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

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Abstract

Colonization of vacant habitats is an important process to support the long term persistence of populations and species. Barrier removal projects provide opportunities to study the colonization process to identify the source, abundance, spatial extent and rate of colonization. Salmonid fishes provide interesting experimental opportunities where colonization can be studied under the influence of artificial breeding programs and life history polymorphisms. We used a before after experimental design to follow the colonization process of anadromous *Oncorhynchus mykiss* 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 was established. 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 with adult anadromous *O. mykiss* expanding into the upper basin sites 3 to 4 years after barrier removal.

Key Words: colonization, barrier removal, stream restoration, artificial propagation, fish passage, effectiveness monitoring

Introduction

Colonization of unoccupied habitat is an ecological process that is important to the long term persistence of species. Two conceptual theories are often used to explain the demographic processes related to range expansion and migration. The metapopulation theory suggests that populations are mediated by localized extinction and colonization processes. Local populations support each other in a source-sink dynamic (Hanski and Gilpin 1996, Rieman and Dunham 2000). The member-vagrant theory suggests that members of a population have local adaptations that provide a selective advantage over vagrants from other populations (Garant et al. 2000, Primmer et al. 2006). Both theories have some empirical support suggesting that environmental stability may mediate the population demographic process (Garant et al. 2000). Yet, understanding the underlying demographic process becomes important when predicting the response of a population or species to a management action such as barrier removal.

Direct removal or damage to habitat threatens 50% of species in the United States (Richter et al. 1997). Water diversions are cited as having the greatest adverse effect on aquatic fauna in California (Moyle and Williams 1990). Passage barriers such as dams or culverts limit access of fish species into historically accessible natal habitats isolating populations (Neville et al. 2006). In Washington State over 7,700 km of historical salmon habitat are inaccessible to migratory fishes because of impassable culverts or road crossings (Roni et al. 2002). 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), and oftentimes are opportunistic after unpredicted catastrophic events like volcanic eruptions or the release of toxic chemicals (Leider 1989, Demairias et al. 1993).

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 and the need for artificial stocking of desirable populations and/or stocks are typical management questions. 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 due to their threatened and endangered status in the U.S. (McClure et al. 2003). Salmonid systems which are largely driven by spawners homing to natal streams do not appear to support exploration of new environments necessary for population expansion and colonization. Several species of trout or salmon 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 (Araki et al. 2007, Parker et al. 2001, Docker and Heath 2003, Weigel et al. in review). 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).

Artificial propagation also directly impacts migration and reproductive success of trout and salmon (Araki et al. 2007, McLean et al. 2004a, McLean et al. 2004b, Miller et al. 2004). These hatchery produced fish provide an over-abundant source population to colonize unoccupied habitats. Yet, hatchery salmon and steelhead are documented to have lower relative reproductive success in the natural stream environments (Araki et al. 2007, Miller et al. 2004, McLean et al. 2004b). Therefore, hatchery fish may not be a desirable source population for the colonization of newly opened habitats, and their role or impacts to the colonization process are not well-understood. Hatchery trout and salmon are documented to have higher rates of straying (Quinn 1993). The demographic effect of hatchery fish on the colonization process due to these high abundances and larger straying rates could reduce or eliminate the contributions from naturally produced trout or salmon. 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.

Genetic data are often used to monitor the effect of colonization as it identifies inter-breeding groups (or local populations) and source population (Gaggiotti et al. 2003, Demairias et al. 1993, Garant et al. 2000, 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, Narum et al. 2006, Neilsen et al. 2009). Variation in these short term sampling efforts can arise from sampling bias and/or demographic effects. Over longer time periods (>20 years), temporal variation has been found to explain about 2% of molecular variation in *O. mykiss* populations, an amount similar to the variation among these populations (Beacham et al. 1999, Heath et al.

2002). These long term studies are generally influenced by genetic drift and changes in habitat condition, hatchery and harvest practices over these time frames.

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 are providing demographic stability. In this study, we use genetic data to determine if the anadromous population 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 large hatchery effects. Trout were allowed to naturally colonize the unoccupied habitat. Individual migrations and movements were monitored with passive integrated transponder tags (PIT tags) and readers. The objective of our study is 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 genetic responses occurred; and 3) identify if a population of anadromous *O. mykiss* was successfully established in Beaver Creek.

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 located on the east side of the Methow Basin and flows west into the Methow River 57 km upstream from the mouth (Fig 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 100-year flood on Beaver Creek is estimated at 13.5 m^3/s (Ruttenberg et al.

2009). 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 or 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 present throughout the Beaver Creek basin 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 (Martens and Connolly 2010, Ruttenberg et al. 2009). The most downstream irrigation diversion was a 2.0 m high concrete diversion dam that was modified to allow fish passage after the fall 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 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 upstream blocked by the dam. The Wenatchee, Entiat, Methow and Okanagan 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 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 of Wenatchee, WA. This brood 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 tributaries upstream of Grand Coulee Dam (Chapman et al. 1994). This brood was later 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.

Currently hatchery releases into the Methow from both the state and federal programs are to release 450,000 – 550,000 *O. mykiss* smolts per year. *O. mykiss* are spawned and 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, WDFW, personal communication). Hatchery *O. mykiss* are released as age 1 smolts in the upper 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.

Adult returns

Hatchery produced steelhead comprise more than 80% of the returning adults to Wells Dam, the nearest adult counting location to the Methow Basin. The low counts of wild adult summer steelhead led to the National Marine Fisheries Service concluding that the hatchery stock is critical to recovery of the species and included the hatchery stock as protected under the Endangered Species Act (McClure et al. 2003). Between 1999 and 2010, the wild steelhead returns range from 5 to 18% of the run (Fig 2). During our study (2005-2008), wild steelhead returns range from 9% in 2005 to 18% in 2008 (C. Snow, WDFW unpublished data).

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. 1). This location was chosen for access and stream channel topography allowing the successful operation of the equipment. This fish trap was two directional capturing trout moving upstream or downstream. The trap was operated from March 20 to May 9, May 14 to December 5, 2005, February 13-May 1, June 27-November 27, 2006, February 24 to March 30, May 25 to November 29, 2007 and February 24 to May 3, July 11 to July 30, September 2 to December 10, 2008. The date, direction of movement, fork length (mm), weight (g), sex and wild or hatchery origin were recorded for adult trout. The hatchery *O. mykiss* were collected from the Wells Hatchery (brood years 2005 and 2006) hatchery x hatchery crosses provided by the WDFW.

Juvenile *O. mykiss* were sampled at 6 sites located on Beaver Creek (Fig. 1). One site was downstream the lowest diversion dam (ds dam), 2 sites were located between the various diversion dam modifications (UBR1, UBR2) and 3 sites (CMP, UBR4, SFB) were upstream from the diversion dam modifications (Fig. 1). Sites were selected to represent the stream habitats longitudinally in the basin and located where access was permitted from private land owners. Juvenile sites were sampled to represent the population genetics spatially across the Beaver Creek basin and tributaries. Before barrier treatment collections were made during the fall of 2004 or the summer 2005 sampling age 1+ juvenile *O. mykiss* present at collection sites in the stream. After barrier treatment collections were made during the summer or fall 2008 and 2009 sampling age 1+ juvenile *O. mykiss* present at collection sites in the stream. The 4 to 5 year time between the before and after treatment collections is about 1 generation.

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 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.

Trout movements were monitored using a network of stationary PIT tag interrogation stations in Beaver Creek (Fig. 1) and at dams and passage facilities on the mainstem Columbia River. One multi-antenna, multiplex PIT tag interrogation station and two single antenna PIT tag interrogation stations were operated in Beaver Creek (similar to those 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 3 pairwise configurations across the stream bed providing complete coverage at most stream flows. This configuration collects information that can be used 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 lowest single antenna PIT tag interrogation station was operated from September 2004 to December 2008. The multiplex interrogation station was operated from July 20, 2004 to present. The upper single antenna PIT tag interrogation 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 read at any of the Columbia River facilities. Some of these fish returned in successive years. Anadromous *O. mykiss* trout were read on the mainstem Columbia River during upstream and/or downstream migration. Hatchery origin trout were identified from PIT or coded wire tags, fin clips or other marks.

Lab methods

Sixteen microsatellite markers were used to identify individuals. Thirteen of these markers are standardized across the Columbia River basin which allows for data sharing across labs (Stevenson et al. 2009). 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 using LIZ600 (Applied Biosystems) as an internal standard. 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). Primer sets used were Ogo4 (Olsen et al. 1998), Oke4 (Buchholz et al. 1999), Oki23 (Smith et al. 1998), Omy1001 and Omy1011 (Spies et al. 2005), Omy7 (Stephenson et al. 2009), Oneu14 (Scribner et al. 1996), One102 (Olsen et al 2000), Ots100 (Nelson & Beacham 1999), Ots3m (Greig & Banks 1999), Ots4 (Banks et al 1999), Ssa289 (McConnell et al. 1995), Ssa407 and Ssa408 (Cairney et al. 2000), Omm1036 and Omm1046 (Rexroad et al 2002).

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 extract dried down in a 96 well plate. Cycling conditions included initial denaturation of 15 min at 95°C, followed by 28 cycles of 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 of 30 min at 60°C. Multiplex A contained Oki23, Oke4, Oneu14, Ssa289, Ssa408; Multiplex B contained Ots4, Omy7, Ogo4, One102, Omm1046, Ssa407; Multiplex C contained Ots100, Omy1011, Omy1001, Ots3m, Omm1036.

Amplification products were diluted (usually with 10ul DNA grade water) and one ul of each dilution added to 10 ul of LIZ/formamide solution (30ul LIZ600 to 1ml formamide) in a 96 well half-skirted PCR plate compatible with AB3730 genetic analyzer. A few samples from each plate were run on the AB3730 to test fluorescent strength of peaks. Adjustments were then made to sample dilution and volume, along with voltage and time settings for sample injection on the AB3730, to achieve optimum strength fluorescent peaks for the actual full plate run of 96 samples. Completed 3730 runs were analyzed automatically using Genemapper, followed by manual analysis of all peaks to verify correct peak calling. All homozygous results were checked for small allele dropout and large allele dropout. Peaks were also visually checked for conformity to expected profiles. Peaks were scored following the Stevan Phelps Allele Nomenclature (SPAN) convention (Stephenson et al. 2009). Lab error rates for the 13 standardized loci are <2% (Stephenson et al. 2009). Duplicate samples run for our study indicate lab error rates <1%.

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 was used to test the frequency of statistical significance due to non-treatment related factors (such as finite sampling). Multiple tests were also used as much as possible to confirm the significance of the before after comparisons detected at the sites.

Prior to statistical tests, full siblings were identified and removed from the data set using MLRelate (Kalinowski 2006). Exact tests of Hardy Weinberg Equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0.10. Expected heterozygosity was 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). Exact tests for Fst 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 (HxH crosses brood years 2005 and 2006, 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 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.

285 Results

286 Difficulties running the weir during spring stream flows resulted in inconsistencies
287 between capture efficiencies and counts across the years of our study. Fluvial *O. mykiss* were
288 particularly numerous during 2006 with nearly 3 times the number of adult migrants than the
289 other years of our study (Fig. 3). Over the 4 years of our study, 34 individual fluvial rainbow
290 trout >200mm were documented during the spawning run in Beaver Creek. Males were the
291 largest proportion of this life history type comprising 76% of these trout; females and unknown
292 determinations were 6% and 18%, respectively. The fluvial *O. mykiss* were documented entering
293 Beaver Creek up to 3 consecutive years during our study with 32% of the individuals entering
294 the creek multiple years.

295 Capture efficiency was high for adults during 2005 and 2006 with only two individuals in
296 2005 and one individual in 2006 known to be missed in our sample. However, the weir was not
297 run for the entire spawning seasons during 2007 and 2008 reducing the ability to count the wild
298 anadromous *O. mykiss* entering the stream during these years (Fig. 3). Numerous hatchery *O.*
299 *mykiss* were read at the Beaver Creek interrogation stations during these years, and the counts
300 based on PIT tags would be biased toward hatchery trout.

301 PIT tags from adult *O. mykiss* in the stream migrated higher into the basin during the later
302 two years of the study indicating that trout were still expanding into the upper basin (Fig. 4). PIT
303 tags from parr in Beaver Creek indicate an increase in outmigration during these years in the
304 upper basin. However, PIT tag outmigration from the middle reach (UBR1) remained relatively
305 constant indicating that juvenile *O. mykiss* were expressing an anadromous life history from this
306 reach prior to barrier treatment (Fig. 5). In 2007, parr tagged in Beaver Creek during the study
307 began to return as adults (n=1 2007, n=3 2008, n=4 2009) indicating a local population of

anadromous *O. mykiss* was established and homing back to the stream. 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 mouth of the Methow River. Eight adults (21%) were detected in Beaver Creek and 4 (33%) were detected in other tributaries (Twisp and upper Methow rivers). One-third of the adults that were detected entering natal tributaries in the Methow Basin were known strays into other streams.

The total number of alleles detected at each locus ranged from 7 to 28. 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 pattern to these pairs of loci and this disequilibrium could be a result of non-random mating or mixing of populations in the hatchery breeding practices.

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 1). The proportion of hatchery admixture showed confounding results with some sites increasing and some decreasing before-after the barrier removal. The comparisons of proportion of hatchery admixture among consecutive years were consistent except for the South Fork Beaver Creek (SFB) comparison. The campground site downstream from the mouth of the South Fork Beaver Creek showed an increase of proportion of hatchery admixture when comparing the 2005 to 2009 samples. Pair wise Wilcoxan rank tests before and after comparisons and temporal comparisons of the proportion of hatchery admixture

were not significant ($p>0.05$) except for the comparison between 2005 and 2008 SFB site ($p=0.02$).

Comparisons of genetic differentiation (Fst and allele frequency) showed significant differences at the two most downstream sites in the basin (Table 1). Both of these variables show significant differences indicating consistency across these measurements supporting the conclusion that population genetics have 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 (2004 and 2008) were significant, but was 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 collections. All of the temporal tests on the consecutive years did not show any significant tests for Fst or allelic frequencies (Table 1).

Discussion

Adult *O. mykiss* entered Beaver Creek during the first spawning season after barrier removal. Hatchery *O. mykiss* were a small proportion of these colonizing adults despite high abundances due to local fishery management programs. Anadromous parr tagged in Beaver Creek returned to the study area as adults in 2008 indicating that an anadromous population was established in the newly opened habitat. Comparisons of population genetic parameters before and after barrier treatment indicate significant changes in the lowest two monitoring sites in the basin (downstream from the dams and UBR1). The other sites did not show significant changes. Hatchery admixture was not significantly different in the before and after comparisons. Temporal tests of the population genetic parameters found no significant differences between pair wise comparisons over consecutive years.

353 Adult anadromous *O. mykiss* did not increase during the first 4 years after barrier
354 removal. Counts of wild and hatchery anadromous *O. mykiss* declined from 2005 to 2007 and
355 then increased slightly. This followed the trend of adult counts into Wells Dam. Fluvial rainbow
356 trout were a variable portion of the run and inter-breed with the anadromous *O. mykiss* (Weigel
357 et al. in review). Although Anderson et al. (2010) found rapid colonization and steadily
358 increasing abundances of coho salmon (*O. kisutch*) during the first 3 years after passage was
359 restored at a dam, Demarias et al. (1993) found that re-colonization occurred much slower in the
360 Virgin River chub (*Gila seminuda*) after an accidental release of rotenone, a fish poison. After
361 29 months, the population genetic attributes of the Virgin River chub had returned to the pre-
362 poison conditions at the site closer to the unaffected (source) population (30km), but was
363 significantly different at a more distant site (>60km away) indicating that this population was
364 still disconnected. The rate of colonization is mediated by the abundance, distance and
365 connectivity to source populations; therefore, different species and locations may vary in
366 response to connectivity projects.

367 Few hatchery *O. mykiss* entered Beaver Creek despite high proportions of hatchery trout
368 in the returns to the basin. Leider (1989) also found different proportions of hatchery *O. mykiss*
369 between a hatchery counting site lower in the basin and a natal tributary. Hatchery fish may
370 have a preference to return to release locations or the hatchery site with a smaller proportion
371 straying into the newly opened habitat. In addition, other survival differences may affect the
372 proportion of hatchery fish between the ladder at Wells Dam and the natal tributaries, such as
373 selective harvest. Hatchery admixture did not significantly change in our before – after pairwise
374 comparisons. In addition, only one juvenile parr from Beaver Creek was found to be spawned by

a hatchery *O. mykiss* indicating very low reproductive contribution from this population (Weigel et al. in review).

Several parr tagged in Beaver Creek returned as adults in 2007 through 2011 indicating that an anadromous population is established in the newly opened habitat. Some straying of these returning adult *O. mykiss* occurred during the study and 66% of these adults returned to the natal area. All the strays were detected in tributaries upstream from Beaver Creek. *O. mykiss* were found to stray into tributaries upstream from the natal tributary after the volcanic eruption on Mt. St. Helens, WA (Leider 1989). Additional adult *O. mykiss* tagged as parr in Beaver Creek were last detected migrating upstream on 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 natal stream undetected or returned to Beaver Creek downstream from the lowest tag interrogator. The adult *O. mykiss* from Beaver Creek had a substantially higher rate of straying (33%) than documented in other studies (7.7%) (Hendry et al. 2004).

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, finite population sizes and finite sampling. Therefore, some tests could show significant differences and be unrelated to the treatment. Similar to other studies, our populations were generally 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 (Narum et al. 2004, Narum et al. 2006, Heath et al. 2000, Neilsen et al. 2009). Therefore, we expect a less than 5% rate of significant temporal tests due to random or unmeasured effects.

The lower two sites show significant differences in allele frequency and Fst values, but not in proportion of hatchery admixture. We did not expect to see a change in the lowest site downstream from the dams because this site was accessible to *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 population with the resident populations that were residing upstream from the diversion dams. Hatchery *O. mykiss* did not appear to contribute to the colonization of the study area; therefore, this reduction in hatchery admixture could be due to the source of the successful (non-hatchery) colonizers. The first site upstream from the diversion dam treatments (UBR1) had the greatest shift in Fst and allele frequencies which were significantly different before and after treatment. This site had only a slight, not significant increase in the proportion of hatchery admixture indicating little hatchery influence at this site.

The sites further upstream did not show changes in population genetics when comparing before and after treatment samples. Tag data indicate that few spawners made it to these upper reaches of the basin during the first 4 years after barrier removal. Although outmigration 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 Fst 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 pairwise 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.

Successful colonization requires that source populations are available that can provide colonizers into the newly opened habitat; connectivity between the source population(s) and the newly opened habitat and adequate habitat conditions to establish and support the colonizing species. The barrier removal resulted in connectivity in Beaver Creek allowing individuals to access the creek from other (source) populations. Adults that entered Beaver Creek successfully reproduced (Weigel et al. in review) and anadromous *O. mykiss* established a population in the basin. The sites in the lower reaches of Beaver Creek had significant changes in genetic differentiation when comparing before and after the barrier removal. Colonization and expansion of anadromous *O. mykiss* was a slower process than expected with adult anadromous *O. mykiss* expanding into the upper basin sites during the later years of the study.

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Table 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), private alleles (PA) and proportion hatchery admixture (%H), population differentiation (Fst) and allele frequency exact test (Pval).

	Before						**After**							
Site	year	n	H	AR	PA	%H	year	n	H	AR	PA	%H	Fst	Pval
Downstream 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	45.2	2008	28	0.82	7.2	0.22	47.6	0.021*	<0.001*
UBR1	2004	19	0.78	6.4	0.26	45.2	2009	26	0.82	7.0	0.29	47.0	0.027*	<0.001*
Campground	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 Legends

Figure 1. Study location and sampling sites in Beaver Creek, Methow Basin, Washington.

Figure 2. Counts of wild and hatchery adult *O. mykiss* returns to Wells Dam, Columbia River, Washington (1999-2010). Data provided by Washington Department of Fisheries and Wildlife.

Figure 3. Adult *O. mykiss* counts into Beaver Creek 2005-2008. Counts include *O. mykiss* captured at the weir by population and life history (hatchery (H in weir), wild (W anad in weir) and fluvial (fluv in weir)) and tagged trout read at interrogation stations in Beaver Creek and not captured at weir (hatchery (anad tag) and fluvial (fluv tag)).

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

Figure 5. Number of parr outmigrants recorded migrating downstream past the tag interrogation stations in Beaver Creek. Parr were tagged at sites located upstream of the interrogation stations in the middle reach of Beaver Creek (UBR1) and upper Beaver Creek (UBR2, SFB, CMP and UBR4). The interrogation stations were installed during the summer and fall 2004. Therefore, the counts for 2004 are not complete enumeration of annual parr outmigrants.

Figure 6. STRUCTURE output for the 6 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 Washington Department of Fisheries and Wildlife.