Fluvial rainbow trout contribute to the colonization of steelhead (*Oncorhynchus mykiss*) in a small stream

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Abstract Life history polymorphisms provide ecological and genetic diversity important to the long term persistence of species responding to stochastic environments. *Oncorhynchus mykiss* have complex and overlapping life history strategies that are also sympatric with hatchery populations. Passive integrated transponder (PIT) tags and parentage analysis were used to identify the life history, origin (hatchery or wild) and reproductive success of migratory rainbow/steelhead for two brood years after barriers were removed from a small stream. The fluvial rainbow trout provided a source of wild genotypes to the colonizing population boosting the number of successful spawners. Significantly more parr offspring were produced by anadromous parents than expected in brood year 2005, whereas significantly more parr offspring were produced by fluvial parents than expected in brood year 2006. Although hatchery steelhead were prevalent in the Methow Basin, they produced only 2 parr and no returning adults in Beaver Creek. On average, individual wild steelhead produced more parr offspring than the fluvial or hatchery groups. Yet, the offspring that returned as adult steelhead were from parents that produced few parr offspring, indicating that high production of parr offspring may not be related to greater returns of adult offspring. These data in combination with other studies of sympatric life histories of *O. mykiss* indicate that fluvial rainbow trout are important to the conservation and recovery of steelhead and should be included in the management and recovery efforts.

Keywords Colonization · Parentage · Barrier removal · Fitness · Life history polymorphism

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Introduction

Many species of salmonids exhibit multiple life history strategies (Behnke 1992; Hendry et al. 2004). These strategies may involve extensive migrations to access more resources (such as food or space) and attain greater size. Rainbow trout (*Oncorhynchus mykiss*) are particularly diverse in life history, migratory and rearing strategies (McPhee et al. 2007). Anadromous rainbow trout (called steelhead) emerge as young and rear in freshwater for 1 to 4 years, migrate to the ocean to rear an additional 1 to 4 years, and then migrate back to the natal stream to spawn and complete their life cycle. Resident forms of rainbow trout remain in freshwater for the entire life cycle. A non-migratory form of the resident life

history rears in the smaller tributary stream habitat. The migratory forms of the resident life history will rear in larger rivers (called fluvial) or in lakes (called adfluvial) (Behnke 1992). Populations of anadromous and resident life history forms are often sympatic and may inter-breed (Zimmerman and Reeves 2000; Docker and Heath 2003; McPhee et al. 2007; Christie et al. 2011).

Retention of divergent life history strategies within common taxonomic units is interpreted as an evolutionary strategy that promotes adaptive flexibility in stochastic environments (McPhee et al. 2007). In a modeling study, Parker et al. (2001) found that a common gene pool among alternate life histories resulted in faster population recovery after disturbance, and in unstable environments, this strategy is predicted to be the evolutionary stable state. As anadromous populations or stocks have declined (e.g. McClure et al. 2003), extensive efforts have been underway to modify or restore critical habitats. When sympatric, resident rainbow trout and steelhead are a common population; therefore, restoration efforts should focus on restoring habitats that promote the full expression of life history diversity (McPhee et al. 2007). Yet, these alternative life history strategies are generally not considered in management or recovery of steelhead. Furthermore, triggers for migration or life history expression result from complex interactions between environmental and genetic factors that are not fully understood (Hendry et al. 2004; McPhee et al. 2007).

Barrier removal projects provide opportunities to study colonization which can provide insight into the role of life history diversity in response to environmental disturbance. Hatchery steelhead are also present across most of the range historically accessible to anadromous migrations. Therefore, understanding the role of life history diversity and hatchery steelhead in colonization of re-opened habitat provides insight into the ecological significance of life history diversity in stream and population recovery efforts. In this study, we used genetic data and tag movement detections to document the reproductive success of fluvial rainbow trout, wild steelhead and hatchery steelhead after barrier removal. The objectives of our study were to: 1) determine genetic variation, genetic differentiation and gene flow among fluvial rainbow trout, wild steelhead and hatchery steelhead in the study area; 2) identify the relative reproductive success of each population or life history; and 3) follow the offspring from the colonizing

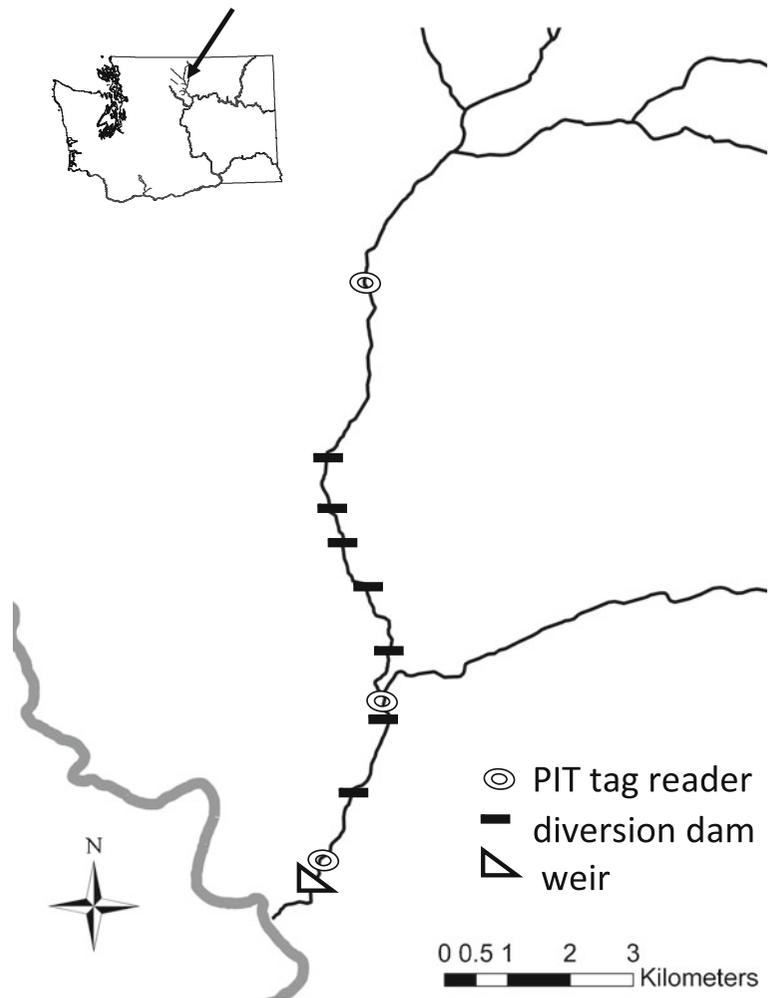migratory *O. mykiss* to determine if they successfully return as adult steelhead.

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 and flows west into the Methow River 57 km upstream from the mouth (Fig. 1). The Beaver Creek watershed is 290 km² with basin elevations that range from 463 to 1,890 m and stream flows that ranged from 0.05 to 4.7 cms during the study (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) resulting in fragmented resident *O. mykiss* populations within the basin (Weigel et al. 2013a). Resident rainbow trout were present throughout Beaver Creek and tributaries prior to implementing the barrier removal projects. 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 consolidated and modified to five 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. Access for migratory steelhead/rainbow trout was restored to Beaver Creek for the spring 2005 spawning season.

Hatchery releases

State and federal programs rear and release hatchery steelhead in the Methow Basin as fisheries mitigation for hydropower facilities on the Columbia River. Broodstock for the programs in this area 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

Fig. 1 Study area and location of fish trap and PIT tag readers in Beaver Creek, Methow Basin, Washington. The *arrow* on the inset map points to the location of Beaver Creek in relation to the State of Washington and the Columbia River

hatchery broods have been managed as demographically independent stocks.

Hatchery mitigation in the Methow during our study included a release of 450,000–550,000 steelhead smolts per year that are spawned and reared at Wells Hatchery (rkm 830.1) 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 (Snow et al. 2010). Hatchery steelhead are released as age 1 smolts in the Methow and Chewuch rivers upstream from the town of Winthrop, WA. All hatchery origin steelhead 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. During our study (2005–

2008), hatchery steelhead returns ranged from 82 % in 2008 to 91 % in 2005 (Snow et al. 2010).

Methods

Life history, migrations and survival were identified by monitoring the individual migrations and movements with passive integrated transponder (PIT) tags and tag reading stations located throughout the Columbia River and tributaries. To understand population genetic characteristics and gene flow among the relevant life histories and populations, we compared fluvial rainbow trout and wild steelhead captured at the weir to samples from the local Wells Hatchery brood. To understand individual reproductive success, we used parentage analysis to

identify offspring from candidate spawners migrating into the study area.

Fish capture and tagging

Adult and juvenile *O. mykiss* moving upstream or downstream were captured in Beaver Creek using a picket weir installed 1.3 km upstream from the mouth (Fig. 1). Upstream and downstream moving fish were held separately in a trap box. 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. 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 based on tags and external marks. A tissue sample was removed from the caudal fin and stored in 95 % non-denatured ethanol. 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 using a needle and syringe-style tag implanter. PIT tags were full duplex, 12 mm size tag.

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. 1) and at dams and passage facilities on the mainstem Columbia River. Migratory life history (anadromous or fluvial) of the adult trout was identified from the locations of the PIT tag detections. 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. The comparison of weir captures and PIT tag detections in the stream indicated that nearly all adult migrants were sampled in brood years 2005 and 2006. Total parr collection at the trap was incomplete due to trap inefficiencies or environmental conditions. However, the collection was considered random and representative of juvenile parr outmigrating from the spawning populations in the stream because the trap

captured juveniles during periods when outmigration was highest. Examination of outmigration phenotypes of full siblings found a wide range of outmigration timing and no significant relationship between family and timing of parr outmigration (Weigel 2013) further supporting unbiased collection of parr. Most of the *O. mykiss* parr migrated out of Beaver Creek during the first fall after spawning (age 0) when stream flows were lower and trap operation was most successful.

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 collected at the weir and from the hatchery brood samples. Thirteen of these markers are standardized across the Columbia River Basin and are described 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 have previously been reported to be <2 % (Stephenson et al. 2009). Duplicate samples indicate laboratory error rates <1 % for our study.

Statistical analysis

Adult rainbow/steelhead collected at the weir were compared to hatchery samples from the Wells Hatchery brood to assess the population genetic attributes for each source group. Life history was compared across the different years in the sample. Adult rainbow/steelhead collected at the weir were grouped by year, population/life history. Exact tests of Hardy Weinberg Equilibrium and linkage disequilibrium, and calculations of heterozygosity, genetic differentiation and F_{ST} were performed 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).

Reproductive success was measured by assigning offspring captured in Beaver Creek 2005 through 2008 to candidate parents for brood years 2005 and 2006. 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 impossible. 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). *O. 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. A chi-square test was used to determine if the number of offspring by source was significantly different than would be expected by chance. This test was performed without hatchery steelhead because hatchery steelhead provided such a small number of offspring ($n=2$ in 2005 and 0 in 2006) in comparison to the fluvial rainbow trout and wild anadromous steelhead resulting in low expected values.

Results

Tag detections identified that more than 90 % of the migratory adult *O. mykiss* captured at the weir were natural-origin steelhead/rainbow trout during the first two brood years entering Beaver Creek. 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). Migration timing of the two life history strategies overlapped. In 2005, fluvial rainbow trout were captured in the fish trap between April 2 and April 15 and steelhead were captured between March 25 and May 14. In 2006, fluvial rainbow trout were captured in the fish trap between March 15 and April 21 and steelhead were captured between March 24 and April 6. The anadromous and fluvial life histories separated by size with steelhead >500 mm (Table 1). Only 7.6 % ($n=5$) of our adult captures in Beaver Creek for brood years 2005 and 2006 were identified as hatchery steelhead, and none of the fluvial trout were of hatchery origin.

The steelhead and rainbow trout showed similar genetic variation across groups in our sample (Table 2) and did not violate the assumptions of Hardy Weinberg or linkage equilibrium. 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). Tests of Hardy Weinberg Equilibrium did not detect significant departures in the 2005 or 2006 steelhead or fluvial rainbow trout 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 wild steelhead/rainbow trout collected at the weir in 2006 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.

Table 1 Sample size (N), range (mean) of size for fluvial and anadromous adult *O. mykiss* captured at the fish trap in Beaver Creek during 2005 and 2006

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

Genetic differentiation tests indicated that hatchery steelhead were significantly different from the wild steelhead and the fluvial rainbow trout, but the fluvial rainbow trout were not significantly different from the wild steelhead. We did not detect significant differences in allele frequencies between years for the groups (wild steelhead, fluvial rainbow trout and hatchery steelhead); therefore, we combined the samples from the 2 years for comparisons among these groups. Genetic diversity was similar across all the life histories and years in the sample (Table 2). Exact tests of genetic differentiation were not significant comparing the wild anadromous and fluvial samples ($p=0.049$), but were significant comparing the wild anadromous and Wells Hatchery samples ($p=0.001$) and the fluvial rainbow trout and Wells Hatchery samples ($p<0.001$). F_{ST} estimates across these groups showed little differentiation (wild to hatchery steelhead = 0.002, fluvial to hatchery = 0.006, wild steelhead to fluvial = 0.004) and indicated that the fluvial rainbow trout were most different from the hatchery steelhead.

Parentage analysis assigned 243 (16 %) offspring to one or two parents in brood years 2005 and 2006 from a total of 1,544 parr. 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). 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. In 2005, three steelhead 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 steelhead were missing from our sample one due to failed DNA extraction and the other was recorded in the tag readers.

Table 2 Genetic diversity (unbiased heterozygosity (H), average allelic richness (AR), and average private alleles (PA)) and sample size (N) for wild anadromous and fluvial *O. mykiss* collected in the weir in Beaver Creek and Wells Hatchery brood years 2005 and 2006

Source	Brood year	N	H	AR	PA
Years separate					
Wild anadromous	2005	27	0.81	7.2	0.5
Wild anadromous	2006	23	0.83	7.4	0.5
Wells hatchery	2005	49	0.81	7.0	0.4
Wells hatchery	2006	50	0.82	7.6	0.3
Fluvial	2005	9	0.83	7.7	0.6
Fluvial	2006	20	0.81	7.1	0.5
Years combined					
Wild anadromous	2005, 2006	50	0.83	7.4	1.4
Wells hatchery	2005, 2006	99	0.82	7.0	1.2
Fluvial	2005, 2006	29	0.82	7.3	1.5

Wells Hatchery samples were hatchery x hatchery crosses provided by the WDFW

Examining mating pairs by life history indicated that breeding was common among the life history types (Fig. 2). No offspring from fluvial x fluvial crosses were detected in our samples.

Reproductive success across the life history types and populations varied during the two brood years in the study. On average, steelhead parented more parr offspring (2005 avg=11.3 SD=15.6; 2006 avg=0.9 SD=2.1) than fluvial rainbow trout (2005 avg=1.0 SD=2.0; 2006 avg=3.0 SD=6.3) or hatchery steelhead (2005 avg=0.7 SD=1.1; 2006 0 offspring) in our sample. Individual reproductive success varied across groups from 0 to 58, 23 and 2 parr for wild steelhead, fluvial rainbow trout and hatchery steelhead, respectively (Fig. 3). Steelhead parented 97 % of the matching parr in 2005 (n=270), but only 18 % in 2006 (n=19). Fluvial rainbow trout parented 3 % of the matching parr in 2005 (n=9) and 81 % in 2006 (n=83). Hatchery steelhead only parented 1 % of the matching parr in 2005 (n=2) and no matching parr in 2006. Number of offspring produced was lower in 2006 than 2005, and more offspring matched to fluvial rainbow trout than to steelhead in 2006 (Fig. 3). The number of offspring by group was significantly different than expected by chance for the fluvial and wild anadromous adults (p<0.001 df=1). Significantly more parr offspring were produced by anadromous parents than expected in brood year 2005, whereas

significantly more parr offspring were produced by fluvial parents than expected in brood year 2006.

PIT tag detections from the matching offspring identified two adults from brood year 2005 and one adult from brood year 2006 that successfully returned to the Columbia River. 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 fitness may not be related to the number of parr produced. Additionally, none of the adult returns were wild steelhead x steelhead crosses even though this parent cross comprised 46 % of the sample.

Discussion

During the colonization of the re-opened habitat, wild fluvial rainbow trout directly contributed to the production of smolts and adult steelhead that returned to Beaver Creek. Breeding and gene flow among fluvial rainbow trout and wild steelhead was observed in the allele frequency and parentage tests. Fluvial rainbow trout captured in Beaver Creek were wild-origin, and the significant difference in allele frequencies between the fluvial rainbow trout and the hatchery steelhead indicate

Fig. 2 Proportion of one and two parent matches by life history and source population for brood years 2005 and 2006
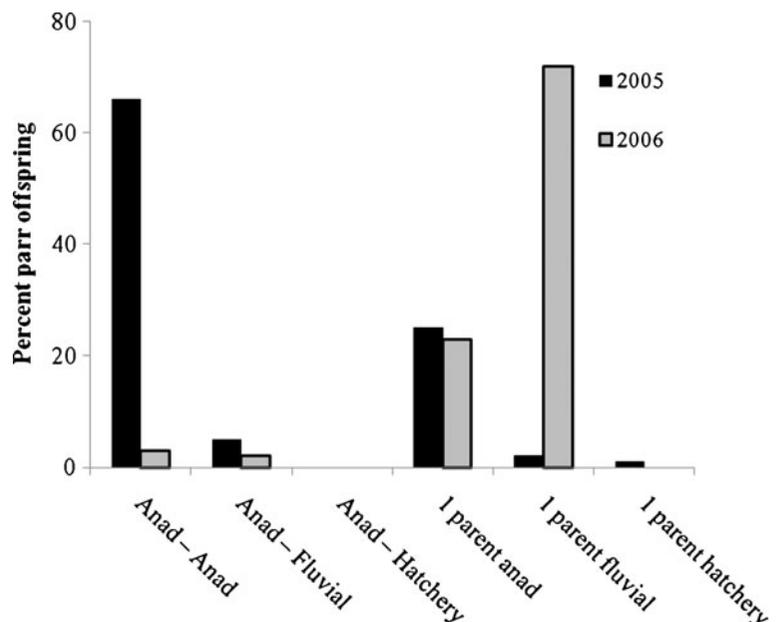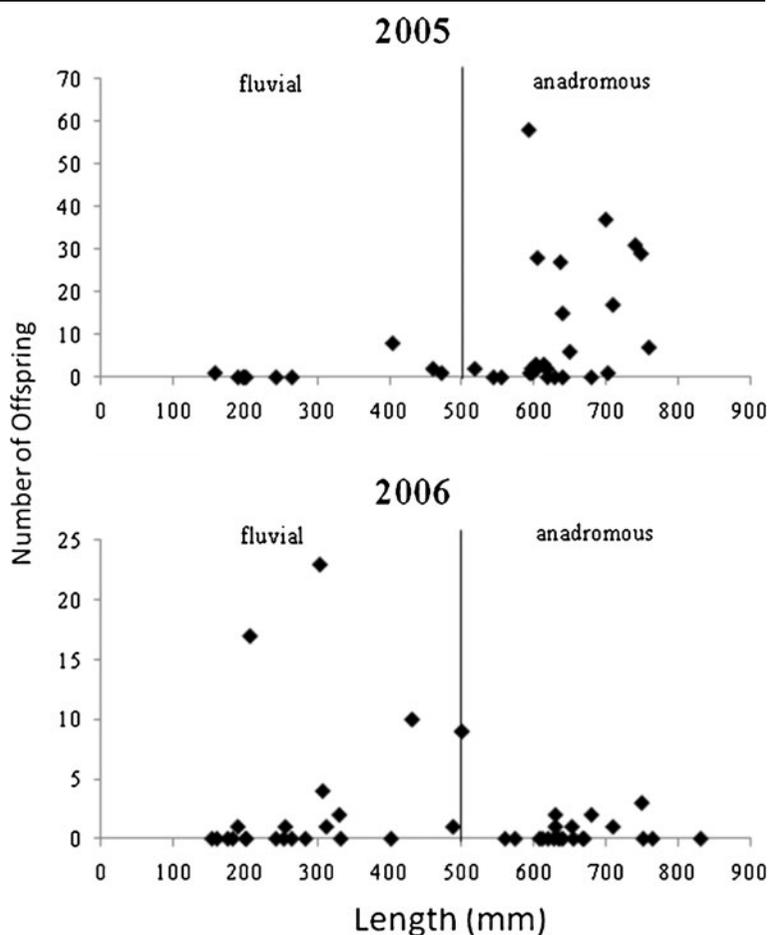
Fig. 3 Number of offspring
matched by parent fork
length (mm) for 2005 and
2006 adult rainbow/steel-
head. The *horizontal line* in-
dicates the length that sepa-
rates the fluvial and anadro-
mous adults in the study.
Note the different Y-axis
scales

that fluvial rainbow trout in our study are not hatchery residuals. Hatchery steelhead were a very small component of the successful spawners colonizing Beaver Creek during the first 2 years after barrier removal and did not produce any offspring that returned as adults. Although parr from non-migratory resident parents can produce smolt outmigrants, all returning adult steelhead had at least one migratory (fluvial or anadromous) parent.

Fluvial rainbow trout and steelhead successfully spawned and produced anadromous offspring. Successful mating among rainbow trout and steelhead has been documented in other populations (Zimmerman and Reeves 2000; Docker and Heath 2003; Araki et al. 2007a; Christie et al. 2011). The allele frequency test and parentage data indicates that gene flow was high among the wild steelhead and fluvial rainbow trout. Similarly, Christie et al. (2011) estimated from pedigree analyses that 20 % of the steelhead genes arise from the resident life history in Hood River, Oregon. Therefore,

in the Methow Basin populations, the wild steelhead exchange genes with the fluvial rainbow trout. The wild steelhead are then incorporated into the Wells Hatchery population through artificial breeding. Although hatchery steelhead did not contribute to the colonization of Beaver Creek during the first two brood years, breeding between hatchery and wild steelhead could occur naturally in other locations or during later stages of colonization.

Non-migratory resident rainbow trout were not the focus of our parentage analysis; although, parr from these crosses smolt, they have not been documented to return as adult steelhead (Thrower et al. 2004; Weigel et al. 2013b). Lack of selection from the ocean environment on the genotype from this life history has been suggested as an explanation for low ocean survival (Thrower et al. 2004). High rates of missing parents in steelhead studies are thought to be the result of matings with unsampled non-migratory rainbow trout, and unmatched parr in our study were more numerous than

those documented in Seamons et al. (2004). 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. 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.

The composition of the life history of the parents that produced offspring were substantially different between 2005 and 2006, perhaps related to the unusually high spring flows in 2006. In 2006, fluvial rainbow trout 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 the magnitude than during the other years of our study (Ruttenberg 2007; USGS 2012). Maximum stream flow between March 15 and May 20 in Beaver Creek was 2.6 cms (91 cfs) on May 10, 2005 and 8.9 cms (313 cfs) on May 18, 2006. In 2006, stream flows exceeded 2.6 cms (91 cfs) from April 29 through May 20 when the stream flow measuring equipment was lost. These high flows may have scoured redds, caused mortality in early emergent fry or prevented the later returning adults from spawning. Overall reproduction was lower during this spawning season indicated by the lower number of offspring produced by individual adults and reduced age 0 densities at monitoring sites in the stream (Weigel 2013). Alternate life history strategies can provide demographic stability in less stable environments (Parker et al. 2001) and genetic compensation to the breeding population when steelhead abundances are low (Araki et al. 2007a). During colonization, this inter-breeding among the multiple life history polymorphisms should increase the number of breeders and genetic variation.

Most of the fluvial rainbow trout captured in the weir were male indicating a gender bias for this alternate life history polymorphism (Weigel et al. 2013b). Therefore, this interaction between life history and gender would result in high proportions of breeding among the life history strategies. Interestingly, we did not document any fluvial x fluvial crosses indicating that it may be more common for these fluvial rainbow trout to attempt to mate with an anadromous or resident partner.

Similarly, Christie et al. (2011) found significantly more steelhead matings occur between an anadromous female and a resident male. Male steelhead, unlike the females, may have less to gain from anadromy particularly when considering iteoparity. In Beaver Creek, one-third of the fluvial rainbow trout returned in successive years with one individual returning four spawning seasons (Weigel et al. 2013b). Iteoparity and higher post-spawning adult survival of the males utilizing this strategy could increase the lifetime fitness while reducing the risks of an anadromous migration particularly in populations with long migration distances to the ocean.

When examining individual reproductive success, the wild steelhead produced more offspring which is expected based on the reproductive biology of salmonids, where larger size is directly related to greater egg numbers and egg size for females (Beacham and Murray 1993; Fleming 1998) and greater ability to fight and maintain dominance during courtship for males (Gross 1985; Taborsky 1998). Moreso, even though the wild steelhead x steelhead cross was almost twice as abundant in our sample, none of these parr survived to migrate back into the Columbia River as adult steelhead. Although our sample size is too small to draw conclusions, the adult steelhead that returned from the two brood years sampled had some unknown or resident parent. Although greater parr/smolt production is presumed to increase the chances of adult returns from the individual's offspring pool, these data suggest that there are other underlying mechanisms determining which smolts survive to the adult life stage. Hatchery steelhead have been found to have much lower relative reproductive success than wild steelhead (Miller et al. 2004; Araki et al. 2007a, b, 2008), and hatchery steelhead could be spawning successfully in some areas of the Methow and potentially reducing reproductive success of the wild steelhead. Another possible explanation could be that the fluvial life history is introducing genetic variability creating an effect similar to hybrid vigor (Allendorf and Luikart 2007). Inbreeding depression can be the cost of the high degree of homing observed in salmon and steelhead (Wang et al. 2002). Life history diversity with gender bias may reduce the risks of inbreeding particularly if the different life histories and/or genders have differing straying rates. Certainly, more studies coupling genetic and migratory behavior in steelhead populations focusing on life history diversity and hatchery populations are

necessary to elucidate whether this is a localized or more widespread effect across the species range.

Hatchery steelhead were a very small component of the adult returns to the colonizing population. One hatchery steelhead produced only two parr that did not return as adults indicating no detectable genetic contribution to the initial colonization in Beaver Creek despite high abundances of hatchery-origin adult steelhead returning to the Methow Basin. We could not determine why the hatchery escapement and reproduction was low in our study, but it appears that low hatchery contributions were largely a result of low numbers of hatchery-origin trout into the stream. The proportions of hatchery to wild steelhead 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 release site. Leider (1989) also found substantially higher proportions of hatchery steelhead at a counting location near the mouth of the larger mainstem stream when compared to a natal tributary further upstream.

Life history diversity has been challenging for fisheries scientists to understand and the coupling of genetic and tagging methods in this study has provided insight into the relative importance of the alternate life history strategies and hatchery populations. In summary, fluvial rainbow trout were natural-origin and contributed smolts and adults to the colonization of Beaver Creek after barrier removal. This strategy provides a genetic preserve and source of variation particularly important in years with low abundances. The fluvial life history strategy was important in the early colonization process and during environmental disturbance when stream flows reduced the reproductive success of the anadromous spawners in 2006. Considering the complex life history and gender biases in *O. mykiss*, populations of steelhead would benefit from stream restoration efforts on larger riverine systems focusing on high quality rearing habitat and abundant prey species. Further research into the inter-action between genetic and environmental factors on the expression of life history diversity and the resulting lifetime fitness will better inform resource managers about where stream restoration efforts will be most productive toward steelhead population recovery.

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