitats by fish has not been widely studied. Salmon and trout (family Salmonidae) generally have extensive migrations for rearing and spawning and home to natal areas. Straying is thought to be minimal in most salmonid species and this maintains the genetic structure and local adaptations. However, colonization requires individuals that will stray into newly recovered or opened habitats. Straying is documented to be higher in hatchery-reared salmonids. In addition, hatchery salmonids have been documented to have substantially reduced relative reproductive success in the natural environment. Therefore, habitat re-opened by barrier removal projects could could be colonized disproportionally by these less fit and oftentimes highly abundant hatchery trout and salmon.

This dissertation uses population genetic markers coupled with passive integrated transponder (PIT) tags to examine the effect of small irrigation barriers on native steelhead/redband trout (*Oncorhynchus mykiss*) populations in a small tributary to the Methow River, Washington, and the colonization of anadromous *O. mykiss* (steelhead) after barrier removal. Chapter 1 includes a literature review of salmonid migrations, behavior, population genetic structure and impacts from introduced stocks and species. Chapter 2 uses parentage analysis to identify the source, phenotypes and individual fitness of *O. mykiss* colonizing Beaver Creek after barrier removal. Chapter 3 uses a before-after comparison of monitoring sites to document the source, life history and abundances of *O. mykiss* migrating into Beaver Creek during the first four spawning seasons, the rate and spatial extent of colonization, and changes in population genetic measures. Chapter 4 examines the effect of small irrigation diversion dams on contemporary migration rates in Beaver Creek prior to modification of the diversion

dams. This chapter compares migration patterns and population genetic measures in Beaver Creek to two nearby reference basins, and identifies which variables create resistance to migration in *O. mykiss*.

Chapter 1

Literature Review

Introduction

Species conservation and persistence relies on the goal of providing numerous, well-distributed populations or sub-populations that can demographically support each other through connected landscapes. Metapopulation theory is the foundation of conservation biology proposing that local populations will demographically support each other through local extinction-recolonization or source-sink processes. This theory suggests that populations or groups of species reside in areas of favorable habitat and connect with one another through migratory corridors (Hanski and Gilpin 1996). Three conditions define metapopulations: 1) habitat consists of discrete patches; 2) the dynamics of occupied patches are not perfectly synchronous; and 3) dispersal among the component populations influences the dynamics and/or the persistence of the metapopulation or at least some of the local populations (Rieman and Dunham 2000).

The metapopulation model predicts that given unstable environmental conditions, the evolution of locally adapted gene pools is restricted due to recurring local extinctions (Garant et al. 2000). However, this framework of demographic and genetic support has been difficult to empirically verify in stream dwelling salmonids (Rieman and Dunham 2000). A competing hypothesis called the member-vagrant model predicts that population structure evolves as a consequence of selective forces promoting precise

homing which would result in local adaptation and strong genetic structure among populations (Garant et al. 2000; Primmer et al. 2006). The member-vagrant model would result in significant isolation by distance and temporally stable population structure whereas isolation by distance would be absent or weak under the meta-population model (Primmer et al. 2006).

Trout and salmon (family Salmonidae) are known to have high levels of genetic differentiation (measured as F_{ST} values) across fairly small geographic areas; however anadromous species have lower F_{ST} values than resident species (Waples 1998). Many studies also have found significant isolation by distance in stream dwelling salmonids (Heath et al. 2002; Costello et al. 2003; Narum et al. 2008; Neilsen et al. 2009). Furthermore, studies indicate that salmonid species generally resist introgression from outside populations (Utter 2000). Therefore, the salmonid literature tends to support a member-vagrant model. Yet, some studies also indicate that the member-vagrant model may be important during periods of environmental and population stability, whereas the metapopulation model may be important after catastrophic environmental events or in locations of environmental instability (Rieman and Dunham 2000; Garant et al. 2000). Regardless of the underlying demographic model, scientists and natural resource managers will benefit most from understanding the underlying demographic and evolutionary processes such as dispersal, connectivity, and phenotypic diversity (Rieman and Dunham 2000).

The high genetic differentiation and structuring in stream dwelling salmonids is thought to be associated with local adaptations that are developed as an evolutionary interaction between genotypes and environmental conditions. These local adaptations are

maintained by a complex behavioral system that includes homing to natal streams and assortative mating (Hendry and Stearns 2004). This behavioral system can reduce effective population sizes resulting in increased rates of genetic drift. Anthropogenic activities can disrupt the behavioral mating system and subsequently the population genetic structure of salmonid species. Hatchery fish result in an increase in migration from an outside population and can increase genetic diversity and decrease genetic differentiation, whereas habitat alteration can create fragmentation, population isolation, and reduce migration (Fig. 1.1).

The three major components determining population genetic structure are: system of mating, genetic drift, and gene flow. Genetic drift and gene flow (mediated by inter-population migration) are opposing processes with drift increasing and gene flow decreasing inter-population differentiation. The balance between drift and gene flow largely determines population genetic structure in a species. Yet, assortative mating can be a strong micro-evolutionary force selective to specific phenotypic traits and subsequently associated genotypes (Templeton 2006). Assortative mating is a behavioral reproductive trait that can act selectively on specific loci when the trait is genetically heritable. Assortative mating and selection can determine the level of fitness of an individual, and hence, reproductive contribution to future generations. Drift and selection are believed to be fairly strong evolutionary processes. Mutation is not believed to be a strong process in determining genetic variability at contemporary population scales due to slow and random mutation rates that tend to occur in a single location in the genome (Allendorf and Luikart 2007).

Genetic differentiation in species is created by sub-division and isolation of populations. The degree of isolation influences the rate of migration between populations. When migration rates among the populations are low, genetic drift becomes the dominant process creating genetic variability between the different populations. Various behavioral and environmental factors affect the degree of isolation (or conversely connectivity) among the populations, and act simultaneously with genetic processes (such as drift, selection and mutation). Therefore, these genetic, behavioral and environmental processes collectively create and maintain genetic variability in species (Fig. 1.2).

Demographic processes influenced by historic and current events also will influence genetic diversity and differentiation. The rate and magnitude of variance in genetic drift is inversely related to population size (Templeton 2006; Allendorf and Luikart 2007). Genetic drift leads to random fixation of alleles over time which will reduce genetic diversity. In addition, whenever population abundance is low such as colonization after a geologic (founder effect) or catastrophic event (bottleneck effect), the reduced genetic diversity from these events will remain in the contemporary population genotypes (Templeton 2006; Allendorf and Luikart 2007). Therefore, these types of populations can show signs of reduced genetic diversity and isolation despite current landscape conditions.

In salmonids, biological factors that maintain genetic differentiation include species and life history isolating mechanisms such as: spatial and temporal segregation in spawning, migration patterns for rearing (or life history strategy), homing to natal areas, and assortative mating. Environmental isolating factors include: barriers that

impede or reduce migration between populations and habitat heterogeneity. These behavioral and environmental factors also interact to determine the reproductive effect of hatchery populations on wild populations. The role of these various factors on wild and hatchery salmonids are discussed further below.

Behavioral and Environmental Factors Influencing Genetic Diversity

Most of the behavioral factors appear to have some genetic control, such as life history, maturation, and run timing (Quinn and Dittman 1990; Silverstein and Hershberger 1992; Fleming 1998; Thorpe and Morgan 2006). However, there is also considerable evidence that most of these factors are phenotypically plastic and highly influenced by environment (Thorpe 1994; Vollestad et al. 2004; Fleming 1998; Taborsky 1998). Therefore, experimentally identifying the underlying reasons and triggers for these factors in fish populations has been difficult. Factors that increase population isolation will tend to increase genetic differentiation between populations or demes, whereas factors that decrease population isolation will tend to decrease genetic differentiation.

Life History

Oncorhynchus mykiss may establish multiple life history strategies including a freshwater resident form that remains in smaller tributary streams, a freshwater fluvial form that migrates between smaller and larger river systems, and an anadromous form

that spawns and rears in freshwater and migrates to the estuary or ocean. This species is iteoparous and may survive for multiple spawning events (Behnke 1992). Several other species of salmonids exhibit similar overlapping life history strategies with iteoparous resident and anadromous forms, such as sockeye/kokanee (*Oncorhynchus nerka*), Atlantic salmon (*Salmo salar*), and brook trout (*Salvelinus fontinalis*). These species generally maintain some level of inter-breeding between life history types which likely is an evolutionary assurance to unpredictable environmental conditions (Parker et al. 2001).

Oncorhynchus mykiss exhibits the greatest diversity of life history strategies for salmonids native to North America which includes multiple return times for adults during spawning migrations, varying periods of fresh water and ocean residency, and plasticity of life history between generations (WDFW 2008). Juvenile steelhead rear in freshwater for 1 to 4 years and rear 1 to 4 additional years in the ocean. This variable life history can result in 13 different combinations of fresh water and ocean ages with extensive overlap between generations. Age combinations of 2 to 3 years in streams and 2 or 3 years in the ocean are the most common (Meehan and Bjornn 1991).

Reproductive strategies in *O. mykiss* include semelparity (one spawning event preceding mortality), iteoparity (multiple spawning events spanning multiple years), and precocity (early maturation) (Behnke 1992). Precocity is more prevalent in resident male *O. mykiss*, and anadromous female *O. mykiss* have a greater tendency toward iteoparity than the anadromous males (Wertheimer and Evans 2005; Narum et al. 2008). Anadromous *O. mykiss* have been documented to spawn up to 4 times; however, the expression of iteoparity and survival of post-spawned adults is inversely related to the

migration distance and has been estimated to be less than 5% in the upper reaches of the Columbia Basin (Whitt 1954; Wertheimer and Evans 2005).

Salmonids are known to home as adults to natal streams to spawn which is considered to be related to adaptations to these localized habitats (Quinn and Dittman 1990; Quinn 1993). Homing in combination with assortative mating is thought to reduce gene flow between populations of salmonids (Quinn and Dittman 1990). Numerous phenotypic characteristics have been found to have some genetic control, such as age at maturity, date of spawning, egg size and developmental rate, disease resistance, agnostic behaviors, and rheotactic responses (Quinn and Dittman 1990). Some evidence exists that homing in Chinook salmon (*O. tshawytscha*) is a heritable trait (McIsaac and Quinn 1988). Another possible explanation is that homing is based on familiarity to the stream habitat where the cost of searching is minimized (Fleming 1998). Low proportions of trout and salmon have been detected straying, and straying from natal areas is thought to be a behavior to avoid unfavorable habitat conditions and/or colonize new habitats (Quinn 1993). However, the relative occurrence and reproductive success of strays is largely unknown, though wild steelhead have been measured to have very low straying rates (<3%) (Quinn 1993).

There is no taxonomic distinction between the resident and anadromous life history types (Docker and Heath 2003); however, microsatellite markers can detect various degrees of genetic differentiation between the life history types (Narum et al. 2004; Narum et al. 2008). Interbreeding between life history types occurs, and in some basins numerous returning steelhead (up to 40 percent) have had at least one non-anadromous parent (WDFW 2008). Resident redband trout can produce smolt

outmigrants; however their survivability to return as adults is lower than the other types of crosses (Thrower et al. 2004; WDFW 2008). The steelhead-steelhead cross produces the largest proportion of smolt outmigrants (WDFW 2008). Otolith microchemistry has been used to identify the life history of an individual and their maternal parent using strontium to calcium ratios between the center and outer portions of the otolith. In the Deschutes River, Oregon, all steelhead tested were derived from a steelhead mother and all rainbow trout tested were derived from a rainbow trout mother. However, in the Babine River, Canada, 4% of steelhead tested had otolith microchemistry indicating a resident mother, and 22% of the resident rainbow trout had otolith microchemistry indicating a steelhead mother (Zimmerman and Reeves 2000). Although these results indicate some life history crossing in the Babine River, the otolith only indicates one parental mode of behavior leaving the paternal information unknown which could also influence the migration pattern of the offspring.

Basin size and stream order are indicative of the larger stream habitats that steelhead will utilize that are different from the smaller redband trout (Hartman and Gill 1968; Bjornn and Reiser 1991). Steelhead dominate in streams with drainage areas exceeding 130 km^2 (50 mi^2), however they can occur in streams with drainage areas as small as 13 km^2 (Hartman and Gill 1968). Steelhead are present in streams ranging from third to fifth order, whereas rainbow trout may be present in streams as small as second order (Bjornn and Reiser 1991). These general trends in stream size and drainage position are verified with landscape studies incorporating genetic data in *O. mykiss* where the anadromous life history type tends to be more prevalent in the larger, lower elevation stream habitats (Heath et al. 2002; Narum et al. 2004; Narum et al. 2008). This trend in

habitat utilization and size can result in spatial segregation between these life history types.

Segregation between life history types can arise from spatial and/or temporal isolation in reproductive habitats. Although spatial and temporal segregation has been shown to occur in *O. mykiss* populations, these studies have indicated that there is some overlap both spatially and temporally that may not create complete isolation between these life history types (Zimmerman and Reeves 2000; McMillan et al. 2007). Spatial segregation arises from the differences in fish size between the resident and anadromous life history types, where larger fish will utilize larger habitats (Bjornn and Reiser 1991). In addition, steelhead used significantly larger sediment sizes and deeper water during spawning than rainbow trout in the Deschutes River, Oregon; however, water velocity at the redd site was not significantly different (Zimmerman and Reeves 2000). This difference in micro-habitat selection of spawning habitat significantly contributed to spatial separation between the two life history types (Zimmerman and Reeves 2000). Another study of *O. mykiss* on the Olympic Peninsula, Washington determined that there was significant spatial separation between tributaries where spawning steelhead and rainbow trout were observed, however this separation was weaker than the temporal segregation (McMillan et al. 2007).

Temporal segregation in reproductive behavior is common in numerous taxa which can create isolation between earlier and later reproducing individuals called isolation by time (Hendry and Day 2005). The resident and anadromous life history types have been found to exhibit some temporal segregation in spawning timing (Zimmerman and Reeves 2000; McMillan et al. 2007) with temporal segregation being a

stronger factor than spatial segregation in *O. mykiss* (McMillan et al. 2007). In the Deschutes River, Oregon, steelhead had a shorter spawning season ranging from the middle of March through the end of May. Rainbow trout had a longer spawning season from the end of March through the end of August. The spawning date for 50% spawned trout was significantly earlier for steelhead (Zimmerman and Reeves 2000). Although significant spatial and temporal segregation has been detected in *O. mykiss*, mating behaviors exhibited by this variable species allows for considerable overlap between spawning individuals, life history types and generations.

Temporal segregation can also occur by run timing in salmonids, particularly for the anadromous life history. This timing factor can be stream flow dependent where high flows could either cause the trout to expend too much energy to hold in suitable spawning locations and/or deter successful external fertilization. The redds of earlier spawning females may benefit from little competition for the highest quality spawning habitat, but these redds may also be harmed by the possible occurrence of very high flows that can scour redds, digging from later spawning females and higher predation before other food items become more seasonally available (Fleming 1998).

Salmonid Mating Systems

Salmonids have attracted substantial scientific attention due to their complex mating systems and behavior (Gross 1984; Foote and Larkin 1988). The female salmonid chooses a redd site and may be attended by numerous males. The female typically deposits eggs into more than one redd, and fertilization is external (Gross 1984; Chapman

1988; Reiser and Bjornn 1991; Fleming 1998). Both of these behaviors create opportunities for male competition during the mating events, and the sex ratio at the redd site is male biased (numerous males attending a single female) (Fleming 1998). Generally, larger females are preferred for greater fecundity that is the result of a direct relationship between body size, egg number and egg size (Beacham and Murray 1993; Fleming 1998). Larger eggs also tend to produce larger fry which could present an advantage when selecting preferred sites during early rearing (Fleming 1998). In addition, the larger female can dig a deeper redd that could be less susceptible to disturbance by later spawning females and predation by conspecifics or other species. Therefore, trait selection on the female generally arises from attaining the largest size possible to have the greatest reproductive success (Theriault et al. 2007).

Male salmonids have developed several reproductive strategies that will determine their success in courting the ripe female. The males establish a size based dominance hierarchy with the largest attendant male gaining the most access to the female during courtship and the spawning event (Gross 1984; Taborsky 1998). The dominant male fights for this position with other competing males requiring substantial energy expenditure. Therefore, a second mating strategy has developed in male salmonids called sneaking. Sneaking males are usually small, and oftentimes, precocious. This mating strategy relies on a hiding-in-wait behavior while the dominant male courts the female. When the female releases eggs for the spawning event, the subdominant, sneaker males rush to the redd and release sperm simultaneously with the dominant male. The success of this strategy is largely reliant on the proximity to the redd site (Gross 1985). Habitat complexity has been found to be associated with the success

of subdominant males in fertilization events (Gross 1985). Therefore, the larger males create a size-based hierarchy based on fighting, and the smaller males create a second size-based hierarchy based on hiding and sneaking. Hiding and sneaking also reduces the costly competitive, aggressive encounters for these males on the spawning bed.

The complex and diverse life history strategies in most salmonids like *O. mykiss* results in three types of males that can be present during spawning. The largest dominant males will be anadromous fish that have achieved larger size through longer (2-3 year) ocean residence. These males will develop sexual characteristics like hook jaws or hump backs that are used to visually increase size to competitors and improve competition in aggressive interactions (Gross 1985; Taborsky 1998). The smallest size of males will be precocious parr that mature early putting more growth into reproductive organs rather than somatic growth (Gross 1984; Foote and Larkin 1988). Therefore, two size dominance hierarchies develop at the spawning bed: one for the fighting, larger bodied males, and one for the precocious, sneakers males (Fleming 1998; Kosaki and Maekawa 2000). The fighting males do not appear to perceive the sneaker males as a threat, so few aggressive interactions are directed toward them (Kosaki and Maekawa 2000). These precocious parr do not develop the secondary male characteristics which could result in appearing more female and less of a competitor (Taborsky 1998). In addition, an intermediate size male may be present. This male could be anadromous with a shorter ocean residence (called a jack), or could also be a fluvial trout that has attained a larger size in the mainstem, freshwater habitats. The intermediate male is believed to have the least success for spawning since they have a size disadvantage for both fighting and sneaking. Therefore, selection for male spawning characteristics and behaviors is

thought to be disruptive (or asymmetrical), with the larger and the smaller size male favored over the intermediate size (Gross 1984, 1985; Foote and Larkin 1988).

On the spawning bed, the largest attendant male gains the most access to the female during courtship and the spawning event (Taborsky 1998). However, subdominant males and parr will release sperm during the spawning event and have been found to fertilize a proportion of the eggs (Blanchfield and Ridgway 1999; Kosaki and Maekawa 2000; Therialt and Bernatchez 2007). The precocious parr due to the much smaller size often sneaks just underneath the mating pair and also can successfully fertilize some of the female's eggs. Precocious parr have a greater gonadosomal index and higher sperm motility than the larger, dominant males which are traits thought to boost sperm competition and reproductive success for this strategy (Fleming 1998; Taborsky 1998). Parr, specifically, have been found to participate in more than 50% of observed spawning events with an individual parr fertilizing up to 34% of the eggs in masu salmon (*O. masou*) and brook trout (Fleming 1998; Kosaki and Maekawa 2000; Theriault et al. 2007). The larger, fighting male is typically considered to be at an advantage guarding and spawning the largest available female despite high energetic costs to this behavior due to equal or better success fertilizing eggs with this more fecund female (Taborsky 1998), and parr have been found to be excluded from spawning events when more than one anadromous male is present (Kosaki and Maekawa 2000).

Assortative mating is non-random selection of phenotypic traits during reproduction and can be a powerful micro-evolutionary process (Templeton 2006). Female salmonids have been found to exhibit some mate choice (Foote 1989; Fleming 1998) and even delay a spawning event when attended by a smaller male, spawning after

a larger male appears at the spawning site (Taborsky 1998). Although numerous behavioral studies indicate strong size dominance hierarchies in most species of salmonids, the recent application of non-invasive genetic techniques during the last 10 years has provided interesting information to determine the population level effect of various mating behaviors. In general, genetic studies have verified that salmonid mating is complex with spawning efforts including individuals who displayed monogamy, polygamy, polyandry and polygynandry (Seamons et al. 2004). Multiple matings across different parent pairs is common with males tending to have more partners than females. Size was not found to be significantly related to the number of surviving offspring produced (Seamons et al. 2004; Dickerson et al. 2004).

A parentage study conducted on steelhead in a tributary in western Washington failed to detect size assortative mating, but did find that steelhead frequently produced offspring with multiple partners and that run timing was significantly related to successful parent pairing (Seamons et al. 2004). Male steelhead arrived before the female with which he successfully paired, but there was no relationship between the date of arrival at the sampling weir and reproductive success (i.e. the relative date of arrival within the spawning season was not significant).

The lack of genetic evidence for assortative mating could have several explanations. Genetic studies seem to verify that the dominant male typically does gain reproductive access to the female he is courting, and does contribute to a substantial portion of her fertilized eggs. For example, although size assortative mating was not detected in pink salmon (*O. gorbuscha*), a significant relationship between male dominance score and offspring contribution was detected (Dickerson et al. 2004).

However, the complex male mating system with subdominant and sneaker males allows opportunities for these subdominant males to steal reproductive opportunity in synchrony with the dominant male, and these opportunities likely also contribute offspring. In addition, males have been found to mate with up to 10 different females, and they may have a prolonged spawning window depending on their energy reserves. Certainly, long return migration in combination with dominance fighting can reduce the energy reserves of the largest males, thereby leaving some of the smaller males able to participate in repeated spawning events. When considering the cumulative effect of all of these factors at the population scale, the mixed mating hierarchies and multiple mating partners may reduce the effects of assortative mate selection particularly if there is little to no realized fitness size advantage for male salmonids.

Another possible explanation for the differences between size assortative behavioral observations and genetic parentage of resulting offspring can be related to other factors such as predation. A study in anadromous and resident brook trout found that predation of spawned eggs by subdominant male brook trout increased significantly when the male brook trout was smaller than the female (Blanchfield and Ridgway 1999). The strategy of offspring sampling varies widely in these studies from the sampling of adult returns, smolt outmigrants and juvenile parr. This sampling strategy could indicate a different mating strategy than what behaviorally occurred at the redd. In other words, paternity at the spawning event could be missed based on the effect and time frame of progeny sampling.

Size and other phenotypes such as run timing and maturation have been found to be heritable traits (Quinn and Dittman 1990; Taborsky 1998). Heritability studies have

certainly indicated variable levels of inheritance in several species of salmonids (e.g. Heath et al. 1994; Heath et al. 2002; McCarthy et al. 2003; Dickerson et al. 2005). Maturation timing in male salmon has been widely studied due to the less desirable effects on fish size for commercial and hatchery operations. Male parr maturity has been found to be heritable both paternally and maternally (Iwamoto et al. 1984; Silverstein and Hershberger 1992; Fleming 1998). However, a transplant study indicates that there is phenotypic plasticity in the maturation of male masu salmon parr during transplant studies (Morita et al. 2009). Heritability in most salmonids is thought to involve a genotype interaction with the incubation and rearing environment thereby making detection of genotypic and environmental correlates unclear.

In addition, reluctance of a female salmonid to spawn with smaller males is thought to suggest that an important genetic component underlies this behavior with the female choosing a genetically superior male (Taborsky 1998). The general evaluation of the effects of various hatchery brood management practices lends some insight into the complexity of genotype and environmental triggers. One example is the lack of breeding opportunity afforded to jacks in both the natural and hatchery environments, yet this life history trait not only continues to remain in these populations but in some instances increased in frequency indicating other triggers than genetics can determine life history (Vollestad et al. 2004).

The multiple life histories combined with spatial and temporal segregation should result in isolated populations that could evolve into separate species. Yet, inter-breeding at either higher or lower levels seems to maintain both life history types within a drainage oftentimes only partially segregated (Zimmerman and Reeves 2000; Docker and Heath

2003; McPhee et al. 2007). Therefore, the resident life history form is believed to be a genetic protection (or reservoir) against harsh environment or limited access in the more isolated habitats occupied by the species. In this way, the locally derived genotypes of the species would provide genetic protection to the anadromous life history type in possible situations where this more risky life history strategy may be less successful (Brannon et al. 2004). This resident life history may be induced by genetic predisposition or due to low temperatures that discourage smoltification (Brannon et al. 2004).

Many salmonids appear to maintain a mixed life history strategy as an evolutionary stable strategy (Parker et al. 2001). Therefore, it is hypothesized that under stable environmental conditions, there is a selective advantage in habitat specialization tending toward isolation and subsequent speciation. However, under unpredictable environmental conditions, a mixed life history strategy will avoid extinction and recover from perturbation faster, reestablishing the equilibrium conditions (Parker et al. 2001).

These variable life history traits combined with extensively overlapping generations in *O. mykiss* are thought to provide some genetic compensation for periods when population abundances are low (Narum et al. 2008). In addition, anadromous *O. mykiss,* as well as other species of salmonids, are known to have considerable variation in reproductive success. Araki et al. (2007) found that resident *O. mykiss* provided genetic compensation when the anadromous spawning abundances were lower. Reduced variability in reproductive success when population sizes are low also has been found to provide some protection to expected losses in genetic diversity (Ardren and Kapuscinski 2003).

Habitat Heterogeneity

Heterogeneity in the environment can create population isolation through spatial habitat preferences and/or inhospitable conditions for rearing or migration. Certainly, water temperature and water chemistry can deter migration or rearing of salmonids in streams (Bjornn and Reiser 1991). In addition, landscape heterogeneity such as gradient, elevation and temperature could affect the willingness or energy expenditure for an individual to pass through various locations or habitats (Bjornn and Reiser 1991). Due to the complex interaction between genotype and environment in determining various phenotypic and migratory behaviors in salmonids, the spawning, incubation and early rearing habitats as well as the access for returning adults can be important in determining the population genetics.

Environmental factors are closely connected with the dominant life history strategy through inter-related, continuous variables such as elevation, temperature and stream gradient. Elevation, gradient, air temperature, precipitation, and upstream distance explained 79% of the variation in expected heterozygosity of juvenile steelhead trout sampled in the Klickitat Basin, Washington. Interpolation of the PCA axes in this analysis indicated that steelhead did not occur upstream of a second higher gradient reach (estimated 3-4%) in the mainstem Klickitat River suggesting that adult steelhead may be limited in their ability to navigate higher gradient, higher volume river reaches during spawning migrations (Narum et al. 2008).

In salmonids, habitat gradients can be important during the freshwater rearing phase or during the adult return migration to the spawning habitat. These habitat gradients can determine the available growth rate and food resources, and subsequent balance between the risk and benefits of migration (Thorpe 1994; Brannon et al. 2004). In addition, the interaction between genotype and environment can play an important role in determining the life history patterns across a landscape. For example, returning adult steelhead may have access blocked to certain habitats due to obstructions, flow levels or gradients and associated water velocities (Narum et al. 2006b; Narum et al. 2008). Since each life history type is somewhat determined by parental genetics, the extent of upstream migration and location of suitable sized habitats for spawning will influence the distribution of the life history types across a basin.

Numerous studies have examined various basin and environmental attributes associated with the distribution of genetic diversity and differentiation in salmonids including bull trout (*Salvelinus confluentus*) (Costello et al. 2003; Meeuig et al. 2010), cutthroat trout (*O. clarkii*) (Neville et al. 2006a), Chinook salmon (Neville et al. 2006b), steelhead (Narum et al. 2008), and white spotted charr (*Salvelinus leucomaenis*) (Morita et al. 2009). Most of these studies have found that basin or spatial attributes explained more genetic variation than environmental attributes. For example, Costello et al. (2003) found that basin attributes (stream network) explained 29-45% whereas environmental variables only explained 7-9% of the genetic variation and diversity. The basin components were significant in this study whereas the environmental components were not, and barriers, distance from mouth and temperature were significant variables examined (Costello et al. 2003). Morita et al. (2009) found that precocious maturation in

male white spotted charr (male resident life history) was more strongly and inversely related to stream width than stream temperature. Certainly, temperature, elevation and distance to mouth often are highly correlated variables making it very difficult to identify the underlying deterministic mechanisms. Yet, spatial or basin position can explain a substantial amount of genetic patterns at the basin and landscape scale.

Distance, spatial position and basin (same/different) should be inter-dependent variables. Distance between populations or sampled groups would be highly reliant on the ability of the study organism to travel and disperse (Bohanak and Jenkins 2003). The greater the distance, oftentimes, the lower the probability of migration between two sites. In this way, isolation by distance is thought to work in a 'stepping stone' model where individuals between adjacent populations or demes have a greater probability of exchanging successful migrants.

Connectivity and Migration

Barriers strongly influence the ability of individuals to migrate between various sites in a landscape (or connectivity), and thereby often explain much of the variation in genetic diversity. Waterfall barriers have been found to be the best explanatory variable in population genetic diversity in bull trout (Costello et al. 2003; Meeuig et al. 2010). However, in these studies, barriers will likely have both an historic (geologic) and contemporary effect on population connectivity and resulting gene flow processes. In addition to waterfalls, beaver dams may also form natural barriers to fish migration in streams. The genetic effect of waterfall barriers that function over geologic timescales is

fairly well understood with isolated populations upstream of the waterfalls exhibiting lower heterozygosity, lower allelic richness, and greater genetic differentiation due to genetic drift (Allendorf and Luikart 2007).

Anthropogenic barriers, such as dams, culverts or stream dewatering, also exist on most landscapes and effect migration, inter-population interactions and life history expression in salmonids. These barriers are oftentimes incomplete and work in shorter timescales (numerous decades) than geologic barriers. The effect of these barriers on population genetic diversity is relatively unknown, particularly since these barriers are often incomplete and temporary (Neville et al. 2006a). In addition, stream barriers may allow uni-directional (downstream) gene flow (Meeuig et al. 2010) and infrequent passage based on climatic conditions. Many factors can influence the degree of genetic effect that these barriers may have on fisheries populations, such as number populations upstream of the barrier, number of individuals in population(s), amount of migration, and genetic similarity between the migrating populations.

Colonization Process

When populations are extirpated by anthropogenic activities or natural disasters, the vacant habitat is eventually re-colonized. The time scale of this colonization process will rely on the length of time it takes for the habitat to recover (habitat suitability) and distance, dispersal capabilities and densities of the nearest source populations. Under this scenario, there may be no detectable difference between the metapopulation model or the

member-vagrant model, as new colonizers will likely have similar traits and may be motivated by similar factors to investigate the vacant habitat.

Re-colonization requires several critical elements: 1) source populations that can provide colonizers into the newly opened habitat; 2) connectivity between the source population(s) and the newly opened habitat; and 3) adequate habitat conditions in the newly opened habitat to establish and support the species of interest. If a source population is close and the habitat is suitable, colonization of the habitat can occur fairly quickly. For example, Kiffney et al. (2009) studied the colonization of coho salmon (*O. kisutch*) after installing a fish passage ladder at a dam on the Cedar River, Washington. The dam blocked fish migration and coho were excluded from this habitat for more than 100 years. Coho salmon occupied the stream habitat downstream from the dam and the salmon immediately migrated and spawned in the re-opened habitat upstream of the dam. In this case, migration, spawning and reproduction began during the first year that the stream was reconnected.

Studies of re-colonization of habitat are largely opportunistic attempting to describe changes in populations after natural catastrophic events, such large floods, forest fire, or volcanic eruptions, or after alterations of anthropogenic barriers or habitat alteration, such as dams or releases of toxic chemicals. Garant et al. (2000) observed that a population of Atlantic salmon displaced after a large summer flood in a tributary of the Sainte-Marguerite River, Canada, showed a substantial genetic change. They conclude that this observation indicates that the site was not colonized by returning adults that originated from the natal site, but from salmon originating from other populations in the stream. However, large genetic change could also result from small breeding

populations. Therefore, it is difficult to conclude the source of the colonizers at this site without supporting migratory data.

The volcanic eruption of Mt. St. Helens in 1980 provided an opportunity to observe the response of steelhead trout to a catastrophic event. The eruption left tributaries of the Cowlitz River, Washington, too degraded in water quality to support returning adult steelhead trout (Leider 1989). Steelhead trout destined to return to these tributaries were thought to stray into the Kalama River, a nearby, less impacted stream. This study found that the number of returning adult summer steelhead counted at a weir in the Kalama increased more than 200% from 1980 to 1982, and out-of-basin strays increased from 16% prior to the eruption to 52% and 42% after the eruption in 1980 and 1981, respectively. In addition, this study indicated that the dominant anadromous age class of two year ocean rearing strayed less than the other ocean rearing age classes represented in the data. Although substantially more out-of-basin steelhead trout were detected at the weir, the study cannot determine whether these fish attempted to spawn in the Kalama or subsequently migrated to other basins (Leider 1989). Furthermore, this study did not directly monitor the changes in migration and abundance in the impacted Cowlitz and Toutle River basins.

In 1988, a portion of the range of endangered Virgin River chub was lost during a rotenone poisoning treatment that spread downstream killing all the fish outside the intended treatment site. A population genetics study documented the before and after genetic characteristics of the Virgin River chub (*Gila seminuda*) (Demarais et al. 1993). Before treatment, this species did not show spatial or temporal genetic structure indicating high levels of gene flow. After treatment, 2 of 3 tested loci were significantly

different when compared to the before treatment data indicating substantial genetic alterations after the treatment which created a subdivision between the populations in the stream and substantially reduced the number of breeders. After 29 months, the upstream site about 30 km from the source population was genetically similar to the before treatment data; however, the most downstream site that was 60 to 100 km from a source population was not recolonized. This lack of recolonization could also have been influenced by intermittent stream flow reducing connectivity between the source population and the lower study site (Demarais et al. 1993).

A study comparing populations of rainbow trout across forest areas with different lengths of time after forest fire found no change in population genetics measures across variously aged fire impacts (Neville et al. 2009). Connectivity among the burned areas was maintained, and therefore, individuals and gene flow continued afterward relatively uninterrupted. However, this study did find that genetic distances were greater upstream from culverts that prevented passage (Neville et al. 2009). These data indicate that trout are able to survive in streams impacted by forest fires or leave the area and return later. Forest fires are natural events, similar to flooding, and it is possible that species have evolved over time to adjust to these natural processes.

Several other factors likely influence the rate and success of colonization of newly opened habitat such as: the distance between the habitat and the source populations, the migratory or dispersal ability of the species, the density of individuals in the source population(s), and the fitness of the colonizing adults in the new habitat. Colonizing (or founding) individuals may exhibit an affinity to assortatively mate with individuals from the same source population for greater genetic compatibility, or conversely, avoid

assortatively mating with individuals from the same source population possibly avoiding related individuals. A study examining grey seals (*Halichoerus grypus*) indicated that two of nine possible source populations largely contributed individuals to a new colony and mating between founding individuals was not random. These data also indicated a strong relationship between the population genetics in the newly established colony with source populations at a closer geographic distance. In addition, there was a positive relationship with productivity in the source populations, but this variable was weaker than distance (Gaggiotti et al. 2004). This study indicates that several environmental and population demographic factors may influence the colonization of vacant habitats, and that these factors may have different levels of relative influence on the resulting population genetics.

Lastly, the level of fitness of colonizing individuals into a reopened habitat will determine the success of the colonizers as well as the population genetic response. Environmental conditions such as run timing and developmental rates are thought to be adaptive traits in salmonids (Brannon et al. 2004). Given that species and populations exhibit local adaptations to specific environmental conditions, then genotypes and phenotypes likely evolved to maximize fitness given selective environmental conditions. Therefore, this suggests that not all colonizing individuals will contribute to the founding gene pool. Hatchery salmonids oftentimes represent a source population with numeric advantages over natural or wild fish in many anadromous populations; yet, they likely exhibit reduced reproductive success (Araki et al. 2007; see section below for more details).

Little research has been done in fish populations on selective gradients during the colonization process. Anderson et al. (2010) studied the selective gradients of coho salmon colonizing the Cedar River the first 3 years after passage was installed at a dam. This study found that selective gradients were different for the different genders of salmon and shifted in direction and magnitude during this short timeframe. This study found a strong selective gradient on size that increased over time for both sexes. Selection on early run timing was strong and directional the first spawning season after passage was restored; however in the next two seasons run timing was a stabilizing selective gradient (Anderson et al. 2010). Adult abundances into the newly opened habitat increased rapidly each year after passage was restored (Kiffney et al. 2009). Shifting gradients could be a response to increasing abundances and intra-specific competition for resources.

Genetic Effect of Hatchery Introgression

Anadromous salmonid populations are often supported with additional production from hatchery programs with the goal to increase the numbers of returning adult fish and support commercial and recreational harvest. Fish that escape harvest either return to the hatchery or will stray into the natural populations in the stream environments. Because straying is believed to demographically support the natural populations, policy has listed many hatchery populations of anadromous fish under the Endangered Species Act shifting the goals of some hatcheries to include conservation of declining populations. The harvest and the conservation goals can conflict when managing these fish.

Intra-species Introgression

The main concern regarding hatchery salmonids is the potential effects of inter-breeding and competition in the natural environments. Hatcheries create separate breeding populations that are directly influenced by brood management practices. The hatchery strays increase migration from the hatchery population to self-supporting, wild populations in the basin. The source and number of individuals used to create a brood stock strongly influences the genotypic and phenotypic characteristics. The subsequent brood practices additionally alter genotypes and phenotypes. These hatchery strays can either increase or decrease genetic diversity in wild populations depending on the underlying source genetics of the brood stock and the success of the hatchery fish in the natural environment. Continual mixing of wild and hatchery populations can occur intentionally in the hatchery mating or unintentionally in the natural environment. The continued inter-breeding of various populations would be expected to alter the genotypes resulting in the continual break down of locally adapted gene complexes and loss of native genotypes. In addition, continual mixing of populations makes genetic monitoring and management of native populations challenging.

Although hatchery strays are fairly abundant in the natural environment, most recent studies indicate little successful inter-breeding between the hatchery and natural populations. Two studies examined the effect of summer run steelhead on native winter run steelhead. Although the summer steelhead were found to contribute to the smolt production in these streams, little inter-breeding was detected likely due to these different

stocks having distinctly separate migration and breeding timing (Kostow et al. 2003; Narum et al. 2006b). In Forks Creek, Washington, hatchery and wild steelhead maintained distinct population groups (F_{ST} = 0.02) where correct population assignment to each group was >85% (Houser et al. 2006). However, the populations in this basin are largely maintained separately where the hatchery fish are typically removed from the wild run at a fish collection weir (Houser et al. 2006). Two out of three populations of adult steelhead were significantly different than the local hatchery population in the Grande Ronde Basin, Oregon where hatchery fish are known to stray into the natural populations (F_{ST}=0.01) (Narum et al. 2006a).

Reduced fitness by hatchery fish in the wild can be due to genetic incompatibilities that result in lower offspring survival (outbreeding depression) or selection against hatchery fish by natural-origin breeders during spawning. The genetic incompatibilities are thought to be a result of losses in local adaptations or breakdown of co-adapted gene complexes. Scientific research in steelhead has focused on the effect of parent source (hatchery versus natural) on production of offspring. In Hood River, Oregon, pedigree analysis was used to estimate the relative reproductive success of hatchery and naturally produced steelhead in the natural environment, and this study was able to follow first and second generation hatchery steelhead. In this study, the hatchery fish spawning in the natural environment were estimated to have about 38% loss in reproductive success in relation to the natural population (Araki et al. 2007). This study began to indicate the possibility of underlying genetic incompatibilities occurring in relation to the hatchery environment that pass to the subsequent generations.

Additional evidence of genetic incompatibilities is supported by a study that created crosses between hatchery and wild male and females. The eggs were reared in the hatchery and the fry were released into tributaries to Lake Superior (Miller et al. 2004). This study estimated the relative reproductive success of all the crosses to the natural x natural cross, and found a significant maternal effect where the crosses with a naturally produced female trout had higher survival. The hatchery x hatchery cross had the lowest relative survival (21%). This study also found that after 1 year in the natural stream environment, the natural x natural cross was significantly smaller (Miller et al. 2004). Further research into these populations determined that stocking fry into the natural stream environment in sufficient numbers significantly increased the relative contribution of hatchery females to naturally produced females (Caroffino et al. 2008). In addition, the genotype data indicated a shift in allele frequencies during two generations of hatchery brood management with significantly different F_{ST} values between the hatchery and the natural population and significantly lower allelic richness in the hatchery population (Caroffino et al. 2008).

Outbreeding depression is a concern in hatchery salmonids inter-breeding with wild salmonids. Fraser et al. (2010) found that increased genetic and phenotypic differences (such as collective environmental and life history differences, genetic divergence and geographic distance) between the parental sources of Atlantic salmon (*Salmo salar*) resulted in greater reductions in fitness in the natural environment. Backcrossing the hatchery salmon to the wild did not restore the phenotypes to values consistent with the wild population. Accidental releases of hatchery salmon into the wild

is a concern in this study area, and the potential impacts of these hatchery fish can cause losses in fitness in the depressed, wild populations.

In addition, other phenotypic traits can be altered in the hatchery populations. For example, the adipose fin is clipped on most anadromous hatchery fish in the Columbia Basin as a permanent mark for identification during harvest or hatchery activities. Although this mark is generally thought to have little effect on the performance of the fish, the fin has been identified to increase swimming performance and linked to male sexual characteristics. Male sexual characteristics such as increased body size, jaw size, and adipose fin increase in size during the breeding season. Female brown trout (*Salmo trutta*) have been shown to significantly select a male mate based on the size of the adipose fin (and other sexual characteristics), but there was no significant difference found in adipose fin size between the successfully spawned and not spawned males (Petersson et al. 1999). The effects of the adipose fin is suggestive of mate selection, a study comparing fin present with fin absent has not been conducted to identify the effect of excision.

In summary, research indicates substantial evidence of reduced relative reproductive success of hatchery salmonids. However, effects can vary widely depending on the environment, release strategies, strains, source brood (native vs. non-native) and success in the wild (Kostow et al. 2003; Narum et al. 2006b; Araki et al. 2007). Future direction of brood management suggests minimizing the number of generations in the hatchery. In addition, Caroffino et al. (2008) found promising results testing fry releases; however, the positive and negative effects of alternative release strategies should be thoroughly studied before being applied in larger scale programs.

Inter-species Hybridization with Cutthroat Trout

Interspecies hybridization occurs when species isolating mechanisms are disrupted. When hybrid progeny are fertile and readily backcross with the parental taxa, genetic mixing can be extensive. This genetic introgression can create a hybrid swarm where all the individuals in a population have hybrid ancestry and both parental genotypes are lost. Hybridization can occur naturally due to range expansion or natural disturbances that increase contact between previously isolated species. However, these natural evolutionary processes tend to create narrow hybrid zones limited by the organism dispersal distance and habitat-fitness relationship (Barton and Hewitt 1985).

Interspecies hybridization also occurs from non-native introductions threatening many native taxa (Rymer and Simberloff 1996). Non-native rainbow trout readily hybridize with native cutthroat trout resulting in fertile hybrids. This introgressive hybridization is thought to be the greatest threat to the conservation of several subspecies of native cutthroat trout (Allendorf and Leary 1988). Hybrid zones between native cutthroat trout and non-native rainbow trout tend to extend over broad geographic areas (Carmichael et al. 1993; Rubidge et al. 2001; Hitt et al. 2003; Weigel et al. 2003).

The native range of westslope cutthroat trout historically included basins in northern Idaho, western Montana and portions of Canada with several disjunct populations in Washington (Wenatchee, Methow and Yakima basins) and Oregon (John Day basin). However, the historic distribution of this subspecies is not certain (Behnke 1992). A small portion of the range of westslope cutthroat trout overlaps with native

rainbow/steelhead trout within the Columbia River Basin (Behnke 1992; Weigel et al. 2003), and these species were believed to be separated spatially and temporally (Behnke 1992). Westslope cutthroat trout are believed to be native to the Methow Basin in northcentral Washington, and would have co-evolved with rainbow/steelhead trout. Westslope cutthroat trout are also thought to be native to Lake Chelan, the basin adjacent to the Methow. Westslope cutthroat trout from Lake Chelan were propagated in a local hatchery since 1903 and fish from this brood were stocked in Washington (Behnke 1992). The Methow Basin and other local basins in north central Washington were extensively stocked with rainbow trout, steelhead trout, westslope cutthroat trout, and cutthroat X rainbow trout hybrids (Behnke 1992; Ostberg and Rodriguez 2006; Washington Department of Fish and Wildlife, personal communications).

Where native westslope cutthroat trout and steelhead populations overlap, hatchery steelhead trout and rainbow trout were stocked in the same streams and multiple strains of hatchery rainbow trout have been stocked during the last century (Weigel et al. 2003). In addition, hatchery rainbow trout are often stocked into high elevation streams and lakes, many of which were historically fishless (Weigel et al. 2003; Ostberg and Rodriguez 2006). The stocking of rainbow or cutthroat trout into these fishless high elevation habitats can create populations of non-native trout which can be a source of non-native parental fish (Ostberg and Rodriguez 2006).

Hybridization has been shown to be directional in some studies, and reciprocal in others (Ostberg et al. 2004; Ostberg and Rodriguez 2006). Mate selection in hybridized populations has not been well studied and could be affected by numerous factors such as size differences, spawning timing, abundance and availability of a suitable mate. Several

studies indicate bimodal distribution of hybrid genotypes indicating a mating preference or selection for hybrids closer to either parental genotype (Weigel et al. 2003; Ostberg et al. 2004). Size assortative mating is likely a factor in habitats where cutthroat trout and steelhead overlap. In coastal cutthroat X steelhead hybrids, first generation hybrids were produced by a female steelhead mating with a male cutthroat trout (Ostberg et al. 2004). However, it is also possible that size assortative mating could occur in the other direction when hatchery male steelhead intermediate in size may be less successful competing with larger steelhead for a conspecific mate could out-compete smaller male cutthroat trout.

Population Genetic Diversity in Steelhead

Heterozygosity and allelic richness are measures of diversity. *O. mykiss* populations have a wide range of values, but most commonly have values of heterozygosity around 0.6-0.8 and allelic richness between 4.0 and 12.0 in tested microsatellite loci, though more extreme values are documented (Heath et al. 2002; Narum et al. 2004; Narum et al. 2006a; Narum et al. 2008; Nielsen et al. 2009).

Population genetic differentiation is commonly measured with F_{ST} values. These values allow comparison within and across species (Templeton 2006; Allendorf and Luikart 2007). F_{ST} values are a reflection of the drift-migration process, and provide an indication of the relative relationship and exchange between sites or population groups. Anadromous fish have F_{ST} values that average around 0.10, which is intermediate between marine species and freshwater species (Waples 1998). *Oncorhynchus mykiss* populations tend to have F_{ST} values ranging between 0.10 to 0.20; however, values as

high as 0.38 have been documented (Heath et al. 2002; Narum et al. 2004; Narum et al. 2006a; Narum et al. 2008; Nielsen et al. 2009).

Life history is related to the level of genetic diversity and differentiation detected in *O. mykiss* populations. Resident populations tend to have greater isolation and hence lower genetic diversity and higher genetic differentiation as a result of genetic drift (Narum et al. 2008; Nielsen et al. 2009). Values of genetic differentiation and diversity of resident *O. mykiss* populations (Neville et al. 2009) are similar to other resident salmonids such as bull trout (Costello et al. 2003) and cutthroat trout (Neville et al. 2006).

Comparison of genetic diversity and differentiation between sites in North America with sites in Kamchatka, Russia (McPhee et al. 2007) indicate that the *O. mykiss* populations in Kamchatka have lower genetic diversity and similar genetic differentiation (Table 1.1). It also appears that populations with higher hatchery influences, such as the Grande Rhonde population (Narum et al. 2006a) may have excessive heterozygosity and allelic richness. Increases in allelic richness from hatchery fish were documented in *O. mykiss* in Lake Michigan (Bartron and Scribner 2004).

Population genetic measures in *O. mykiss* populations have been found to be temporally stable over the short term (<=1 generation). Several studies found that sampling in subsequent years did not result in differences in population genetic measures (Narum et al. 2004; Heath et al. 2002). Yet longer term studies found mean number of alleles and heterozygosity measures were not significantly different, but population differentiation (F_{ST}) values were significantly different over a 40 year period (Heath et al. 2002). Studies encompassing these longer timeframes (several decades) could detect

genetic differences caused by drift and changes in habitat condition, hatchery and harvest practices.

The Upper Columbia and the Methow Basin Steelhead

The Grand Coulee Fish Maintenance Project mitigated for the construction of Grand Coulee Dam during the 1930s. Hatchery activities intended to replace lost production of anadromous salmon and steelhead from tributaries blocked upstream of the dam. The Wenatchee, Entiat and Methow subbasins are located downstream of Grand Coulee Dam and are utilized to rear and release salmon and steelhead for this extensive hatchery mitigation program. The U. S. Fish and Wildlife Service manages this program in the Leavenworth Complex which includes several fish hatcheries located in each of these basins. In addition, the State of Washington also manages several hatcheries as fisheries mitigation programs in these basins. In the Methow, the State manages two hatcheries one located at Wells Dam on the Columbia near the mouth of the Methow, and another in the upper basin near the town of Winthrop, WA.

The stock for all these hatcheries originated from collections on the Columbia River at Rock Island Dam, downstream of Wenatchee, Washington. These collections are believed to have been utilized for the original brood inter-breeding the returning adults from each of the major tributaries upstream. This brood was then used to establish local broods in each of the basins. Therefore, the hatchery stock is often considered to be a genetically homogenized brood that would have little local genetic attributes or

adaptations. In recent years, the Methow and the Wenatchee hatchery broods have been managed as demographically independent stocks.

Adult steelhead run returns are dominated by hatchery produced steelhead comprising more than 80% of the run (WDFW 2008). This led to including the hatchery stocks as protected under the ESA in the Upper Columbia as they are believed to be critical to the recovery of the species (McClure et al. 2003). There is considerable belief that the effect of the hatchery brood management in addition to the large proportion of hatchery returns to the basin has resulted in a loss of any localized genotypes and hence local adaptations. However, it is recognized that resident *O. mykiss* inter-breed with anadromous *O. mykiss* which could have maintained some native genotype in the basin. Examination of allozyme data collected from 10 populations in the Upper Columbia (3 from the Methow, 1 from Wells Hatchery) indicate that some site based genetic structure existed between the sites within a basin, but not between the basins. Additional analyses of these data indicate that the interpretation of these data is unclear (i.e. although there is no evidence for population structure, the data do not rule out the possibility of genetic structure) (NMFS 2001). Genetic studies in the Methow Basin have focused on hatchery and predominantly anadromous habitats located in the lower portions of the upper half of the basin (Twisp, Chewuch, and Methow rivers). Sampling in these areas does not allow the consideration of the range of life history types of *O. mykiss* in the Methow Basin to identify the role and the spatial relationships of genetic structure that could be maintained particularly in the smaller tributary stream habitats.

Barrier Removal and Irrigation Projects in Natal Tributaries

Although various programs related to the Columbia Basin Biological Opinions have created habitat restoration projects in the basin, the relative impact of the existing condition and expected response in population genetic diversity is unexplored. The intended response to barrier re-design projects is to increase available natal habitat and hence increase population size and reproductive habitats available to anadromous *O. mykiss* targeted for recovery under the Endangered Species Act. However, the unintended effects of high numbers of hatchery adults in the Methow basin combined with the potential reduced fitness of hatchery fish in the natural environment could create opportunities for the straying of these less fit fish into the re-opened accessible habitats. Barriers oftentimes protect native species from the effects of hatchery or other exotic species. Genetic effects of barrier removal projects has not been investigated in *O. mykiss*.

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Table 1.1. Comparison of population genetic measures from this study to other published values for *O. mykiss*. Table values include: total number of sites in study, number of sites that were anadromous life history (anad. sites), number of loci tested, expected (He) or observed (Ho) heterozygosity, allelic richness (AR), genetic differentiation (F_{ST}), significant isolation by distance (IBD, yes/no) and citation.

Stream	Total sites	Anad. Sites	No. Loci	He(Ho)	AR	F_{ST}	Sig IBD	Citation
Beaver, Libby, Gold Methow R., WA	19	8	16	0.67-0.83	4.53-6.88	0-0.18	Y anad, N resid	Chapter 3
Klickitat R., WA	20	7	13	0.46-0.82	2.8-9.0	0-0.38	Y anad, N resid	Narum et al. (2008)
Grande Rhonde R., OR	4	4	20	(0.76-0.81)	11.2-12.4	0.005-0.016		Narum et al. (2006a)
Walla Walla R, Touchet R., WA	14	12	6	0.8, 0.78	14.5, 13.7	0.001-0.018		Narum et al. (2004)
Snake R., ID	79	75	11	0.55-0.73	4.1-6.2	0.003-0.05	Y	Nielsen et al. (2009)
Skeena, Nass, Dean R., BC, Canada	10	10	6	0.75-0.85		Avg. 0.04	Y	Heath et al. (2002)
Kamchatka, Russia	7	5	10	0.24-0.54	1.9-9.8	0-0.19	Y	McPhee et al. (2007)

Figure 1.1. Diagram of genetic processes in trout and salmon.

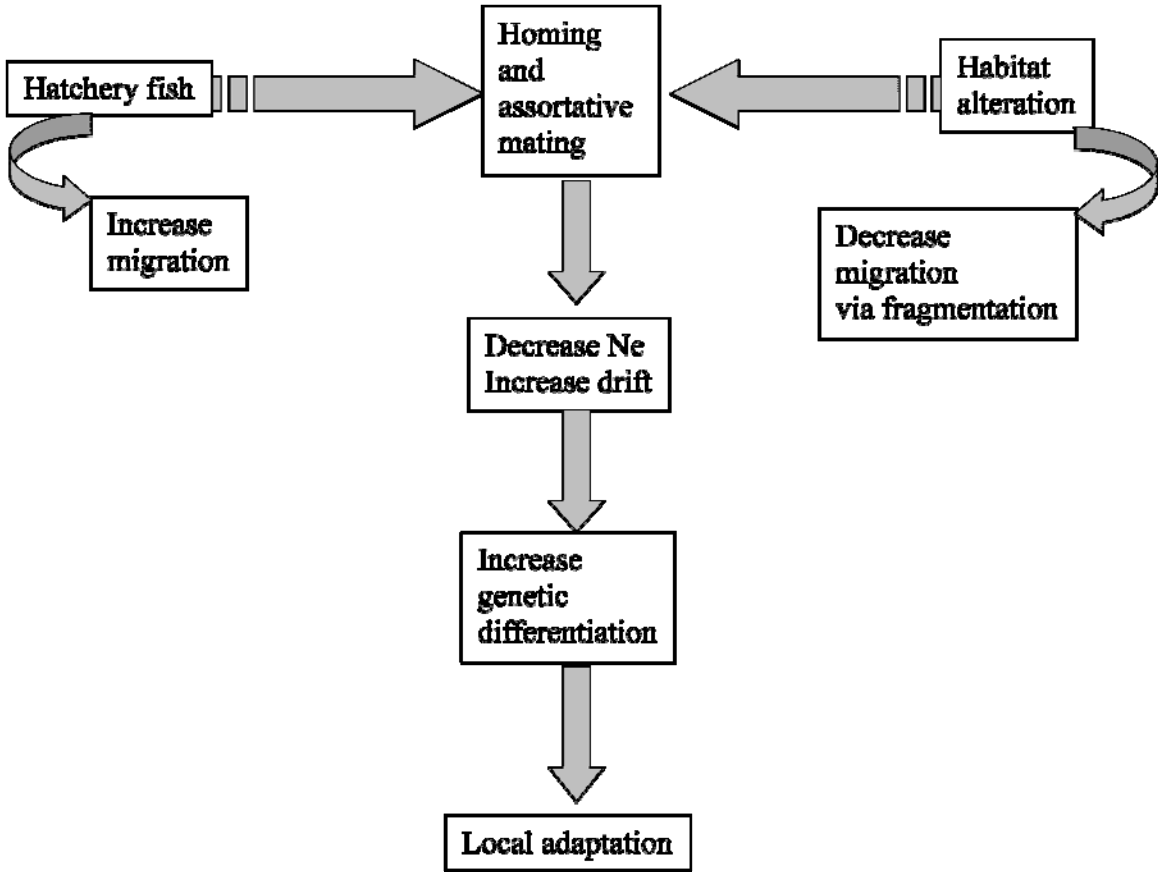
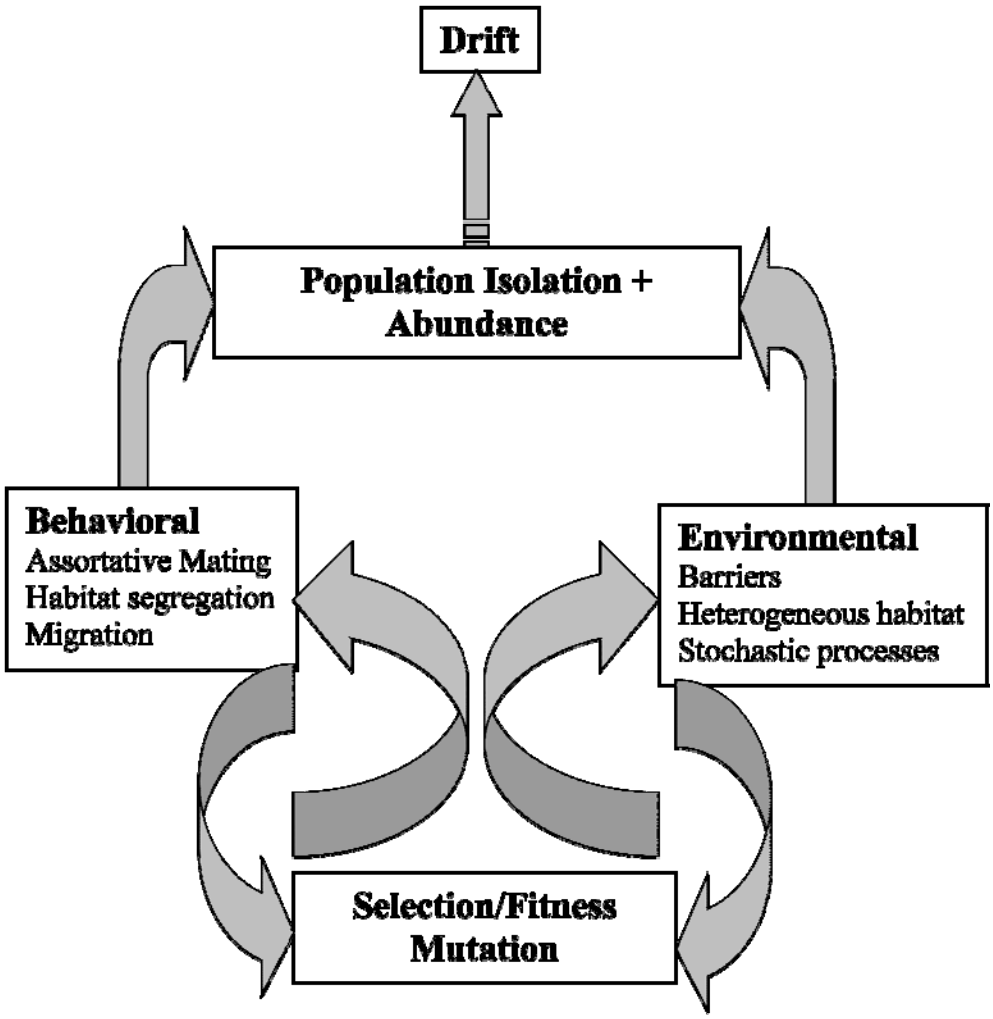


Figure 1.2. Diagram of evolutionary and genetic processes influencing genetic structure.



Chapter 2

Individual Fitness and Phenotypes of Steelhead (*Oncorhynchus mykiss*) Colonizing a Natal Stream After Barrier Removal

Abstract

Colonization and range expansion are important to the long-term persistence of populations and species. We used a parentage analysis to identify the source and phenotypes of successful colonizers of *Oncorhynchus mykiss* in a natal stream where fish passage was restored. Hatchery *O. mykiss* produced few (n=2) parr offspring and these did not return as adults. The proportion of hatchery admixture was not related to spawner success, but timing of adult migration was significantly related to spawner success. Assortative mating occurred by migration date, with earlier arriving adults producing more offspring that survived to parr. Offspring that returned as anadromous adults from these brood years were produced by parents that had only a few matching parr indicating that individual reproductive success may not be related to the number of parr produced by a parent. The fluvial life history polymorphism provided genetic compensation boosting the abundance and number of successful spawners particularly during 2006 when stream flow conditions were unusually high.

Introduction

Colonization and range expansion are important to the long-term persistence of populations and species. The meta-population theory states that localized colonization and extinction processes are ongoing in a landscape and populations interact in a source-sink dynamic (Hanski 1999). As a consequence of environmental variability, species and population ranges are continually expanding or contracting. The colonization process is often difficult to study in a natural setting as it is associated with unpredicted environmental disasters such as landslides (Lamberti et al. 1991), volcanic eruptions (Leider 1989), floods (Garant et al. 2000), forest fires (Neville et al. 2009) or chemical spills (Demarias et al. 1993).

In a catastrophic event, habitat can become unsuitable for rearing and/or reproduction for a period of time displacing individuals or causing widespread mortality. Eventually, individuals from the existing population or other population(s) return to the vacant habitat to re-colonize (Garant et al. 2000; Gaggiotti et al. 2004; Neville et al. 2009). Compatible local adaptations developed in a nearby and similar environment likely influence the success of the colonizer (Garant et al. 2000). However, other factors could determine the source and success of the colonizers, such as density of the source population and distance between the source population and the vacant habitat (Gaggiotti et al. 2004; Pess 2009).

Salmonids provide an opportunity for understanding the role of colonization under conditions of high levels of local adaptation resulting from fidelity to natal sites

combined with well-documented and complex mating system behaviors (Hendry and Stearns 2004). In addition, life history variation and hatchery populations contribute additional factors to the population demographic process (Araki et al. 2007a; Christie et al. 2011) that could affect colonization. Several species of trout and salmon have multiple life history strategies co-occurring in natal streams, such as resident (stream-rearing), fluvial (river-rearing) and anadromous (ocean-rearing) (Behnke 1992; Hendry and Stearns 2004). These various life history strategies are known to provide demographic and genetic support to species in variable or unstable environments and inter-breeding between these life history types is widely documented (Parker et al. 2001; Docker and Heath 2003; Araki et al. 2007a; Christie et al. 2011).

Artificial propagation also directly impacts migration and reproductive success of trout and salmon (Miller et al. 2004; Araki et al. 2007a, 2007b, 2008). Hatchery-produced fish provide an over-abundant source population available to colonize unoccupied habitats. Yet, hatchery steelhead are documented to have lower relative reproductive success in natural stream environments (Miller et al. 2004; Araki et al. 2007a, 2007b, 2008). Therefore, hatchery fish may not be a desirable source population for the colonization of newly opened habitats, but their role and potential contributions to the colonization process are not well understood. Hatchery steelhead and salmon are documented to have higher rates of straying (Quinn 1993). The demographic effect of hatchery fish on the colonization process due to high abundance and higher straying rates could reduce or eliminate the contributions from naturally produced fish. Yet, this demographic advantage is likely countered by the reduced fitness that could even result in unintended genetic or fitness effects on the colonizing population.

Colonizing individuals may also be subject to different biological constraints or processes due to low densities in the vacant habitat. For example, low numbers of colonizers could result in reduced genetic diversity, which could limit population viability over the long term (Allendorf and Luikart 2007). Early colonizers could be subject to different selective gradients (Anderson et al. 2010). Individual fitness could be increased or decreased depending on the compatibility of selective phenotypes in the vacant habitat combined with reduced densities (Lamberti et al. 1991; Kiffney et al. 2009).

Barrier removal projects create opportunities to study the colonization process (Kiffney et al. 2009; Anderson et al. 2010). Trout and salmon are typically target species for habitat restoration projects due to their threatened and endangered status in the U.S. (McClure et al. 2003). Barrier removal is oftentimes targeted toward increasing population distribution and abundance of the anadromous life history due to extensive impacts from harvest, hydropower, and variable ocean conditions (McClure et al. 2003). Barrier removal in combination with the co-existing life history strategies and hatchery populations of *O. mykiss* creates an opportunity where colonization can be studied while the resident *O. mykiss* populations are providing demographic stability. In this study, we used genetic data to document the reproductive success of colonizing *O. mykiss* trout after the modification of several small irrigation dams in Beaver Creek, a natal tributary to the Methow River, Washington. Trout were allowed to naturally colonize the restored habitat. Individual migrations and movements were monitored with passive integrated transponder (PIT) tags and tag reading stations. The objectives of our study were to: 1) identify abundance and source of colonizing steelhead; 2) determine individual

reproductive success of colonizing adult steelhead; and 3) identify attributes of successful colonizers.

Study Area

The Methow River is located on the east side of the Cascade Mountain Range in north-central Washington, and is a tributary of the Columbia River located about 843 km upstream from the estuary. Beaver Creek is a 3rd order natal tributary located on the east side of the Methow Basin that flows west into the Methow River 57 km upstream from the mouth (Fig 2.1). The Beaver Creek watershed is 290 km^2 with basin elevations that range from 463 to 1,890 m and stream flows that ranged from 0.05 to 4.7 m^3/s during our study, 2004-2008 (Martens and Connolly 2010).

Access for migratory fish into Beaver Creek was disconnected due to water withdrawal and diversion structures for more than 100 years (Martens and Connolly 2010). Resident *O. mykiss* were present throughout Beaver Creek and tributaries prior to implementing the barrier removal projects. Anadromous *O. mykiss* (steelhead) and Chinook salmon were present downstream from the lowest diversion dam (Martens and Connolly 2010). From 2000 to 2004, seven small irrigation diversion dams (1.0 to 2.0 m high) were modified to Rosgen vortex weirs that allow fish passage (Ruttenberg 2007; Martens and Connolly 2010). The most downstream irrigation diversion was a 2.0 m high concrete dam that was modified to allow fish passage after the fall 2004.

Hatchery Releases

The Grand Coulee Fish Maintenance Project provides mitigation for the construction of Grand Coulee Dam during the 1930s. The Wenatchee, Entiat, Methow and Okanagan rivers are located downstream of Grand Coulee Dam and salmon and steelhead are released into these rivers for this extensive hatchery mitigation program. The State of Washington also manages a hatchery program to mitigate for other hydropower facilities on the Columbia River.

The stock for all these hatcheries originated from collections on the Columbia River at Rock Island Dam, downstream from Wenatchee, WA. This brood was established from the returning adults to this dam assumed to be migrating to the major tributaries upstream (Chapman et al. 1994). This brood was later used to establish local broods for each of the separate river basins. In recent years, the Methow and the Wenatchee hatchery broods have been managed as demographically independent stocks.

Hatchery mitigation strategies in the Methow during our study included a release of 450,000 – 550,000 *O. mykiss* smolts per year that are spawned and reared at Wells Hatchery on the Columbia River (rkm 830.1) downstream from the mouth of the Methow River. The hatchery-origin steelhead are crossed with natural-origin steelhead and progeny from these crosses are primarily released into the Methow River and tributaries (C. Snow, Washington Department of Fisheries and Wildlife, personal communication). All hatchery-origin steelhead were marked with an internal tag (such as a PIT tag), external tag (such as an elastomer tag) and/or fin clip. Hatchery-origin adults comprised

the majority of the adult returns to the basin. During our study (2005-2008), hatchery steelhead returns to Wells Dam ranged from 82% in 2008 to 91% in 2005 (Snow et al. 2010).

Methods

Stream Sampling

Adult and juvenile *O. mykiss* were captured in Beaver Creek using a picket weir installed 1.3 km upstream from the mouth that captured fish moving upstream and downstream (Fig. 2.1). The trap was operated from March 20 to May 9 and May 14 to December 5 during 2005; February 13 to May 1 and June 27 to November 27 during 2006; February 24 to March 30 and May 25 to November 29 during 2007; and February 24 to May 3, July 11 to July 30 and September 2 to December 10 during 2008. Gaps in weir collection during May, June, December and January were due to high stream flows or stream icing preventing weir operation. In 2008, the weir was not operated during August because data from previous years indicated little downstream movement by juveniles during this month. The date, direction of movement, fork length (mm), and weight (g) were recorded for adult and juvenile *O. mykiss*. In addition, gender and wild or hatchery origin were recorded for adults. A tissue sample was removed from the caudal fin and stored in 95% non-denatured ethanol. Fish were searched for tags and external marks. If the trout did not have a PIT tag, one was inserted in the dorsal sinus cavity for adult trout or the body cavity for juvenile trout.

Movements of *O. mykiss* trout were monitored using a network of stationary PIT tag reading stations in Beaver Creek (as described in Connolly et al. 2008) (Fig. 2.1) and at dams and passage facilities on the mainstem Columbia River. Migratory life history (anadromous or fluvial) of the adult trout was identified using PIT tags. Fluvial individuals left Beaver Creek and were not detected at any of the Columbia River facilities. Anadromous individuals were read on the mainstem Columbia River during upstream migration. Adult collections were nearly complete in brood years 2005 and 2006 to allow for parentage analysis of offspring. Although parr collection at the trap was incomplete due to periods when the fish trap was inoperable, the collection was considered random and representative of juvenile parr outmigrating from the spawning populations in the stream.

Laboratory Methods

Tissue samples from the Wells Hatchery brood years 2005 and 2006 (hatchery x hatchery crosses) were provided by the Washington Department of Fisheries and Wildlife (WDFW). Sixteen microsatellite loci were used to identify individuals. Thirteen of these markers are standardized across the Columbia River Basin and are cited in Stephenson et al. (2009). Additional primer sets analyzed were: *One102* (Olsen et al. 2000), *Omm1036,* and *Omm1046* (Rexroad et al. 2002).

DNA was isolated from fin clips preserved in ethanol using Qiagen DNEasy tissue extraction kits following standard manufacturer's protocols. Sixteen microsatellite loci were amplified using the polymerase chain reaction (PCR) in three multiplex

reactions using Qiagen Multiplex PCR Master Mix on Applied Biosystems GeneAmp PCR System 9700 thermal cyclers in 96 well plates. PCR products were run on an Applied Biosystems 3730 genetic analyzer. Peaks were scored using GeneMapper version 3.7 software (Applied Biosystems, Foster City, California), and labeled following the Stevan Phelps Allele Nomenclature (SPAN) convention (Stephenson et al. 2009). Forward primers were fluorescently labeled (Applied Biosystems).

Amplification (PCR) reactions consisted of 5 ul reactions containing 2.5 ul Qiagen Multiplex PCR Master Mix, five or six primer sets and water, added to 2 ul of extract dried down in a 96 well plate. Cycling conditions included initial denaturation for 15 min at 95°C, followed by 28 cycles for 30 s at 94°C, 90 s at 51°C (Multiplex A) or 57°C (Multiplex B and Multiplex C), and 60 s at 72°C, followed by a final cycle for 30 min at 60°C. Multiplex A contained *Oki23*, *Oke4*, *Oneu14*, *Ssa289*, and *Ssa408*; Multiplex B contained *Ots4*, *Omy7*, *Ogo4*, *One102*, *Omm1046*, and *Ssa407*; Multiplex C contained *Ots100*, *Omy1011*, *Omy1001*, *Ots3m*, and *Omm1036*.

Amplification products were diluted with 10 ul DNA grade water and 1 ul of each dilution was added to 10 ul of LIZ/formamide solution (30 ul LIZ600 to 1 ml formamide). Completed runs were analyzed automatically using Genemapper, followed by manual analysis of all peaks for verification. All homozygous results were checked for small allele dropout and large allele dropout. Peaks were also visually checked for conformity to expected profiles. Laboratory error rates for the 13 standardized loci are <2% (Stephenson et al. 2009). Duplicate samples indicate laboratory error rates <1% for our study.

Statistical Analysis

An exclusion analysis for all candidate parents and offspring was performed for brood years 2005 and 2006 collected from Beaver Creek during 2005 through 2008 using CERVUS version 3.0.3 (Marshall et al. 2002). One mismatch allele was allowed for genotyping error or null alleles. Individuals with 1 mismatching allele were a small portion (4%) of the sample (n=7 of 165 in brood year 2005 and n=3 of 75 in brood year 2006). Due to the complex life history of *O. mykiss*, complete sampling of parents was not possible. Therefore, we did not attempt to use other methods to infer parentage and expected one parent matches to be common in the data set. The probability of exclusion over all loci was >0.9999 (Marshall et al. 2002). *Oncorhynchus mykiss* sampled during the spawning period as small as 150 mm were included in the parentage analysis as candidate parents to search for small precocious males. However, *O. mykiss* <180 mm were not included in further analyses to avoid including juvenile parr (immature) that would result in excessive individuals that produced 0 offspring in the sample. Only one small male parent less than 180 mm was identified, and excluding this individual from further analyses did not affect the results.

Adult trout collected at the weir were grouped by life history (anadromous or fluvial) and compared to hatchery samples from the Wells Hatchery brood. Life history was compared across the different years in the sample. Exact tests of Hardy Weinberg Equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0.10. Heterozygosity, genic differentiation and F_{ST} were calculated using GENEPOP version

4.0.10 (Raymond and Rousset 1995). Unbiased estimates of allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). All comparisons were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

Parent samples were grouped by year and by whether they produced offspring or not. The offspring and no offspring groups were compared for each year for heterozygosity and genic differentiation using GENEPOP version 4.0.10 (Raymond and Rousset 1995). Unbiased estimates of allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). All comparisons were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

The proportion of hatchery genotype was estimated for each adult *O. mykiss* in our sample by analyzing the data from adults sampled at the weir with known hatchery steelhead from Wells Hatchery (n=99) using STRUCTURE version 2.3.3 (Pritchard et al. 2000). The admixture model was run in STRUCTURE using 10,000 iterations for burn in and 100,000 iterations using a Markov Chain Monte Carlo resampling algorithm as described in Prichard et al. (2000). Number of populations (K) was set to 2 and all other settings were run using default values. Program settings were verified by examining the output from multiple runs of the data for consistency. Ten independent runs were performed and the run with the lowest log likelihood was chosen as the best run.

Adults that produced offspring were compared to adults that did not produce offspring in our sample using a Wilcoxon Rank Sum test in R (R Development Core Team 2010). A generalized linear model (GLM) using a negative binomial distribution was used to predict the number of offspring produced by individual adult *O. mykiss* from phenotypes. The independent variables for the GLM were selected based on Pearson

correlations and relationships between the variables. Predictor variables that were highly correlated (r>0.80) were not used in the model to avoid multicollinearity. Therefore, we included weight as an indicator of size and life history in the model. The date past the fish trap was assigned the number of days counted from January 1 of the brood year that the adult *O. mykiss* was recorded past the weir. Independent variables used in the models were: weight (g), day past weir, proportion hatchery genotype, and interactions between these variables.

A global model included all of the candidate predictor variables and interaction variables. From this global model, various combinations of predictor variables were chosen for comparison to the global model. The relative plausibility of the models were compared using Akaike's Information Criteria (AIC, Akaike 1973; Burnham and Anderson 1998) with the best fitting model having the lowest AIC value. A goodness-of-fit test was used on the best fitting model to test whether the data could plausibly arise from the model. All GLMs and goodness-of-fit tests were performed using R (R Development Core Team 2010). Assortative mating was tested on successful spawning pairs using a Spearman correlation in R.

Results

Weir captures in combination with the stationary tag readers allowed some estimation of missing adults in the sample. In 2005, 36 adult *O. mykiss* were captured in the weir between March 25 and May 7. In 2006, 43 candidate adult *O. mykiss* were sampled at the weir between March 15 and April 7. In 2005, 3 adult anadromous *O.*

mykiss were known to be missing from our sample. One adult was not captured but recorded in the PIT tag readers, and DNA could not be extracted from two tissue samples. In 2006, two adult anadromous *O. mykiss* were missing from our sample one due to failed DNA extraction and the other was not captured but recorded in the tag readers.

The anadromous adult returns to Beaver Creek were fairly consistent in 2005 (n=27) and 2006 (n=23), whereas the number of adult fluvial returns were more variable (n=9 in 2005 and n=20 in 2006). In 2005, fluvial *O. mykiss* were captured in the fish trap between April 2 and April 15 and anadromous *O. mykiss* were captured between March 25 and May 14. In 2006, fluvial *O. mykiss* were captured in the fish trap between March 15 and April 21 and anadromous *O. mykiss* were captured between March 24 and April 6. These dates overlap in migration timing of the life history strategies. The anadromous and fluvial life histories separated by size with anadromous *O. mykiss* >500 mm (Table 2.1). Only 7.6% of our adult captures in Beaver Creek for brood years 2005 and 2006 were identified as hatchery *O. mykiss*, and none of the fluvial trout were hatchery origin.

Number of alleles per locus ranged from 7 to 30 in our sample and average allelic richness ranged from 7.0 to 7.7 (Table 2.2). Tests of Hardy Weinberg Equilibrium did not detect significant departures in the 2005 or 2006 adult *O. mykiss* captured at the weir. Exact tests for the Wells Hatchery samples indicate the samples were in Hardy Weinberg Equilibrium. Tests for linkage disequilibrium found two pairs of loci in the 2006 adult *O. mykiss* data and 6 pairs of loci in the Wells Hatchery samples that were significant; however, there was no pattern to these pairs of loci and these numbers do not exceed the number of significant tests expected by chance.

We did not detect significant differences in genetic differentiation between years for anadromous, fluvial or hatchery *O. mykiss*; therefore, we combined the samples from the two years for comparisons among these groups. Genetic diversity was similar across all the life histories and years in the sample (Table 2.2). Exact tests of genetic differentiation were not significant comparing the anadromous and fluvial samples ($p=0.049$), but were significant comparing the anadromous and Wells Hatchery sample ($p=0.001$) and the fluvial and Wells Hatchery samples ($p<0.001$). F_{ST} estimates across these groups showed little differentiation: wild anadromous to hatchery =0.002, fluvial to hatchery = 0.006, wild anadromous to fluvial = 0.004.

A total of 1,544 candidate offspring were tested for parentage from which 243 (16%) offspring matched to a parent in brood years 2005 and 2006. Most of the matching offspring were collected as age 0 or age 1 parr outmigrants with age 2 and older parr only providing 10% of the total number of matches. A total of 168 parr tested matched to brood year 2005 with 43 individuals matching only one parent. A total of 75 parr matched to brood year 2006 parents with the majority (n=71) of these matching only one parent (Fig. 2.2). One-parent matches are the result of missing adults from the sample, either missed at the fish trap, failed DNA extraction, or originating from resident populations in the stream. The complex life history interactions and spring spawning during the higher stream flows makes complete sampling of candidate parents in studies of *O. mykiss* difficult.

The number of successful mates ranged from 1 to 3, yet more than 50% of the two-parent matches only had one mate per individual. Two individual *O. mykiss* (1 male and 1 female) produced offspring with three different mates in the same spawning season.

The adult that produced the most offspring (n=58) was an anadromous wild male (593 mm) who bred with two large females (700 mm and 749 mm) in 2005. One anadromous wild female spawned in both years and matched to 37 offspring in 2005 and 3 offspring in 2006. Matings by life history indicated that inter-breeding was common among the life history types. Proportions between these groups varied drastically between 2005 and 2006 (Fig. 2.2). Interestingly, no offspring from fluvial x fluvial crosses were detected in our samples.

Due to differences between the two years, we did not combine the 2005 and 2006 samples for most statistical comparisons. Heterozygosity (He) was similar for all the groups and years (Table 2.3). Allelic richness and private alleles were slightly lower in the adults that produced parr offspring in the 2005 samples, and private alleles were slightly lower in the 2006 adults that produced parr (Table 2.3). The 2005 adults that produced parr were significantly larger than adults that did not produce parr (Fig. 2.3, length $p=0.017$, weight $p=0.001$). The 2006 adults that produced parr were not significantly different in size than those that did not produce parr (Fig. 2.3, length $p=0.27$, weight $p=0.42$). The day past the weir was not significantly different for adults that produced parr from those that did not produce parr in either year (Fig. 2.3, 2005 $p=0.46$, 2006 $p=0.92$).

The number of matching parr offspring per individual parent ranged from n=0 to 58 (mean=7.0, SD=13.0) in 2005 and from n=0 to 23 (mean=1.7, SD=4.4) in 2006. Larger anadromous steelhead produced most of the matching offspring in 2005, whereas the smaller fluvial rainbow trout produced most of the matching offspring in 2006 (Figure 2.4). Adults captured in Beaver Creek ranged from 4 to 95% hatchery admixture.

Model selection found that the models combining length, weight, proportion hatchery admixture, and the interactions between these variables to be equally plausible predicting the number of parr produced by males for brood year 2005 (Table 2.4). Model selection found that the model using day past the weir was twice as likely as the next best fitting model predicting the number of parr produced by females for brood year 2005 (Table 2.4). The best model predicting the number of parr produced by individual parents in the 2006 data set showed the model using length as the best fitting predictor for number of parr offspring for males, however the proportion hatchery admixture and day were also plausible models (Table 2.5). Models using various combinations of proportion of hatchery admixture, day past the weir and length were equally plausible predicting the number of parr offspring for females in brood year 2006 (Table 2.5). The goodness-of-fit tests for each year were not significant ($p=0.18$ and 0.89 for 2005 and 2006, respectively).

Correlations between the spawning pairs that successfully produced parr offspring from the 2005 and 2006 sample indicated disassociation in male and female size. Length was not significant ($p=0.11$, rho=-0.40) and weight was not significant ($p=0.08$, rho= -0.43). Day past the weir had a significant positive association ($p<0.001$, rho=0.84) and the proportion of hatchery admixture had a positive association that was not significant ($p=0.35$, rho=0.24). If only examining the 2005 spawning pairs, size had a disassociation that was significant (length, $p=0.009$, rho=-0.69; weight $p=0.005$, rho=-0.72). Day past the weir was significant ($p=0.006$, rho=0.72) and proportion of hatchery admixture was not significant ($p=0.56$, rho=0.18). These data generally indicate that successful spawning pairs were different sizes, and individuals that arrived near the same date

spawned together. The proportion of hatchery admixture does not appear to be related to successful spawning pairs.

Instream PIT tag readers identified two adults that successfully returned to the Methow Basin from brood year 2005 and one adult in brood year 2006. Two of these adults were offspring from one-parent matches (one anadromous, one fluvial) and one adult was the offspring from an anadromous female and fluvial male parents. The parents from these three adult returns produced few parr offspring (n=1, 4 and 6) detected in our sample, indicating that individual reproductive success may not be directly related to the number of parr produced. These surviving offspring all outmigrated from Beaver Creek in October 2005 (age 0) or September 2007 (age 1 and 2). All of the matching offspring in brood year 2006 were tagged allowing us to estimate parr-to-adult survival for this brood year at 1.3%.

Discussion

Expected heterozygosity and average allelic richness were similar to values documented for *O. mykiss* in other studies across North America (Heath et al. 2002; Narum et al.2004, 2006, 2008; Nielsen et al. 2009). In addition, the *O. mykiss* in our study had similar spawning behavior and reproductive success as documented in another established population of steelhead (Seamons et al. 2004). Resident or fluvial rainbow trout have been found to contribute substantially to the anadromous populations indicating that these life history strategies do not function as separate populations (Araki et al. 2007a; Christie et al. 2011). Lastly, hatchery steelhead were a very small

component of the adult returns to the colonizing population and produced only two parr neither of which return as adults, indicating no detectable genetic contribution to the re-establishment of the steelhead during our study.

Colonization and Spawning Attributes

Migratory adult *O. mykiss* produced offspring in Beaver Creek during the first spawning season after barrier removal. Matching offspring in our study were lower (16%) than other studies performed on *O. mykiss* where 50% of the candidate offspring sample matched to parents. In our study and another steelhead parentage study, one-parent matches were common (Seamons et al. 2004). Factors such as the location of the collections, the number of migratory *O. mykiss* in relation to the number of resident rainbow trout, and the spatial extent of the resident populations will affect the proportion of no-parent and one-parent matches in the samples. Sampling strategy and the extent of the resident populations in Beaver Creek are not comparable to other parentage studies on *O. mykiss*. Although allowing only one mismatching allele for the exclusion assignments might reduce the number of matching parr, we found this was minimal in our sample and suspect that the extensive resident population in combination with a few migratory adults entering the newly opened habitat likely influenced the proportion of parent-offspring matches in the sample. The sampling strategy of collection of parr at the weir resulted in collections from all populations upstream. We used wide ranges of size classes to query samples for the brood year analyses due to variable growth rates longitudinally in the

stream and overlapping generations. Therefore, we analyzed a wider range of samples than we matched to ensure inclusion of all possible matches in our analysis.

Our results on mating behavior and individual fitness are similar to other studies in streams with established populations of anadromous *O. mykiss* (Seamons et al. 2004; Araki et al. 2007a). *O. mykiss* also had multiple spawning partners similar to another study providing additional genetic variation (Seamons et al. 2004). Migration date was the only variable that was significantly and positively correlated with the successful pairing of adult *O. mykiss* in our study, which was also found in Seamons et al. (2004). The successful mating pairs had a disassociation with size that was significant for the 2005 brood year parents. The successful matings were found to have a larger female or a larger male so there was no identifiable patterns in the data between size and gender. Proportion of hatchery admixture was not related to mate choice. The complex spawning relationships between individual adults and life history strategies would add genetic variation to the small spawning population, and reduces the effect of assortative mating which could be a strong micro-evolutionary process (Templeton 2006).

Individual adult *O. mykiss* in our study ranged from producing 0 to 53 parr offspring. Interestingly, the majority of the offspring in our study (84% in 2005 and 75% in 2006) were produced by a few adults (n=8 in 2005, n=4 in 2006) indicating unequal and varying levels of genetic contributions from adults in the population. However, individual reproductive success is measured to the adult life stage. PIT tag detections of adult returns to Beaver Creek from brood years 2005 and 2006 were low (n=3), so we cannot estimate individual reproductive success. Yet, these returning adults were not

offspring from the highest producing *O. mykiss* in the sample, and those that did return indicated a large contribution of the fluvial life history to these returning adults.

Size in adult salmonids has been shown to be related to higher fecundity in females and greater dominance in males (Gross 1984; Beacham and Murray 1993; Fleming 1998; Taborsky 1998; Theriault and Bernatchez 2007). Therefore, it is believed that size will be directly related to spawning success of the individual adult. However, more recent genetic studies found that size is not significantly related to the number of surviving offspring (Seamons et al. 2004; Dickerson et al. 2004). In our study, adults from brood year 2005 that successfully produced offspring were significantly larger (length and weight) than adults that did not produce offspring. But, in brood year 2006, size (length, weight) was not significantly different between the adults that produced offspring and those that did not, likely due to the higher success of the smaller fluvial adults. The GLM results indicate that size was positive in 2005 and negative in 2006 depending on the success of the anadromous component of the population. Therefore, the results on size were confounded.

Run timing is considered an important factor in spawning success (Anderson et al. 2010). Although earlier spawning adults could give offspring an advantage with earlier emergence, developmental timing is also related to stream temperatures. Therefore, the length of egg development could be shorter for later spawning *O. mykiss* as stream temperatures are warming during the springtime months. Day past the fish trap was not a significant variable in our data set, but did indicate interaction with the other variables in the GLM for the 2005 data.

Adaptive evolution likely increases selection on colonizers in the newly opened habitat. Anderson et al. (2010) found that selection values on body size and migration date were greater during colonization of coho salmon (*O. kisutch*) after restoring fish passage at a dam compared to other studies. Early spawners had an advantage during the first year after colonization; however, intermediate arrival dates had a greater advantage in the second and third years (Anderson et al. 2010). In our study, early spawners had produced more parr during the first year after passage was restored to Beaver Creek. However, it is important to note that selection models can change during colonization potentially changing some of these relationships (Anderson et al. 2010).

Role of Life History

Fluvial and anadromous *O. mykiss* have not been shown to be separate populations within the same basin (Zimmerman and Reeves 2000; Docker and Heath 2003), and our data indicate no significant difference between allele frequencies in these two groups. Even though we detected significant differences in allele frequencies between the hatchery steelhead and the wild rainbow/steelhead, F_{ST} values among these groups are close to zero indicating little differentiation. However, when using highly polymorphic microsatellite loci, the population differentiation measures derived from heterozygosity (such as F_{ST} and G_{ST}) can incorrectly indicate zero differentiation (Jost 2008). Fluvial rainbow trout and hatchery steelhead had significantly different allele frequencies, whereas fluvial rainbow trout and wild steelhead were not significantly different. Our parentage results indicate breeding between the wild steelhead and the

fluvial rainbow trout, and hatchery practices maintain gene flow between the wild and hatchery steelhead. Fluvial rainbow trout captured in Beaver Creek were all naturally-produced, and the significant difference in allele frequencies between the fluvial rainbow trout and the hatchery steelhead support that fluvial rainbow trout in our study are not hatchery residuals.

The composition of the life history of the parent matches were substantially different between 2005 and 2006, perhaps related to the extremely high spring flows in 2006. In 2006, fluvial *O. mykiss* and one-parent matches were a larger proportion of the data set, and post-spawning stream flows in the study area and the Methow Basin were three times higher in magnitude than during the other years of our study (Ruttenberg 2007; USGS 2012). Alternate life history strategies are thought to provide demographic stability in less stable environments (Parker et al. 2001). Interestingly, we did not document any fluvial x fluvial crosses indicating that it may be more common for these fluvial *O. mykiss* to attempt to mate with an anadromous or resident partner. Araki et al. (2007a) found that resident *O. mykiss* were providing genetic compensation during years when the anadromous adult abundances were low. Christie et al. (2011) estimated that 20% of anadromous *O. mykiss* genes arise from the resident life history and significantly more anadromous *O. mykiss* matings occur with a resident male. During colonization, this inter-breeding among the multiple life history polymorphisms should increase the number of breeders and genetic variation. Most of the fluvial *O. mykiss* captured in the weir were male indicating a sex bias for this alternate life history polymorphism. Therefore, this interaction between life history and gender would result in high proportions of cross breeding among the life history strategies.

The effective population size is important to understanding the expected rate of genetic drift and, hence, maintenance of genetic diversity in the population over time (Allendorf and Luikart 2007). The effective number of breeders is used for populations with overlapping generations (Heath et al. 2002). Nunney (1992) suggests that the effective population size to total population size should be approximately 0.5 under most natural conditions. In our study, the ratio of the number of breeders to the total number of adults is 0.64 and 0.34 for brood years 2005 and 2006, respectively (average 0.49). Other studies have estimated similar effective breeder to total population size ratios in *O. mykiss*: 0.53 (Ardren and Kapuscinski 2003) and 0.13 to 0.54 (Araki et al. 2007a). In addition, Araki et al. (2007a) document the contribution of the fluvial *O. mykiss* to the effective number of breeders. In Beaver Creek, the initial population of anadromous adult *O. mykiss* into the study area is relatively low (<25 individuals), however the genetic compensation from the fluvial life history provided considerable boost to the breeding population resulting in adequate breeder to total number of spawner ratios. These ratios indicate that genetic diversity is likely adequate during the colonization of Beaver Creek even though population abundances were low.

Role of Hatchery Fish

Hatchery fish were a very small component of the colonizing adult *O. mykiss* to Beaver Creek even though they represent a large majority of the adult returns to Wells Dam (n=3 in 2005 and n=3 in 2006). The proportions of hatchery to wild *O. mykiss* can change from counting points lower in the basin to those in natal tributaries. For example,

hatchery fish may be harvested in the recreational fishery or may be returning to other stream locations closer to a hatchery or to a release site. Lieder (1989) also found substantially higher proportions of hatchery *O. mykiss* at a counting location near the mouth of the larger mainstem stream when compared to a natal tributary further upstream. Too few hatchery fish returned to Beaver Creek to estimate relative reproductive success. In our study, only one hatchery adult produced parr (n=2) and none of these parr were detected as adults. I could not determine why the hatchery escapement and reproduction was low in this study, but it appears that low hatchery contributions were largely a result of low numbers of hatchery-produced trout into the stream.

The hatchery program releases smolts from wild x hatchery crosses into the Methow River and tributaries creating a high amount of admixture between the hatchery and wild population complicating genetic detection. The proportion of hatchery admixture was not significantly related to the success of spawning *O. mykiss* in this study; yet, there is substantial documentation that hatchery *O. mykiss* have a lower relative reproductive success (Miller et al. 2004; Araki et al. 2007a, 2008). The proportion of hatchery admixture was not related to the number of parr produced nor the selection of a successful mate, although the GLM indicates interaction between variables measured. The allele frequencies were significantly different between both the anadromous and fluvial returns to the weir and the Wells Hatchery fish; yet, the STRUCTURE output indicated some admixture in all the individuals in the sample. In general, these results indicate that hatchery admixture is not related to the number of parr produced; however the Methow system may not provide a good model for direct

comparison of relative reproductive success due to the high levels of admixture and intentional cross breeding in the hatchery.

In summary, our study found that anadromous *O. mykiss* successfully reproduced the first spawning season after the habitat was re-connected. Spawning behavior, attributes of successful spawners, and the individual fitness of the colonizing *O. mykiss* were generally similar to those documented for other well established, equilibrium populations of *O. mykiss* indicating similar spawning dynamics were occurring during colonization in Beaver Creek. A relatively low number of anadromous adults migrated into Beaver Creek and a small number of adults contributed to the majority of outmigrating parr offspring. Although hatchery *O. mykiss* were prevalent in the Methow Basin, they produced few parr and no returning adults in Beaver Creek. The fluvial component of the *O. mykiss* population provided genetic compensation boosting the abundance and number of successful spawners particularly during 2006 when springtime stream flows were exceptionally high. Therefore, the contribution of the fluvial component of *O. mykiss* populations in colonization and other demographic and genetic processes should not be discounted particularly in unstable environmental conditions.

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Table 2.1. Range (mean) of fork length (mm) and weight (g) for fluvial and anadromous adult *O. mykiss* captured at the fish trap in Beaver Creek during 2005 and 2006.

Year	N	Length (mm)	Weight (g)
Fluvial			
2005	9	189-472 (291.7)	69.8-1134 (388.9)
2006	20	182-500 (293.9)	60.4-1270 (340.2)
Anadromous			
2005	27	518-700 (630.3)	1120-2903 (2191.8)
2006	23	560-832 (662.2)	1440-3767 (2481.9)

Table 2.2. Genetic diversity (unbiased heterozygoisty (H), average allelic richness (AR), and average private alleles (PA)) for adult anadromous (AW) and fluvial (FW) *O. mykiss* collected in the fish trap in Beaver Creek and Wells Hatchery (WH) brood years 2005 and 2006. Wells Hatchery samples were hatchery x hatchery crosses provided by the WDFW.

Years separate				
Group	Number	H	AR	PA
AW05	27	0.81	7.2	0.5
AW06	23	0.83	7.4	0.5
WH05	49	0.81	7.0	0.4
WH06	50	0.82	7.6	0.3
FW05	9	0.83	7.7	0.6
FW06	20	0.81	7.1	0.5
Years combined				
AW	50	0.83	7.4	1.4
WH	99	0.82	7.0	1.2
FW	29	0.82	7.3	1.5

Table 2.3. Genetic diversity (unbiased heterozygosity (H), average allelic richness (AR), and average private alleles (PA)) for colonizing adult *O. mykiss* who produced or did not produce parr offspring for brood years 2005 and 2006.

	Number	H	AR	PA
2005				
No offspring	13	0.82	7.6	2.7
Offspring	23	0.82	7.2	2.4
2006				
No offspring	38	0.82	7.1	2.4
Offspring	20	0.82	7.1	2.3

Table 2.4. Models variables, AIC score, number of parameters (K), adjusted AIC score (AICc), model weights and evidence ratio for brood year 2005 by gender. Variables included in the model are: weight (g), fork length (mm), day past weir, and proportion hatchery admixture (proph).

	AIC	K	AICc	Δ_i	$£(g_i \mid x)$	w_i/w_t	evid ratio
Males							
weight*day*proph	61.6	8	65.7	0	1	0.31	
length*day	67.7	4	66.3	0.64	0.72	0.22	1.38
day*proph	68.2	4	66.8	1.14	0.56	0.18	1.78
weight*day	68.6	4	67.2	1.54	0.46	0.14	2.17
length*day*proph	64.6	8	68.7	3.0	0.22	0.07	4.48
Females							
day	92.4	2	90.2	0	1	0.54	
day*proph	93.8	4	91.6	1.4	0.50	0.27	2.01
length	96.3	2	94.2	3.9	0.14	0.08	7.03
length*day	96.3	4	94.2	3.9	0.14	0.08	7.03
proph	98.7	2	96.5	6.3	0.043	0.02	23.33
length*proph	99.4	4	97.3	7.0	0.03	0.02	33.11

Table 2.5. Models variables, AIC score, number of parameters (K), adjusted AIC score (AICc), model weights and evidence ratio for brood year 2006 by gender. Variables included in the model are: weight (g), fork length (mm), day past weir, and proportion hatchery admixture (proph).

	AIC	K	AICc	Δ_i	$£(g_i \mid x)$	w_i/w_t	evid ratio
Males							
length	26.7	2	24.5	0	1	0.36	
proph	27.8	2	25.6	1.1	0.58	0.21	1.73
day*proph	27.9	4	25.7	1.2	0.55	0.20	1.82
length*day	29.4	4	27.2	2.7	0.26	0.09	3.86
length*proph	29.6	4	27.4	2.9	0.23	0.08	4.26
day	30.1	2	27.9	3.4	0.18	0.06	5.47
length*day*proph	32.8	8	34.1	9.53	0.01	0.00	117.25
Females							
proph	27.5	2	25.7	0	1	0.24	
day*proph	29.3	4	28.3	1.10	0.58	0.14	1.73
length*proph	29.9	4	28.9	1.12	0.57	0.14	1.75
length*day	33.4	4	32.4	1.26	0.53	0.13	1.88
day	37.2	2	35.4	1.38	0.50	0.12	1.99
length	37.5	2	35.7	1.39	0.50	0.12	2.00
length*day*proph	31.7	8	37.1	1.44	0.49	0.12	3.05

Figure 2.1. Study area and location of fish trap and PIT tag readers in Beaver Creek, Methow Basin, Washington.

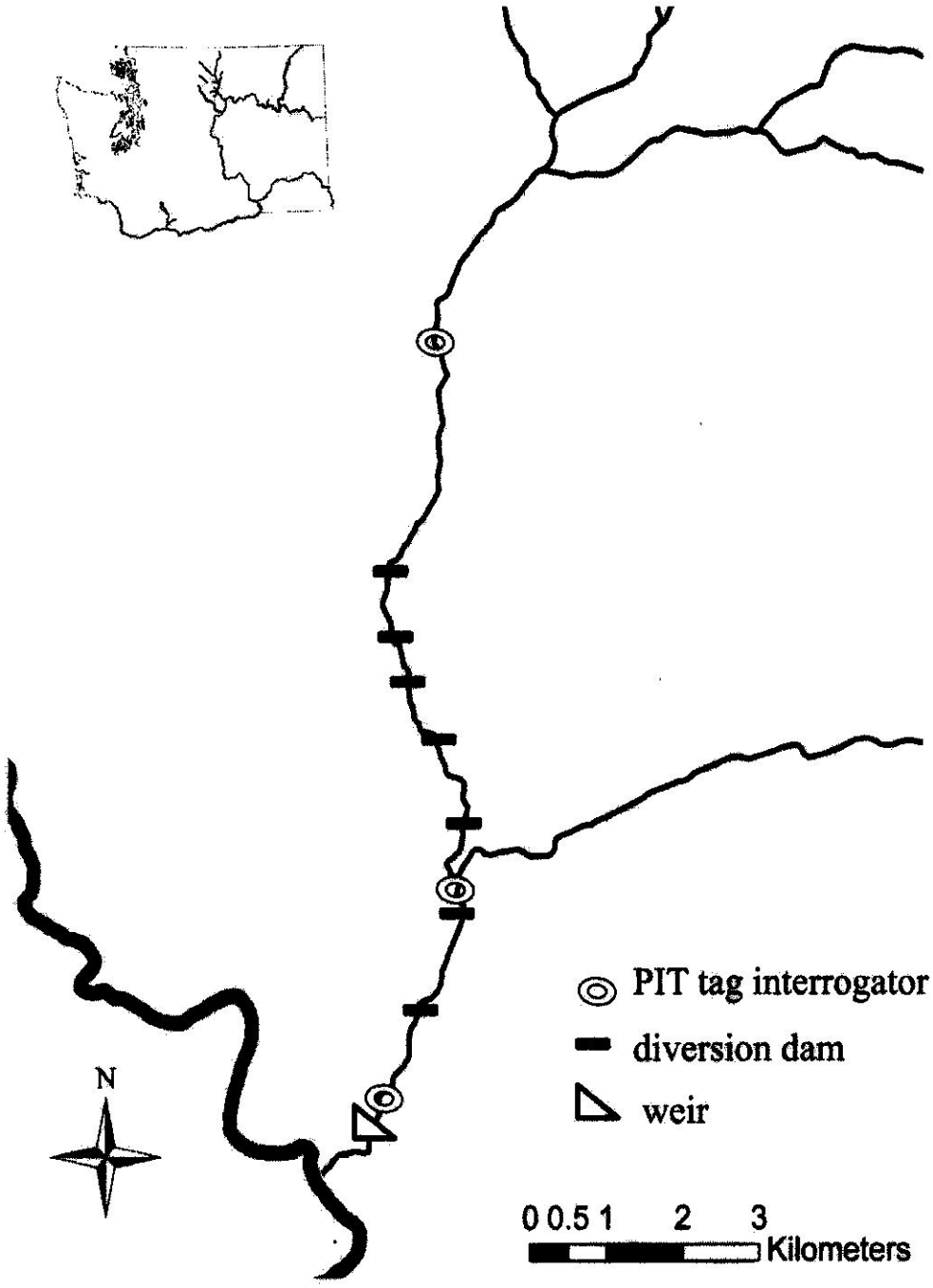


Figure 2.2. Proportion of one and two parent matches by life history and source population for brood years 2005 and 2006.

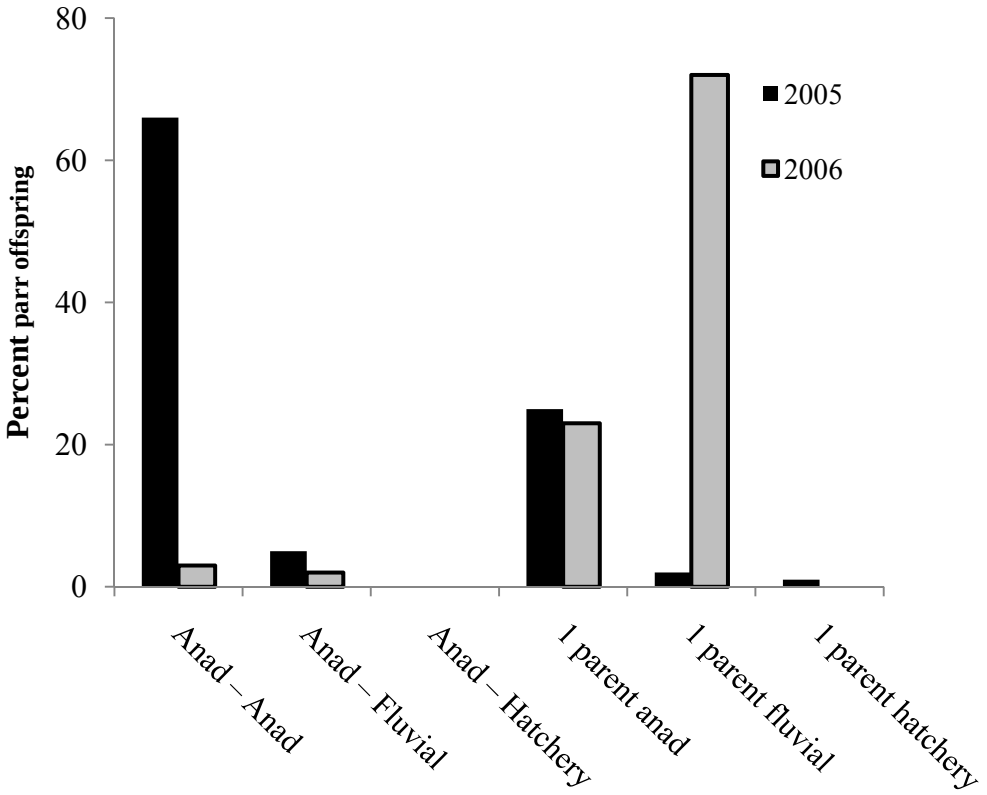


Figure 2.3. Comparison of fork length (mm), weight (g) and day past the weir of adult *O. mykiss* that produced or did not produce parr offspring in brood years 2005 and 2006. Significant tests are length and weight for brood year 2005 comparisons.

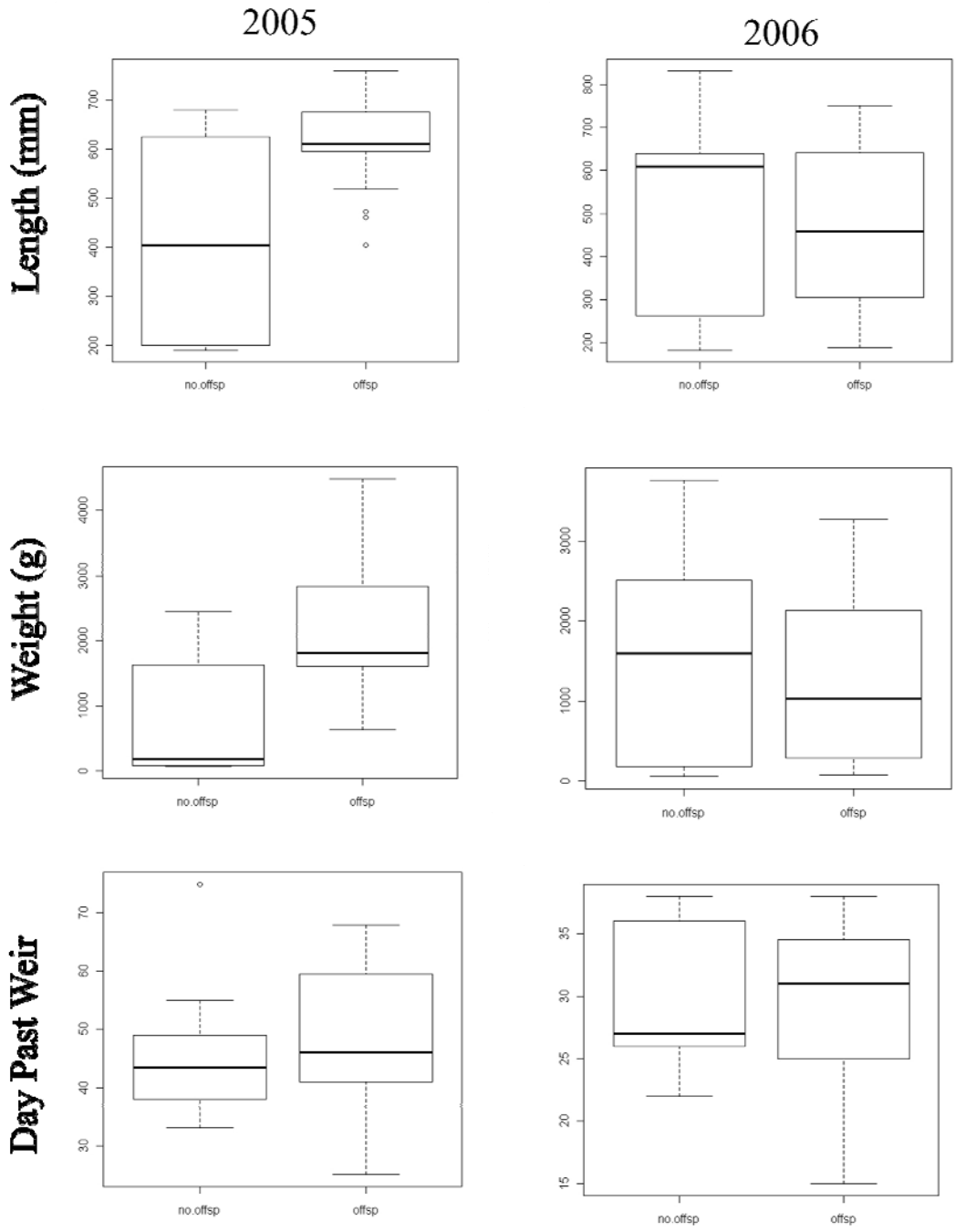
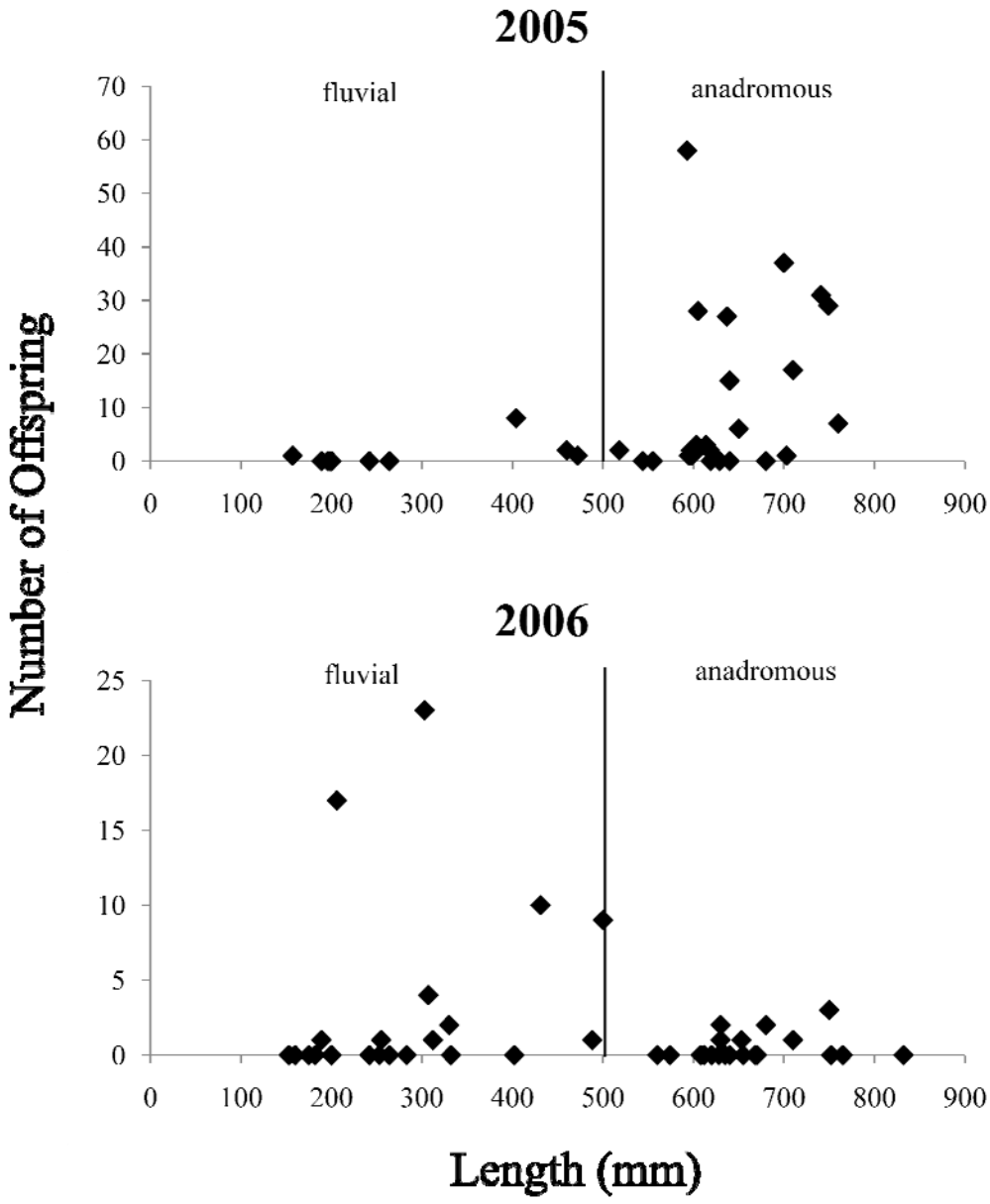


Figure 2.4. Number of offspring matched by parent fork length (mm) for 2005 and 2006 adult rainbow/steelhead.



Chapter 3

Colonization of Steelhead (*Oncorhynchus mykiss*) in a Natal Stream After Barrier Removal

Abstract

Colonization of vacant habitats is an important process to support the long term persistence of populations and species. We used a before-after experimental design to follow the colonization process of anadromous *Oncorhynchus mykiss* at six monitoring sites in a natal stream after the modification or removal of numerous stream passage barriers. Passive integrated transponder tags and stationary interrogation stations were used with population genetic sampling to determine the source, extent and success of the barrier removal projects. Adult anadromous *O. mykiss* migrated into the study area the first spawning season after passage barriers were removed. Hatchery *O. mykiss*, although comprising more than 80% of the adult returns to the basin, did not appear to influence the early colonization process in the study area. Parr outmigration increased during the first four years after barrier removal from the upper sites in the basin, and population genetic measures significantly changed in the lower two monitoring sites in the basin. Colonization and expansion of anadromous *O. mykiss* was a slower process than expected when compared to other barrier removal projects with adult anadromous *O. mykiss* beginning to migrate into the upper basin sites 3 to 4 years after barrier removal.

Introduction

Direct removal or damage to habitat threatens 50% of species in the United States (Richter et al. 1997). Small barriers, such as diversion dams and culverts, adversely impact aquatic fauna and are more numerous and widely distributed across the landscape than the larger mainstem dams (Moyle and Williams 1990; Sheer and Steel 2006). As numerous species of fish have declined over the last several decades, extensive efforts have been made to remove or modify these barriers to allow passage of target fish species (Bernhardt et al. 2005). These management actions are aimed at re-connecting unoccupied habitats to re-establish populations of threatened or endangered species that collectively will increase production. Few studies have collected data during the colonization process of fish in stream environments (Bernhardt et al. 2005).

Barrier removal projects create opportunities to study the colonization process using before-after treatment experimental design (Kiffney et al. 2009; Anderson et al. 2010). The rate of colonization will be dependent on the dispersal capability of the species as well as distance and density of the unoccupied habitat to candidate source populations (Gaggiotti et al. 2004). Barrier removal projects implemented in streams with populations of target species downstream of the structure are documented to rapidly colonize with volunteers when passage is restored (Kiffney et al. 2009; Anderson et al. 2010).

Trout and salmon are typically target species of restoration actions due to their threatened and endangered status in the U.S. (McClure et al. 2003). Yet, salmonid systems are largely supported by spawners homing to natal streams and the development

of local adaptations which can appear to hinder population expansion and colonization processes. Several species of salmonids have multiple life history strategies co-occurring in the natal streams, such as resident (stream-rearing), fluvial (river-rearing) and anadromous (ocean-rearing) (Behnke 1992). These various life history strategies are known to provide demographic and genetic support to species in variable or unstable environments and inter-breeding between these life history types is widely documented (Parker et al. 2001; Docker and Heath 2003; Araki et al. 2007; Christie et al. 2011). Barrier removal is oftentimes targeted toward increasing population distribution and abundance of the anadromous life history which has severely declined due to extensive impacts from harvest, hydropower, and variable ocean conditions (McClure et al. 2003).

Release of hatchery-reared conspecifics can directly impact migration and reproductive success of trout and salmon (Miller et al. 2004; Araki et al. 2007). These hatchery produced fish provide a potential over-abundant source population to colonize unoccupied habitats. Yet, hatchery salmon and steelhead are documented to have lower relative reproductive success than those naturally produced in the stream environments (Miller et al. 2004; Araki et al. 2007, 2008). Therefore, hatchery fish may not be a desirable source population for the colonization of newly opened habitats, and their role and impact to the colonization process are not well-understood. Hatchery trout and salmon are documented to have higher rates of straying than naturally reared conspecifics (Quinn 1993). The demographic effect of hatchery fish on the colonization process due to these greater abundances and high straying rates could reduce or eliminate the contributions from naturally produced trout or salmon. Yet, this demographic advantage

is likely countered by fitness reductions and negative genetic consequences imposed by colonizing hatchery populations.

Genetic data are often used to monitor the effect of colonization to identify inter-breeding groups (or local populations) and source population (Demairias et al. 1993; Garant et al. 2000; Gaggiotti et al. 2003; Bartron and Scribner 2004). Studies have indicated that populations of *O. mykiss* are generally stable (no significant differences in population genetic measures) over short time periods ranging from several months to 5 years (Heath et al. 2002; Narum et al. 2004, 2006; Nielsen et al. 2009). Over longer time periods (>20 years), temporal variation has been found to explain about 2% of molecular variation within *O. mykiss* populations, an amount similar to the variation among populations (Beacham et al. 1999; Heath et al. 2002). This genetic variation measured in these long term studies are generally influenced by genetic drift and changes in habitat condition, hatchery and harvest practices.

Barrier removal in combination with the co-existing life history strategies and hatchery populations of *O. mykiss* creates an experiment where colonization can be examined while the resident *O. mykiss* populations provide demographic stability. In this study, we use population genetic measures and movement data to determine if the anadromous life history of *O. mykiss* was successfully established after the modification of several small irrigation dams in Beaver Creek, a natal tributary to the Methow River, Washington. We are particularly interested in the colonization process in *O. mykiss* because it has complex and co-occurring life history strategies combined with potentially large hatchery effects. Migratory *O. mykiss* and other species of fish were allowed to naturally colonize the unoccupied habitat. Individual migrations and movements were

monitored with passive integrated transponder tags (PIT tags) and readers. Because the different life history types inter-breed, the PIT tag information was used to identify the life history of individuals during the study. The objectives of our study were to: 1) identify the source and abundance of colonizers (anadromous, hatchery or fluvial) during the first four years after barrier removal; 2) identify if and where in the basin detectable changes occurred to population genetic measures; and 3) identify if the anadromous life history was successfully established by identifying the adult return of parr spawned in Beaver Creek establishing the first generation after barrier removal.

Study Area

The Methow Basin is located on the east side of the Cascade Mountain Range in north-central Washington. The Methow River is a tributary of the Columbia River located about 843 km upstream from the estuary. Beaver Creek is a 3rd order natal tributary that flows west into the Methow River 57 km upstream from the mouth (Fig 3.1). The Beaver Creek basin is 290 km^2 with basin elevations that range from 463 to 1,890 m and stream flows that ranged from 0.05 to 4.7 m^3/s during the study (Martens and Connolly 2010). The upper portion of the basin is managed forest land administered by state or federal agencies. The lower portion of the basin is irrigated, privately-owned farm and ranch land.

Access for fish into Beaver Creek was disconnected due to water withdrawal and associated structures for more than 100 years (Martens and Connolly 2010). Resident *O. mykiss* were the most abundant species of salmonid throughout the Beaver Creek basin

prior to implementing the barrier removal projects. Anadromous *O. mykiss* (steelhead) were present downstream from the lowest diversion dam (Martens and Connolly 2010). From 2000 to 2004, seven small irrigation diversion dams (1.0 to 2.0 m high) were modified to rock vortex weirs that allow fish passage (Ruttenberg et al. 2009; Martens and Connolly 2010). The most downstream irrigation diversion was a 2.0 m high concrete diversion dam that was modified to allow fish passage after the fall of 2004. Access for migratory *O. mykiss* trout was restored to Beaver Creek for the spring 2005 spawning season.

Hatchery Releases

The Grand Coulee Fish Maintenance Project provides mitigation for the construction of Grand Coulee Dam during the 1930s. Hatchery activities are intended to replace lost production of anadromous salmon and steelhead from tributaries upstream blocked by the dam. The Wenatchee, Entiat, Methow and Okanagan rivers are located downstream of Grand Coulee Dam and are utilized to rear and release salmon and steelhead for this extensive hatchery mitigation program. The State of Washington also manages a hatchery program to mitigate for other hydropower facilities on the Columbia River.

The anadromous *O. mykiss* stock for all these hatcheries originated from collections on the Columbia River at Rock Island Dam, downstream of Wenatchee, WA. This brood stock was established from the returning adults to this dam assumed to be migrating to the major tributaries upstream, such as the Wenatchee, Entiat, Methow,

Okanagan and other tributaries upstream of Grand Coulee Dam (Chapman et al. 1994). This original brood stock was later used to establish local brood stocks in each of the basins. In recent years, the Methow and the Wenatchee hatchery brood stocks have been managed as demographically independent stocks.

Currently state and federal hatchery programs in the Methow Basin release 450,000 – 550,000 *O. mykiss* smolts per year. Returning adult *O. mykiss* are spawned and the eggs are reared at Wells Hatchery on the Columbia River downstream from the mouth of the Methow River. Current practices include intentional breeding between hatchery and naturally produced adults, and progeny from these crosses are primarily released in the Methow River basin (C. Snow, Washington Department of Fisheries and Wildlife, personal communication). Hatchery *O. mykiss* are released as age 1 smolts in the Methow and Chewuch rivers upstream from the town of Winthrop, WA. All hatchery origin *O. mykiss* were marked with an internal tag (such as PIT tag), external tag (such as elastomer tag) and/or fin clip. Hatchery origin adults comprised the majority of the adult return to the basin. Between 1999 and 2010, the hatchery steelhead returns ranged from 82 to 95% of the run (Fig. 3.2). During our study (2005-2008), hatchery steelhead returns ranged from 82% in 2008 to 91% in 2005 (Snow et al. 2010).

Methods

Fish Collections and Movements

Adult *O. mykiss* were captured in Beaver Creek using a picket weir installed 1.3 km upstream from the mouth (Fig. 3.1). This location was chosen for accessibility and stream channel topography. This trap captured fish moving upstream or downstream. The trap was operated from March 20 to May 9 and May 14 to December 5 during 2005; February 13-May 1 and June 27-November 27 during 2006; February 24 to March 30 and May 25 to November 29 during 2007; and February 24 to May 3, July 11 to July 30 and September 2 to December 10 during 2008. The date, direction of movement, fork length (mm), weight (g), sex and population origin (wild or hatchery) were recorded for adult trout.

Juvenile *O. mykiss* were sampled at 6 sites on Beaver Creek (Fig. 3.1). One site was downstream of the lowest diversion dam (DS Dam), 1 site was located between the various diversion dam modifications (UBR1) and 4 sites (UBR2, CMP, UBR4, SFB) were upstream from the diversion dam modifications (Fig. 3.1). Before barrier treatment collections were made during the fall of 2004 or the summer of 2005 sampling age 1+ juvenile *O. mykiss* in the stream. After barrier treatment collections were made during the summer or fall 2008 and 2009 sampling age 1+ juvenile *O. mykiss* present in the stream. The 4 to 5 years between the before and after collections represents about 1 generation for *O. mykiss*.

Juvenile *O. mykiss* were collected using a backpack electrofisher (Smith Root Inc. LR-24). Trout were measured to the nearest mm fork length and weighed to the nearest 0.01g using a digital scale (Ohaus, Scout Pro SP 400). Juvenile and adult trout were scanned for PIT tags and coded wire tags and inspected for any other external tags (such as fin clips, elastomer tags, etc.). If the trout did not have a PIT tag, a tag was inserted in the dorsal sinus cavity for adult trout or the body cavity for juvenile trout >65 mm (12.5mm tag, full duplex 134.2 kHz). A tissue sample was removed from the caudal fin of juvenile and adult trout and stored in 95% non-denatured ethanol.

Movements of *O. mykiss* trout were monitored using a network of stationary PIT tag reading stations in Beaver Creek (Fig. 3.1) and at dams and passage facilities on the mainstem Columbia River. One multi-antenna, multiplex PIT tag reading station and two single antenna PIT tag reading stations were operated in Beaver Creek (described in Connolly et al. 2008; Martens and Connolly 2010). Briefly, the multiplex unit was operated with a Digital Angel Model FS-1001 transceiver connected to 6 custom made antennas and a DC power source. The antennas were arranged in three arrays across the stream bed with each array having two antennas that extend across the stream bed providing redundancy and complete coverage at most stream flows. This configuration allowed us to determine direction of movement and efficiency of detection. The single antenna interrogation stations were operated using a 2001F-iso Digital Angel PIT-tag reader powered by a 12-volt battery connected to a single custom made antenna. The most downstream single antenna PIT tag reading station was operated from September 27, 2004 to December 2, 2008. The multiplex reading station was operated from July 20,

2004 to present. The upper single antenna PIT tag reading station was operated from August 1, 2004 to November 12, 2008.

Migratory life history (anadromous or fluvial) of the adult trout was identified using PIT tags. Fluvial *O. mykiss* trout left Beaver Creek and were not detected at any of the Columbia River facilities. Some of these fish returned in successive years. Anadromous *O. mykiss* trout were detected on the mainstem Columbia River dams during upstream and/or downstream migration. Hatchery origin trout were identified from PIT or coded wire tags, fin clips or other marks.

Laboratory Methods

Tissue samples from the Wells Hatchery brood years 2005 and 2006 (hatchery x hatchery crosses) were provided by the Washington Department of Fisheries and Wildlife (WDFW). Sixteen microsatellite markers were used to identify individuals. Thirteen of these markers are standardized across the Columbia River Basin and are cited in Stephenson et al. (2009). Additional primer sets analyzed were: *One102* (Olsen et al. 2000), *Omm1036* and *Omm1046* (Rexroad et al. 2002).

DNA was isolated from fin clips preserved in ethanol using Qiagen DNEasy tissue extraction kits following standard manufacturer's protocols. Sixteen microsatellite loci were amplified using the polymerase chain reaction (PCR) in three multiplex reactions using Qiagen Multiplex PCR Master Mix on Applied Biosystems GeneAmp PCR System 9700 thermal cyclers in 96 well plates. PCR products were run on an Applied Biosystems 3730 genetic analyzer. Peaks were scored using GeneMapper

version 3.7 software (Applied Biosystems, Foster City, California), and labeled following the Steven Phelps Allele Nomenclature (SPAN) convention (Stephenson et al. 2009). Forward primers were fluorescently labeled (Applied Biosystems).

Amplification (PCR) reactions consisted of 5 ul reactions containing 2.5 ul Qiagen Multiplex PCR Master Mix, five or six primer sets and water, added to 2ul of DNA extract dried down in a 96 well plate. Cycling conditions included initial denaturation for 15 min at 95°C, followed by 28 cycles for 30 s at 94°C, 90 s at 51°C (Multiplex A) or 57°C (Multiplex B and Multiplex C), and 60 s at 72°C, followed by a final cycle for 30 min at 60°C. Multiplex A contained *Oki23*, *Oke4*, *Oneu14*, *Ssa289*, and *Ssa408*; Multiplex B contained *Ots4*, *Omy7*, *Ogo4*, *One102*, *Omm1046*, and *Ssa407*; Multiplex C contained *Ots100*, *Omy1011*, *Omy1001*, *Ots3m*, and *Omm1036*.

Amplification products were diluted with 10 ul DNA grade water and 1 ul of each dilution was added to 10 ul of LIZ/formamide solution (30 ul LIZ600 to 1 ml formamide). Completed runs were analyzed automatically using GeneMapper, followed by manual analysis of all peaks for verification. All homozygous results were checked for small allele dropout and large allele dropout. Peaks were also visually checked for conformity to expected profiles. Lab error rates for the 13 standardized loci were <2% (Stephenson et al. 2009). Duplicate samples indicate lab error rates <1% for our study.

Statistical Analysis

The before-after analysis relies on the assumption that temporal genetic diversity is stable so that a detectable response can be attributed to the treatment. To test the

temporal stability of the genetic diversity and variation, we used pair-wise comparisons between consecutive years. Therefore, pair-wise comparisons between the before-after samples were used to detect changes due to the instream treatments whereas pair-wise comparisons between consecutive years were used to test the frequency of statistical significance due to non-treatment related factors (such as finite sampling). Before-after comparisons were tested twice against different years for 3 out of 5 sites to confirm the significance and repeatability of the before-after comparisons (Table 3.1).

Prior to statistical tests, full siblings were identified and removed from the data set using ML-RELATE (Kalinowski 2006). Exact tests of Hardy Weinberg Equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0.10 (Raymond and Rousset 1995). Expected heterozygosity was calculated using GENEPOP. Unbiased estimates of allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). Exact tests for F_{ST} were performed using ARLEQUIN v3.5 (Excoffier and Lischer 2010). All comparisons were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

The proportion of hatchery admixture was estimated for each *O. mykiss* collected at each site and year in the sample with known hatchery steelhead from Wells Hatchery (n=99) using STRUCTURE version 2.3.3 (Pritchard et al. 2000). The hatchery brood years were not statistically different and were combined for our analysis. STRUCTURE is a Bayesian based model that clusters individuals according to allelic frequencies minimizing Hardy Weinberg and linkage disequilibrium. The model allows for admixture between population groups. The admixture model was run in STRUCTURE using 10,000 iterations for burn in and 100,000 iterations using a Markov Chain Monte

Carlo resampling algorithm as described in Prichard et al. (2000). All other settings were run using default values. Ten independent runs were made for each site and the run with the lowest log likelihood was selected as the best run for estimation of hatchery admixture. The average of the percent hatchery admixture was calculated for each site and collection year.

Results

Difficulties running the weir during high springtime stream flows resulted in inconsistencies between capture efficiencies and counts across the years of our study (Fig. 3.3). Fluvial *O. mykiss* were particularly numerous during 2006 with nearly three times the number of adult migrants than the other years of our study (Fig. 3.4). Over the 4 years of our study, 34 individual fluvial rainbow trout >200 mm were documented during the spawning run in Beaver Creek. Males were the largest proportion (67%) of this life history type; females and unknown determinations were 6% and 18%, respectively. Individual fluvial *O. mykiss* were documented entering Beaver Creek up to four consecutive years during our study with 26% of the individuals entering the creek multiple years.

Capture efficiency at the fish trap was high for adults during 2005 and 2006 with only two individuals in 2005 and one individual in 2006 known to be missed in our sample (Fig. 3.3). However, the weir was not run for the entire spawning seasons during 2007 and 2008 reducing the ability to count the wild anadromous *O. mykiss* entering the stream during these years. Numerous hatchery *O. mykiss* were read at the Beaver Creek tag reading stations during these years, and the counts based on PIT tags would be biased

toward hatchery trout due to the greater number of tags inserted in hatchery trout in the basin during these years.

Adult *O. mykiss* with tags migrated further upstream in Beaver Creek during the latter two years of the study (Fig. 3.5) indicating that adult *O. mykiss* were still expanding into the upper basin. Parr tagged at sites upstream from the diversion dams were detected as smolts in the Columbia River dams during all years of the study. Twelve percent of the tags released in parr during 2004 at UBR1, the site between the diversion dams, were detected during downstream migration on the Columbia River. These data provide definitive evidence that juvenile *O. mykiss* from this reach were expressing an anadromous life history prior to barrier treatment; yet, none of these parr returned as adults. The percent of tagged parr that were detected as smolts from the site between the diversion dams (UBR1) after barrier removal varied annually with no apparent trend (8% in 2005, 5% in 2006, 6% in 2007, 8% in 2008, 6% in 2009 and 14% in 2010). Parr from the sites further upstream had 1 to 2% of tagged parr detected as smolts on the Columbia River and these rates remained constant during the study period indicating no change in life history at these sites. None of the parr tagged at the upper sites (UBR2, CMP, UBR4 and SFB) returned as adults.

Between 2007 and 2011, 38 adult *O. mykiss* that were tagged as parr in Beaver Creek were detected migrating upstream. Most (68%) of these adults were last detected on the Columbia River or at the PIT tag reading station at the mouth of the Methow River. Eight adults (21%) were detected in Beaver Creek (n=1 2007, n=3 2008, n=4 2009) and 4 (33%) were detected in other tributaries (Twisp and upper Methow rivers). These tagged returning steelhead show that anadromous *O. mykiss* progeny from early

colonizers in the basin successfully homed back to Beaver Creek. Yet, one-third of these returning adults were detected in other tributaries in the Methow Basin.

The total number of alleles detected at each locus ranged from 7 to 28 with the average allelic richness ranging from 4.9 to 7.2 by site and collection date (Table 3.1). Tests of Hardy Weinberg Equilibrium and linkage disequilibrium did not detect significant departures in the juvenile samples from the sites on Beaver Creek. Tests on the Wells Hatchery samples did not detect any significant departures from Hardy Weinberg Equilibrium but did detect linkage disequilibrium at 6 pairs of loci. There was no discernible pattern to these pairs of loci.

The genetic diversity parameters indicated some changes in the before-after comparisons with the temporal tests remaining stable for expected heterozygosity and allelic richness. Private alleles did vary across the comparisons (Table 3.1). The STRUCTURE output indicates that the upstream sites have less Wells Hatchery admixture in the samples (Figs. 3.6 and 3.7). The Wells Hatchery practices intentionally inter-breed hatchery and wild adults that return to Wells Dam. Therefore, there are contributions of non-hatchery alleles shown in the Wells Hatchery brood samples. The proportion of hatchery admixture decreased at site DS Dams, downstream from the diversion dams. The proportion of hatchery admixture generally increased at sites upstream from the diversion dams, except for site UBR4 where proportion of hatchery admixture decreased. Pair-wise Wilcoxon rank tests for before-after comparisons were significant for both comparisons at UBR1 ($p<0.003$) and for only one comparison (2005 and 2008) at the SFB site ($p=0.02$), all other comparisons were not significant. Proportion of hatchery admixture was fairly consistent for the temporal comparisons

(2008 to 2009) except for site SFB where hatchery admixture declined. The pair-wise Wilcoxon rank tests for all the temporal comparisons of the proportion of hatchery admixture were not significant ($p>0.05$).

Comparisons of genetic differentiation (F_{ST} and allele frequency) showed significant differences at the two most downstream sites in the basin (Table 3.1). Both of these measures show significant differences indicating consistency across these measurements and supporting the conclusion that population genetics changed at these sites after barrier removal. Interestingly, the site downstream from the dams showed significant change even though it was accessible prior to the barrier removal treatments. The genetic differentiation tests at UBR4 were significant comparing 2004 and 2008, but not significant for the comparison between 2004 and 2009. It is possible that this significance could be a result of finite sampling or non-random mating or tissue collections. All of the temporal tests on the consecutive years did not show any significant tests for comparisons of F_{ST} or allele frequencies (Table 3.1).

Discussion

Following the initial number of adult migrants into Beaver Creek in 2005, adult *O. mykiss* did not increase in the subsequent 3 years after barrier removal. Counts of wild and hatchery steelhead declined from 2005 to 2007 and then increased slightly. This followed the trend of adult counts into Wells Dam. Fluvial rainbow trout were a variable portion of the run and reproduced with the steelhead. Although Anderson et al. (2010) found rapid colonization and steadily increasing abundances of coho salmon (*O. kisutch*)

during the first 3 years after passage was restored at a dam, Demarias et al. (1993) found that re-colonization occurred much slower in the Virgin River chub (*Gila seminuda*) after an accidental release of rotenone, a fish poison. The rate of colonization is mediated by the abundance, distance and connectivity to source populations; therefore, different species and locations may vary in response to connectivity projects or disturbance events.

Few hatchery steelhead entered Beaver Creek despite high proportions in the returns to the Wells Dam. Leider (1989) also found different proportions of hatchery steelhead between a hatchery counting site lower in the basin and a natal tributary. Hatchery fish may return to release locations or the hatchery site near the release location. In addition, other survival differences may affect the proportion of hatchery fish between the ladder at Wells Dam and the natal tributaries, such as selective harvest.

Several parr tagged in Beaver Creek returned as adults in 2007 through 2011 indicating that the complete life cycle of the anadromous life history was established in the newly opened habitat. Some straying of these returning adult steelhead occurred during the study and 66% of these adults returned to the natal area. All the strays detected in the Methow Basin were recorded in tributaries upstream from Beaver Creek. Steelhead were found to stray into tributaries upstream from the natal tributary after the volcanic eruption on Mt. St. Helens, WA (Leider 1989). Additional steelhead tagged as parr in Beaver Creek were last detected migrating upstream in the Columbia River or the mouth of the Methow River. These adults were not detected again entering a natal tributary, and the fate of these adults is unknown. These trout either died, entered another stream undetected or returned to Beaver Creek downstream from the lowest tag reader. The steelhead from Beaver Creek had a substantially higher rate of straying (33%) than

documented in other studies (7.7%) (Hendry et al. 2004). Our data do not indicate why this high straying rate was observed, but this could be part of the early colonization process prior to establishing a viable population and associated local adaptations. Although this number is only based on 4 adult strays from Beaver Creek, we consider this number to be a conservative estimate of straying because there are many locations in the basin where strays would not be detected.

The temporal stability of the population genetic measures is important to identify when attempting to detect a treatment effect. Population genetic measures can vary due to genetic drift from finite population sizes (Allendorf and Luikart 2007). Therefore, some tests could show significant differences and be unrelated to the treatment. Similar to other studies, our populations were temporally stable over short term comparisons. Similar tests ranging from collections <1 to 5 years apart found that only 1 out of 21 comparisons was significantly different (Heath et al. 2002; Narum et al. 2004, 2006; Nielsen et al. 2009). Therefore, we expect a less than 5% rate of significant temporal tests due to random or unmeasured effects.

Oncorhynchus mykiss from the two most downstream sites showed significant differences in allele frequency and F_{ST} values. We did not expect to see a change in the site downstream from the dams because this site was accessible to migratory *O. mykiss* prior to the barrier treatments. Interestingly, there was also a reduction in the proportion of hatchery admixture at this site after barrier removal, another unexpected result. This shift in genetic parameters may be due to individual trout moving downstream from upstream sites for rearing or possibly due to the mixing of the anadromous or fluvial *O. mykiss* with the resident individuals that were residing upstream from the diversion dams.

The reduction in hatchery admixture could result from the higher contribution of the wild *O. mykiss* spawning in the newly opened habitat.

The first site upstream from the diversion dam treatments (UBR1) had the greatest shift in F_{ST}, allele frequencies and hatchery admixture which were significantly different before and after treatment. This site had some parr outmigration occurring prior to the barrier treatments and also hatchery admixture estimate of about 27%. The increase in hatchery admixture is interesting considering that the hatchery steelhead that colonized the stream during 2005 and 2006 produced very few offspring. However, resident *O. mykiss* can adopt anadromous life history and gene flow into the hatchery from the populations upstream from Wells Dam is very high. Therefore, the hatchery admixture is also tied to the anadromous life history through hatchery brood practices. Parr were exhibiting an anadromous life history from site UBR1 prior to removal of the diversion dam. Life history is plastic in *O. mykiss* and can be growth or genetically related. It is uncertain if a few adult steelhead were accessing this site prior to barrier removal, or whether this site was converting more parr to smolts due to favorable growing conditions.

The sites further upstream did not show changes in population genetics when comparing before and after treatment samples. Tag data indicate that few spawners migrated to these upper reaches of the basin during the first 4 years after barrier removal. Although outmigration (measured as smolts moving past the lower Columbia River dams) increased from tags released at these sites during the study indicating an increase in anadromy, removal of the related individuals from the analysis will require more adults to spawn in these reaches of stream before genetic response will be detectable. The UBR4 site showed a significant change in F_{ST} and allele frequencies when comparing the

2004 to 2008 samples, but this comparison was not significant when comparing the 2004 to 2009 samples. Since the pair-wise comparisons were not similar across the different years, we considered that the significant comparison did not indicate clear genetic changes due to the treatment. Similarly, the SFB site had an increase in allelic richness and private alleles when comparing the 2005 to 2008 samples, but not when comparing the 2005 to 2009 samples. These shifts in population genetic measures could be the result of genetic drift from finite population size of breeders, non-random mating, finite sampling, or result from a few new migrants in 2008 that did not migrate into this area in 2009.

Although it is possible that the genetic shifts at sites in Beaver Creek could be due to increased migration among resident *O. mykiss* from the monitoring sites, the tag data recorded adult steelhead moving higher into the basin during the study. Additionally, the increases in proportion of hatchery admixture and significant changes in population genetics indicate that resident movements are not the likely explanation for the observed results. Further, barriers in streams may allow passage downstream, but prevent passage upstream allowing migration and gene flow in the downstream direction. It is also likely that the resident sites produce a small number of anadromous or fluvial outmigrants that can navigate downstream the diversion dams. However, if this was the case then significant changes in allele frequencies would not be expected.

In summary, adult *O. mykiss* entered Beaver Creek during the first spawning season after barrier removal and parr from these first brood years return to Beaver Creek indicating that the complete life cycle of steelhead was established. In addition, tag movement data indicated that adult *O. mykiss* were moving to the upper monitoring sites

in the 3rd and 4th years after barrier removal. Hatchery steelhead were a small proportion of these colonizing adults despite high abundances from releases by local fishery management programs. Abundances of adult *O. mykiss* did not increase during the four years the weir was operated. Because hatchery fish did not comprise a majority of the run into Beaver Creek and they are expected to have substantially reduced reproductive success (Miller et al. 2004; Araki et al. 2007), the low numbers of the wild steelhead into the Methow Basin are likely limiting colonization of this life history type in the stream. Colonization and expansion of steelhead was a slower process than expected with adult steelhead beginning to expand into the upper basin sites during the later years of the study. Monitoring of the population and colonization process should continue until the anadromous life history reaches a stable distribution and abundance in the basin. Additionally, as the colonization process continues, it is possible that relationships may shift, such as abundances of hatchery steelhead into the stream.

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Table 3.1. Genetic variation for pair-wise before-after treatment comparisons and temporal tests on consecutive years for sites in Beaver Creek sampled between 2004 and 2009. Sites are listed from the most downstream to the most upstream. Repeated pair wise tests were done to test repeatability of results. Parameter include: sample size (n), expected heterozygosity (H), average allelic richness (AR), average private alleles (PA), average proportion of hatchery admixture (%H), population differentiation (F_{ST}) and allele frequency exact test (Pval).

	Before						**After**							
Site	Year	n	H	AR	PA	%H	Year	n	H	AR	PA	%H	F_{ST}	Pval
DS Dam	2005	28	0.80	7.1	0.42	40.0	2009	23	0.81	7.2	0.35	35.6	0.014*	0.001*
UBR1	2004	19	0.78	6.4	0.26	27.4	2008	28	0.82	7.2	0.22	47.6	0.021*	<0.001*
UBR1	2004	19	0.78	6.4	0.26	27.4	2009	26	0.82	7.0	0.29	47.0	0.027*	<0.001*
CMP	2005	36	0.76	6.3	0.04	6.0	2009	21	0.78	6.3	0.06	12.6	0.002	0.047
UBR4	2004	15	0.70	4.9	0.03	6.3	2008	28	0.69	5.2	0.05	3.2	0.011*	0.009*
UBR4	2004	15	0.70	4.9	0.03	6.3	2009	23	0.68	5.3	0.03	5.0	-0.002	0.558
SFB	2005	28	0.72	5.5	0.03	1.8	2008	33	0.77	6.0	0.09	8.3	0.004	0.121
SFB	2005	28	0.72	5.5	0.03	1.8	2009	21	0.73	5.5	0.04	4.0	0.002	0.276
Temporal tests														
UBR1	2008	28	0.82	7.2	0.22	47.6	2009	26	0.82	7.0	0.29	47.0	-0.003	0.253
UBR2	2008	29	0.80	6.7	0.11	9.8	2009	22	0.80	6.6	0.18	9.0	-0.004	0.880
UBR4	2008	28	0.69	5.2	0.05	3.2	2009	23	0.68	5.3	0.03	5.0	<-0.001	0.147
SFB	2008	33	0.77	6.0	0.09	8.3	2009	21	0.73	5.5	0.04	4.0	0.005	0.568

* indicates statistical significance after Bonferroni correction

Figure 3.1. Study location and sampling sites in Beaver Creek, Methow Basin, Washington.

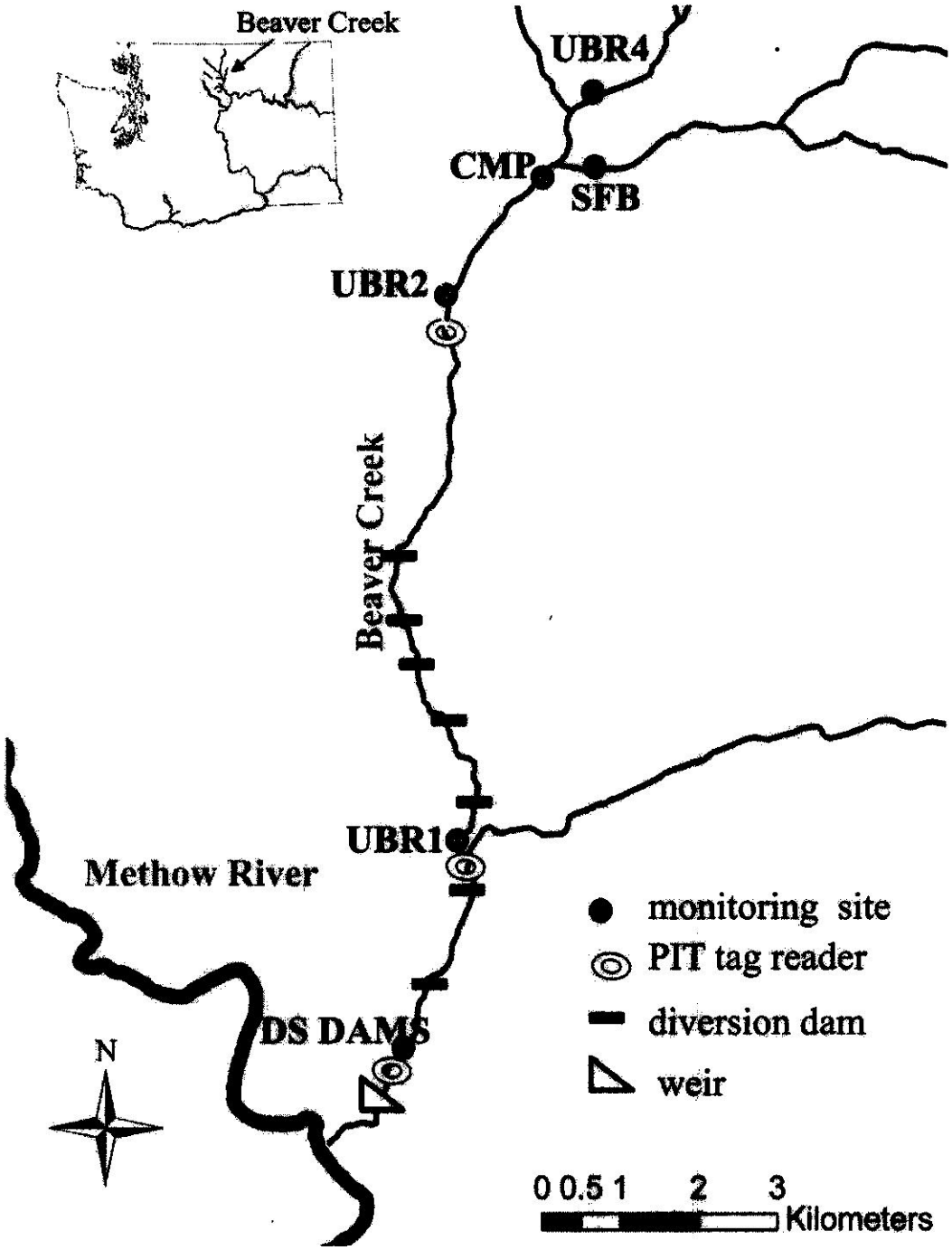


Figure 3.2. Counts of wild and hatchery steelhead returns to Wells Dam, Columbia River, Washington (1999-2010). Data compiled from Snow et al. (2010).

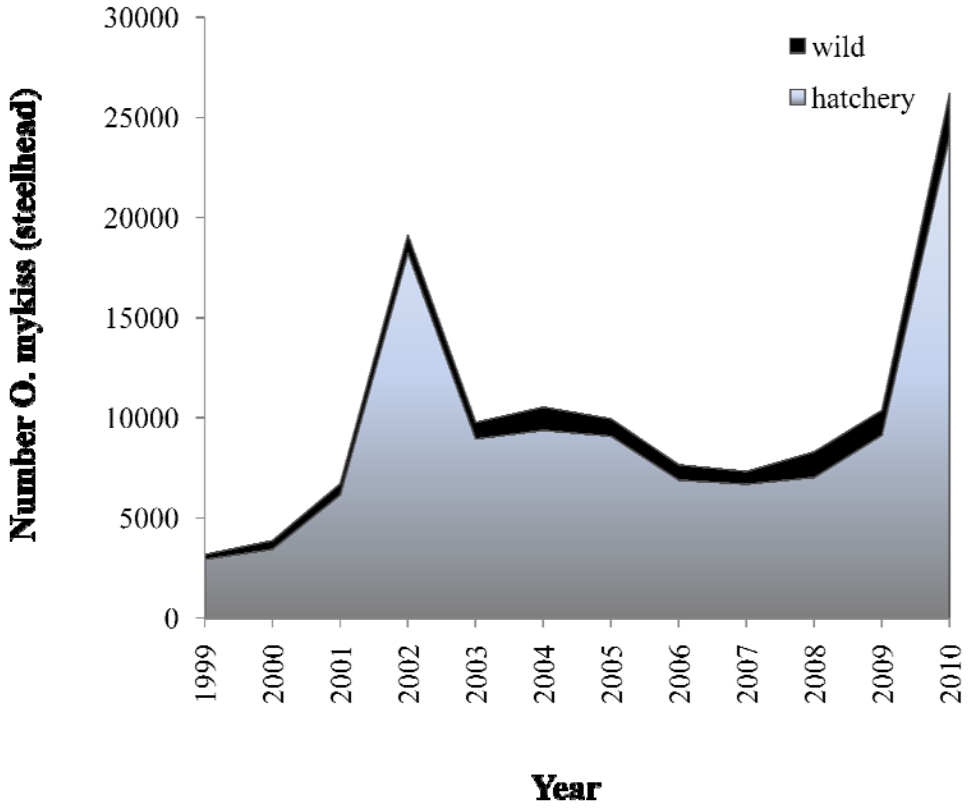


Figure 3.3. Individual adult steelhead captured at the weir (weir) and adult steelhead read in Beaver Creek not captured at the weir (tag) by year.

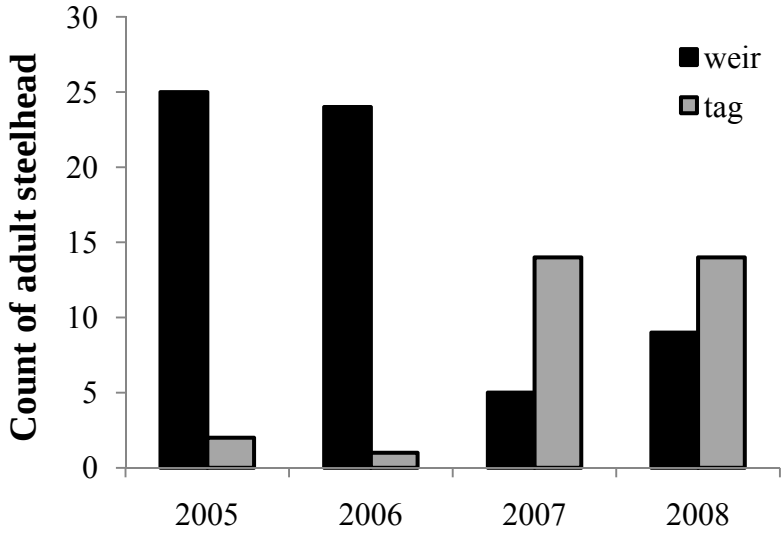


Figure 3.4. Adult *O. mykiss* counts into Beaver Creek 2005-2008. Counts for each source (hatchery, wild anadromous and fluvial) are shown separately in the colored bars. The white bar represents the total count of spawners from the weir and PIT tag reading stations.

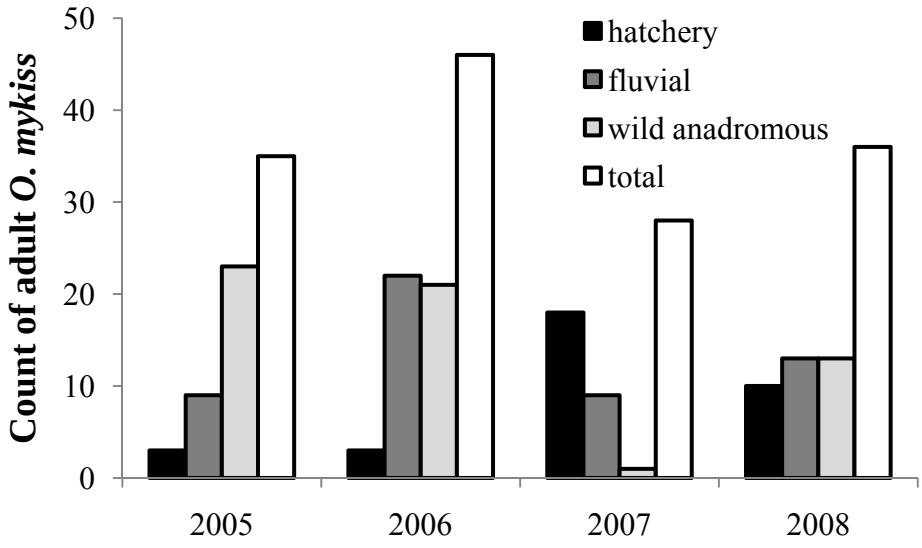


Figure 3.5. Number of adult *O. mykiss* trout counted at tag reading stations located at rkm 4 and rkm 12 migrating upstream during spawning season in Beaver Creek 2005-2008.

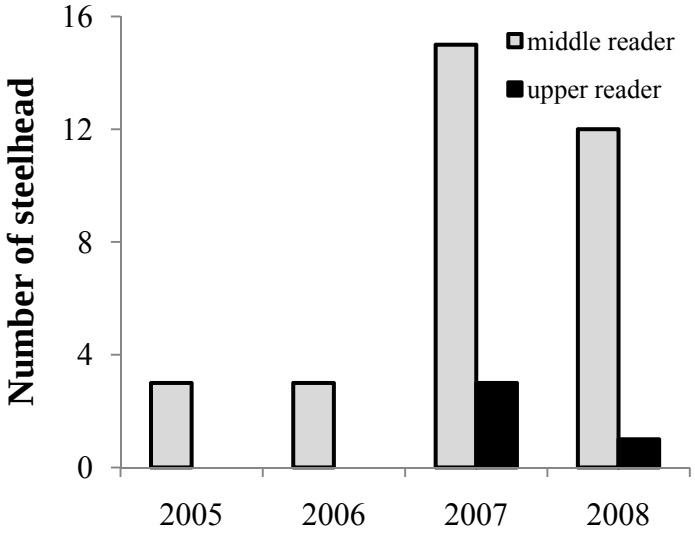


Figure 3.6. Output from STRUCTURE showing population admixture for the lowest 3 monitoring sites in Beaver Creek. The Wells Hatchery steelhead were used as a reference for the hatchery population (HxH crosses, brood years 2005 and 2006). Hatchery samples were provided by WDFW.

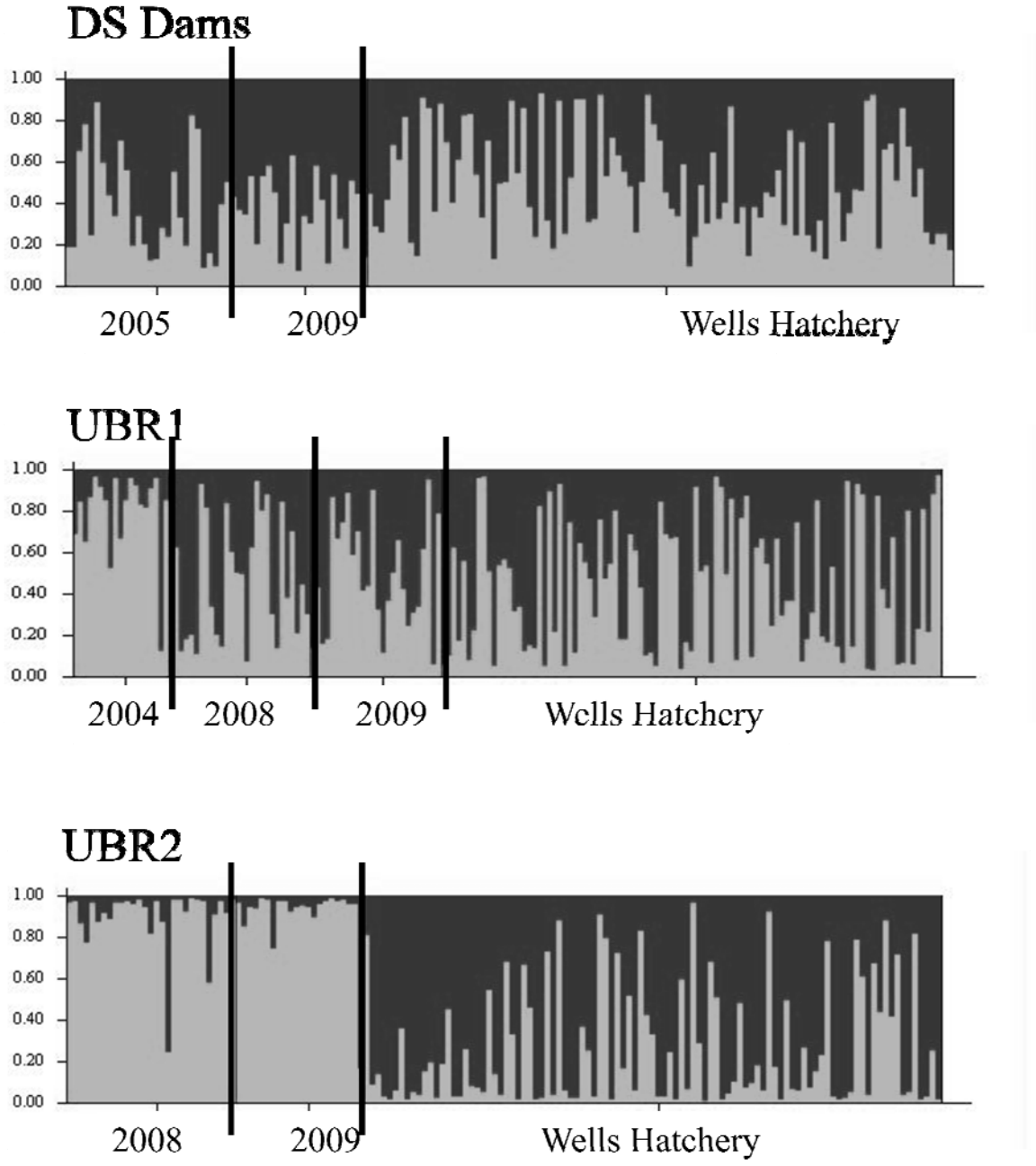
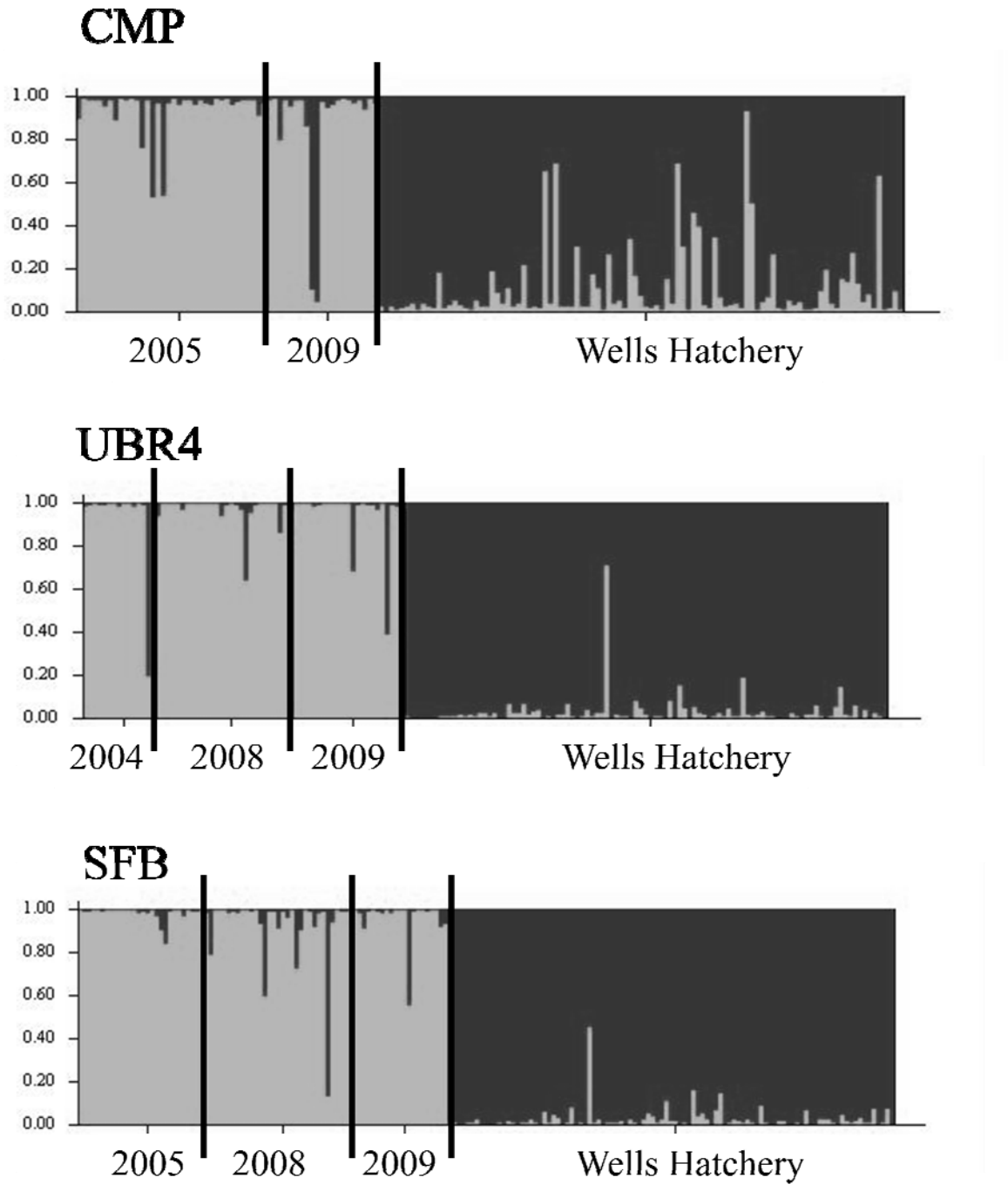


Figure 3.7. Output from STRUCTURE showing population admixture for the upper 3 monitoring sites in Beaver Creek. The Wells Hatchery steelhead were used as a reference for the hatchery population (HxH crosses, brood years 2005 and 2006). Hatchery samples were provided by WDFW.



Chapter 4

The Impact of Small Irrigation Diversion Dams on the Contemporary Migration Rates of Steelhead and Redband Trout (*Oncorhynchus mykiss*)

Abstract

Barriers to migration and gene flow interrupt ecological and evolutionary processes which may reduce fitness and increase the risk of extinction through loss of adaptive potential. Barriers to migration are numerous in stream environments and can occur from anthropogenic activities (such as dams and culverts) or natural processes (such as beaver dams or log jams). Identification of barriers can be difficult when obstructions are temporary or incomplete providing intermittent passage. We examined the effects of several small irrigation diversion dams on the contemporary migration rates of *Oncorhynchus mykiss* in three tributaries to the Methow River, Washington. We compared migration rates and associated environmental variables in Beaver Creek to nearby reference streams, Libby and Gold Creeks. Migration was asymmetrical among pair-wise sites. The three basins had different contemporary migration patterns: Beaver Creek did not have any contemporary migration between sites, Libby Creek had two-way migration between sites and Gold Creek had downstream migration between sites. Wilcoxon tests between sites with migration and sites without migration found significant differences in distance, number of obstructions, obstruction height to depth ratio and maximum stream gradient in Libby and Gold Creeks. When comparing sites without

migration in Beaver Creek to sites with migration in Libby and Gold Creeks, the number of obstructions was the only significant variable. Multinomial logistic regression identified obstruction height to depth ratio and maximum stream gradient and their interaction as the best fitting model to predict the level of migration among sites. Small irrigation diversion dams were limiting population interactions in Beaver Creek and collectively blocking anadromous *O. mykiss* migration into the stream. Variables related to stream resistance (gradient, obstruction number and obstruction height to depth ratio) were better predictors of contemporary migration rates than distance, and can provide important insight into migration and population demographic processes in lotic species.

Introduction

Populations in a landscape interact to exchange individuals providing demographic support and genetic variation. Meta-population theory is often used to describe this inter-dependence between populations where local populations support each other in a source-sink dynamic important to the long term persistence of species in stochastic environments (Hanski and Gilpin 1996; McCullough 1996). The emergence of this theory in conservation biology resulted in resource management strategies that focus on maintaining and improving connectivity between populations (Crooks and Sanjayan 2006; Kettunen et al. 2007). Barriers to migration and gene flow interrupt these ecological and evolutionary processes which may reduce fitness (Reed and Frankham 2003) and/or increase the risk of extinction through loss of adaptive potential (Swindell and Bouzat 2005). Yet, barriers can also have the beneficial effect of preventing the invasion of non-native species or strains that may inter-breed with native species or stocks (Novinger and Rahel 2003; Fausch et al. 2009).

Hybridization with introduced species threatens many species of invertebrates, fish, birds and mammals (Rhymer and Simberloff 1996). Hybridization can spread widely and become an uncontrollable problem for scientists trying to protect native species (Rhymer and Simberloff 1996; Allendorf et al. 2001). In aquatic environments, hatchery fish are widely stocked for conservation and mitigation purposes to support recreational and commercial fisheries (e.g. Thurow et al. 1997). These introduced fish oftentimes reproduce in the natural environment which, in some cases, can directly reduce fitness of the native stock (e.g. Epifanio et al. 1999; Miller et al. 2004). Although

barriers can protect the native genotypes from these threats, this situation is not ideal because these populations are still subject to the effects of fragmentation and isolation.

In streams and rivers, direct removal or damage to habitat threatens 50% of species in the United States (Richter et al. 1997) and passage barriers prevent access to a significant amount of preferred habitat in the Columbia River Basin (Sheer and Steel 2006). Small barriers, such as diversion dams and culverts, are more numerous and widely distributed across the landscape than the larger mainstem dams (Sheer and Steel 2006), and water diversions are cited as having the greatest adverse effect on aquatic fauna in California (Moyle and Williams 1990). As numerous species of fish have declined in abundance over the last several decades, extensive efforts have been made to remove or modify these barriers to allow passage of target fish species (Bernhardt et al. 2005).

Genotypic data can be used to identify migrants and estimate migration rates between populations. These methods have become more popular with the development of genotypic markers that allow non-invasive genetic sampling and the emergence of disequilibrium methods to identify migrants (Rannala and Mountain 1997; Pritchard et al. 2000). Recent migration rates within the last one to two generations can be estimated using the disequilibrium method described in Wilson and Rannala (2003). The disequilibrium method captures genotypic relationships between samples on recent time scales allowing assessment of the current conditions on a landscape.

In this study, we use population genetic parameters to compare *O. mykiss* populations in a tributary stream basin with numerous irrigation diversion dams to two nearby tributary basins to the Methow River, Washington. *O. mykiss* are a spring

spawning species migrating during peak flows which can alter temporary barriers such as beaver dams, log jams and small irrigation diversions creating passage opportunities over or around these obstacles. Therefore, determining the level of connectivity or pass-ability of an obstacle may be difficult. Direct observations of tagged individuals may be impractical if passage is periodic (such as every few years) and/or very small. In addition, movement (or dispersal) of individuals based on tag information does not provide an estimate of successful migration (or genotypic exchange) between sites. The objectives of our study were to: 1) estimate the level of migration; 2) identify the relative proportion of hatchery admixture; and 3) identify the relative effect of stream obstructions and distance on migration rates and hatchery admixture in *O. mykiss* from Beaver, Libby and Gold creeks, tributaries to the Methow River, WA.

Study Area

The Methow Basin is located on the east side of the Cascade Mountain Range in north-central Washington. The Methow River is a tributary of the Columbia River located about 843 km upstream from the estuary. Beaver Creek is a 3rd order tributary that flows west into the Methow River 91.1 km upstream from the mouth (Fig 4.1). Libby and Gold Creeks are 3rd and 4th order tributaries, respectively, that flow east into the Methow River. Libby Creek is 42.5 km and Gold Creek is 35.1 km from the mouth of the Methow River. Basin areas for Beaver, Libby and Gold basins are 290.1, 104.4 and 230.5 km^2, respectively. The upper portions of these basins are managed forest land

administered by state or federal agencies. The lower portions of these basins are irrigated, privately-owned residences and farms.

Access for fish into Beaver Creek was disconnected due to water withdrawal and associated structures for more than 100 years (Martens and Connolly 2010). Several small irrigation diversion dams (1.0 to 2.0 m high) were located along Beaver Creek. Six of these structures were "push up" dams made of various materials such as wood, rock and plastic sheets or tarps. The most downstream irrigation diversion was a 2.0 m high concrete diversion dam. These irrigation diversion structures were modified to allow fish passage from 2000 to 2004. Although Libby and Gold Creek Basins support irrigation withdrawals, residences and road systems, these streams maintained connectivity for spring migrating *O. mykiss*. *O. mykiss* were the most abundant species of salmonid throughout the Beaver, Libby and Gold Creek Basins.

Hatchery Releases

The Grand Coulee Fish Maintenance Project provides mitigation for the loss of fish habitat and production from the construction of Grand Coulee Dam during the 1930s. Hatchery production was intended to replace lost natural production of anadromous salmon and steelhead from tributaries upstream blocked by the dam. The State of Washington also manages a hatchery program to mitigate for other hydropower facilities on the Columbia River.

Currently state and federal hatchery programs in the Methow Basin release 450,000 – 550,000 *O. mykiss* smolts per year. Returning adult *O. mykiss* are spawned

and the eggs are reared at Wells Hatchery on the Columbia River downstream from the mouth of the Methow River. Current practices include intentional breeding between hatchery and naturally produced adults, and progeny from these crosses are primarily released in the Methow River basin (C. Snow, Washington Department of Fisheries and Wildlife, personal communication). Hatchery *O. mykiss* are released as age 1 smolts in the Methow and Chewuch Rivers upstream from the town of Winthrop, WA. All hatchery-origin *O. mykiss* were marked with an internal tag (such as a PIT tag), external tag (such as an elastomer tag) and/or fin clip.

Methods

Fish Collections and Movements

Juvenile *O. mykiss* were sampled at 6 sites in the Beaver Creek Basin, 4 sites in the Libby Creek Basin and 11 sites in the Gold Creek Basin (Fig. 4.1). This study is intended to assess the connectivity of populations of *O. mykiss* in Beaver Creek prior to the completion of diversion dam modifications that would improve fish passage in the stream particularly for an anadromous life history. In Beaver Creek, one site was downstream of the lowest diversion dam (LBC), one site was located between the various diversion dam modifications (UBR), three sites were located upstream from the diversion dams (CMP, SFB, BCusLC) and one site was located on a tributary that flows into Beaver Creek between the diversion dams (FRA) (Fig. 4.1). Sites were selected in a stratified random design to spatially represent populations located in these basins.

Collections were made during the fall of 2004 or the summer of 2005 sampling age 1+ juvenile *O. mykiss* in the stream.

Juvenile *O. mykiss* were collected using a backpack electrofisher (Smith Root Inc. LR-24). Trout were measured to the nearest mm fork length and weighed to the nearest 0.01g using a digital scale (Ohaus, Scout Pro SP 400). Juvenile and adult trout were scanned for PIT tags and coded wire tags and inspected for any other external tags (such as fin clips, elastomer tags, etc.). If the trout did not have a PIT tag, a tag was inserted in the body cavity for trout >65 mm (12.5 mm tag, full duplex 134.2 kHz). A tissue sample was removed from the caudal fin of juvenile and adult trout and stored in 95% non-denatured ethanol. Anadromous outmigration rates were estimated for each site based on the proportion of PIT tags read at passage facilities on the mainstem Columbia River out of the total number released at a site.

Stream segments between the mouth of each creek and each site were walked to measure obstructions and gradients. Obstructions were beaver dams, log jams, culverts and diversion dams that could prevent adult *O. mykiss* passage in the stream. The obstruction type, height and jump pool depth were measured. Maximum stream gradient was measured between each site using a clinometer. Stream temperature was measured at each site using Hobo tidbit loggers reading every 30 mins. during the summer 2009. Elevation at each site and stream distances were measured using GIS.

Laboratory Methods

Tissue samples from the Wells Hatchery brood years 2005 and 2006 (hatchery x hatchery crosses) were provided by the Washington Department of Fisheries and Wildlife (WDFW). Sixteen microsatellite markers were used to identify individuals. Thirteen of these markers are standardized across the Columbia River Basin and are cited in Stephenson et al. (2009). Additional primer sets analyzed were: *One102* (Olsen et al. 2000), *Omm1036* and *Omm1046* (Rexroad et al. 2002).

DNA was isolated from fin clips preserved in ethanol using Qiagen DNEasy tissue extraction kits following standard manufacturer's protocols. Sixteen microsatellite loci were amplified using the polymerase chain reaction (PCR) in three multiplex reactions using Qiagen Multiplex PCR Master Mix on Applied Biosystems GeneAmp PCR System 9700 thermal cyclers in 96 well plates. PCR products were run on an Applied Biosystems 3730 genetic analyzer. Peaks were scored using GeneMapper version 3.7 software (Applied Biosystems, Foster City, California), and labeled following the Stevan Phelps Allele Nomenclature (SPAN) convention (Stephenson et al. 2009). Forward primers were fluorescently labeled (Applied Biosystems).

Amplification (PCR) reactions consisted of 5 ul reactions containing 2.5 ul Qiagen Multiplex PCR Master Mix, five or six primer sets and water, added to 2 ul of extract dried down in a 96 well plate. Cycling conditions included initial denaturation for 15 min at 95°C, followed by 28 cycles for 30 s at 94°C, 90 s at 51°C (Multiplex A) or 57°C (Multiplex B and Multiplex C), and 60 s at 72°C, followed by a final cycle for 30

min at 60°C. Multiplex A contained *Oki23*, *Oke4*, *Oneu14*, *Ssa289*, and *Ssa408*; Multiplex B contained *Ots4*, *Omy7*, *Ogo4*, *One102*, *Omm1046*, and *Ssa407*; Multiplex C contained *Ots100*, *Omy1011*, *Omy1001*, *Ots3m*, and *Omm1036*.

Amplification products were diluted with 10 ul DNA grade water and 1 ul of each dilution added to 10 ul of LIZ/formamide solution (30 ul LIZ600 to 1 ml formamide). Completed runs were analyzed automatically using Genemapper, followed by manual analysis of all peaks for verification. All homozygous results were checked for small allele dropout and large allele dropout. Peaks were also visually checked for conformity to expected profiles. Laboratory error rates for the 13 standardized loci are <2% (Stephenson et al. 2009). Duplicate samples indicate laboratory error rates <1% for our study.

Statistical Analysis

Passage over obstacles for adult *O. mykiss* require a 1 to 1.25 height to pool depth ratio based on jumping ability and hydraulics (Bjornn and Reiser 1991). Therefore, we considered stream obstacles exceeding this 0.8 ratio obstructions. The number of obstructions was standardized to stream distance. For migration rate analyses, all environmental variables were summarized pair-wise between sites within each tributary basin. For hatchery admixture analyses, all environmental variables were summarized from the mouth of the tributary to the site. Stream temperature data were averaged between July 1 and September 10, 2009 for relative maximum summertime temperature across sites.

Prior to statistical tests, full siblings were identified and removed from the data set using ML-RELATE (Kalinowski 2006). Exact tests of Hardy Weinberg Equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0.10 (Raymond and Rousset 1995). Expected heterozygosity and exact tests for allele frequencies were calculated using GENEPOP. Unbiased estimates of allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). All comparisons were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

Migration rates were estimated between each site within each of the tributary basins using Bayesian Inference of Migration Rates (BIMR) (Faubet and Gaggiotti 2008). Sites without significant allele frequency differences were not included in this analysis and one site was used to represent all the similar comparisons. In Libby Creek, site LLC was not included in the migration rate analysis. In Gold Creek, sites LGC, LSF, LFD, UGC were not included in this analysis. BIMR was run using 2 million iterations for burn in and 20 million iterations for sampling with a thinning interval of 2,000 using a Markov Chain Monte Carlo resampling algorithm as described in Wilson and Rannala (2003). The default values were used for all other parameter settings. Ten runs were performed for each basin and the best run was selected with the lowest log likelihood. We also calculated the deviance as described in Faubet et al. (2007) to identify runs that did not converge. Sites with migration were compared to sites without migration using a Wilcoxon test. To avoid excessive numbers of sites with 0 migration estimates, we analyzed the Beaver Creek sites separately. Therefore, sites with and without migration were compared in Gold and Libby Basins, and then sites with migration from Gold and Libby Basins were compared to Beaver Creek.

Multinomial logistic regression was used to predict migration rates between each site within a tributary basin from measured environmental variables. Migration estimates were grouped into one of three categories for the response variable: no migration, low migration (0.001-0.07) and high migration (0.10-0.32). The purpose of these models was to evaluate the relative model fit for isolation by distance to isolation by resistance (obstruction number, obstruction height to depth, maximum gradient). Average maximum summertime stream temperature was used as an alternative variable to distance that would have an expected longitudinal gradient in the stream. A global model included the candidate predictor variables for distance and resistance and interaction variables. From this global model, subsets of predictor variables were chosen for comparison to the global model. The relative plausibility of the models were compared using Akaike's Information Criteria with the small sample adjustment (AIC, Akaike 1973; Burnham and Anderson 1998) with the best fitting model having the lowest AICc value. Model weights and evidence ratios were calculated as described in Burnham and Anderson (1998). A goodness-of-fit test was used on the best fitting model to test whether the data could plausibly arise from the model. All GLMs and goodness-of-fit tests were performed using R (R Development Core Team 2010).

The proportion of hatchery admixture was estimated for each *O. mykiss* collected at each site in the sample with known hatchery steelhead from Wells Hatchery (n=99) using STRUCTURE version 2.3.3 (Pritchard et al. 2000). The two hatchery brood years were not statistically different and were combined for our analysis. STRUCTURE is a Bayesian based model that clusters individuals according to allelic frequencies minimizing Hardy Weinberg and linkage disequilibrium. The admixture model was run

in STRUCTURE using 10,000 iterations for burn in and 100,000 iterations using a Markov Chain Monte Carlo resampling algorithm as described in Prichard et al. (2000). The number of populations (K) was set to 2 and all other settings were run using default values. Ten runs were performed for each site and the run with the lowest log likelihood was selected as the best run. Percent hatchery admixture for each individual was averaged for each sample collection at each site. Logistic regression was used to predict percent hatchery admixture from measured environmental variables, and models were compared using AICc values (Akaike 1973).

Results

The total number of alleles detected at each locus ranged from 7 to 24 with the average allelic richness ranging from 4.5 to 6.8 (Table 4.1). Tests of Hardy Weinberg Equilibrium detected significant departures in two comparisons (One 102 at SFL and Omy 1011 at CTR). Linkage disequilibrium detected significant departures at 6 pairs of loci from the samples from Beaver, Libby and Gold Creek sites. Tests on the Wells Hatchery samples did not detect any significant departures from Hardy Weinberg Equilibrium but did detect linkage disequilibrium at 6 pairs of loci. There was no discernible pattern to the pairs of loci in the linkage disequilibrium tests.

Percent tag outmigration was used as an indication of the dominant life history at a site. Percent tag outmigration and percent Wells Hatchery admixture both followed a declining trend from the largest values at the most downstream sites in each tributary basin and smallest values in the upstream sites and were highly correlated ($r>0.80$) (Table

4.1, Fig. 4.2). Genetic differentiation (F_{ST}) ranged from 0.02 to 0.15 in Beaver Creek. Libby and Gold Creeks had slightly lower F_{ST} values ranging from 0 to 0.09 (Table 4.2). Allele frequency exact tests were significantly different for all pairs of sites in Beaver Creek. In Libby Creek, the two most downstream sites were not significantly different (LLC and LCI). In Gold Creek, the mainstem sites (LGC, GCdsMF, UGC), LSF and LFD were not significantly different. All other comparisons were significant (Table 4.2).

Pair-wise migration estimates indicated no migration between sites for the generation prior to 2004 in Beaver Creek (Table 4.3). Libby Creek maintained migration in both directions between the three tested sites in this basin (Table 4.4). In Gold Creek, there was migration from sites in the upper tributaries into the mainstem Gold Creek sites and lower South Fork Gold and lower Foggy Dew (Table 4.5). However, there was no detectable migration upstream to these upper sites from the mainstem in Gold Creek. When the level of migration among sites in Libby and Gold creeks were categorized, 75% of the sample had no migration, 17% of the sample had low migration and 8% of the sample had high migration.

Wilcoxon rank sum tests comparing sites in Libby and Gold Creeks with migration to sites without migration found significant differences in distance, number of obstructions, obstruction height to depth, and gradient ($p<0.02$) (Figs. 4.3 and 4.4). When comparing the Beaver Creek sites to the sites in Libby and Gold that had migration, the number of obstructions was the only significantly different comparison ($p<0.03$). Model selection found that the model with obstruction height to depth and maximum gradient was the best fit predicting the level of migration with an AICc of 23.66 (Table 4.6). Evidence ratios indicated this model was 294 times more likely than

the next best fitting model (Table 4.6). Obstruction height to depth ratio and maximum gradient were inversely related to increased migration, and the interaction term was positively related to migration (Table 4.7). The goodness-of-fit test for this model was not significant ($p>0.98$).

The models predicting percent hatchery admixture did not clearly indicate relationships in the data. The ΔAICc values for the top four models were less than 1 providing little support for any of the tested predictors. None of these predictors were significant. The goodness-of-fit tests for the top two models were not significant ($p=1.0$).

Discussion

Disconnected or fragmented habitats can impact the demographic exchange and genetic diversity among populations by restricting gene flow and increasing the effects of genetic drift (Allendorf and Luikart 2007). Loss of genetic diversity is associated with losses in fitness (Reed and Frankham 2003) and reduced adaptive potential (Swindell and Bouzat 2005). Overall, the *O. mykiss* in our study had similar genetic measurements as those in other studies of the species (Heath et al. 2001; Narum et al. 2004, 2008; Neilsen et al. 2009). Our study did not include populations upstream of waterfalls, therefore we documented slightly lower maximum F_{ST} values and slightly higher minimum heterozygosity than Narum et al. (2008). Pre-treatment barrier effects indicate the highest F_{ST} and lowest heterozygosity in the upper Beaver Creek sites (FRA and BCusLC), little to no recent migration and reduced Wells Hatchery admixture among sites in the basin. The reference streams in our study showed connectivity among sites

throughout the basins, and migration was generally biased in the downstream direction. There was high migration of individuals between the lower two sites in Libby Creek (LLC, LLI) and the five lower-most sites in Gold Creek (LGC, GCdsMF, UGC, LSF, LFD).

Longitudinal trends in streams are directly correlated with environmental variables such as distance from the mouth, elevation, temperature, width, depth, and channel gradient. These longitudinal gradients of environmental variables are typically correlated with species distributions (Weigel and Sorensen 2001 and citations therein). Similarly, life history of *O. mykiss* has a longitudinal gradient in the stream with anadromous sites located lower in the tributary basins and resident sites higher in the basins (Narum et al. 2004, 2008). Sites intermediate between these have a moderate level of anadromy. Gradients in the landscape and the associated environment may result in spatial autocorrelation in the data (Legendre 1993; Smouse and Peakall 1999; Neville et al. 2006b). I addressed non-independence among variables in our data by standardizing variables to distance prior to statistical analysis, using variables with little direct correlation with elevation or distance from the mouth of the stream and using only select variables with longitudinal gradients in a hypothesis testing framework. Maximum gradient was used instead of average channel gradient to represent the most difficult obstacle a fish had to pass when traveling upstream between sites, and this variable is related to underlying geology moreso than the longitudinal trend in the channel.

In our study, we used the percent of tags that outmigrated as an index of anadromy at a site. This variable has a longitudinal gradient and also is highly correlated with the percent of Well Hatchery admixture (r=0.80). Percent hatchery admixture is

linked to anadromy via the local hatchery brood practices. Although matings between hatchery and wild anadromous *O. mykiss* are possible in the natural habitat, they were rare in Beaver Creek and did not result in offspring that survive to return as an adult. Other studies indicate drastic reductions in relative reproductive success when hatchery *O. mykiss* spawn in the natural stream habitat (Araki et al. 2007). In addition, parentage data indicates incomplete isolation between the fluvial and anadromous life history (Araki et al. 2007; Christie et al. 2011). In Beaver Creek, the wild anadromous *O. mykiss* is the link between the hatchery population (with intentional cross breeding in the hatchery) and the fluvial population. Interestingly, the percent tag outmigration data indicates an anadromous life history present at site UBR even though there was very little to no recent migration into the site. The anadromous life history can arise from a resident mother (Zimmerman and Reeves 2000), so juvenile outmigration (anadromy) could have occurred at this site prior to barrier treatment.

Asymmetrical migration is documented for numerous species including humans (*Homo sapiens*) (Faubet and Gaggiotti 2008), plants (*Centaurea corymbosa*), wolves (*Canis lupis)* (Wilson and Rannala 2003) and cutthroat trout (*O. clarkii hewshawi*) (Neville et al. 2006). Stream habitats lend to asymmetrical movement due to the longitudinal gradient with larger habitats in downstream areas, as well as the resistance that the stream flow presents for upstream movement. This resistance results in greater energy expenditure to travel against the current and climb in elevation. Previous studies of stream barriers examined waterfalls that exceed the jumping ability of the study species. These studies treat the barriers as complete or non-existent with a binomial response variable (Costello et al. 2003; Meeuig et al. 2010). Yet, barriers (or

obstructions) can also be incomplete or temporary. Incomplete barriers are passable under specific stream flow conditions whereas temporary barriers would eventually move or deteriorate.

Obstructions can occur naturally (such as log jams or beaver dams) or arise from anthropogenic activities (such as culverts or irrigation diversion dams). These types of smaller obstructions are often more numerous on the landscape than waterfalls and can have cumulative effects on the migration and dispersal of aquatic species. In our study, migration was biased in the downstream direction among the sites in the reference streams, Libby and Gold creeks. Libby Creek had higher levels of two directional migration among sites whereas Gold Creek had migration solely from the upper sites into the mainstem and lower sites. However, it is important to note that the lower-most sites were lumped for this analysis due to indistinguishable population genetic differences indicating high migration rates among these sites.

Faubet and Gaggiotti (2008) similarly combine populations with no detectable genetic differences. Simulation studies indicate that migration estimation can be inaccurate particularly when genetic differentiation is low (F_{ST}=0.01); however, the estimation can be fairly accurate when differentiation is higher (Faubet et al. 2007; Faubet and Gaggiotti 2008). Higher migration rates (>0.3 Wilson and Rannala 2003; >0.7 Faubet and Gaggiotti 2008) are also difficult to detect. These inaccuracies influence the parameter estimates, increase unexplained variation and result in greater posterior probability intervals (Faubet et al. 2007; Faubet and Gaggiotti 2008). In this study, potential inaccuracies in the exact estimated rates are addressed by using categorical classifications of the relative rates of estimated migration. We also only analyzed sites

with detectable genetic differences, thereby avoiding the source of some of these inaccuracies.

Geographic distance is commonly correlated with genetic distance (Wright 1943). Distance is related to the dispersal ability of the organism. Isolation by distance is commonly detected in anadromous *O. mykiss* populations (Heath et al. 2001; Narum et al. 2008; Neilsen et al. 2009). Basin is also often associated with genetic distances in salmonids with sites from different basins having greater genetic distances (Costello et al. 2003; Narum et al. 2004, 2008; Neilsen et al. 2009). Although distance often provides good predictive models, ecologists are often striving for more mechanistic relationships that could drive an organism's preference for habitats or ability to survive and reproduce. Resistance has been used to explain the path and associated likelihood of movement by organisms (Cushman et al. 2006; Spear et al. 2010). In this hypothesis, certain pathways may be less preferred but still available and characteristics of the site are associated with a resistance or permeability value (Spear et al. 2010).

In anadromous fish, resistance is potentially important due to the long migration distances traveled by adults returning to natal areas that deplete limited energy reserves. This could influence the distance or the ability to navigate obstructions in the stream environment. In our analysis, we compared the level of migration between sites using three resistance variables (number of obstructions, obstruction height to depth ratio and maximum upstream gradient) to the null hypothesis of isolation by distance. In the reference streams, we found that isolation by resistance was a better predictor of the level of migration than distance. The resistance variables that provided the best fit for the data included obstruction height to depth ratio, maximum gradient and their interaction.

Interestingly, the percent Wells Hatchery admixture did not provide as clear model results when comparing resistance variables to distance. We suspect that this is a result of the hatchery brood practices that link the wild anadromous alleles to the hatchery alleles resulting in an association between these alleles and the anadromous gradient longitudinally in the stream. Alternatively, it is possible that the migration of hatchery *O. mykiss* into these sites is unrelated to the variables tested resulting in a spurious correlation, where hatchery *O. mykiss* that successfully spawn at sites in the study area use other cues such as presence of a mate or presence of spawning gravel.

In summary, small irrigation diversion dams were limiting population interactions in Beaver Creek and collectively blocking anadromous *O. mykiss* migration into the stream. However, these barriers also limited the percent Wells Hatchery admixture in this stream providing some protection to native genotypes in the basin. The patterns of migration and associated environmental variables were different when comparing Beaver Creek to the reference streams indicating that the higher level of anthropogenic impacts in the creek resulted in fragmentation of the *O. mykiss* population. Variables related to stream resistance, such as obstruction height to depth ratio and maximum gradient, were better predictors of the level of migration than stream distance. This important finding may provide a better understanding of factors related to stream connectivity and population interactions and should be investigated in other lotic species.

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Table 4.1. Sample sizes (n), expected heterozygosity (He), average allelic richness (AR), average private alleles (PA), percent PIT tags that outmigrated, percent hatchery admixture and elevation (m) for sample sites in Beaver, Libby and Gold creeks.
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	n	He	AR	PA	%tag out	% hatchery	elev (m)
Beaver	**Creek**						
LBC	28	0.81	6.64	0.21	7.0	40	474.5
UBR	19	0.79	6.01	0.17	6.0	27.4	547.5
CMP	36	0.76	5.91	0.05	0.4	6	803.1
SFB	28	0.73	5.25	0.08	1.0	1.8	888.3
BCabLC	22	0.70	4.79	0.07	0.0	0.9	1028.2
FRA	25	0.67	4.53	0.10	0.0	1.8	851.7
Libby	**Creek**						
LLC	36	0.82	6.84	0.13	8.0	31.5	425.9
LCI	23	0.81	6.81	0.19	3.0	25.6	486.7
LCBen	32	0.78	6.06	0.11	0.4	14.1	778.7
SFL	15	0.72	4.67	0.12	0.0	0.6	1052.5
Gold	**Creek**						
LGC	46	0.82	6.78	0.16	6.0	42.6	401.5
GCdsMF	30	0.82	6.77	0.13	6.0	37.1	486.7
UGC	16	0.83	6.81	0.23	5.0	28	669.2
GCusCC	25	0.79	6.10	0.20	2.0	9.5	790.9
LSFG	28	0.81	6.55	0.11	6.0	56.9	644.9
USFG	39	0.77	5.84	0.05	2.0	13.7	876.1
RNY	19	0.74	5.14	0.09	1.0	3.8	754.4
MFG	15	0.76	5.49	0.15	0.0	9.6	681.4
LFD	35	0.83	6.88	0.26	3.0	29.3	730.1
UFD	25	0.79	5.86	0.22	0.0	3.3	882.2
CTR	7	0.82	6.69	0.55	2.0	3.8	924.8

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Table 4.2. Pairwise F_{ST} values among sample sites in Beaver, Libby and Gold creeks.

	LBC	FRA	UBR	CMP	SFB	BCusLC	LLC	LCI	LCB	SFL
FRA	0.091									
UBR	0.018	0.1089								
CMP	0.0269	0.0743	0.0455							
SFB	0.0464	0.1125	0.0625	0.0289						
BCusLC	0.0868	0.1466	0.0965	0.0567	0.0989					
LLC	0.0039*	0.0866	0.0211	0.028	0.0447	0.0741				
LCI	0.0122	0.0763	0.0247	0.0196	0.0344	0.0708	0.0046*			
LCB	0.0432	0.1091	0.0393	0.0408	0.051	0.0842	0.0282	0.0178		
SFL	0.1	0.1818	0.1075	0.1067	0.1165	0.1515	0.0916	0.0931	0.08	
LGC	0.0063*	0.0825	0.025	0.0164	0.0416	0.0565	0.0000*	0.0048*	0.0263	0.0935
LSF	0.0138	0.0953	0.0267	0.0191	0.0411	0.061	0.0043*	0.01	0.0191	0.0896
USF	0.0268	0.0979	0.0387	0.027	0.0537	0.0674	0.0235	0.0263	0.0476	0.1108
RNY	0.0407	0.1188	0.0491	0.0486	0.0631	0.099	0.0317	0.0407	0.0467	0.1078
UGC	0.006	0.0713	0.0255	0.0247	0.0488	0.0831	0.0067*	0.0047*	0.0301	0.0946
LFD	0.019	0.0884	0.0337	0.0274	0.0453	0.073	0.0077	0.0085	0.0265	0.087
UFD	0.0237	0.094	0.0418	0.0308	0.0497	0.089	0.0208	0.0191	0.0397	0.0991
GCusCC	0.065	0.1229	0.0776	0.0633	0.0887	0.0904	0.045	0.0488	0.0516	0.1048
CTR	0.0605	0.1497	0.0823	0.0821	0.0966	0.1254	0.0525	0.0665	0.085	0.0917
MFG	0.0504	0.0974	0.0564	0.0485	0.0639	0.1036	0.0373	0.0393	0.0516	0.1389
GCdsMF	0.0016*	0.08	0.0235	0.0142	0.0363	0.0615	0.0007*	0.0054*	0.0255	0.0905

* denotes not significant allele frequency exact test after Bonferroni correction

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Table 4.2. continued

LSF	0.0000*									
USF	0.0137	0.011								
RNY	0.0327	0.0253	0.0437							
UGC	0.007*	0.0077	0.0224	0.0406						
LFD	0.0074	0.0055	0.0277	0.0351	0.009*					
UFD	0.0182	0.0166	0.0356	0.0447	0.0253	0.0177				
GCusCC	0.0408	0.0445	0.0672	0.0722	0.0477	0.0397	0.0541			
CTR	0.0582	0.0642	0.0747	0.0859	0.0424	0.0541	0.0769	0.0637		
MFG	0.0324	0.0361	0.0544	0.0488	0.0318	0.0335	0.0509	0.0705	0.0941	
GCdsMF	0.000*	0.003*	0.0198	0.0324	0.0054*	0.0056*	0.0114	0.0397	0.0586	0.0367

* denotes not significant allele frequency exact test after Bonferroni correction

Table 4.3. Mean pairwise migration estimates and 95% posterior probabilities in parentheses among sites in Beaver Creek.

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from/into	LBC	UBR	SFB	BCusLC	CMP	FRA
LBC	1 (1.0, 1.0)	0 (5.8×10^{-12}, 5.7×10^{-10})	0 (5.6×10^{-12}, 1.1×10^{-9})	0 (4.1×10^{-12}, 7.4×10^{-10})	0 (1.1×10^{-12}, 6.7×10^{-10})	0 (6.7×10^{-12}, 5.7×10^{-10})
UBR	0 (1.9×10^{-12}, 1.5×10^{-9})	1 (1.0, 1.0)	0 (7.4×10^{-12}, 1.1×10^{-9})	0 (3.3×10^{-12}, 7.0×10^{-10})	0 1.1×10^{-12}, 6.3×10^{-10})	0 (8.3×10^{-12}, 5.7×10^{-10})
SFB	0 (1.8×10^{-12}, 1.5×10^{-9})	0 (6.1×10^{-12}, 5.8×10^{-10})	1 (1.0, 1.0)	0 3.3×10^{-12}, 7.3×10^{-10})	0 (9.3×10^{-13}, 6.7×10^{-10})	0 (5.5×10^{-12}, 5.9×10^{-10})
BCusLC	0 (2.2×10^{-12}, 1.5×10^{-9})	0 (4.8×10^{-12}, 5.6×10^{-10})	0 (6.5×10^{-12}, 1.2×10^{-9})	1 (1.0, 1.0)	0 1.1×10^{-12}, 6.6×10^{-10})	0 (5.7×10^{-12}, 5.9×10^{-10})
CMP	0 (2.0×10^{-12}, 1.4×10^{-9})	0 (4.2×10^{-12}, 5.8×10^{-10})	0 (7.0×10^{-12}, 1.1×10^{-9})	0 4.0×10^{-12}, 8.1×10^{-10})	1 (1.0, 1.0)	0 (6.0×10^{-12}, 5.9×10^{-10})
FRA	0 (2.4×10^{-12}, 1.4×10^{-9})	0 (5.0×10^{-12}, 5.7×10^{-10})	0 (6.3×10^{-12}, 1.2×10^{-9})	0 (4.0×10^{-12}, 7.5×10^{-10})	0 8.3×10^{-13}, 6.4×10^{-10})	1 (1.0,1.0)

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Table 4.4 Mean pairwise migration estimates and 95% posterior probabilities in parentheses among sites in Libby Creek.

from/into	LCI	LCB	SFL
LCI	0.63	0.18	0
	(0.43, 0.82)	(0.04, 0.32)	($3.6x10^{-9}$, 0.01)
LCB	0.32	0.75	0
	(0.13, 0.50)	(0.58, 0.91)	($3.7x10^{-9}$, 0.01)
SFL	0.06	0.07	1
	(0.01, 0.13)	(0.02, 0.15)	(0.98, 1.0)

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Table 4.5. Mean pairwise migration estimates and 95% posterior probabilities in parentheses among sites in Gold Creek.

from/into	CTR	UFD	GCusCC	GCdsMF	MFG	RNY	USF
CTR	1 (1.0, 1.0)	0 ($1.0X10^{-14}$, $3.8X10^{-10}$)	0 ($9.1x10^{-12}$, $1.3x10^{-9}$)	0.02 ($6.5x10^{-4}$, 0.08)	0 ($4.9x10^{-13}$, $4.8x10^{-11}$)	0 ($5.0x10^{-12}$, $9.0x10^{-10}$)	0 ($2.0x10^{-12}$, $1.5x10^{-10}$)
UFD	0 ($5.3X10^{-12}$, $1.0X10^{-9}$)	1 (1.0, 1.0)	0 ($7.0x10^{-12}$, $1.3x10^{-9}$)	0.19 (0.06, 0.35)	0 ($4.6x10^{-13}$, $4.6x10^{-11}$)	0 ($6.0x10^{-12}$, $1.0x10^{-9}$)	0 ($1.4x10^{-12}$, $1.7x10^{-10}$)
GCusCC	0 ($5.2X10^{-12}$, $1.1X10^{-9}$)	0 ($8.3X10^{-15}$, $4.2X10^{-10}$)	1 (1.0, 1.0)	0.08 (0.01, 0.17)	0 ($4.6x10^{-13}$, $4.3x10^{-11}$)	0 ($6.4x10^{-12}$, $9.7x10^{-10}$)	0 ($1.3x10^{-12}$, $1.7x10^{-10}$)
GCdsMF	0 ($4.2X10^{-12}$,$1.2X10^{-9}$)	0 ($6.4X10^{-15}$, $3.8X10^{-10}$)	0 ($9.2x10^{-12}$, $1.4x10^{-9}$)	0.46 (0.27, 0.69)	0 ($4.1x1^{0-13}$, $4.6x10^{-11}$)	0 ($5.3x10^{-12}$, $1.0x10^{-9}$)	0 ($9.3x10^{-12}$, $1.9x10^{-10}$)
MFG	0 ($5.3X10^{-12}$, $1.1X10^{-9}$)	0 ($8.6X10^{-15}$, $4.0X10^{-10}$)	0 ($8.2x10^{-12}$, $1.3x10^{-9}$)	0.07 (0.005, 0.17)	1 (1.0, 1.0)	0 ($6.7x10^{-12}$, $1.0x10^{-9}$)	0 ($1.6x10^{-12}$, $1.7x10^{-10}$)
RNY	0 ($5.3X10^{-12}$, $1.0X10^{-9}$)	0 ($8.7X10^{-15}$, $3.7X10^{-10}$)	0 ($7.6x10^{-12}$, $1.4x10^{-9}$)	0.03 ($8.0x10^{-4}$, 0.09)	0 ($3.9x10^{-13}$, $4.6x10^{-11}$)	1 (1.0, 1.0)	0 ($1.6x10^{-12}$, $1.6x10^{-10}$)
USF	0 ($4.8X10^{-12}$, $1.1X10^{-9}$)	0 ($5.6X10^{-15}$, $3.8X10^{-10}$)	0 ($7.0x10^{-12}$, $1.3x10^{-9}$)	0.15 (0.04, 0.29)	0 ($3.7x10^{-13}$, $4.4x10^{-11}$)	0 ($7.0x10^{-12}$, $9.7x10^{-10}$)	1 (1.0, 1.0)

Table 4.6. Multinomial logistic regression results and model comparison values.

Variables	AIC	K	AICc	Δ_i	£(g_i|x)	w_i/w_t	evid ratio
ob_htd, max grad	27.16	3	23.66	0	1	0.99544	
grad, temp	39.20	4	35.03	11.37	0.003	0.00338	294.9
ob_htd, ob_km, max grad	43.15	8	37.15	13.49	0.001	0.00117	849.8
ob_htd, temp	50.16	4	45.99	22.33	1.4E-05	1.4E-05	70732.9
ob_km, grad	61.02	4	56.85	33.19	6.2E-08	6.2E-08	16137667
temp	65.82	2	63.07	39.41	2.8E-09		
dist	70.13	2	67.38	43.72	3.2E-10		
ob_km, ob_htd, grad, temp	70.56	16	64.89	41.23	1.1E-09		
dist, ob_km, ob_htd, grad	71.75	16	66.08	42.42	6.1E-10		

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Table 4.7. Model coefficients and standard errors for the multinomial models predicting the level of migration in Libby and Gold creeks.

Model	Variable	Coeff	Std Error
Low Migration	Intercept	220.25	36.49
	ob_htd	-99.39	49.15
	max_grad	-45.37	19.35
	interaction	13.82	8.34
High Migration	Intercept	219.33	36.5
	ob_htd	-92.57	56.53
	max_grad	-45.17	19.34
	interaction	13.15	9.13

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Figure 4.1. Study area and sites in Beaver, Libby and Gold creeks, tributaries to the Methow River, Washington.

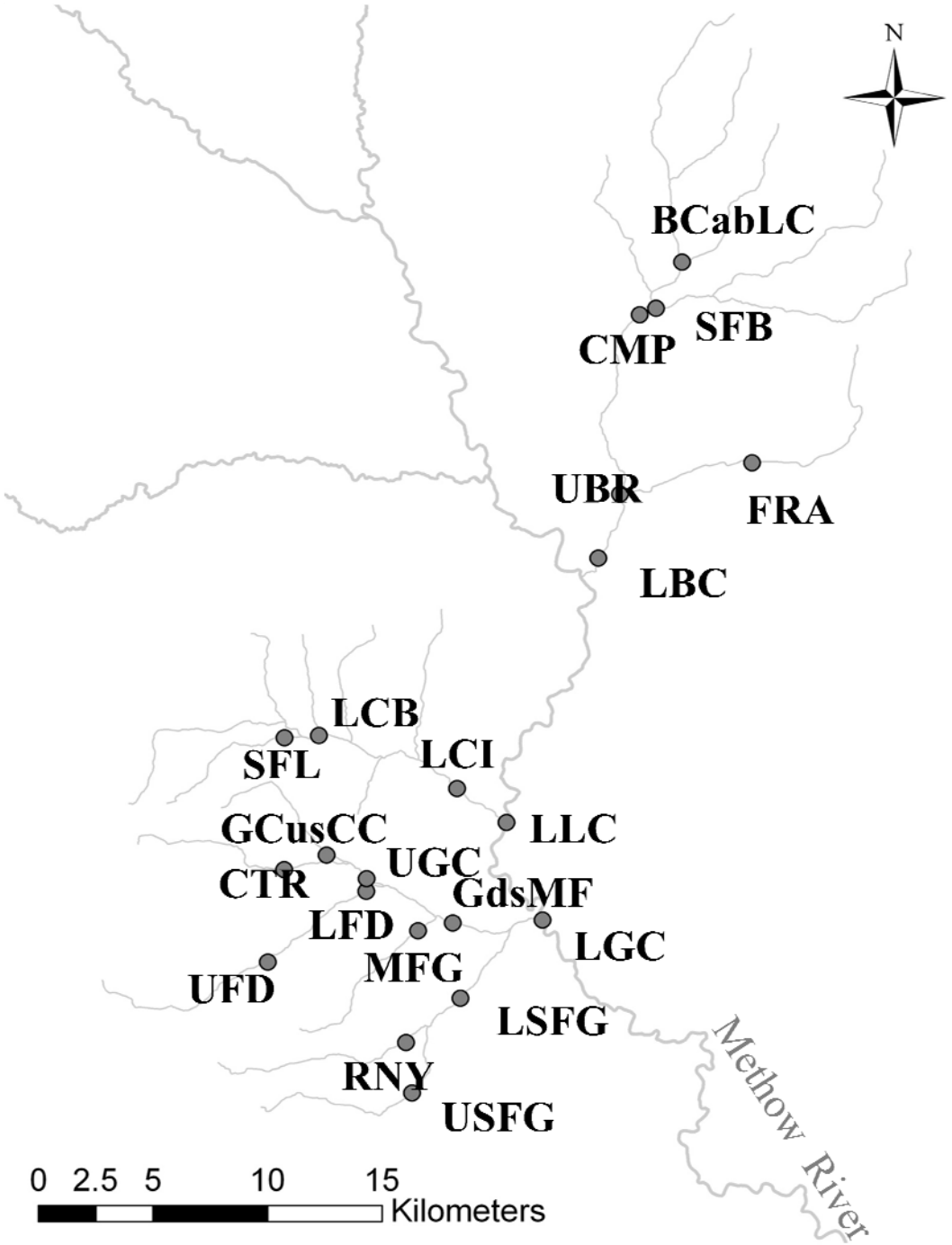


Figure 4.2. Percent hatchery admixture and percent *O. mykiss* with PIT tags that outmigrated. Outmigration was determined as anadromous outmigration when tags were detected on the Columbia River downstream from the Snake River confluence.

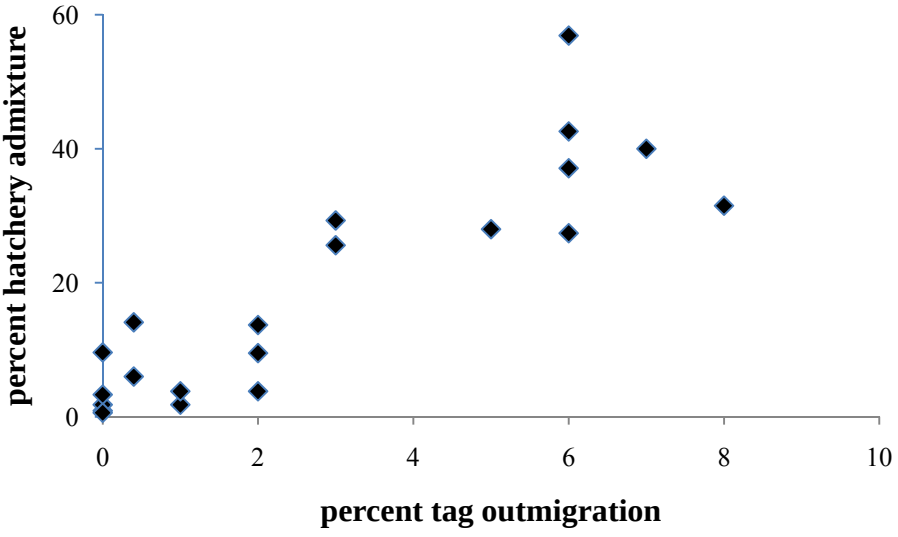
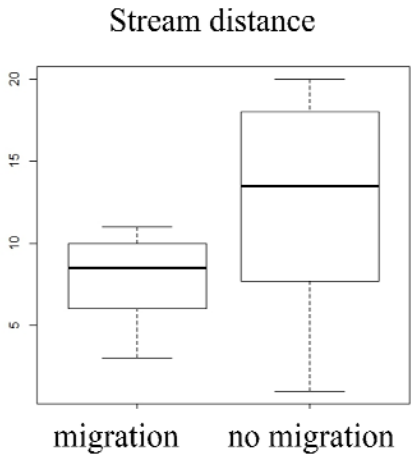


Figure 4.3. Stream distance (km) and stream gradient (%) at sites with migration and sites without migration.



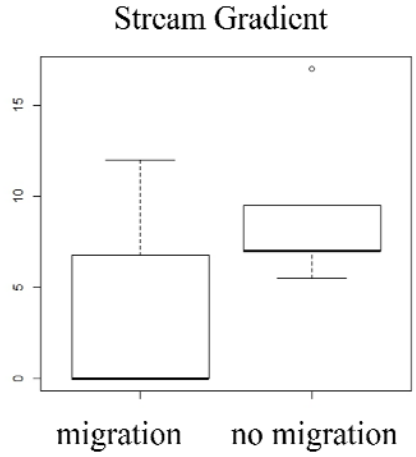


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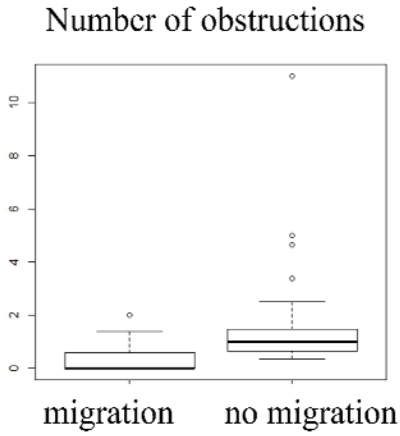
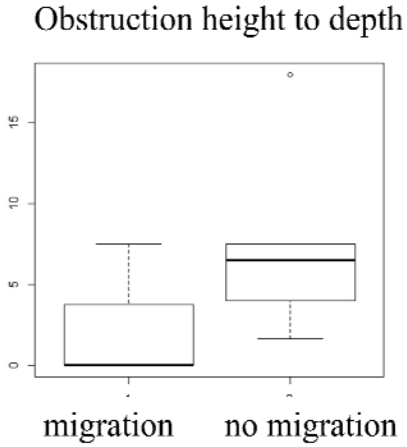
Figure 4.4. Number of obstructions per km and obstruction height to depth ratio for sites with migration and sites without migration.





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Chapter 5

Summary

Colonization is an essential process for the persistence of species in stochastic environments. Salmonids have diverse and overlapping life history strategies that balance the risk of migration with the benefits to fitness. Salmonids are believed to have developed precise homing to natal habitats to ensure successful reproduction after extensive migrations. Connectivity is important for access to essential spawning habitat, and demographic support to increase population size and genetic diversity, as discussed throughout this dissertation. However, I also recognize that connectivity is important for rearing *O. mykiss*, particularly juveniles which may spend up to 4 years in freshwater habitats before smolting. Connectivity during rearing allows juvenile trout and salmon to freely move among the stream habitats to optimize growth and survival.

This dissertation identified several key attributes to population interactions in *O. mykiss* relevant to the management of these populations and habitats. Chapter 2 identified that fluvial *O. mykiss* successfully inter-breed with anadromous *O. mykiss*, and the fluvial component of the colonization of Beaver Creek was substantial. Hatchery *O. mykiss* did not produce offspring that returned to Beaver Creek as adults. Parr outmigrated from Beaver Creek primarily at age 0 and age 1 and some of these parr returned to Beaver Creek as adults. Most of the age 0 parr did not outmigrate until the second spring after leaving Beaver Creek and the exact rearing location for these parr between detections at the Beaver Creek weir and the mainstem Columbia River

downstream from the Snake River confluence is unknown. Parr to adult survival for the first two brood years was estimated at 1.3%.

Chapter 3 identified that both anadromous and fluvial *O. mykiss* were iteoparous. The fluvial life history had higher rates of consecutive spawning events in Beaver Creek, and appears to be a primarily male biased alternative life history strategy. Barrier removal resulted in changes in adult and juvenile migration and population genetic measurements. Percent hatchery admixture increased with the increase in anadromous spawning upstream from the barrier removal projects. Colonization was still progressing into the upper sites in Beaver Creek one generation after barrier removal. Chapter 4 identified that small irrigation diversion dams were limiting recent migration patterns in Beaver Creek, and there was a small effect of population isolation and drift at the two upper-most sites in Beaver Creek. The alternative hypothesis of isolation by resistance was a much more plausible model of migration in Libby and Gold creeks, the two reference streams, than isolation by distance. Percent hatchery admixture was not clearly related to any specific distance or resistance variable tested, and exhibited a longitudinal gradient similar to the measured percent tag outmigration.

Effectiveness Monitoring Results

Barrier removal in Beaver Creek successfully opened what appears to be unoccupied habitat upstream from the diversion dams, and successfully established anadromous offspring produced in this newly opened habitat that returned as adults after ocean residency. Shifts in the populations including adult and juvenile migrations and

population genetic attributes occurred after the habitat was opened. Colonization by hatchery *O. mykiss* and the associated loss of fitness was a concern prior to barrier removal; however, hatchery fish did not successfully colonize Beaver Creek during the first two brood years.

The success and rates of colonization are influenced by the distance to source populations, abundance of the source populations and the suitability of the source populations to local adaptations in the unoccupied habitat (Gaggiotti et al. 2004). In Cedar Creek, WA, coho salmon (*Oncorhynchus kisutch*) nearly doubled each year for the first three years after passage was installed at a dam. This stream had a population of coho salmon spawning downstream from the dam prior to the passage project, and has much shorter migration distances between the ocean and stream habitats than the Methow Basin (Kiffney et al. 2010). In Beaver Creek, it appears that adult anadromous *O. mykiss* were limited with a relatively small number of anadromous spawners passing through the weir ($n<30$). The lack of an increase in the adult run during this time was unexpected, and could be due to the low abundance of the wild anadromous *O. mykiss* returning to Wells Dam coupled with the removal of about 200 individuals for hatchery breeding. Some years when wild returns are low, the hatchery does not have sufficient numbers of wild *O. mykiss* to meet hatchery brood goals.

The colonization process was still underway at the end of this study about five years after barrier removal. Agency commitments for longer term studies and funding are needed to fully understand the effects of various resource management actions on the population growth and survival. Ideally, the colonization dynamic could be studied until the spatial expansion of the anadromous life history appears to reach equilibrium. This

could then be compared to models predicting distributions of species based on habitat attributes to gain a better understanding of where future habitat actions would have the most benefit. In addition, the colonization dynamic could shift over time. For example, the role or effect of hatchery *O. mykiss* in Beaver Creek could change, or selective gradients for traits could change as documented by Anderson et al. (2010) in salmon from Cedar Creek. I detected higher rates of straying in the adults returning to Beaver Creek from the first two brood years after barrier removal. Therefore, we do not know if the straying rate is comparable to other populations and the earlier literature underestimates straying due to a lack of ability to track "unseen" fish, or whether the straying rate will increase or decrease as the population develops. The new instream PIT tag readers help us track these individuals for their complete life cycle. Continued monitoring using the network of PIT tag reading stations newly established in the Methow Basin can provide a better understanding of movements and straying.

Monitoring could be effectively continued on a small scale including continuing the operation and maintenance of the stationary PIT tag interrogation stations, tracking smolt conversion rates at the designated monitoring sites by continuing to deploy PIT tags in juvenile *O. mykiss* and other target species, and sampling tissue for genetic analysis once per generation at the designated monitoring sites. Continued monitoring of abundances (adult escapement) into Beaver Creek would be ideal to track the full life history expressions into the study area; however, it may be more cost effective to work with adult tagging efforts at Priest Rapids Dam and perform run decomposition estimates for Beaver Creek.

Member Vagrant versus Meta-Population

The member-vagrant theory is founded on the concept of local adaptations providing the members of a population an advantage over migrants from other populations. The closer the population is to critical attributes related to reproduction and survival, the more successful a migrant may be in a new population. Evidence presented in this dissertation supporting the member-vagrant theory includes: fitness consequences to hatchery *O. mykiss*, natal homing of wild fluvial and anadromous *O. mykiss*, and fitness advantages of these wild anadromous and fluvial spawners.

The hatchery, on the other hand, is managed as a meta-population of all the steelhead arriving at Wells Dam. This results in some degree of panmixia within the hatchery population and also links the hatchery alleles to the anadromous alleles via artificial spawning. It is likely that most of this brood originates from the Methow/Okanagan Basins upstream of the dam; however, strays from other basins in the Columbia/Snake Rivers have been detected and likely have been incorporated in the brood. Out-crossing can have unintended fitness effects resulting from outbreeding depression. Outbreeding depression has been attributed to two mechanisms: loss of local adaptation (ecological outbreeding depression) and disruption of co-adapted gene complexes (Allendorf and Luikart 2007 and citations therein). The first mechanism is related to disrupting local adaptation that is under selective pressure in a certain environment. The second mechanism is thought to disrupt traits that are coded for by multiple genes that have evolved co-adaptation to expression. These co-adapted gene complexes are often attributed as the reason why the measurable effects of outbreeding

depression may not develop until the F_2 generation. It is hypothesized that first generation hybrids may not be affected because both full sets of co-adapted genes are present. However, these co-adapted complexes are broken down during recombination in the subsequent generations (Allendorf and Leary 2007 and citations therein).

After examining the genetic measurements from hatchery X hatchery brood from Wells Hatchery during this study, it appears that the population has a high level of genetic diversity and a sufficient effective population size such that inbreeding or drift may not be major concerns. Drift in the hatchery population can be countered by incorporating just a few wild *O. mykiss* per generation into the hatchery brood. Removal of high numbers of wild anadromous *O. mykiss* returning to the Methow/Okanagan Basins could be excessive. For example, one wild individual per year could be sufficient to meet hatchery genetic goals. In addition, the high numbers of wild X hatchery crosses currently being released in the Methow Basin could reduce the fitness in the wild populations from outbreeding depression. If the progeny from these hatchery X wild crosses have reduced fitness or outbreeding depression, a loss in productivity to the wild populations could be an unintended impact of the hatchery brood management practices due to a loss of higher productive individuals to the population. Losses in fitness from the F_1 wild X hatchery crosses could be greatest in the subsequent generations when these hatchery reared adults attempt to spawn in the wild.

Genetic Processes in *O. mykiss*

The strong homing behavior of salmonids tends to result in inter-breeding groups that have some degree of relatedness at the spawning sites. Although this is relatively unstudied, *O. mykiss* seem to spawn randomly but may have behavior mechanisms to prevent mating between siblings. The strong homing behavior to natal areas is thought to speed the development of local adaptations that maximize survival. Therefore, the species genetically balances itself outside of the effects and constraints of inbreeding while maintaining very limited gene flow (Wang et al. 2002). Relatedness increased with an increase in resident *O. mykiss* at sites in our study (i.e. higher elevation sites had greater proportions of related individuals). Although relatedness may be detectable at spawning sites, trout and salmon are tetraploids, therefore, they can maintain more genetic diversity when population abundances decline (Wang et al. 2002).

The relative effects of habitat alteration on these genetic processes is relatively unknown, and is difficult to separate from the genetic effects of hatcheries or harvest in the Columbia Basin. My data from Beaver Creek indicates that gene flow is moving from the wild anadromous *O. mykiss* into the hatchery via the hatchery brood practices, and then also from the fluvial *O. mykiss* life history to the wild anadromous spawners. Therefore, the fluvial *O. mykiss* appear to represent an important reserve of the native genotype. This life history also appears to be a male-biased alternative life history strategy. I suspect that there is also gene flow between the resident and fluvial life history types; however, my study was not designed to answer this question.

Interestingly, genetic studies of *O. mykiss* continue to support random mating which is in direct conflict with the behavioral literature that indicates strong size assortative mating. Parentage studies have documented male and female individuals that successfully spawn with multiple partners, and our data as well as data from Seamons et al. (2004) document similar spawning dynamics. The spawning event occurs quickly and multiple trout are present; therefore, it could be difficult to determine which or how many males fertilize a female's eggs from behavioral observations. In addition, females may be retaining a portion of eggs to build additional redds and prolong and diversify the spawning effort. These individuals may appear like new spawners in a behavioral study if they are not uniquely tagged and tracked.

Behavioral literature indicates disruptive selection on male size with the lowest fitness advantage on the intermediate sized males (such as jacks). This information suggests intermediate sized males do not have an advantage in either spawning strategy (fighting or sneaking). My data indicates the intermediate sized males, both anadromous and fluvial, seem to have higher fitness than the largest males. We do not have enough data to examine this relationship; however, I suspect that the lifetime fitness of the fluvial male life history has the highest fitness. These individuals seem to have more multiple spawning opportunities, both within and across spawning years. The largest anadromous males are not observed kelting (spawning in multiple years), and it is suggested that the long migration distance coupled with energy expended fighting subdominant males may result in mortality. These large males could also be subject to higher rates of predation while defending the female.

Recovery of *O. mykiss* Under the Endangered Species Act

Presently, the hatchery populations of *O. mykiss* in the upper Columbia are listed as threatened under the Endangered Species Act. The hatchery populations are considered critical for the recovery of the anadromous life history of the species (McClure et al. 2003). However, our data and several other studies conducted on *O. mykiss* in the last 8 years indicate considerably reduced relative reproductive success of hatchery *O. mykiss* in natural environments (Araki et al. 2007, 2008). Therefore, this leaves the question of what is the feasibility of hatchery *O. mykiss* for the conservation and recovery of the species. Further, my data and other studies (McPhee et al. 2007; Christie et al. 2011) suggests that the alternate life history type of fluvial *O. mykiss* is a critical and substantial component contributing to the genetics of the spawning population of anadromous *O. mykiss*. Therefore, the biologically more important (sub)population of the species is currently not protected.

Inter-breeding between life history types had been recognized prior to the decision to list only the anadromous life history form as a distinct population segment (see Zimmerman and Reeves 2000; Docker and Heath 2003). The listing decision for *O. mykiss* (steelhead) was made using the NMFS 1991 Policy on the Definition of Species Under the Endangered Species Act. This policy has two criteria to qualify as a distinct population segment: 1) it must be substantially reproductively isolated from other nonspecific population units; and 2) it must represent an important component in the evolutionary legacy of the species (Sullins 2001). In 1979, when Congress reconsidered the DPS amendment, they recognized the potential for abuse of this designation, and

clearly directed the Services to use the designation "sparingly" and only when the biological evidence indicates that such action is warranted (Senate Report 151, 96th Congress, 1st session).

The *O. mykiss* life history types do not represent substantially isolated reproductive units, and therefore, the exclusion of the resident or fluvial life history types is biologically unsound (McPhee et al. 2007). Conservation for this species certainly hinges on the protection of the full expression of life history types recognizing that the species has variable lengths of freshwater and ocean rearing making it more susceptible than most other anadromous species to land and aquatic based impacts.

Citations

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Appendix A. Description of Data Analysis and Data Flow

Data analyses were conducted throughout this dissertation to examine the effects of stream passage barriers and barrier removal on population genetic measures. Genetic exchange (migration) is expected when populations are freely connected. Migration is also an indication of reproductive contributions which defines successful colonization required to establish a local population based on "new" individuals.

Microsatellite data are expressed as sizes of fragments or number of base pairs. Peaks on electropherograms were binned in apriori size ranges previously defined for each species and locus. Each allele is identified by size. Genetic data (unique alleles per locus) were examined for outliers, missing data and sources of lab error. Alleles may be identified by a two digit code or by the size of the fragment depending on the input requirements of the analytical program. In either method, each allele is labeled with an identifying code. Alleles are examined within and among sample sites (or populations) with frequency based analyses. Many programs have been developed to summarize and calculate standard population genetic measures. The programs and associated settings used in this dissertation are summarized in Table A.1.

The parentage analysis was based on strict exclusion criteria allowing only one mismatching allele. Although I recognize that it is standard practice to allow more mismatching alleles or even use likelihood based assignment methods, the number of missing parents in *O. mykiss* studies precludes confidence in these types of assignment methods. In addition, the standardized laboratory methods and well researched markers

has resulted in minimal lab error for this species across the Columbia River Basin. I was certain of the matches included in the data set based on these criteria. I examined allowing two mismatching alleles, but this only provided a few additional parr-parent matches (n=9, <4% total matches). All but one of these parr was a one parent match, and the one parr that had two matching parents had 2 mismatched alleles for each identified parent. Because these observations did not add information to our analysis, they were not included. I suspect that parent matching rates were low due to missed parents in the sample, inter-breeding among life history types, lack of sampling effort for resident rainbow trout spawners and the large number of resident rainbow trout prevalent in the study area and sample collections at the time of colonization.

The hatchery admixture and migration rate analyses used in this dissertation are based on linkage disequilibrium methods. These analyses group the genetic data by individual to minimize linkage disequilibrium which is an indication of mixing populations. The program STRUCTURE was used in an unconventional application in Chapter 2 to investigate whether individual steelhead/rainbow trout may choose a mate based on hatchery or wild origin, an indication of non-random mate selection. Although we recognize that individual estimates of percent hatchery origin for an individual may not be precise, I felt that it was important to investigate this relationship considering the extensive hatchery history in the study area. Proportions of hatchery admixture were mostly consistent for individuals and averaged across sites indicating some reliability of estimation used in this application.

Figure A.1. Diagram of statistical analyses of population genetic data for each dissertation chapter.

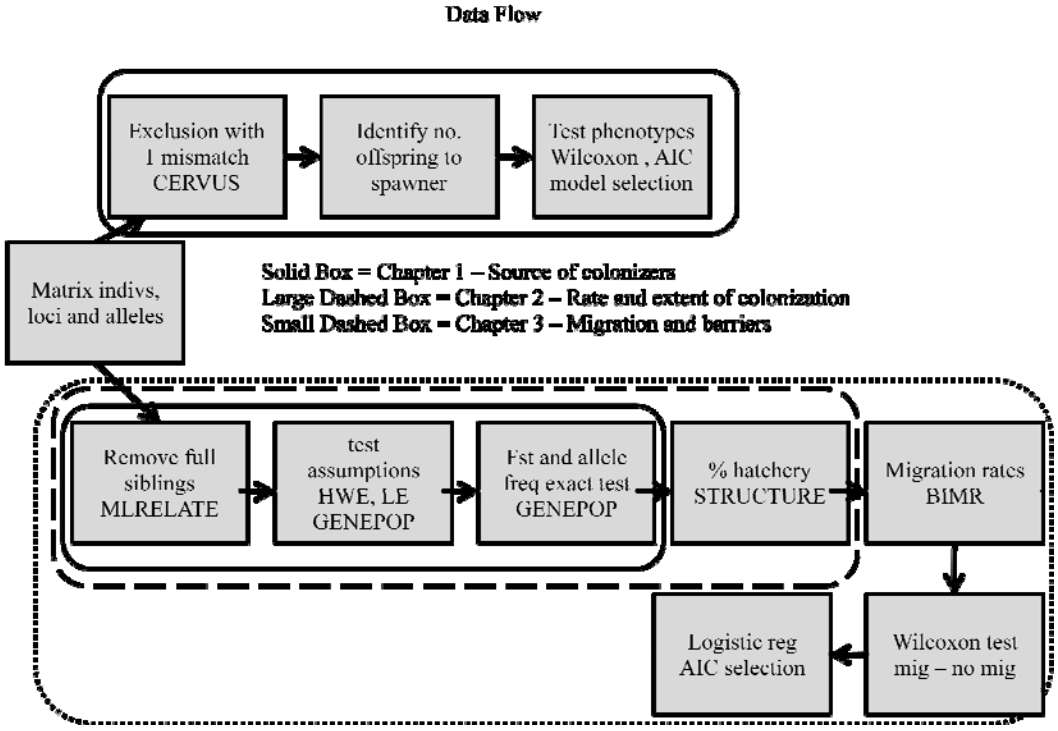


Table A.1. List of statistical test, dissertation chapter where test was used, program version, citation and settings.

Statistical Test	Chapter	Program	Citation	Settings
Parentage	2	CERVUS ver. 3.0.3	Marshall et al. 2002	Exclusion, 1 mismatch
Allelic richness, private alleles, Expected heterozygosity	2, 3, 4	HP RARE	Kalinowski 2005	
Assumptions of HWE, LE	2, 3, 4	GENEPOP ver. 4.0.10	Raymond and Rousset 1995	default
Genetic differentiation (allele frequency exact test, F_{ST})	2, 3, 4	GENEPOP ver. 4.0.10	Raymond and Rousset 1995	default
Wilcoxon Rank Sum	2, 4	R ver. 2.15.1	R Development Core Team 2010	
Negative Binomial GLM	2	R ver. 2.15.1	R Development Core Team 2010	
Sibling identification	3, 4	ML RELATE	Kalinowski 2006	
F_{ST} exact tests	3	ARLEQUIN	Excoffier and Lischer 2010	
Hatchery admixture	2, 3, 4	STRUCTURE ver. 2.3.3	Pritchard et al. 2000	10,000 burn in, 100,000 iterations MCMC, K=2, correlated allele model
Migration rates	4	BIMR ver. 1.0	Faubet and Gaggiotti 2008	2 million burn in, 20 million iterations, 2,000 thinning interval MCMC
Multinomial logistic regression	4	R ver. 2.15.1	R Development Core Team 2010	

Appendix B. Densities of Juvenile *O. mykiss* for Monitoring Sites in Beaver Creek

Figure B. 1. Density of juvenile O. mykiss (trout/m) by year for site UBR1, between the diversion dam treatments. This graph shows the reduced recruitment in the 2006 brood year (age 0). Data from P. Connolly, USGS.

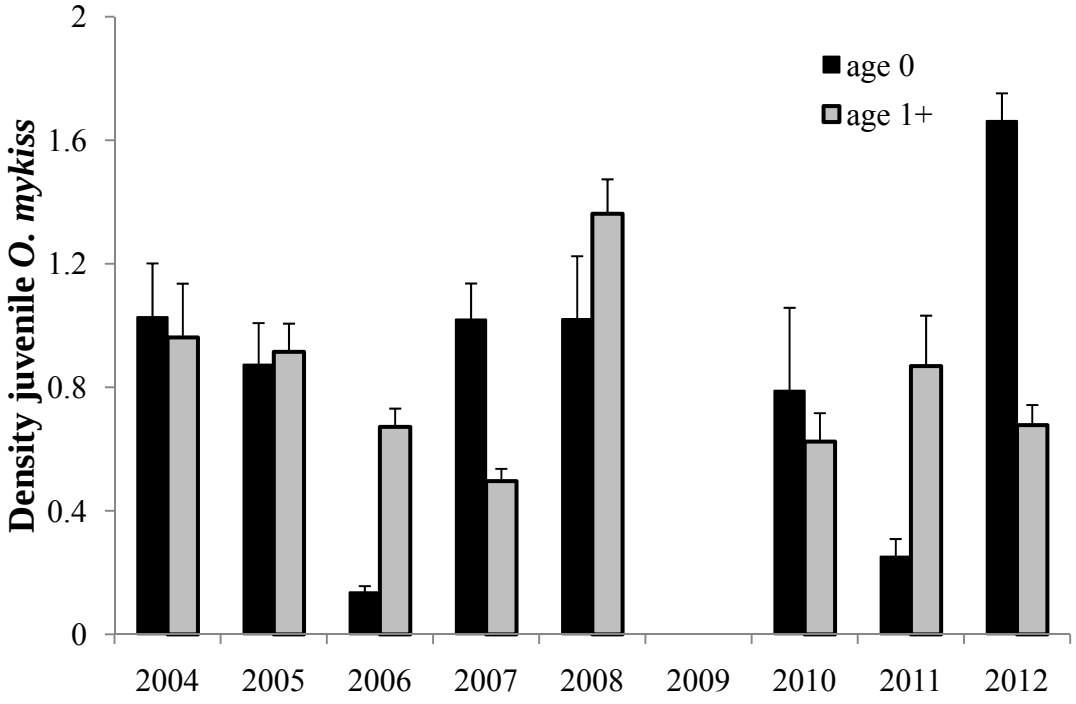


Figure B. 2. Densities of juvenile *O. mykiss* (trout/m) by year for site UBR2, upstream from the diversion dam treatments. This graph shows little to no effect of the reduced 2006 recruitment indicating a different effect in the resident rainbow trout populations higher in the stream. Data from P. Connolly, USGS.

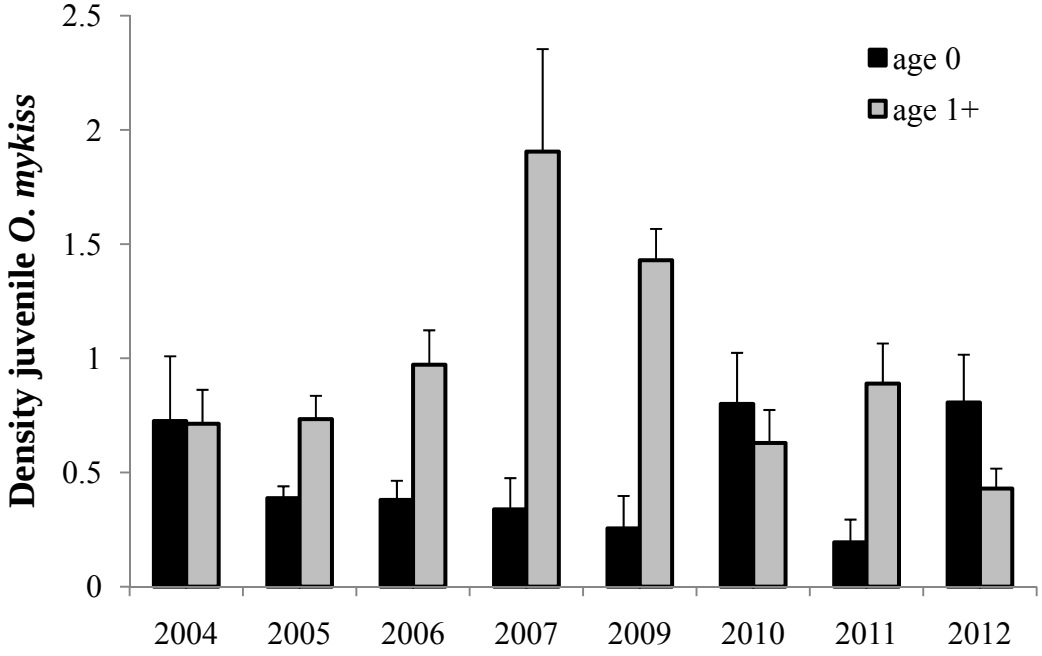
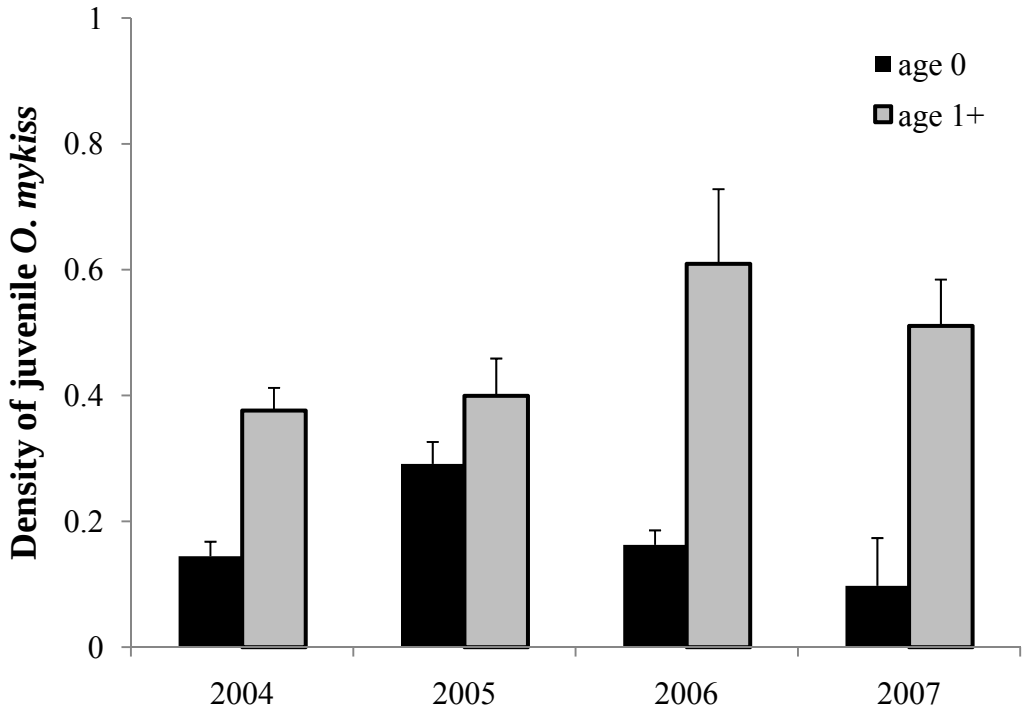


Figure B.3. Densities of juvenile *O. mykiss* (trout/m) by year for site UBR4, upstream from the diversion dam treatments. This graph shows little to no effect of the reduced 2006 recruitment indicating a different effect in the resident rainbow trout populations higher in the stream. Data from P. Connolly, USGS.



Appendix C. Analysis of Sibling Phenotypes

Sibling offspring from the four largest family groups (from parentage analysis in Chapter 2) were compared to test whether parents influenced phenotype expression in these offspring. Phenotypes from full sibling offspring were tested across family groups using a Kruskal Wallis test in R (R Development Core Team 2010). Four family groups had sample sizes greater than 14 full siblings for this test. Families A and B are paternal half siblings. Phenotypes tested were fork length (mm), weight (g) and day past weir. Day past weir was calculated as the number of days after January 1 of the brood year that the juvenile trout was captured in the fish trap. Family significantly explained variation in size in the offspring family groups (length $p=0.01$, weight $p=0.01$), but not significantly related to day of outmigration from Beaver Creek ($p=0.55$).

This test allowed an examination of phenotypes across genetically identical individuals that are rearing in the same stream. Family significantly explained differences in length and weight as expected due to differing parental lengths and/or differing spawn dates. Interestingly, the smaller female parents have the larger mean length at capture at the weir.

Table C.1. Parent fork length (FL mm), percent hatchery and day past the weir for the 4 largest family groups.

Family	FL male	FL female	% H male	%H female	day past weir male	Day past weir female
A	593	700	28	8	74	86
B	593	749	28	23	74	74
C	741	540	15	57	100	123
D	640	605	18	21	111	102

Table C.2. Family, number of full sibling offspring, and phenotype data at outmigration from Beaver Creek (FL=fork length in mm, wt=weight in g, day past weir is number of days from January 1 of the brood year spawning occurred). Mean values are in parentheses.

Family	No. offspring	Offspr FL	Offsp wt	Offsp day past weir
A	29	75-99 (86.5)	3.4-11.5 (6.6)	200-435 (310.8)
B	28	75-100 (91.0)	4.4-10.4 (7.6)	220-424 (307.5)
C	25	75-330 (94.5)	4.3-481 (24.8)	262-1188 (348.2)
D	15	72-135 (94.7)	4.2-25.7 (9.5)	257-545 (348.1)

Appendix D. Individual Data for Fluvial Rainbow Trout

Table D.1. Fluvial rainbow trout ≥ 200 mm fork length captured at the weir and detected at the tag reading stations in Beaver Creek. The weir was operated until 2008 for marking and recapturing, the tag readers were operated until present for detecting tags. Capture date at weir (tag insertion), fork length (mm), tag number, gender (male (M), female (F) or unknown (U)) and spawning years detected in stream. Last detections for tags vary from late March to late July within a spawning season.

				Migration Year				
Capture Date	Length	PIT tag no.	Sex	2005	2006	2007	2008	2009
4/2/2005	303	3D9.1BF20A53F1	M	X				
4/5/2005	243	3D9.1BF20BCFA5	U	X				
4/5/2005	460	3D9.1BF1FDD67F	M	X				
4/10/2005	472	3D9.1BF1FDA52B	F	X				
4/13/2005	404	3D9.1BF1FDA039	M	X				
4/14/2005	247	3D9.1BF20A321D	M	X				
3/15/2006	431	3D9.257C62334B	M		X	X	X	
3/22/2006	264	3D9.257C61A21A	M		X			
3/23/2006	200	3D9.257C62DD33	M		X			
3/24/2006	332	3D9.257C61B230	M		X	X		
3/24/2006	303	3D9.257C631634	M		X	X		
3/26/2006	307	3D9.257C617AFE	F		X			
3/26/2006	200	3D9.257C6301A0	M		X			
3/27/2006	402	3D9.257C6336F7	M		X			
3/27/2006	200	3D9.257C631BDB	M		X			
3/30/2006	255	3D9.257C635A63	M		X	X	X	
3/30/2006	312	3D9.257C5F4890	M		X			
3/30/2006	253	3D9.257C634B18	M		X			
3/31/2006	488	3D9.257C62372E	M		X	X	X	X
4/2/2006	330	3D9.25761E3EA	M		X	X		
4/4/2006	206	3D9.257C62DF89	M		X			
4/6/2006	283	3D9.257C6212CD	M		X			
4/7/2006	242	3D9.257C62CE6C	U		X	X		
3/9/2007	288	3D9.1C2C31C46C	U			X		
3/18/2007	353	3D9.1C2C3102B4	M			X	X	
3/19/2007	365	3D9.1C2C30E392	M			X		
3/6/2008	304	3D9.1C2C311519	U				X	
3/12/2008	226	3D9.1C2C2DA5A6	U				X	
3/18/2008	400	3D9.1C2C31A5C1	M				X	
4/3/2008	330	3D9.1C2C24D0E	M				X	

4/6/2008	344	3D9.1C2C31A76C	M		X	
4/9/2008	253	3D9.1C2C31AD6D	U	X	X	
4/12/2008	370	3D9.1C2C310288	M		X	
4/15/2008	273	3D9.1C2C314CA5	M		X	
4/15/2008	310	3D9.1C2C30EE77	M		X	