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ARTICLE

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Abstract

Colonization of vacant habitats is an important process for supporting the long-term persistence of populations and species. We used a before–after experimental design to follow the process of colonization by steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout) at six monitoring sites in a natal stream, Beaver Creek, after the modification or removal of numerous stream passage barriers. Juvenile *O. mykiss* were collected at monitoring sites by using a backpack electrofisher. Passive integrated transponder tags and instream tag reading stations were used in combination with 16 microsatellite markers to determine the source, extent, and success of migrant *O. mykiss* after implementation of the barrier removal projects. Steelhead migrated into the study area during the first spawning season after passage was established. Hatchery steelhead, although comprising more than 80% of the adult returns to the Methow River basin, constituted a small proportion (23%) of the adult *O. mykiss* colonizing the study area. Adult steelhead and fluvial Rainbow Trout entered the stream during the first spawning season after barrier removal and were passing the uppermost tag reader (12 km upstream from the mouth) 3–4 years later. Parr that were tagged in Beaver Creek returned as adults, indicating establishment of the anadromous life history in the study area. Population genetic measures at the lower two monitoring sites (lower 4 km of Beaver Creek) significantly changed within one generation (4–5 years). Colonization and expansion of steelhead occurred more slowly than expected due to the low number of adults migrating into the study area.

Direct removal of 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 are larger main-stem 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 the passage of target fish species (Bernhardt et al. 2005). These management actions are aimed at reconnecting unoccupied habitats to re-establish populations that collectively will increase production of threatened or endangered species. Few studies have collected data during the fish colonization process in stream environments (Bernhardt et al. 2005), and oftentimes such

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studies are opportunistic, occurring after unpredicted catastrophic events like volcanic eruptions (Leider 1989) or the release of toxic chemicals (Demarias et al. 1993).

Barrier removal projects create opportunities to study the colonization of species or the re-establishment of migratory life histories by using the before–after treatment experimental design (Kiffney et al. 2009; Anderson et al. 2010). The rate of colonization will be dependent on the species' dispersal capability as well as the density and distance of the unoccupied habitat to candidate source populations (Gaggiotti et al. 2004). Barrier removal projects that have been implemented in streams with populations of target species downstream of the structure have proven successful in allowing rapid, volitional colonization by fish when passage is restored (Kiffney et al. 2009; Anderson et al. 2010).

Trout and salmon are typically the target species for restoration actions due to their threatened and endangered status in the USA (McClure et al. 2003); however, salmonid systems are largely supported by spawners homing to natal streams and by the development of local adaptations, which can appear to hinder population expansion and colonization processes. Several salmonid species have multiple life history strategies that co-occur 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 interbreeding between the 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 often targeted toward increasing population distribution and abundance of the anadromous life history form, which has severely declined due to extensive impacts from harvest, hydropower, and variable ocean conditions (McClure et al. 2003).

Despite the advantages of barrier removal, the release of hatchery-reared conspecifics can directly impact the migration and reproductive success of trout and salmon (Miller et al. 2004; Araki et al. 2007). Hatchery trout and salmon are documented to have higher rates of straying than naturally reared conspecifics (Quinn 1993). Hatchery-produced fish provide a potential overabundant source population to colonize unoccupied habitats, but hatchery salmon and steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout) are documented to have lower relative reproductive success than fish that are 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 in and impact on the colonization process are not well understood.

Genetic data are often used to monitor the effect of colonization, thus allowing for the identification of interbreeding groups (or local populations) and source populations (Demarias et al. 1993; Garant et al. 2000; Bartron and Scribner 2004; Gaggiotti et al. 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 the molecular variation within *O. mykiss* populations, an amount similar to the variation among populations (Beacham et al. 1999; Heath et al. 2002). The genetic variation measured in these long-term studies is generally influenced by genetic drift and changes in habitat condition, hatchery practices, and harvest practices.

We used population genetic measures and movement data to determine whether the anadromous life history form of *O. mykiss* (i.e., steelhead) was successfully established after the modification of several small irrigation dams in Beaver Creek, a natal tributary to the Methow River, Washington. We were particularly interested in the process of colonization by *O. mykiss* because this species 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 newly accessible habitat. Individual migrations and movements were monitored with PIT tags and tag readers. Because the different life history types interbreed, 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 anadromous, hatchery, and fluvial adult migrants during the first 4 years after barrier removal; (2) identify whether there were detectable changes in measures of population genetics and, if so, identify the basin areas where the changes occurred; and (3) determine whether the anadromous life history was successfully established by identifying the adult returns of parr that were produced after barrier removal in Beaver Creek.

STUDY AREA

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

Fish access 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 implementation of the barrier removal projects. Steelhead were present downstream from the lowest diversion dam (Martens and Connolly 2010). From 2000 to 2004, seven small

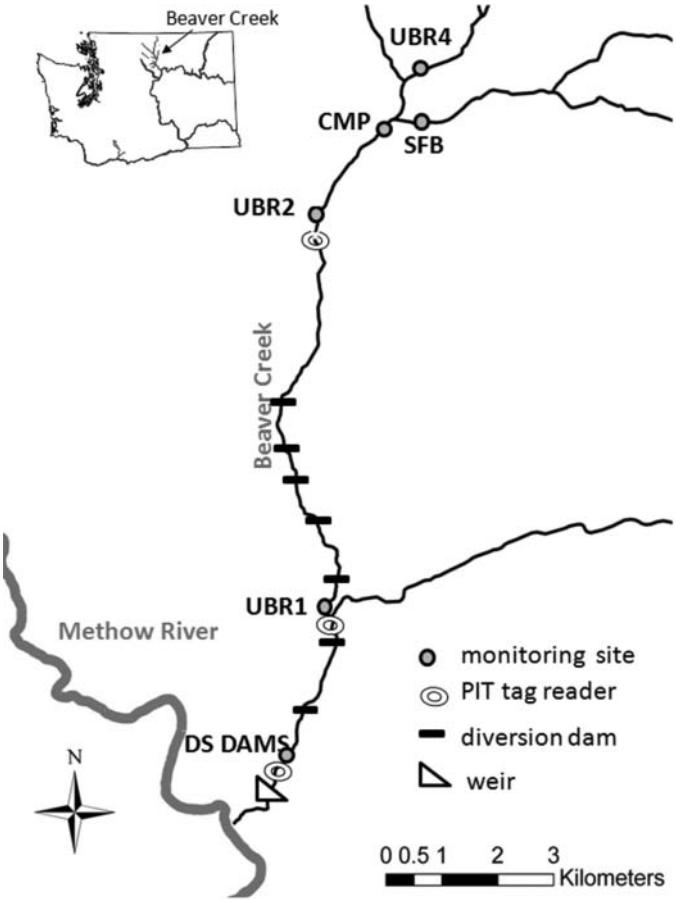

FIGURE 1. Study location and sampling sites in Beaver Creek, Methow River basin, Washington.

irrigation diversion dams (1.0–2.0 m high) were modified into rock vortex weirs that allow fish passage (Ruttenberg et al. 2009; Martens and Connolly 2010). The downstream-most irrigation diversion was a 2.0-m-high, concrete diversion dam that was modified to allow fish passage after the fall of 2004. Access to Beaver Creek by migratory steelhead/Rainbow Trout was restored for the spring 2005 spawning season.

Hatchery Releases

The Grand Coulee Fish Maintenance Project was established to mitigate for the construction of Grand Coulee Dam on the Columbia River during the 1930s. Hatchery activities were implemented to replace lost production of anadromous salmon and steelhead that were blocked from reaching upstream tributaries after the dam was constructed. The Wenatchee, Entiat, Methow, and Okanagan rivers, which are located downstream of Grand Coulee Dam, 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 steelhead stock used in all these hatcheries originated from collections on the Columbia River at Rock Island Dam, located downstream of Wenatchee, Washington. The broodstock was established from returning adults at this dam because these adults were assumed to be migrating to the major tributaries upstream (e.g., Wenatchee, Entiat, Methow, and Okanagan rivers) and other tributaries upstream of Grand Coulee Dam (Chapman et al. 1994). The original broodstock was later used to establish local broodstocks in each of the basins. In recent years, the Methow River and Wenatchee River hatchery broodstocks have been managed as demographically independent stocks.

Currently, state and federal hatchery programs in the Methow River basin release 450,000–550,000 steelhead smolts per year. Returning adult steelhead are spawned, and the eggs are reared at Wells Hatchery (river kilometer 830.1) on the Columbia River downstream from the Methow River mouth. Current practices include intentional breeding between hatchery and naturally produced adults, and progeny from these crosses are primarily released in the Methow River basin (Snow et al. 2011). Hatchery *O. mykiss* are released as age-1 smolts in the Methow and Chewuch rivers upstream from the town of Winthrop, Washington. All hatchery-origin steelhead are marked with an internal tag (e.g., PIT tag), an external tag (e.g., elastomer tag), a fin clip, or a combination of these. Hatchery-origin adults comprised the majority of the adult returns to the Methow River basin. During our study (2005–2008), the percentage of hatchery fish among steelhead returns ranged from 82% in 2008 to 91% in 2005 (Snow et al. 2011).

METHODS

Fish collections and movements.—Adult *O. mykiss* were captured in Beaver Creek using a picket weir installed 1.3 km upstream from the creek's mouth (Figure 1). This location was chosen for its accessibility and stream channel topography. The trap captured fish that were moving upstream or downstream; it was operated from March 20 to May 9, 2005; May 14 to December 5, 2005; February 13 to May 1, 2006; June 27 to November 27, 2006; February 24 to March 30, 2007; May 25 to November 29, 2007; February 24 to May 3, 2008; July 11 to July 30, 2008; and September 2 to December 10, 2008. Gaps in weir collection during May and June were due to high streamflows and debris that washed out the weir. In 2008, the weir was not operated during August because data from previous years indicated little downstream movement by juveniles during that month. Stream icing during December and January prevented trap operation. The date of capture, direction of movement, FL (mm), weight (g), sex, and population origin (wild or hatchery) were recorded for adult trout.

Juvenile *O. mykiss* were sampled at six sites on Beaver Creek (Figure 1): one site downstream of the lowest diversion dam (DS Dam); one site between the various diversion dam modifications (UBR1); and four sites (UBR2, CMP, UBR4, and South Fork Beaver Creek [SFB]) upstream from the diversion dam modifications (Figure 1). Prior to barrier removal, age-1 and older (age-1 +) juvenile *O. mykiss* were sampled in the

stream during fall 2004 or summer 2005. After barrier removal, age-1+ juvenile *O. mykiss* were sampled during the summer or fall of 2008 and 2009. The 4–5 years between the before and after collections represent approximately one generation for *O. mykiss*.

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

Movements of tagged juvenile and adult *O. mykiss* were monitored using a network of in-stream tag reading stations in Beaver Creek (Figure 1) and at dams and passage facilities on the main-stem Columbia River. McNary Dam, located at river kilometer 470 on the Columbia River, was the upstream-most juvenile counting location used during this study. Tagged juvenile *O. mykiss* that were detected at or downstream of this location were considered to be smolts with downstream migrations exceeding 400 km.

The PIT tag reading stations located on Beaver Creek provided information on adult migration into the study area. One multi-antenna, multiplex PIT tag reading station and two single-antenna PIT tag reading stations were operated in Beaver Creek (described by Connolly et al. 2008; Martens and Connolly 2010). Briefly, the multiplex unit was operated with a Digital Angel Model FS-1001 transceiver connected to six custom-made antennas and a DC power source. The antennas were arranged in three arrays across the streambed, with each array having two antennas that extended across the streambed to provide redundancy and complete coverage at most streamflows. This configuration allowed us to determine the direction of movement and increased the probability of detection. The single-antenna interrogation stations were operated using a 2001F-iso Digital Angel PIT tag reader that was powered by a 12-V battery connected to a single custom-made antenna. The downstream-most single-antenna PIT tag reading station was operated from September 27, 2004, to December 2, 2008. The multiplex tag reading station has been operating since July 20, 2004. 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 *O. mykiss* was identified from the PIT tag detections. Fluvial Rainbow Trout left Beaver Creek and were not detected at any of the Columbia River facilities. Some of these fish returned in successive years. Steelhead were detected at main-stem Columbia River dams at river kilometer 470 or farther downstream during their upstream migration, downstream migration, or both. Hatchery-origin *O. mykiss* were identified from PIT tags, coded wire tags, fin clips, or other marks.

Laboratory methods.—Tissue samples from Wells Hatchery brood years 2005 and 2006 (hatchery × hatchery crosses) were provided by the Washington Department of Fish and Wildlife. Sixteen microsatellite markers were used to identify individuals. Thirteen of these markers are standardized across the Columbia River basin and are summarized by Stephenson et al. (2009). Additional primer sets analyzed were *One102* (Olsen et al. 2000), *Omm1036*, and *Omm1046* (Rexroad et al. 2002).

The DNA was isolated from ethanol-preserved fin clips by using Qiagen DNEasy tissue extraction kits in accordance with the manufacturer's standard protocols. Sixteen microsatellite loci were amplified by PCR in three multiplex reactions using Qiagen Multiplex PCR Master Mix in 96-well plates on GeneAmp PCR System 9700 Thermal Cyclers (Applied Biosystems, Foster City, California). The PCR products were run on an Applied Biosystems 3730 Genetic Analyzer. Peaks were scored using GeneMapper version 3.7 (Applied Biosystems) and were labeled by following the Steven Phelps allele nomenclature (SPAN) convention (Stephenson et al. 2009). Forward primers were fluorescently labeled (Applied Biosystems).

Amplification (PCR) consisted of 5-μL reactions containing 2.5 μL of Qiagen Multiplex PCR Master Mix, five or six primer sets, and water, added to 2 μL of DNA extract that was 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 (multiplexes B and C), and 60 s at 72°C, followed by a final cycle for 30 min at 60°C. Multiplex A consisted of *Oki23*, *Oke4*, *Oneu14*, *Ssa289*, and *Ssa408*; multiplex B included *Ots4*, *Omy7*, *Ogo4*, *One102*, *Omm1046*, and *Ssa407*; and multiplex C contained *Ots100*, *Omy1011*, *Omy1001*, *Ots3m*, and *Omm1036*.

Amplification products were diluted with 10 μL of DNA-grade water and 1 μL of each dilution added to 10 μL of LIZ–formamide solution (30 μL of LIZ600 to 1 mL of formamide). Completed runs were analyzed automatically with 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 were less than 2% (Stephenson et al. 2009). Duplicate samples indicated that laboratory error rates were less than 1% for our study.

Statistical analysis.—Detection efficiency at the middle (i.e., multiplex) reader was calculated according to the methods of Connolly et al. (2008; $n = 29$) based on joint probability among the three antenna arrays at this station. Because detection efficiencies for adult steelhead at this reader are high (99.9%), detection efficiencies at the lower tag reader and the weir could be calculated by using a joint probability from detection counts for tagged adult *O. mykiss* that entered Beaver Creek and were recorded as passing the middle tag reading station ($n = 22$; Lady et al. 2003; Connolly et al. 2008). Specifically, site detections

or captures at the lower reader and the weir were coded with a 1 for detection or a 0 for no detection, thus creating three possible pairs of category counts (10, 01, or 11). The program USER version 2.1 (Lady et al. 2003) was used to calculate the probability of detection, with a default starting value of 0.5. The SE was calculated using the delta method as described by Connolly et al. (2008). The CV was calculated as (SE/estimated detection efficiency) × 100. Detection efficiency could not be calculated for the upper tag reader due to small sample size ($n = 3$) and a lack of additional readers upstream from this detection point.

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 pairwise comparisons between consecutive years. Therefore, pairwise comparisons between the before and after samples were used to detect changes due to the barrier removal treatments, whereas pairwise comparisons between consecutive years were used to test the frequency of statistical significance due to non-treatment-related factors (e.g., finite sampling). Before–after comparisons were tested twice against different years for three of the six sites to confirm the significance and repeatability of the before–after comparisons (Table 1).

Prior to statistical tests, full siblings were identified and removed from the data set by using ML-RELATE (Kalinowski 2006); this was done to avoid bias in measuring site-based population genetic measures. Exact tests of Hardy–Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0.10 (Raymond and Rousset 1995). Expected heterozygosity was also calculated in GENEPOP. Unbiased estimates of allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). Exact tests of the genetic differentiation index F_{ST} were performed using ARLEQUIN version 3.5 (Excoffier and Lischer 2010). All comparisons were adjusted for multiple comparisons by using a Bonferroni correction (Rice 1989).

We used STRUCTURE version 2.3.3 (Pritchard et al. 2000) to estimate the proportion of hatchery admixture for each *O. mykiss* collected at each site and year in comparison with the sample of known-hatchery-origin steelhead from Wells Hatchery ($n = 99$). Allele frequencies from 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 while minimizing linkage disequilibrium and deviation from Hardy–Weinberg equilibrium. The model allows for admixture between population groups. The admixture model in STRUCTURE was run by using 10,000 iterations for burn-in and 100,000 iterations with a Markov-chain Monte Carlo resampling algorithm as described by Pritchard et al. (2000). The number of populations (K) was set to 2. 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

Weir captures of *O. mykiss* in Beaver Creek during 2005 and 2006 appeared to be high; only two individuals in 2005 and

TABLE 1. Genetic variation in Rainbow Trout/steelhead sampled at Beaver Creek (Washington) sites between 2004 and 2009, with pairwise before–after treatment comparisons and temporal tests on data from consecutive years. Sites are listed from downstream to upstream (see Figure 1). Two pairwise tests were used to determine repeatability of results when possible. Variables include sample size (n), expected heterozygosity (H), average allelic richness (AR), average number of private alleles (PA), average proportion of hatchery admixture (%H), population genetic differentiation index (F_{ST}), and significance of the allele frequency exact test (P). An asterisk indicates statistical significance after Bonferroni correction.

	Before						After							
Site	Year	n	H	AR	PA	%H	Year	n	H	AR	PA	%H	F_{ST}	P
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

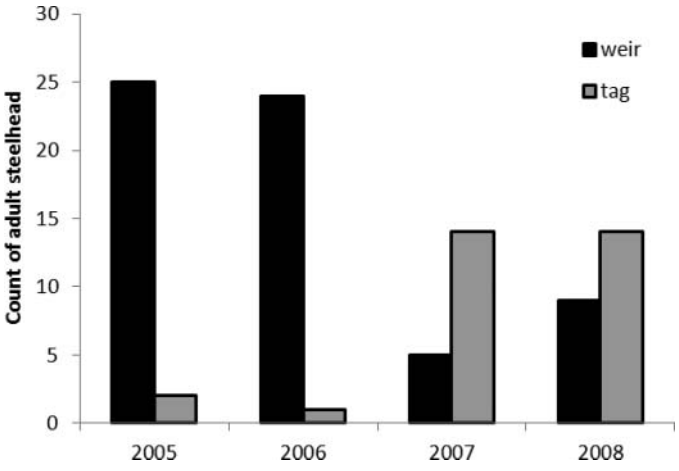

FIGURE 2. Annual counts of individual adult steelhead that were captured at the weir on Beaver Creek (weir) and tagged adult steelhead that were detected in Beaver Creek but not captured at the weir (tag).

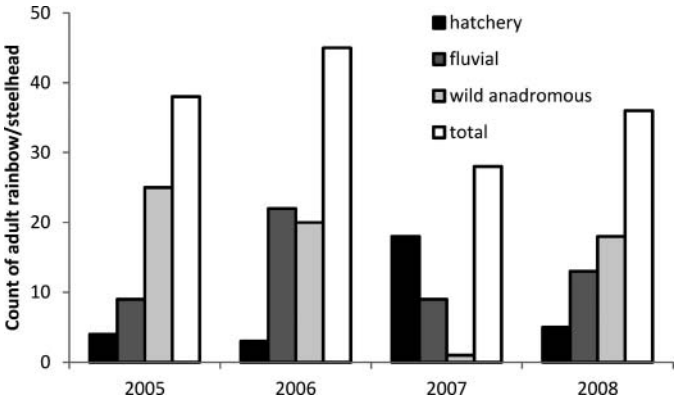

FIGURE 3. Annual counts of adult Rainbow Trout/steelhead entering Beaver Creek by life history type and source (fluvial, wild anadromous, and hatchery) and the total adult count (total), 2005–2008.

one individual in 2006 were known to be missed in our sample based on tag detections. However, the weir was not operational for the entire spawning seasons of 2007 and 2008, reducing our ability to capture and count the wild steelhead that entered the stream during those years (Figure 2). Based on the pattern of steelhead detections and captures, capture efficiency for the weir was estimated at 20% (CV = 45%) for 2005–2008. Attempts to examine years independently failed due to low sample sizes; however, the count data indicated that capture efficiencies during 2005 and 2006 were higher than estimated (Figure 2). Detection efficiency was estimated as 67% (CV = 0.29%) at the lower tag reader and 99.9% (CV = 0.09%) at the middle reader.

Numerous hatchery steelhead were detected at the tag readers in Beaver Creek during the study years, and the counts based on tags would be biased toward hatchery steelhead due to hatchery evaluation programs in the basin during these years. Between 2003 and 2005, tagging of juvenile hatchery steelhead increased from 25% to over 50% in the hatchery programs. The total percentage of tagged hatchery steelhead during these years ranged from 50% to 60% (Washington Department of Fish and Wildlife and U.S. Fish and Wildlife Service, unpublished data). These tagged steelhead returned as adults from 2004 to 2007. Adult hatchery returns into Beaver Creek were primarily comprised Wells Hatchery releases into the Methow River basin; however, one hatchery stray from the Wenatchee River, Washington, and one wild stray from the Tucannon River, Washington, were also detected. The increase in tagged hatchery adults in Beaver Creek during the 2007 spawning season was due to the release of a high number of hatchery smolts in spring 2005. The 2005 release year had the highest proportion of tagged hatchery fish in state and federal hatchery programs, which could explain the increase in hatchery tag returns during 2007. Nearly all of the adult hatchery steelhead that returned to Beaver Creek in 2007 had spent 1 year rearing in the ocean.

Fluvial Rainbow Trout were particularly numerous during 2006, with nearly three times the number of adult migrants than in the other years of our study (Figure 3). Over the four study years, 34 individual fluvial Rainbow Trout larger than 200 mm were documented during the spawning run in Beaver Creek. Males were the largest proportion (67%) of this life history type; females and fish of unknown sex represented 6% and 18%, respectively. Individual fluvial Rainbow Trout were documented as entering Beaver Creek up to four consecutive years, with 32% of the individuals entering the creek in multiple years. Data from tag readers located on Beaver Creek indicated that adult steelhead migrated farther upstream past the tag reader at stream kilometer 12 in Beaver Creek during the latter 2 years of the study (2007 and 2008; Figure 4).

Parr that were tagged at sites upstream from the diversion dams were detected as smolts at Columbia River dams during

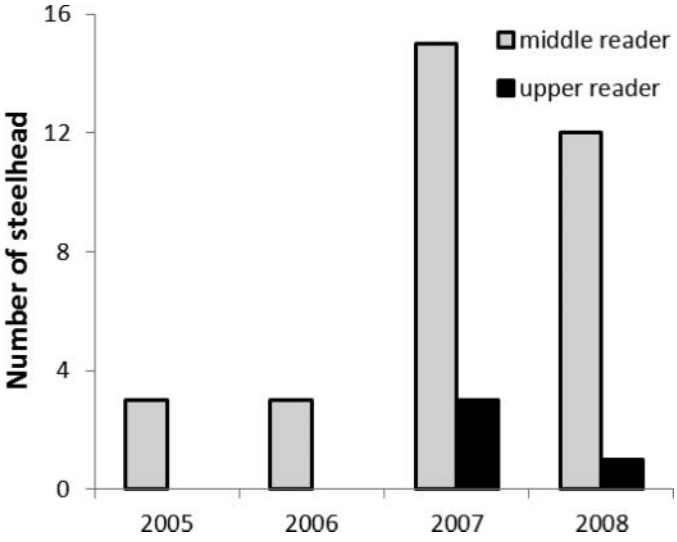

FIGURE 4. Number of upstream-migrating adult steelhead that were counted at tag readers located at river kilometer 4 (middle reader) and river kilometer 12 (upper reader) on Beaver Creek during the spawning season, 2005–2008.

all years of the study. Of the tagged parr that were released during 2004 at UBR1, 12% 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; however, none of these parr returned as adults. The percentage of parr tagged at UBR1 that were detected as smolts 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). Among the parr that were tagged at sites further upstream (i.e., UBR2, CMP, UBR4, and SFB), 1–2% were detected as smolts on the Columbia River; these rates remained constant during the study period, indicating no change in life history at these sites. None of the parr that were tagged at the four upper sites returned as adults.

Between 2007 and 2011, 38 adult steelhead that were tagged as parr in Beaver Creek during previous years were detected as migrating upstream in the Columbia River. Most (68%) of these adults were last detected on the Columbia River or at a tag reader at the mouth of the Methow River downstream from spawning sites in the basin. Eight adults (21%) were detected in Beaver Creek ($n = 1$ in 2007, 3 in 2008, and 4 in 2009), and four adults (33%) were detected in other tributaries (Twisp River and upper Methow River). These tagged returns demonstrated that steelhead progeny from early colonizers in the basin successfully homed back to Beaver Creek; however, one-third of these adults were detected in Methow River basin tributaries other than Beaver Creek.

The total number of alleles detected at each locus ranged from 7 to 28, and the average allelic richness by site and collection date ranged from 4.9 to 7.2 (Table 1). Expected heterozygosity and average allelic richness were similar to values documented for *O. mykiss* in other studies (Heath et al. 2002; Narum et al. 2004, 2006, and 2008; Nielsen et al. 2009). We did not detect significant departures from Hardy–Weinberg equilibrium or significant linkage disequilibrium in the juvenile samples from Beaver Creek sites. Tests on the Wells Hatchery samples did not detect any significant departures from Hardy–Weinberg equilibrium, but linkage disequilibrium was detected at six pairs of loci. There was no discernible pattern to these locus pairs.

The genetic diversity parameters indicated some changes in the before–after comparisons, with the temporal tests remaining stable for expected heterozygosity and allelic richness. The average number of private alleles did vary across the comparisons (Table 1). The STRUCTURE output indicated that Wells Hatchery admixture in the samples decreased from downstream to upstream sites in Beaver Creek (Figures 5, 6). The Wells Hatchery practices include intentional interbreeding of hatchery and wild steelhead that return to Wells Dam. Therefore, contributions of nonhatchery alleles are evident in the Wells Hatchery brood samples. In the comparison of before and after data sets, the proportion of hatchery admixture decreased at the DS Dam site. In the before–after comparisons for sites upstream from the diversion dams, the proportion of hatchery

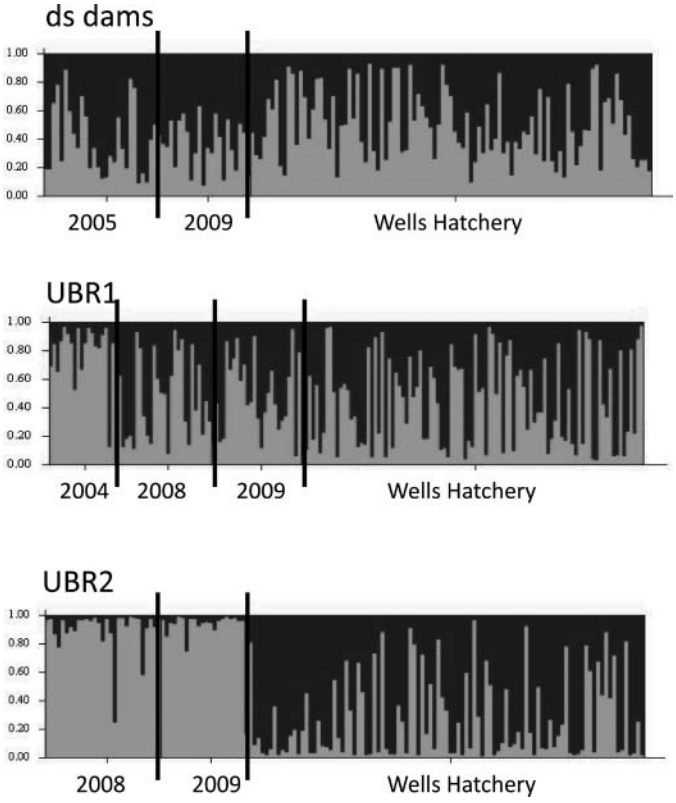

FIGURE 5. Output from STRUCTURE, showing population admixture in Rainbow Trout/steelhead sampled at the downstream-most three monitoring sites in Beaver Creek (Figure 1). Wells Hatchery steelhead were used as a reference for the hatchery population (hatchery × hatchery crosses; brood years 2005 and 2006). Each individual is represented by a bar on the plots (black shading = proportion hatchery alleles; gray shading = proportion wild alleles). Hatchery samples were provided by the Washington Department of Fish and Wildlife.

admixture generally increased after barrier removal; the exception was the uppermost site on Beaver Creek (UBR4), where the proportion of hatchery admixture decreased after barrier removal. Pairwise Wilcoxon rank tests examining hatchery admixture for the before–after comparisons were significant for both comparisons at UBR1 (2004 versus 2008, and 2004 versus 2009; $P < 0.003$) and for only one comparison at the SFB site (2005 versus 2008; $P = 0.02$); the other comparisons were not significant. Proportion of hatchery admixture was fairly consistent in the temporal comparisons (2008 versus 2009) except for SFB, where hatchery admixture declined. None of the pairwise Wilcoxon rank tests for temporal comparisons of hatchery admixture proportion were significant ($P > 0.05$).

Comparisons of genetic differentiation (F_{ST} and allele frequency) showed significant differences at the two downstream-most sites in the basin (Table 1). Both comparisons demonstrated significant differences, indicating consistency across these measurements and supporting the conclusion that population genetics changed at UBR1 after barrier removal. Interestingly, the DS Dam site showed a significant change even though it was

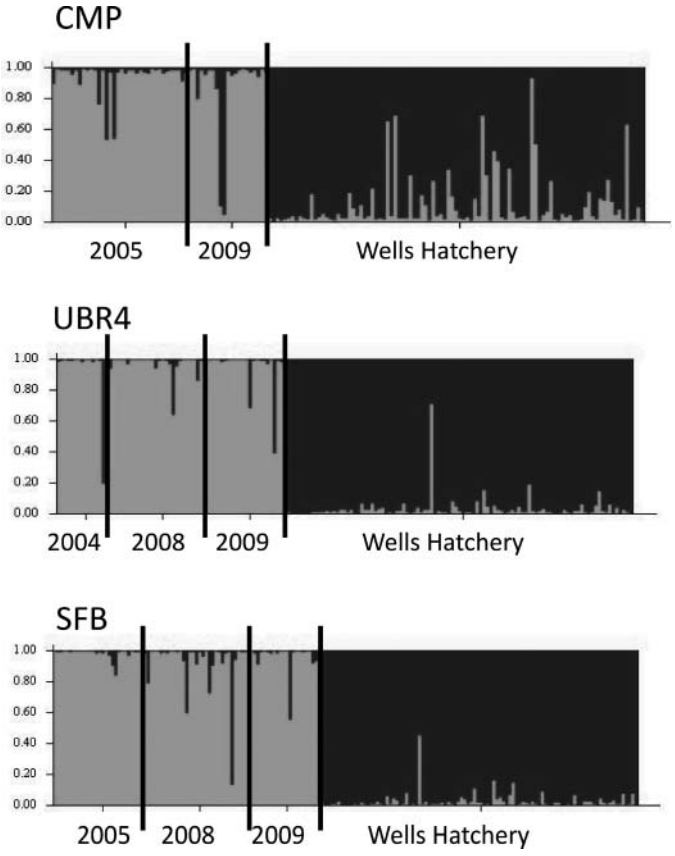

FIGURE 6. Output from STRUCTURE, showing population admixture in Rainbow Trout/steelhead sampled at the upstream-most three monitoring sites in Beaver Creek (Figure 1). Wells Hatchery steelhead were used as a reference for the hatchery population (hatchery × hatchery crosses; brood years 2005 and 2006). Each individual is represented by a bar on the plots (black shading = proportion hatchery alleles; gray shading = proportion wild alleles). Hatchery samples were provided by the Washington Department of Fish and Wildlife.

accessible prior to the barrier removal treatments. The genetic differentiation tests for the uppermost site, UBR4, were significant when comparing 2004 and 2008 but were not significant for the comparison between 2004 and 2009; the significance could be a result of finite sampling or nonrandom mating or tissue collections. The temporal tests comparing F_{ST} or allele frequencies in consecutive years were not significant (Table 1).

DISCUSSION

After the initial number of adult *O. mykiss* migrated into Beaver Creek in 2005, the number of adults 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. The adult steelhead counts at Wells Dam as reported by Snow et al. (2011) were highly correlated with our weir and tag reader counts of adult steelhead entering Beaver Creek ($r = 0.91$), indicating that our counts are following a similar trend. Fluvial Rainbow Trout constituted a variable portion of the run. These adults reproduced with the steelhead and should be considered part of the population (Weigel 2013).

Kiffney et al. (2009) found rapid colonization and steadily increasing abundances of Coho Salmon *O. kisutch* during the first 4 years after dam passage was restored; however, abundances of Chinook Salmon *O. tshawytscha* in that same study declined during the first and second years before increasing during the third and fourth years. Abundances of both Coho Salmon and Chinook Salmon in the Kiffney et al. (2009) study were substantially higher than the abundance of *O. mykiss* in our study. However, the distance colonized by adult Coho Salmon in the Kiffney et al. (2009) study was similar to that of *O. mykiss* in our study within 4 years after passage was restored. Demarias et al. (1993) found that recolonization by Virgin Chub *Gila seminuda* extended as far as 30 km in the Virgin River, Utah, 29 months after an accidental release of rotenone. The rate of colonization is mediated by abundance, distance, and connectivity to source populations; therefore, different species and locations may vary in response to connectivity projects or disturbance events. Wild steelhead and Rainbow Trout constituted 77% of the adults entering Beaver Creek. Abundances of wild steelhead returning to the Methow River basin are low, which likely impacts the number of adults entering Beaver Creek and could slow the population response to barrier removal.

Few hatchery steelhead entered Beaver Creek, despite high proportions of hatchery steelhead in the returns to the Wells Dam. Leider (1989) also found that the proportion of hatchery steelhead differed between a hatchery counting site lower in the basin and a natal tributary. Hatchery fish may return to release locations or to the hatchery site near the release location. In addition, other survival differences (e.g., selective harvest) may affect the proportion of hatchery fish between the ladder at Wells Dam and the natal tributaries. The counts of hatchery-produced steelhead entering Beaver Creek appeared to increase in 2007; however, tagged hatchery steelhead vastly outnumber the tagged wild steelhead in the basin, so without a fully operational weir it is impossible to determine the proportion of wild steelhead that could have entered the stream. Year-to-year variability in population sizes, survival rates, overlapping generations, and numbers of tagged steelhead in the basin precludes our ability to provide a valid estimate of steelhead that were missed at the weir based on these tag counts. Fluvial Rainbow Trout were not tagged by other fisheries sampling programs, and our efforts only tagged those fluvial individuals that were captured at the weir; therefore, the total population size and proportion tagged are also unknown for this life history.

Several parr that were tagged in Beaver Creek returned as adults in 2007–2011, 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, but 66% of these adults returned to the natal area. All of the strays detected in the Methow River basin were recorded in tributaries upstream from Beaver Creek. Steelhead were found to stray into tributaries upstream from

their natal tributary after the volcanic eruption on Mount St. Helens, Washington (Leider 1989). Additional steelhead that were tagged as parr in Beaver Creek were last detected while migrating upstream in the Columbia River or at the mouth of the Methow River; these adults were not detected again as entering a natal tributary, and their fate is unknown. They either died, entered another stream undetected, or returned to Beaver Creek downstream from the lowest tag reader. The rate of straying by steelhead from Beaver Creek (33%) was substantially higher than that documented in other studies (7.7%; see Hendry et al. 2004 and citations therein). Our data do not indicate why this high straying rate was observed, but it could be part of the early colonization process prior to establishing a viable population and associated local adaptations. Although the straying rate in our study is only based on four adult strays from Beaver Creek, we consider this to be a conservative estimate of straying because there are many basin locations where strays would go undetected.

The temporal stability of 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 that are unrelated to the treatment. Similar to results of other studies, our populations were temporally stable over short-term comparisons. Similar tests of collections ranging from less than 1 year apart to 5 years apart found that only 1 of 21 comparisons was significantly different (Heath et al. 2002; Narum et al. 2004, 2006; Nielsen et al. 2009). Therefore, we can expect fewer than 5% of temporal tests to be significant due to random or unmeasured effects.

Steelhead/Rainbow Trout from the two downstream-most sites (DS Dam and UBR1) showed significant differences in allele frequency and F_{ST} values. We did not expect to see a change at the DS Dam site because it was accessible to migratory steelhead/Rainbow Trout prior to the barrier treatments. Interestingly, there was also a reduction in the proportion of hatchery admixture at the DS Dam site after barrier removal. This shift in genetic parameters at the DS Dam site may be due to (1) individual trout moving downstream from upstream sites for rearing or (2) the mixing of anadromous or fluvial *O. mykiss* with the resident individuals occurring 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 UBR1 site had the greatest shift in F_{ST}, allele frequencies, and hatchery admixture, which were significantly different before versus after treatment. The increase in hatchery admixture is interesting since the hatchery steelhead that colonized the stream during 2005 and 2006 produced very few offspring (Weigel 2013). However, resident *O. mykiss* can adopt the anadromous life history, and gene flow into the hatchery from the populations upstream of Wells Dam is high. Therefore, the hatchery admixture is also tied to the anadromous life history through hatchery brood practices. Additionally, parr from UBR1 were exhibiting an anadromous life history prior to modification of the diversion dams. Life history is plastic in *O. mykiss* and can be related to growth or genetics. It is uncertain whether 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. Nevertheless, these data indicate that monitoring of juvenile tag migrations alone may not clearly indicate whether the anadromous life history is established in newly opened stream habitats.

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 during the first 4 years after barrier removal. The UBR4 site showed a significant change in F_{ST} and allele frequencies in the comparison of 2004 with 2008 samples but not in the comparison of 2004 with 2009 samples. Since the pairwise comparisons were not similar across the different years, we felt 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 in the comparison of 2005 and 2008 samples but not in the comparison of 2005 and 2009 samples. These shifts in population genetic measures could be the result of genetic drift from a finite number of breeders, nonrandom mating, or finite sampling; the shifts may also be attributable to the presence of a few new migrants in 2008 that did not migrate into this area in 2009.

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

In summary, adult steelhead entered Beaver Creek during the first spawning season after barrier removal and parr from the initial brood years returned to Beaver Creek, indicating that the complete life cycle of steelhead was established. In addition, tag movement data indicated that adult steelhead were moving to the upper monitoring sites during the third and fourth years after barrier removal. Hatchery steelhead represented a small proportion (23%) of these colonizing adults, despite high abundances of fish from hatchery releases by local fishery management programs. Abundances of adult *O. mykiss* did not increase during the 4 years of weir operation. Because hatchery fish did not comprise a majority of the run into Beaver Creek

and because hatchery fish are expected to have substantially reduced reproductive success (Miller et al. 2004; Araki et al. 2007), the low numbers of wild steelhead entering the Methow River basin are likely limiting colonization of Beaver Creek by this life history type. Colonization and expansion of steelhead were slower 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 attains stable distribution and abundance in the basin. Additionally, as the colonization process continues, relationships such as the relative abundances of hatchery steelhead entering the stream may shift.

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