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ARTICLE

Genetic Population Structure of Willamette River Steelhead and the Influence of Introduced Stocks

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Abstract

Conservation genetics studies are frequently conducted on Pacific salmon *Oncorhynchus* spp. to delineate their population structure and to quantify their genetic diversity, especially for populations that have experienced declines in abundance and are subject to anthropogenic activities. One such group of salmonids is steelhead *O. mykiss* (anadromous Rainbow Trout) from the Willamette River, a tributary of the Columbia River. Within the Willamette River there are multiple steelhead life history and run-timing types, some of which originated from nonnative populations. Late winter-run steelhead and Rainbow Trout are native to the Willamette River, whereas early winter-run and summer-run steelhead have been introduced into the system via releases from artificial propagation efforts. We conducted genetic analyses of Willamette River steelhead to determine the effect that nonnative steelhead released into the Willamette River basin have had on the genetic population structure of native steelhead. We found genetic differentiation among the samples that separated steelhead into four population groups that corresponded to run type. Possibly due to local adaptation, the native run type has retained its genetic distinctiveness from the introduced types, despite there being opportunities for gene flow among all types. Introduced early winter-run steelhead appear to be

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the origin of steelhead inhabiting certain Willamette River tributaries where native steelhead did not historically spawn.

The analysis of genetic population structure within a species has become an essential element in the assemblage of information needed for effective fisheries management. Defining distinct or independent population units to be targeted by management efforts aids in the effective conservation of a species' genetic diversity (Ford 2004; Allendorf and Luikart 2007). Conservation of genetic diversity is critical because genetic variation maximizes a species' evolutionary potential to respond to environmental changes through natural selection (Allendorf and Luikart 2007).

Pacific salmon *Oncorhynchus* spp. are widely studied, in part, because of concerns for their conservation status, and because of their commonly observed subdivided population structure, which is due mainly to their fidelity to return to their natal streams to spawn (Ford 2004). Many populations of Pacific salmon, representing unique ecological, life history, and genetic diversity types, are already believed to have been lost to extinction events (Gustafson et al. 2007). Pacific salmon exhibit life history diversity in traits such as migratory behavior (anadromous versus freshwater resident types), age at emigration to salt water, age at maturity, and timing of spawning (Groot and Margolis 1991). This diversity can often be attributed to local adaptation to environmental conditions (Taylor 1991). Life history diversity is believed to provide long-term stability for salmonid populations in the face of large-scale changes in environmental conditions (Hilborn et al. 2003). For example, Pacific salmon populations are often categorized by the time of year they return to freshwater to spawn, which is usually referred to as run time or run type (Groot and Margolis 1991). Timing of freshwater entry can be critical to a salmon's ability to migrate upstream past barriers that are only passable during certain times of the year due to water flow (Reiser et al. 2006), avoid times of unfavorable water temperatures (Hodgson and Quinn 2002; Goniea et al. 2006), or deposit eggs during a time that will ensure the optimal development and survival of their offspring (Murray and McPhail 1988; Beer and Anderson 2001; Brannon et al. 2004).

Steelhead *O. mykiss* (anadromous Rainbow Trout) exhibit run-timing diversity such that in some river systems spawning migrations occur throughout the entire year in order to access and utilize a variety of spawning habitats (NMFS 2011a). Such diversity is observed within the Columbia River basin, which historically produced up to half a million steelhead adults annually (Chapman 1986), but where now every native steelhead population is listed as threatened under the U.S. Endangered Species Act (ESA; NMFS 2006). Thus, there is a need to better understand their population structure in the interest of conserving genetic diversity, including how they

have been affected by past and present human activities, such as the release of hatchery-raised fish.

In the Willamette River, Oregon, a tributary of the Columbia River, there has been a complex interaction of run-timing diversity, artificial propagation, and anthropogenic activities influencing the genetic population structure among the native and introduced steelhead populations. Three different steelhead run types currently reside within the Willamette River basin: summer run, early winter run, and late winter run (Myers et al. 2006). The late winter run is the only run type native to the Willamette River. This return timing appears to be a unique adaptation that enabled steelhead to ascend steep river gradients and natural barriers, such as Willamette Falls, a 12-m-high natural waterfall located at river kilometer 43, during a short temporal window when the falls were passable (Myers et al. 2006). Since the construction of the first fish ladder at Willamette Falls in 1885 (Kostow 1995) and subsequent further improvements to fish passage facilities at the falls (Frazier 1988), the falls are no longer a limiting factor to upstream migration, and other steelhead run types are able to ascend into the upper Willamette River (Keefer and Caudill 2010). Early winter-run steelhead are not native to the upper Willamette River, as they originate from tributaries to the lower Columbia River, but releases of early winter-run fish have been made throughout the Willamette River system for decades (Myers et al. 2006). Such releases ended in the 1990s above Willamette Falls, but continue below the falls in the Clackamas River (HSRG 2009), a tributary of the lower Willamette River and part of the lower Columbia River steelhead distinct population segment. A distinct population segment is what constitutes a "species" under the ESA and is described as a group of organisms that is separated from other populations of the same taxon because of physical, physiological, ecological, or behavioral factors and that is significant to its taxon (NMFS 2006). Fish passing Willamette Falls prior to February 15 are considered to be early winter-run fish for management purposes (Kostow 1995), but there is overlap between when the early winter run ends and the late winter run begins, creating the potential for temporal overlap between the run types during spawning. Summer-run steelhead are also not native to the Willamette River, but have been and continue to be artificially propagated and released throughout the Willamette River system to provide recreational fishing opportunities as mitigation for the loss of spawning habitat for native winter steelhead that was blocked by 13 high-head dams (Busby et al. 1996; NMFS 2011a). In every year from 1990 to 2012, nonnative summer-run steelhead passing Willamette Falls have outnumbered both the early and late winter runs (ODFW 2013),

leading to concerns about the genetic integrity of native late winter run (NMFS 2011a). The timing and location of spawning summer-run steelhead overlaps with that of the native late winter run (Keefer and Caudill 2010), and summer-run steelhead have been observed spawning in various Willamette River tributaries (Firman and Buckman 2003), so the potential exists for interbreeding between the two run types as well as for competition for food and habitat among juveniles. Winter-run steelhead spawning above Willamette Falls are recognized as a distinct population segment discrete from steelhead spawning below Willamette Falls (NMFS 2006).

The freshwater resident life history type of *O. mykiss*, Rainbow Trout, also resides within the Willamette River basin, including upper river areas above the confluence of the Calapoia River, where steelhead are not believed to have spawned historically (Dimick and Merryfield 1945; Fulton 1970); however, both summer- and winter-run steelhead have been released into these areas (HSRG 2009). In rivers where steelhead and Rainbow Trout co-exist there can be substantial gene flow between them (Docker and Heath 2003; Olsen et al. 2006; McMillan et al. 2007; Heath et al. 2008; Van Doornik et al. 2013). Thus, it is important to understand the reproductive and competitive interactions between these differing life history types, especially in areas not historically utilized by steelhead.

Whereas native steelhead are historically known to inhabit tributaries on the east side of the Willamette River basin, which drain the Cascade Range, there is considerable uncertainty regarding winter-run steelhead that spawn in some of the western tributaries of the Willamette River, which originate in the Oregon Coast Range. Historically, only a few steelhead were believed to have spawned in the western tributaries as the conditions of those rivers were not favorable to steelhead due to summertime low water flows and high temperatures (Parkhurst et al. 1950; Fulton 1970), but presently they contain naturally spawning steelhead populations (Myers et al. 2006). The genetic lineage of the western-tributary steelhead and their relationship to the native steelhead in the eastern tributaries, which originate in the Cascade Range, is unclear. There is historical, anecdotal information that steelhead may have spawned in the Tualatin River in the 1940s (Parkhurst et al. 1950), but it is uncertain if they were a natural or introduced population. A previous analysis based upon allozyme variation suggests that these populations have a genetic affinity with the lower Columbia River steelhead populations (NMFS 1999). Early winter-run steelhead from Big Creek Hatchery, which is located on a tributary to the lower Columbia River, were released into western tributaries from 1958 to 1996 (Myers et al. 2006). In addition, late winter-run steelhead native to eastern tributaries of the Willamette River have also been released into some of the western tributaries. Thus, the steelhead currently residing in the western tributaries could have originated from a number of different sources (HSRG 2009).

Given the potential for complex interactions between the three distinct steelhead run types inhabiting the Willamette River, several management questions exist regarding Willamette River steelhead, including (1) What effect has the continued release of nonnative summer-run steelhead had on native winter-run populations? and (2) Do the distinct population segments identified under the ESA listing (NMFS 2011a, 2011b) accurately describe the population structure of Willamette River steelhead? Thus, our goals were to (1) determine the genetic population structure of steelhead within the Willamette River basin, (2) determine whether the nonnative steelhead released into the Willamette River basin have affected the genetic population structure of native steelhead, and (3) identify the lineage of steelhead residing in the western tributaries of the Willamette River.

METHODS

Sample collection.—We collected 1,047 *O. mykiss* samples from 19 locations in the Willamette River and one in the lower Columbia River (Figure 1; Table 1). Two of the locations were sampled in two different years. A variety of collection methods were used to capture fish to obtain a small amount of fin tissue, which was preserved in 95% ethanol. Adult steelhead were captured at fish collection facilities, such as dams or fish ladders, or by hook-and-line angling. Juveniles were captured in streams either by electrofishing or hook-and-line angling. For samples collected in the 1990s, fin tissue was obtained from lethally collected juveniles frozen whole at -80°C . Adult samples from the 1980s were obtained from dried scale samples archived by the Oregon Department of Fish and Wildlife (ODFW).

We grouped the samples into various configurations for analytical purposes. First, the samples were separated into four groups based upon location: (1) eastern lower and upper Willamette River tributaries, the only locations where native steelhead are historically believed to reside; (2) freshwater resident Rainbow Trout from areas in the upper Willamette River that historically did not contain native steelhead populations; (3) western upper Willamette River tributaries; and (4) Big Creek Hatchery (lower Columbia River), which is one of the early winter-run hatchery stocks that have been propagated and released within the Willamette River basin (Myers et al. 2006). Second, the steelhead samples were separated into three groups based upon run timing: (1) nonnative early winter-run steelhead (i.e., represented by collections from Big Creek Hatchery and Eagle Creek National Fish Hatchery [NFH]); (2) native late winter-run steelhead; and (3) Nonnative summer-run steelhead (i.e., originally derived from Skamania Hatchery stock). In general, individuals with early winter-run timing are observed in the Willamette River from October through February when migrating upstream to spawn, whereas the late winter run returns to the river from February through May (Figure 2) (Murtagh et al. 1992; Kostow 1995). Summer-run

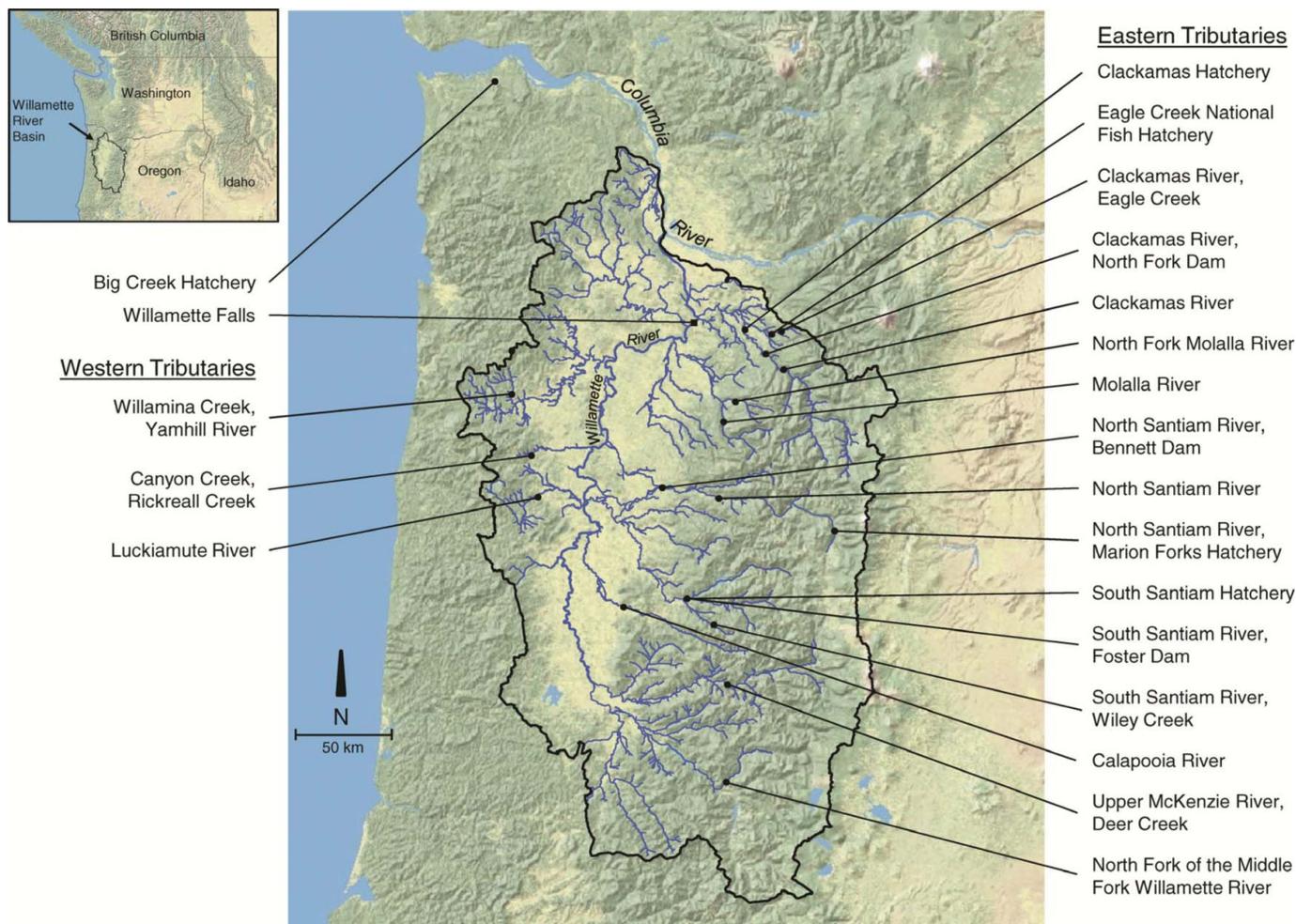


FIGURE 1. The Willamette River basin in Oregon and the approximate locations of steelhead and Rainbow Trout sample collections used in this study. [Figure available online in color.]

steelhead originated from the “Skamania Hatchery stock” and were originally derived from the Klickitat and Washougal rivers on the lower Columbia River (Keefer and Caudill 2010). They are released throughout the Willamette River basin (Myers et al. 2006) and enter the system as returning adults from March through October, and then they remain in freshwater for several months before spawning (Keefer and Caudill 2010).

Data collection.—Genomic DNA was isolated from fin tissue or scales using the Promega Wizard DNA Purification Kit (Promega Corporation) following the manufacturer’s protocol. Samples were genotyped for 15 microsatellite loci: *Ocl1* (Condrey and Bentzen 1998), *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), *Omy77* (Morris et al. 1996), *One14* (Scribner et al. 1996), *Ots3* and *Ots4* (Banks et al. 1999), *Ots100* (Nelson and Beacham 1999), *Ssa289* (McConnell et al. 1995), and *Ssa407* and *Ssa408* (Cairney et al. 2000). Polymerase chain reactions were performed to amplify the loci of interest, and the resulting PCR products were analyzed

via capillary gel electrophoresis using an Applied Biosystems 3100 genetic analyzer (Life Technologies, Grand Island, New York). Individual genotypes were determined using GeneScan and Genotyper software (Life Technologies).

Data analysis.—We used the program COLONY (Jones and Wang 2009) to determine whether full sibling groups were present in any of our juvenile samples, which could bias allele frequency estimates (Allendorf and Phelps 1981). We ran the program for a medium run length with high likelihood precision under the assumption of a polygamous mating system. When full sibling groups were detected, all but one individual from each group was randomly removed from all subsequent analyses. Exact tests for departures from Hardy–Weinberg equilibrium were conducted using GENEPOP (Rousset 2008), and the critical value was corrected for multiple tests using the B–Y false discovery method (Benjamini and Yekutieli 2001; Narum 2006).

Two genetic diversity measures were used to examine within sample diversity. First, expected heterozygosity (H_e) values were calculated for each sample using GenAIEx

TABLE 1. Sample data for steelhead and Rainbow Trout samples: run type, collection year, life stage, number of individuals analyzed (n , after full-siblings were removed), and calculated expected heterozygosity (H_e) and allelic richness (AR). Late winter-run steelhead and resident Rainbow Trout are native to the Willamette River, whereas early winter- and summer-run steelhead are not.

Sampling location	Run type	Collection year	Life stage	n	H_e	AR
Willamette River, eastern tributaries						
Eagle Creek NFH	Early winter	2000	Juvenile	56	0.784	7.5
Calapooia River	Late winter	1997	Juvenile	36	0.669	6.3
Clackamas River	Late winter	2000	Juvenile	41	0.764	7.2
Clackamas River, Eagle Creek	Late winter	2000	Adult	63	0.781	7.5
Clackamas River, North Fork Dam	Late winter	2005	Adult	42	0.780	7.9
North Fork Molalla River	Late winter	1996	Juvenile	49	0.761	7.4
North Santiam River, Bennett Dam	Late winter	2005	Adult	45	0.725	6.8
North Santiam River, Marion Forks Hatchery	Late winter	1998	Juvenile	32	0.707	5.9
South Santiam River, Foster Dam	Late winter	2005	Adult	49	0.734	7.1
South Santiam River, Foster Dam	Late winter	2009	Adult	50	0.719	6.9
South Santiam River, Wiley Creek	Late winter	1997	Juvenile	28	0.760	7.4
Upper McKenzie River, Deer Creek	Rainbow Trout	1998	Juvenile	36	0.570	4.2
North Fork of the Middle Fork Willamette River	Rainbow Trout	1998	Juvenile	29	0.558	4.1
Clackamas Hatchery	Summer	2006	Adult	50	0.759	6.6
Clackamas River	Summer	1986	Adult	84	0.765	6.8
Molalla River	Summer	1988	Adult	46	0.770	6.9
North Santiam River	Summer	1986	Adult	23	0.763	7.3
North Santiam River	Summer	1987	Adult	16	0.768	7.5
South Santiam Hatchery	Summer	2007	Juvenile	45	0.766	6.9
Willamette River, western tributaries						
Canyon Creek, Rickreall Creek	Unknown winter	1997	Juvenile	15	0.700	8.0
Luckiamute River	Unknown winter	1997	Juvenile	24	0.657	6.0
Willamina Creek, Yamhill River	Unknown winter	1997	Juvenile	26	0.728	6.4
Lower Columbia River						
Big Creek Hatchery	Early winter	2000	Juvenile	45	0.779	7.5

(Peakall and Smouse 2006). Second, we computed allelic richness, which takes into account sample size, using the program H-P RARE (Kalinowski 2005).

Among-sample diversity was examined by computing pairwise F_{ST} values with GenAEx. Associated significant values were determined by permuting the data 9,999 times, and the B–Y false discovery method was again used to correct the critical value for multiple tests. An analysis of molecular variance (AMOVA; Excoffier et al. 1992), as implemented in the program Arlequin (Excoffier et al. 2005), was used to analyze genetic diversity among samples in a hierarchical structure. We tested several hierarchical structures to determine which one resulted in the greatest amount of variation among various sample groupings by location, origin, or run type.

The genetic population structure among presumptive population samples was examined by estimating Cavalli-Sforza and Edwards (1967) chord distances with 1,000 bootstrap replicates using PHYLIP (Felsenstein 2005). The resulting distance values were then used to construct a consensus neighbor-joining tree that was depicted using PhyloDraw (Choi et al. 2000). We also conducted a principal

coordinates analysis (PCoA), which plots the major patterns of variation within a multivariate data set, as implemented in GenAEx.

The number of populations represented by our samples was estimated using the program STRUCTURE version 2.0 (Falush et al. 2003), which uses a Bayesian clustering analysis to infer the number of populations present without defining the populations a priori. We varied the number of potential populations (K) from one to eight and ran the program for 100,000 burn-in iterations followed by 200,000 iterations. Each value of K was run for 20 simulations to compute a mean natural log probability value [$\ln P(K)$]. The rate of change of $\ln P(K)$ between successive values of K (ΔK) was calculated following the method of Evanno et al. (2005), and implemented in the program Structure Harvester (Earl and vonHoldt 2012). The true value of K was identified as the one generating the greatest ΔK value. Membership coefficients in each of the K -estimated number of populations were aligned using the Greedy algorithm of CLUMPP (Jakobsson and Rosenberg 2007) with 10^6 random input orders.

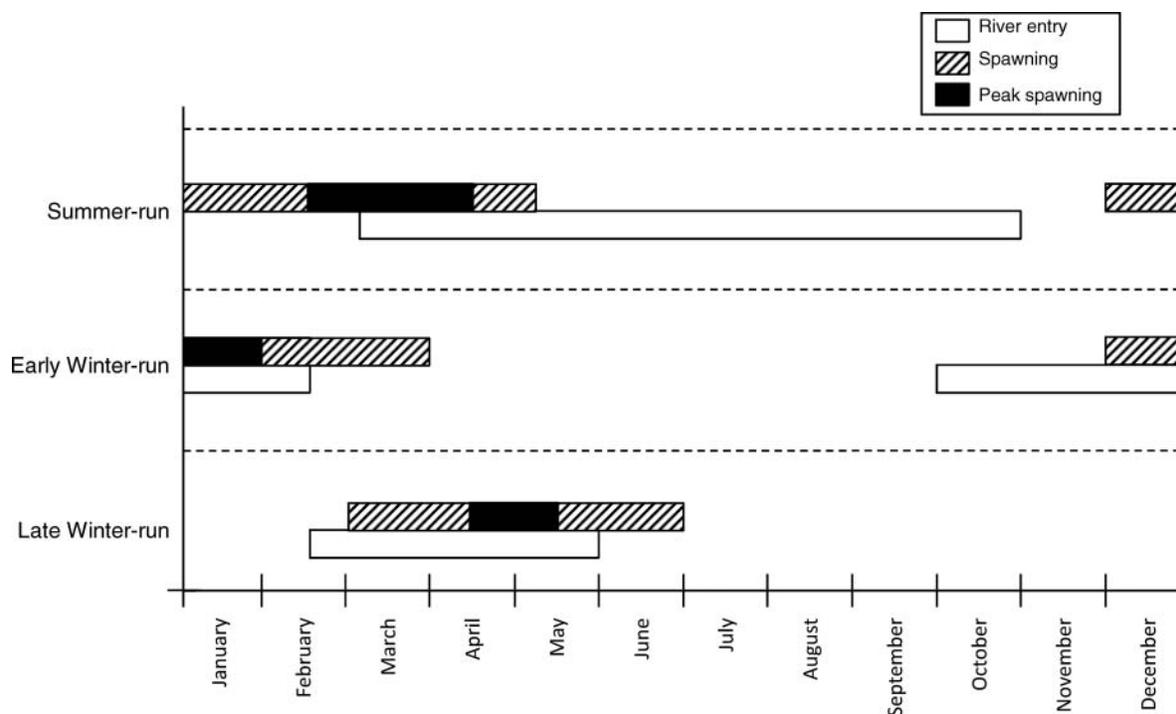


FIGURE 2. Months when adult steelhead of different run types typically return from the ocean to the Willamette River, their range of spawning times, and their peak spawning times. Data were compiled from Howell et al. (1985), Murtagh et al. (1992), McElhaney et al. (2007), ODFW and NMFS (2011a), and ODFW (2013).

RESULTS

Initial Data Check

After identifying full-sib family groups, 20.2% of the juvenile samples were removed from further analyses. The percentage removed from each sample location ranged from 2.0% (North Fork Molalla River) to 55.9% (Canyon Creek). After correcting for multiple tests, 6.1% ($n = 21$) of all Hardy–Weinberg tests were not in equilibrium. The significant tests of Hardy–Weinberg disequilibrium were not confined to a few loci, as they were spread out among 12 different loci. Nor did they show any consistent pattern of heterozygote excess or deficiency, as both cases were represented

throughout the results. However, one-third of the significant tests ($n = 7$) were from the Canyon Creek sample, most likely due to its small sample size.

Genetic Diversity Measures

The resident Rainbow Trout samples had notably lower H_e and allelic richness (AR) values compared with all other run types (Table 1). In other respects, these values were fairly consistent among samples; F_{ST} was 0.056 over all samples. Only 10 of the 253 pairwise F_{ST} comparisons were not significantly different after correcting for multiple tests ($P > 0.008$), seven of which were comparisons

TABLE 2. Results of AMOVA comparing the amount of variation (%) among and within groups of steelhead and Rainbow Trout for various configurations of the samples. For the two- and three-location configurations, the Big Creek Hatchery sample was grouped with the eastern tributaries samples, and for the two- and three-run type configurations, resident Rainbow Trout were grouped with the late winter-run steelhead samples. All values computed were significant ($P < 0.01$).

Comparison	Variation among groups (%)	Variation within groups (%)
Two locations: eastern tributaries and western tributaries	2.8	4.7
Three locations: eastern tributaries, upper Willamette River western tributaries, Clackamas River	1.5	4.8
Two origins: native and nonnative	3.0	4.0
Two run types: summer and winter (early and late)	2.8	4.4
Three run types: summer, early winter, late winter	3.9	3.2
Four run types: summer, early winter, late winter, resident Rainbow Trout	4.5	2.4

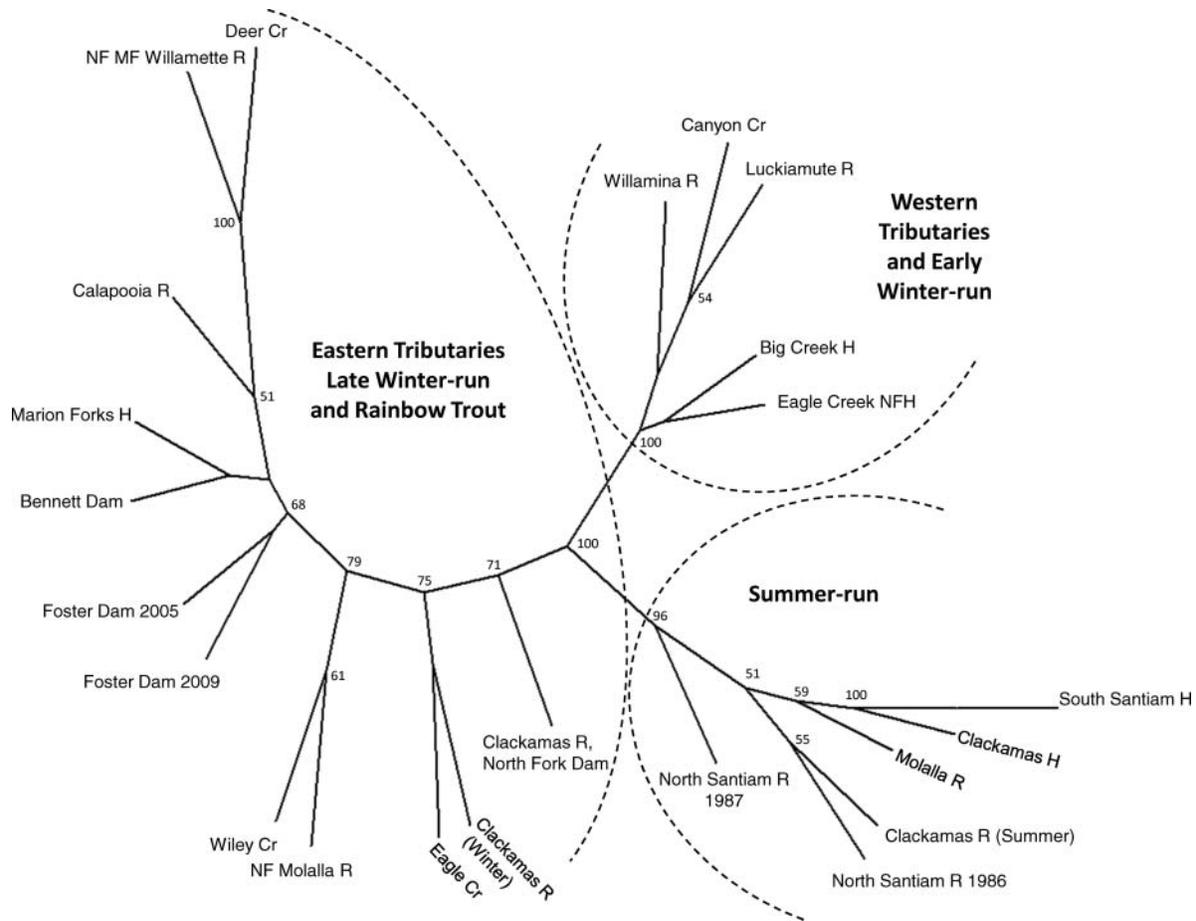


FIGURE 3. Consensus neighbor-joining dendrogram of Cavalli-Sforza and Edwards (1967) chord distances for Willamette River steelhead and Rainbow Trout samples. Bootstrap values are shown at nodes with >50% consensus.

between summer-run samples, two were between late winter-run samples (Foster Dam 2009 and Bennett Dam, and North Fork Mollala River and Wiley Creek), and one was between early winter-run samples (Eagle Creek Hatchery and Rickreall Creek). The AMOVA showed that the greatest amount of genetic variation among sample groups occurred when samples were grouped into four run types: summer, early winter, and late winter steelhead and resident Rainbow Trout (Table 2). The late winter steelhead and resident Rainbow Trout configurations were the only ones where the amount of variation among groups was greater than that within groups.

Population Structure

The dendrogram of genetic distances revealed three main clusters: summer-run samples, winter-run eastern tributary samples, and winter-run western tributaries samples (Figure 3). Within the winter-run, eastern tributaries grouping, the resident Rainbow Trout samples (Deer Creek and North Fork of the Middle Fork Willamette River) cluster together with high

bootstrap support (100%). The winter-run Clackamas River samples (North Fork Dam, Eagle Creek, and Clackamas River winter run 2000) are also in this group. The winter-run, western tributaries group includes the Big Creek Hatchery and the Eagle Creek NFH (which was founded with fish from Big Creek Hatchery) samples.

The results of the PCoA were similar to those of the genetic distance clustering (Figure 4). Four distinct clusters are apparent, corresponding to run type. The first axis accounts for 40% of the variation and divides the native winter-run steelhead and resident Rainbow Trout western tributaries samples from the nonnative winter- and summer-run samples. The second axis accounts for 22% of the variation and divides the summer-run samples from the winter-run, western tributaries samples, and divides the resident Rainbow Trout samples from the winter-run, eastern tributaries samples.

The $\ln P(K)$ and ΔK values from the STRUCTURE analysis showed strong support for four population groupings, since that was the ΔK with the greatest value (Figure 5). Each sample's percentage membership in each of the four

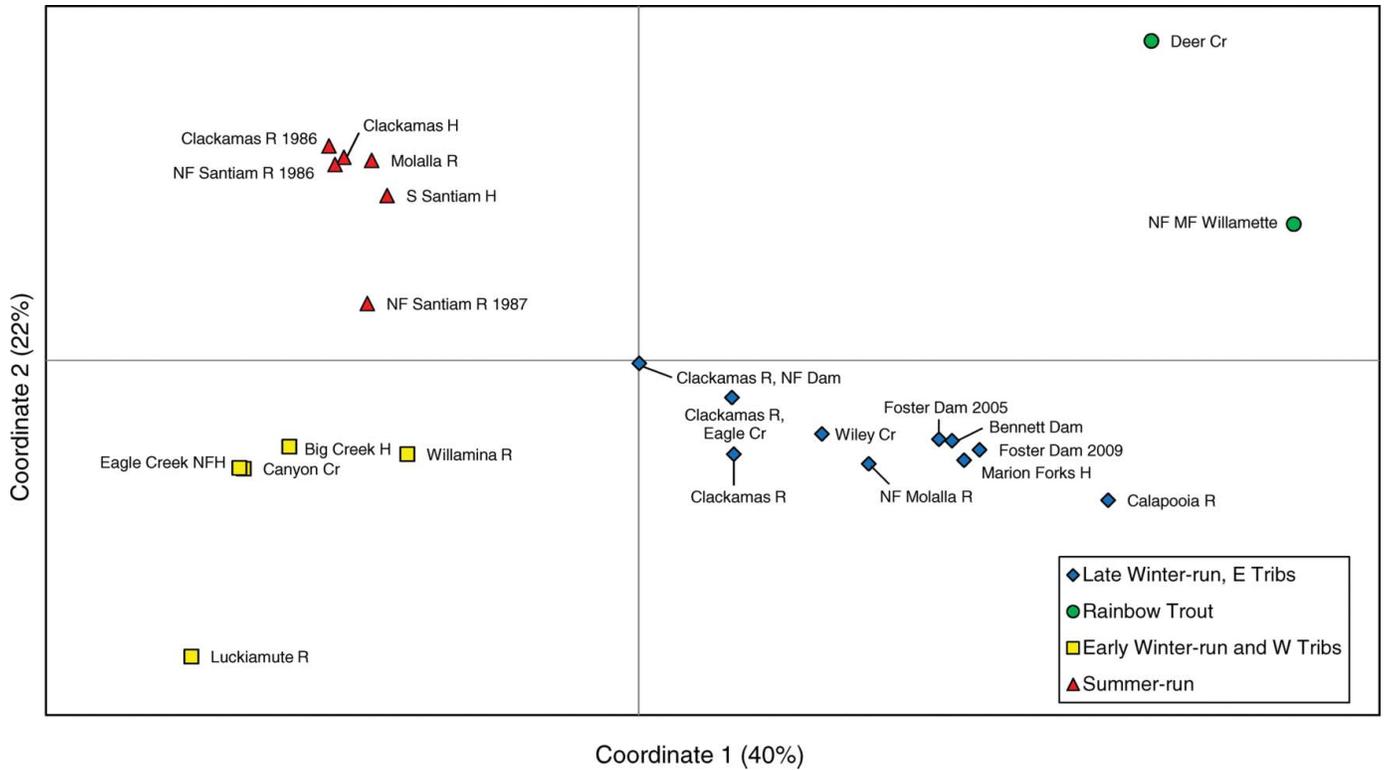


FIGURE 4. Principal components plot of genetic distances among Willamette River steelhead and Rainbow Trout samples. [Figure available online in color.]

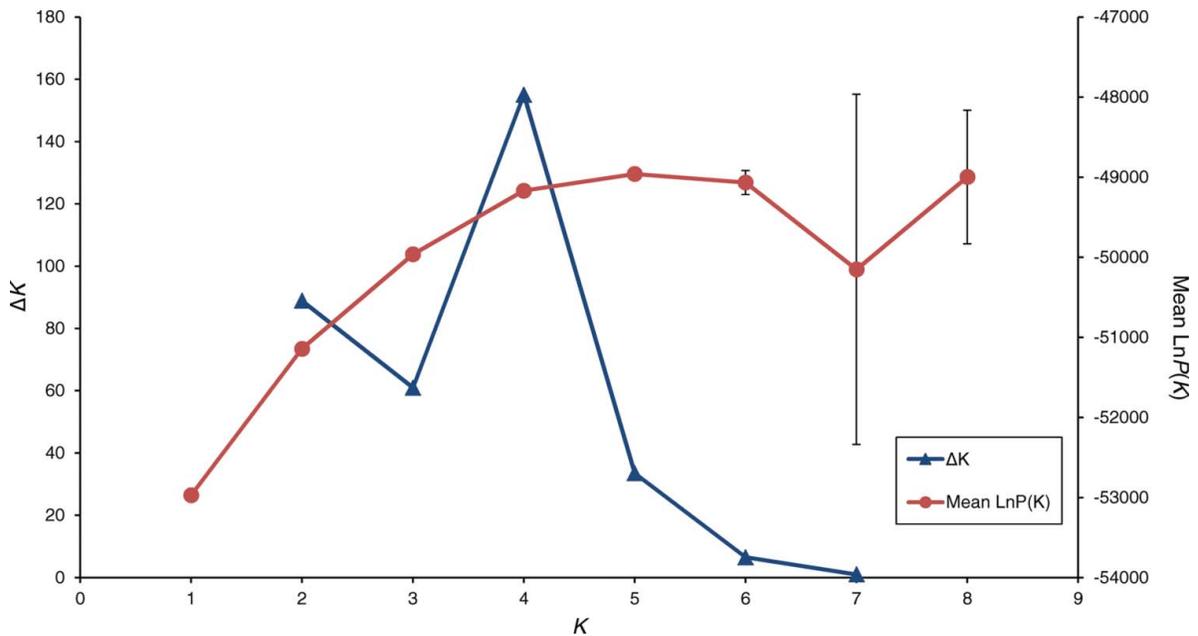


FIGURE 5. Result of STRUCTURE analysis of steelhead and Rainbow Trout samples showing the mean natural log probability for each K value [mean $\ln P(K)$] with 95% confidence intervals, and the rate of change between successive K values (ΔK). [Figure available online in color.]

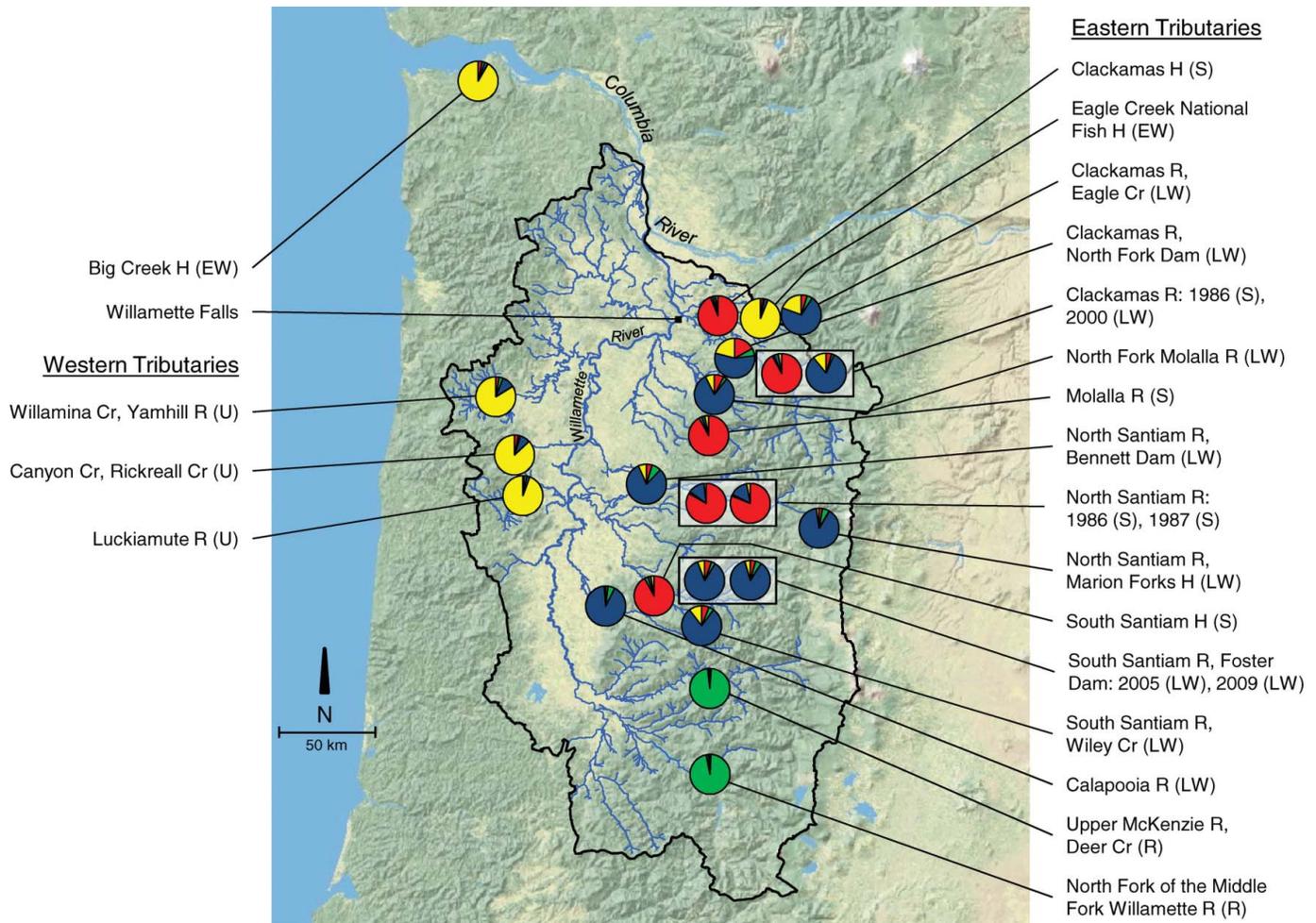


FIGURE 6. STRUCTURE population group membership values for the steelhead and Rainbow Trout sample collections used in this study. Multiple samples from the same location are enclosed in boxes and are labeled from left to right. Collection years are given for locations with multiple years of samples. Abbreviations in parentheses designate the sample's run type: summer steelhead (S), early winter steelhead (EW), late winter steelhead (LW), resident Rainbow Trout (R), or unknown (U). [Figure available online in color.]

population groups provided additional evidence for the presence of major population groups corresponding to run type (Figure 6). The Clackamas River Eagle Creek and North Fork Dam late winter-run samples are the least distinct of the samples, as they are the only samples that do not have at least 75% membership in a single population group. In addition, western tributaries samples have the majority of their membership in the same population group as the early winter-run Big Creek Hatchery sample.

DISCUSSION

Population Structure and Artificial Propagation

We have shown that steelhead populations in the Willamette River are genetically structured on the basis of run type. Artificial supplementation has introduced two nonnative types into the system, but we found that, overall, the native run type

has retained its genetic distinctiveness. Since these two nonnative run types spawn freely within the system (Howell et al. 1985; Keefer and Caudill 2010), there is potential for gene flow among the different types, especially where there is overlap in spawning time and location. Such is the case for all three run types, whose spawning times overlap in March–May (Figure 2) and whose spawning locations also overlap (HSRG 2009; Keefer and Caudill 2010). There are several river systems where summer-run and winter-run steelhead occur together naturally and are genetically distinct from each other (Leider et al. 1984; Nielsen and Fountain 1999; Matala et al. 2009). The late-winter run timing expressed by native Willamette River steelhead is believed to have evolved as an adaptation that allowed fish to access spawning areas upstream from Willamette Falls, which was impassable by steelhead for most of the year until fish passage structures were built (Myers et al. 2006). In addition, later spawn timing is typically observed among steelhead populations that spawn in cold, higher-

elevation headwaters (Van Doornik and Berejikian, in press), such as those in the upper Willamette River. This adaptation may have served to limit introgression of the early winter-run type into the native late winter-run type.

Our results indicate that late winter-run samples from the Clackamas River and from above Willamette Falls are best included in the same genetic population group. This is contrary to the conclusion of a review of west coast steelhead populations for ESA considerations, which identified the upper Willamette River populations as their own distinct evolutionary significant unit (ESU) (currently referred to as a distinct population segment), whereas the Clackamas River populations are included in an ESU with lower Columbia River populations (Busby et al. 1996). That conclusion was based partially on a genetic study by Schreck et al. (1986) that included a late winter-run sample from the Eagle Creek NFH on the Clackamas River. That sample clustered with lower Columbia River samples, including the Big Creek Hatchery early winter-run stock, which is also propagated at the Eagle Creek NFH. It is possible that overlap in adult return times of the two run types to the Eagle Creek NFH has resulted in introgression between the two stocks. On closer examination of our results, an argument can be made that our Clackamas River samples show some separation from the native late-winter upper Willamette steelhead samples in the dendrogram (Figure 3) and PCoA (Figure 4). Also, two of our three wild Clackamas River late winter-run samples did not show as strong a membership to a single population group as did the other samples for the STRUCTURE analysis (Figure 6). Thus, there may be some weak level of genetic differentiation between late winter-run Clackamas River and upper Willamette River populations, which may have been partially caused by limited introgression with the early winter-run stock that has been released into the Clackamas River. The late winter-run Clackamas River samples have a sizeable proportion of their membership in the population group exemplified by the early winter-run samples from Eagle Creek NFH and Big Creek Hatchery (Figure 6), suggesting some level of introgression. Nevertheless, our late winter-run Clackamas River samples are still more genetically similar to the upper Willamette River samples than to the lower Columbia River sample in our study (Big Creek Hatchery). Blankenship et al. (2011) arrived at a similar conclusion from a comprehensive analysis of Columbia River steelhead populations that included several Clackamas River samples. Their Clackamas River samples were genetically more similar to upper Willamette River samples than they were to lower Columbia River samples. It is important to note that the designation of distinct population segments takes into account factors other than genetics, such as habitat characteristics (Busby et al. 1996), which could still support their inclusion in separate distinct population segments.

The resident Rainbow Trout populations we sampled in the McKenzie and Middle Fork Willamette River basins are genetically distinct from other Willamette River samples, but

are most closely related to the native late winter-run steelhead. They also do not show any genetic affinity to the hatchery Rainbow Trout stocks (normally of California origin) that have been released into the system (NMFS 1999). Therefore, they likely represent a unique subpopulation group in tributaries above the confluence of the Calapooia River, where historically steelhead have not spawned. The genetic separation we found between our Rainbow Trout and steelhead samples is similar to river systems where a barrier to upstream migration creates a population of resident Rainbow Trout isolated from steelhead within the same river. In such cases, the Rainbow Trout isolated upstream are genetically distinct from the steelhead below the barrier (Clemento et al. 2009; Pearse et al. 2009; Van Doornik et al. 2013). However, we suspect that in areas below the Calapooia River where Rainbow Trout and steelhead occur in sympatry (areas in which we did not sample Rainbow Trout), there is a greater amount of gene flow between the life history types, making them more genetically similar, a pattern that has been found in other river systems (Docker and Heath 2003; Olsen et al. 2006; McMillan et al. 2007; Heath et al. 2008; Berntson et al. 2011; Van Doornik et al. 2013).

Western Tributaries Origin

Our results indicate that the early winter-run Big Creek Hatchery steelhead released into the western tributaries have been able to establish naturally spawning populations, as all of the western tributary samples clustered within a single subpopulation grouping that includes the Big Creek Hatchery sample. It is important to note that our western tributaries samples were all collected in a single year and included three of the four western tributaries where steelhead have been observed. Steelhead have been observed in the Tualatin River (NMFS 1999), a western tributary, but we did not have samples from that location. Thus, definitive conclusions about their ancestry would benefit from additional sampling and analyses. However, a recent study using radio-tagged Willamette River steelhead did indicate that adult steelhead entering the Yamhill River, a western tributary, had return times characteristic of the early winter run (Jepson et al. 2014). Assuming that the western tributaries populations are derived from early winter-run releases, as our results suggest, we can conclude that this has occurred even though native Willamette River steelhead have had the opportunity to colonize these tributaries. One such opportunity would come when late winter-run steelhead stray from their natal eastern tributaries into the western tributaries, although the aforementioned radio tag study did not find any evidence of this occurring (Jepson et al. 2014). In addition, native steelhead from the eastern tributaries have been released directly into the western tributaries (Myers et al. 2006; HSRG 2009). Perhaps the Big Creek Hatchery steelhead are better adapted than the late winter-run steelhead to the different environmental and hydrological conditions found in the

western tributaries. Other steelhead populations have shown evidence of adaptation to local environmental factors (Narum et al. 2008; Blankenship et al. 2011; Van Doornik and Berejikian, in press). Compared with the eastern tributaries, the western tributaries have lower water flows, lower gradients, hydrographs driven by rain rather than snowmelt, and higher water temperatures in the summer and are smaller in size (Parkhurst et al. 1950; OSGC 1963; Fulton 1970).

CONCLUSIONS

We conclude that native steelhead in the Willamette River remain genetically distinct from introduced stocks, likely due to adaptive differences as well as sufficient temporal and spatial segregation among spawning populations. In other areas, several recent genetic studies have assessed interbreeding of naturally spawning native and introduced stocks of Pacific salmon and have had similar results. For example, Matala et al. (2009) found evidence of restricted gene flow between summer-run and winter-run steelhead in the Hood River, also a tributary of the Columbia River, even though there has likely been accidental mixing between the run types during artificial spawning in a hatchery. Likewise, the introduction of a nonnative lineage of Chinook Salmon *O. tshawytscha* in another tributary of the Columbia River has not resulted in significant introgression into the native lineage, despite overlap in their return times and spawning locations (Smith and Engle 2011). Similarly, a tributary in the lower Columbia River was found to contain a nonnative, naturally spawning Chinook Salmon population that was mostly distinct from the native population, although there was some evidence of gene flow between them (Roegner et al. 2010). It is important to note that in these studies and ours, the persistence of genetically separate lineages does not imply that there has been no introgression between them. Rather, they show that introgression has not occurred at a level substantial enough to genetically homogenize the lineages, likely due to factors such as temporal or spatially segregated spawning, assortative mating, or selection against hybrids of the two lineages. In the Willamette River, potential interbreeding of ESA-protected steelhead with introduced summer-run fish is a continuing concern for fish managers (ODFW and NMFS 2011). Quantifying introgression and natural production of summer steelhead are important next steps to inform management decisions, and to that end additional data collection and analysis efforts are currently being implemented.

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