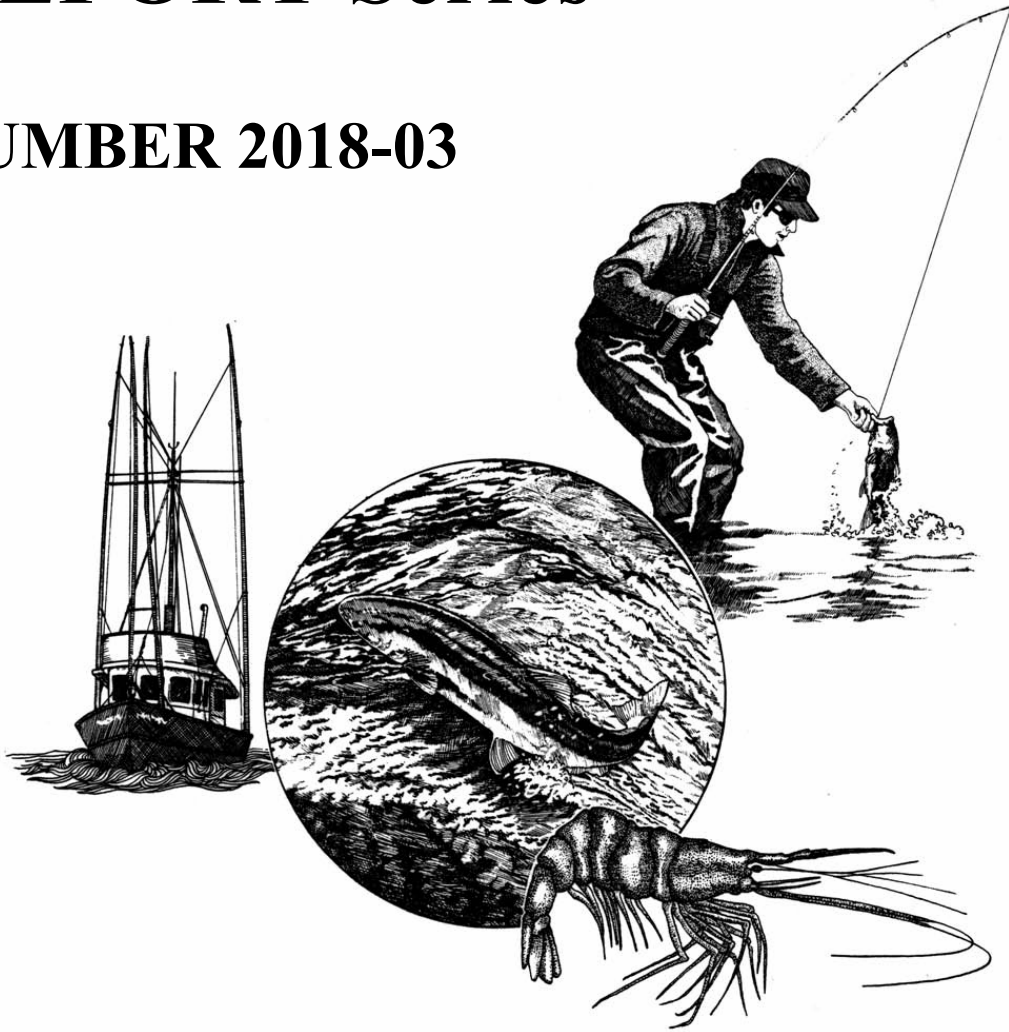# ODFW INFORMATION REPORT Series

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Genetic influence from hatchery stocks on upper Willamette River steelhead *Oncorhynchus mykiss*

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ABSTRACT

In this study we used microsatellite markers to genetically characterize 1,012 unmarked (presumed to be naturally produced), juvenile *Oncorhynchus mykiss*, collected at Willamette Falls in 2009, 2010 and 2011, and from multiple locations throughout the upper Willamette River basin in 2014. From the genetic data, we estimated the proportion of each year's sample that could be assigned as native resident rainbow trout, native winter steelhead, hatchery summer steelhead, the likely descendants of a discontinued hatchery winter steelhead program, or hybrids of these groups. We also attempted to assign individuals to pure or hybrid classes (e.g. F1, F2, backcrosses) and evaluated the predicted accuracy of these class assignments. Although the majority of fish analyzed in each year were assigned as native winter steelhead, we found evidence of natural production by introduced stocks of steelhead. Genetic influence from a discontinued winter steelhead hatchery program was most evident in the Tualatin, Yamhill, Molalla, and below-dam reaches of the North and South Santiam rivers. Pure and hybrid signals from introduced summer steelhead were almost entirely restricted to below-dam reaches of major eastern tributaries and the Molalla River. Evidence of non-native steelhead was nearly absent from above-dam reaches of major tributaries and the Calapooia River. Despite continued hatchery releases of summer steelhead in the Willamette River, we found no compelling evidence for the presence of first-generation hybrid offspring (F1s) that might result from hatchery summer and native winter steelhead matings. However, simulation analyses revealed low statistical power to confidently identify such individuals from our data. We recommend that areas containing native steelhead populations with high genetic integrity receive protection through directed conservation efforts, and continued genetic monitoring should be conducted to inform these efforts and future status reviews.

INTRODUCTION

Both resident and anadromous *Oncorhynchus mykiss*, respectively known as rainbow trout and steelhead, are native to Oregon's upper Willamette River (UWR). Native UWR steelhead return from the ocean as adults during the late winter and early spring (peak migration in late March-April) to spawn (primarily) in the eastern tributaries of the basin that drain the Cascade Mountains and surrounding foothills.

Listed as "threatened" under the U.S. Endangered Species Act (NMFS 2016), UWR steelhead are likely impacted by multiple effects from high-head dams throughout the basin; unfavorable freshwater and ocean conditions; predation from pinnipeds, birds and other fish species; and negative ecological interactions with hatchery steelhead, such as competition and disease transmission (ODFW and NMFS 2011; NMFS 2008). Hatchery steelhead may also spawn in natural environments and potentially interbreed with native UWR steelhead, with unknown but potentially detrimental effects. Recent genetic analyses of *O. mykiss* samples collected from multiple UWR locations revealed four distinct lineages in the basin: native winter steelhead, native rainbow trout, winter steelhead closely related to and likely descended from a Lower Columbia River hatchery population (Big Creek stock 013; hereafter "Big Creek stock") previously released in the basin, and summer steelhead (Skamania stock 024) that continue to be produced and released by ODFW hatchery programs in the UWR (Van Doornik et al. 2015).

Johnson et al. (2013) provided preliminary genetic evidence of natural production by hatchery steelhead in the UWR, and also estimated levels of hybridization between introduced and native *O. mykiss* from samples of juvenile steelhead captured at Willamette Falls. However, that study did not account for the possible presence of stocked, hatchery rainbow trout (i.e. Cape Cod stock 072) or identify those areas within the UWR where natural production by hatchery steelhead occurs. Moreover, Johnson et al. (2013) did not assign individual fish to hybrid classes, as would be necessary to evaluate the effectiveness of

current management at maintaining genetic isolation between native and hatchery stocks. For example, presence of first filial (F1) hybrids would provide strong evidence of recent interbreeding between hatchery and native wild steelhead, though F2 and backcross classes could persist as legacies of interbreeding that occurred in a more distant past.

In this study, we revisited, refined and expanded upon the efforts of Johnson et al. (2013) to:

1. Estimate the proportion of naturally-produced *O. mykiss*, sampled during three years at Willamette Falls, that could be confidently assigned as summer steelhead or hybrids of this stock with others

2. Describe the spatial distributions of *O. mykiss* from the four known genetic lineages, and hybrids of these, within the UWR basin

3. Assign putative hybrids of native winter and hatchery summer steelhead to first filial (F1), second filial (F2) or backcross (BC1 or BC2) generational classes, and estimate statistical confidence of these assignments

We present our findings in the context of ongoing conservation and recovery efforts for UWR winter steelhead, and briefly discuss the implications and limitations of our results.

METHODS

Study Area and Sampling

The Willamette River occurs entirely within the state of Oregon, has 301 km of main stem, an average discharge of 1,060 m^3/s and is a major tributary of the Columbia River. The UWR is defined as those Willamette River tributaries and main stem that occur upstream from Willamette Falls, which is 42 river km upstream from the confluence with the Columbia River. The Sullivan Power Plant is situated at Willamette Falls, and during April and May 2009, 2010 and 2011, we captured, sampled and subsequently released unmarked juvenile *O. mykiss* at this location. Juvenile fish entering the power plant are regularly shunted to a turbine bypass route, where screens can be used to divert them to a holding tank. We anaesthetized fish captured in this tank with $NaHCO_3$ buffered tricaine methanesulfonate (MS-222) and collected a small section (~4 mm^2) of caudal fin tissue, which we then stored in labeled vials filled with 95% EtOH. We recorded the sampling date and fork length of each fish sampled.

In 2014, we captured and sampled unmarked juvenile *O. mykiss* at multiple UWR locations to meet the second objective of our study. To establish sampling sites, we used ArcMap 10 (ESRI 2011) to plot points approximately every two miles along all 2nd to 5th order streams of the following UWR tributaries: Middle Fork Willamette, McKenzie, North Santiam, South Santiam, Yamhill, Tualatin, Molalla, Pudding, and Calapooia rivers. Only sites that occurred on public or major industrial timber lands, and that were within 100 m of a road, were considered. For tributaries that contained high-head dams, we randomly selected 10% of points below these dams and 5% above them, to ensure systematic and adequate sampling in areas where hybridization could (below dams) and would not (above dams) be expected to occur. Similarly, we randomly selected 10% of points in tributaries without high-head dams. This

exercise directed our sampling effort to 198 locations, broadly distributed across major UWR tributaries (Figure 1).

Sampling in 2014 began in early June and ended in the first week of October. We waded streams at each site and used a Smith-Root LR24 backpack electrofisher for a maximum of 30 minutes per site to briefly immobilize and capture juvenile steelhead. Electrofisher settings were determined with the auto-tuning feature (typically between 150 – 300 volts DC, 30 Hz, and 12% pulse rate) and we recorded the total time spent actively shocking at each site, which allowed us to calculate the catch per unit effort (CPUE). We limited our sampling to unmarked *O. mykiss* with fork lengths between 80 and 160 mm, and sampled no more than ten fish from any site to limit over-representation of closely-related individuals in our collection. We recorded the fork length, date, collection location, and stored caudal fin tissues in EtOH, as before.

Genetic and Statistical Analyses

At the National Marine Fisheries Service's Manchester Research Station and at Oregon State University's Marine Fisheries Genetics Laboratory, whole genomic DNA was extracted from fin tissue with either a Promega Wizard DNA Purification Kit (Promega Corp.) or the methods of Ivanova et al. (2006). Polymerase chain reactions were used to amplify 15 microsatellite loci: *Ocl1* (Condrey and Bentzen 1998), *Ogo4* (Olsen et al. 1998), *Oke4* (Buchholz et al. 1999), *Oki23* (Smith et al. 1998), *Omy1001*, *Omy1011* (Spies et al. 2005), *Omy7* (Stephenson et al. 2009), *Omy77* (Morris et al. 1996), *One14* (Scribner et al. 1996), *Ots3*, *Ots4* (Banks et al. 1999), *Ots100* (Nelson and Beacham 1999), *Ssa289* (McConnell et al. 1995), *Ssa407*, *Ssa408* (Cairney et al. 2000). Amplification products were separated by gel electrophoresis on either an Applied Biosystems Inc. 3100 or 3730XL genetic analyzer, and alleles were scored for size with GeneScan, Genotyper or GeneMapper software (Applied Biosystems, Inc.). A subset of samples (n = 69) were analyzed at both laboratories to standardize allele size bins.

The loci chosen for our study were those previously used by Van Doornik et al. (2015) to describe UWR *O. mykiss* population structure, which allowed us to use genotypic data from that study as "baseline" information in our current analyses. Thirteen of these loci (*Ogo4*, *Oke4*, *Oki23*, *Omy7*, *Omy1001*, *Omy1011*, *Oneu14*, *Ots3*, *Ots4*, *Ots100*, *Ssa289*, *Ssa407* and *Ssa408*) are also included in the Stevan Phelps Allele Nomenclature (SPAN) baseline of standardized genotypic data for 25 *O. mykiss* population groups from Oregon and Washington, allowing us to test for genetic signals from out-of-basin immigrants in our dataset.

We analyzed our genotypic data by first identifying and excluding samples of cutthroat trout (*O. clarkii*) and cutthroat-rainbow trout hybrids, based on characteristic allele banding patterns at several loci. We then used the SPAN baseline, supplemented with baseline data for Cape Cod hatchery rainbow trout (ODFW stock 72), and the genetic stock identification (GSI) software ONCOR (Kalinowski et al. 2007) to perform genetic stock assignments for all of our juvenile *O. mykiss* samples that had successfully amplified at eight or more loci. Samples that amplified at fewer than eight loci or that genetically assigned with highest probability to a SPAN group other than the four stocks described from the UWR (Van Doornik et al. 2015) were excluded from subsequent analyses.

We used the Bayesian genetic clustering approach, implemented in the software STRUCTURE (Pritchard et al. 2000), to estimate the proportion, *q*, of individual genomes descended from each of the four
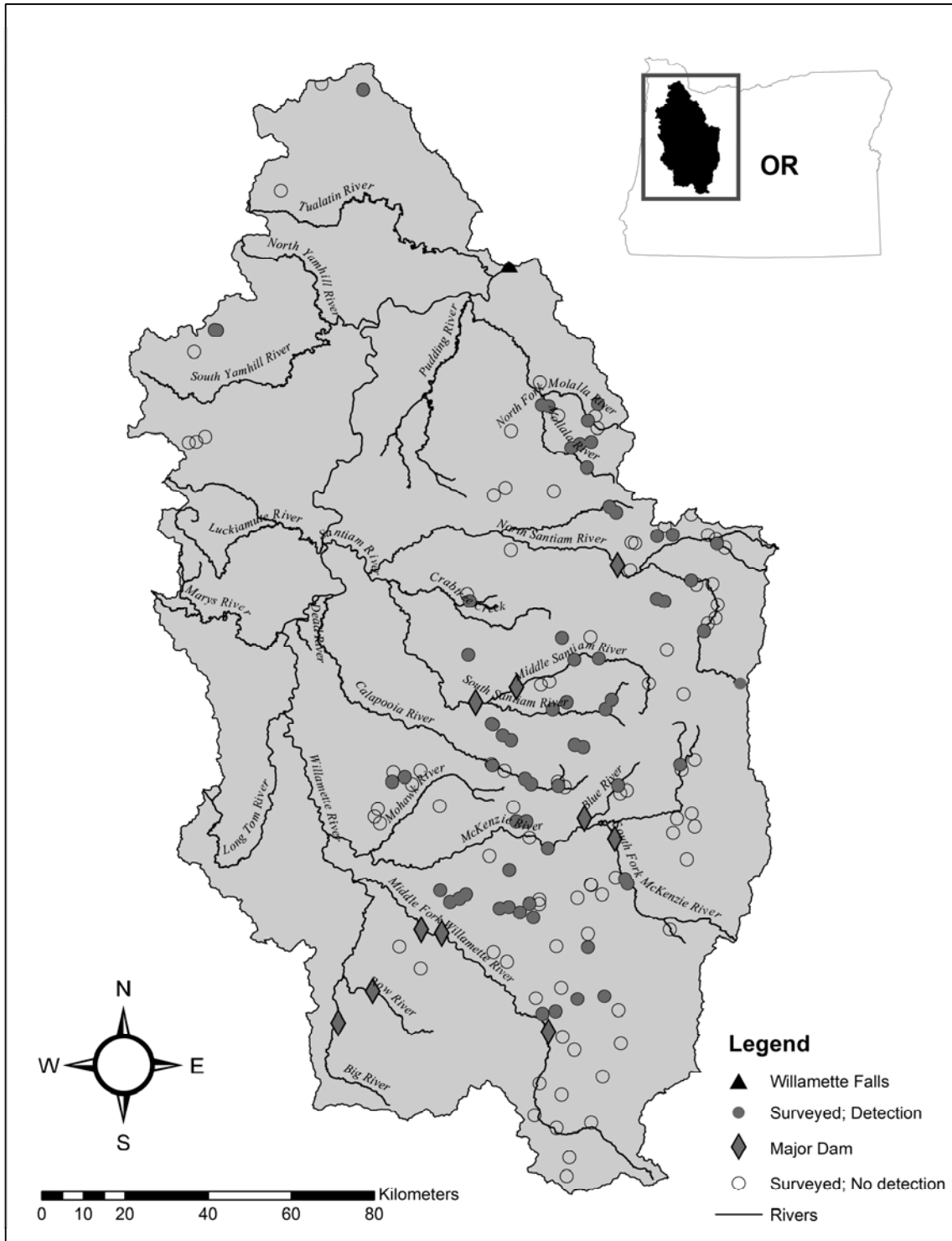
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Figure 1. The upper Willamette River with symbols indicating locations of major dams and sampling sites where juvenile *O. mykiss* were, and were not, captured in 2014.

O. mykiss lineages described by Van Doornik et al. (2015). We classified individuals into "pure" and "hybrid" groups of these lineages according to the following criteria:

> **Pure**: $q > 0.50$ for a single lineage and $q < 0.20$ for all other lineages
> **Two-way hybrid**: $0.20 < q < 0.80$ for each of exactly two lineages
> **Three-way hybrid**: $0.20 < q < 0.80$ for each of exactly three lineages

These q-value thresholds were established to readily classify F1s, F2s and early backcrosses as hybrids, because individuals from these classes would be expected to have genomic contributions $\geq 25\%$ descended from each of two or more groups. Thus, our threshold criterion of $q > 0.20$ for classifying hybrids may be considered liberally inclusive, but protects against false negative (Type II) errors of hybrid identification.

We performed 20 replicate runs of STRUCTURE, each with a burnin of 20,000 followed by 100,000 Markov Chain Monte Carlo simulation repetitions. We set the number of genetic clusters at $K = 4$ and used the LOCPRIOR option of STRUCTURE to identify the four baseline groups in our data, but provided no prior information for juvenile fish of unknown ancestry, including those sampled in 2014, to minimize prior-related influence over results. We used the software CLUMPP (Jakobsson and Rosenberg 2007) to resolve "label switching" – the arbitrary ordering and naming of clusters that occurs during replicate runs of STRUCTURE - and to calculate mean q values in all individuals for each of the K groups.

We then used mean q values and our classification criteria to quantify the number of individuals from pure and hybrid classes in our sample. We constructed histograms depicting the proportion of each year's sample from Willamette Falls (2009-2011) that presented the full possible range of summer steelhead influence. Similarly, we used results from samples collected in 2014 to estimate the percentage of fish captured in areas above and below high-head dams that assigned to pure and hybrid classes of *O. mykiss*.

Fish identified as hybrids within our sample could be the offspring of genetically pure parents from two different lineages, such as crosses between pure native winter and pure hatchery summer steelhead. These first filial (F1) hybrid offspring would be expected to have relatively equal genetic contributions from both pure lineages (P_1, P_2), which could distinguish them from other hybrid classes, such as F2s (F1×F1) and backcrosses (F1× P_1, F1× P_2).

We used the software NEWHYBRIDS (Anderson 2008) to assign juvenile *O. mykiss* genotyped for our study into pure and hybrid classes. Because this software is designed to perform hybrid class assignments for offspring generated from only two genetically distinct groups, we focused this stage of our analysis on individuals previously identified by STRUCTURE as summer steelhead, native UWR winter steelhead, or hybrids of these. Accordingly, we excluded individuals that did not present a q value > 0.8 for one of these two lineages or q values > 0.2 for both lineages. These stringent q thresholds reduced the size of our dataset, but effectively removed putative resident rainbow trout and Big Creek stock steelhead. Similar to our approach with STRUCTURE, we included and identified baseline data for the summer and native winter steelhead lineages in our NEWHYBRIDS analysis, using the z0s and z1s identifiers for these baseline samples, as recommended in the user's guide (available at https://github.com/eriqande/newhybrids). For each fish, NEWHYBRIDS provided a posterior probability of assignment to the following pure and hybrid classes:

P1: pure native winter steelhead
P2: pure summer steelhead
F1: P1 × P2 offspring
F2: F1 × F1 offspring
Backcross 1: P1 × F1
Backcross 2: P2 × F1

There is a known tradeoff between Type I and Type II statistical errors associated with the range of posterior probability cutoff values that might be used to assign individuals to pure and hybrid classes (Burgarella et al. 2009). Should the *P* cutoff for assignment be set "too high", individuals that truly exist within a hybrid class will be erroneously excluded (false negatives). On the other hand, should the *P* cutoff be set "too low", individuals will be inaccurately assigned into classes to which they do not truly belong (false positives). We used the HYBRIDDETECTIVE software package to explore these relationships with our data. We used genotypic data from the purest (*q* > 0.9) hatchery summer (n = 191) and native winter (n = 156) steelhead as input for these analyses. HYBRIDDETECTIVE then performed multiple *in silico* crosses with these data to generate simulated offspring of known hybrid classes, then executed replicate runs of NEWHYBRIDS to assess that software's accuracy of hybrid class assignment across a range of posterior probabilities (Wringe et al. 2017). We conducted three replicates of three simulations with HYBRIDDETECTIVE, using a burnin of 1,000,000 followed by 50,000 sweeps for these analyses.

RESULTS

In 2009, 2010 and 2011, we collected tissue from a total 607 unmarked juvenile salmonids. In 2014, we electrofished at 156 sites in the UWR basin and collected tissues from 464 juvenile fish initially identified as *O. mykiss* at 65 of these (Figure 1). We also received 29 tissue samples collected at two additional UWR sites in 2014 by Oregon State University researchers. Of the total *n* = 1,100 tissues collected, 94% amplified at eight or more microsatellite loci, and 95% of these were found to be *O. mykiss* descended from lineages previously described from the UWR by Van Doornik et al. (2015), as determined through ONCOR population assignments (Table 1). We found no evidence of Cape Cod hatchery stock rainbow trout in our samples.

Hybrid fraction estimates

We classified 1,012 juvenile fish as pure or hybrid individuals of recognized UWR *O. mykiss* stocks (*sensu* Van Doornik et al. 2015), based on mean *q* values obtained through 20 replicate STRUCTURE analyses. As previously reported by Johnson et al. (2013) and presented in Table 2 of this report, *O. mykiss* sampled at Willamette Falls in 2009-2011 were mostly (51.3%) pure native winter steelhead. Only two fish sampled at Willamette Falls assigned as pure resident rainbow trout. Pure winter steelhead likely descended from the Big Creek stock, which was released in the UWR until the mid-1990s, comprised 14.4% of our Willamette Falls sample and pure (non-native) summer steelhead accounted for 10.5% of our sample from that location. Genetic influence from introduced hatchery stocks was also evident through the presence of hybrids among fish sampled at Willamette Falls. We found that hybrids of native and non-native winter steelhead, and hybrids of native winter and introduced summer steelhead, respectively constituted 10.5% and 9.4% of our Willamette Falls collection. The proportion of fish intercepted at Willamette Falls with high summer steelhead influence (*q* > 0.80) ranged from a low of 0.053 (2011) to 0.136 (2010), while the proportion of fish with intermediate summer *q* values (0.20 < *q*

Table 1. The number of unmarked, juvenile fish sampled for this study and, of these, the number that were successfully genotyped at a minimum of eight microsatellite loci, were determined to be cutthroat trout (or hybrids of this species), were from an out-of-basin outlier stock, or were determined to be from a UWR native or introduced hatchery stock and, therefore, analyzed with the program STRUCTURE.

Year	Collection location(s)	Fish sampled	Genotyped at >8 loci	Cutthroat trout	Out-of-basin	Analyzed with STRUCTURE
2009	Willamette Falls	250	247	7	0	240
2010	Willamette Falls	300	298	11	0	287
2011	Willamette Falls	57	57	1	0	56
2014	UWR sites; see Figure 1	493	463	31	3	429
	Total	1,100	1,065	50	3	1,012

Table 2. The number of *O. mykiss* captured at Willamette Falls in 2009-2011 and classified as pure or hybrids of the following genetic lineages: S = hatchery summer steelhead, NW = native winter steelhead, BC = Big Creek stock winter steelhead, RB = native rainbow trout. Classifications were based on STRUCTURE q value criteria described in the text.

Year	S	NW	RB	BC	S×NW	S×RB	S×BC	NW×RB	NW×BC	RB×BC	3×	Subtotal
2009	19	126	1	34	23	1	1	1	31	0	3	240
2010	39	144	1	37	29	0	4	3	25	0	5	287
2011	3	29	0	13	3	0	1	0	5	0	2	56
Total	61	299	2	84	55	1	6	4	61	0	10	583
Percent of Total	10.5	51.3	0.3	14.4	9.4	0.2	1	0.7	10.5	0	1.7	100

<0.80) ranged from 0.125 (2011) to 0.139 (2010) (Figure 2). Very few (*n* = 4) hybrids of native rainbow trout were detected from our Willamette Falls sample.

Genetic analyses of fish captured throughout the UWR in 2014 revealed several clear patterns. First, we observed that the western UWR tributaries, represented by the Tualatin and Yamhill rivers, were dominated by putative Big Creek stock winter steelhead, with some evidence of hybridization between this stock and native winter steelhead in the Yamhill River. This finding is consistent with results from a previous study (Van Doornik et al. 2015) that reported the majority of adult steelhead in western UWR tributaries to be closely related to Big Creek hatchery stock steelhead.

Second, genetic evidence of summer steelhead was nearly absent in areas above UWR dams and in the Calapooia River, with the exceptions of a single hybrid native winter × hatchery summer steelhead detected among the 66 fish sampled above Foster Dam (South Santiam River) and another such hybrid among the 23 fish sampled from the Calapooia. Native winter steelhead was the dominant genetic lineage in the Calapooia, and in above-dam reaches of the North and South Santiam rivers (Table 3). Native rainbow trout comprised the majority of fish sampled above dams in the McKenzie and Middle Fork Willamette rivers, though UWR steelhead were also common above Fall Creek Dam within the Middle Fork basin.

Below dams of the North and South Santiam rivers, we found evidence of natural production and introgression from hatchery stocks. In below-dam reaches of the North Santiam River, pure Big Creek stock winter steelhead, and hybrids of this and the native winter stock, represented 8% (2/26) and 38% (10/26) of that area's total sample. Pure summer steelhead, and hybrids of these with the native winter stock, represented 8% (2/26) and 8% (2/26) of the below-dam sample from the North Santiam River. Relative to the North Santiam, pure summer steelhead represented a slightly smaller percentage of fish sampled below dams in the South Santiam River (5%; 3/58), though summer × native winter hybrids were relatively more common in this system (14% of below-dam sample; 8/58). We did not detect pure Big Creek stock winter steelhead in the South Santiam River. However, hybrids of Big Creek and native winter stock were present above (2% of sample; 1/66) and below (10% of sample; 6/58) Foster Dam (Table 3).

Pure native rainbow trout and pure hatchery stock summer steelhead were the most commonly encountered *O. mykiss* in below-dam reaches of the McKenzie (43% rainbow trout; 15/35) and Middle Fork Willamette (57% summer steelhead; 13/23) rivers, respectively. Both of these rivers are outside the critical habitat designated for UWR native winter steelhead, yet we detected pure native winter steelhead in a below-dam reach of the McKenzie (*n* = 1), as well as in areas above (*n* = 12) and below (*n* = 4) Fall Creek Dam on the Middle Fork Willamette River. The presence of UWR winter steelhead in the Fall Creek drainage of the Middle Fork Willamette basin may be attributable to the local release (and subsequent naturalization) of this stock by ODFW from 1953 until 1999 (Jeff Ziller, ODFW, *personal communication*; ODFW 1992), with the stock's range further expanded by ongoing trap and transport activities of the U. S. Army Corps of Engineers[1]. Among the 26 fish sampled above Fall Creek Dam, we encountered 12 pure native winter steelhead, only a single pure native rainbow trout, and 11 hybrids of these two *O. mykiss* lineages. We also detected two hybrid native winter × Big Creek stock winter steelhead above Fall Creek Dam.

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[1] http://www.nwp.usace.army.mil/news/feature/Article/1311004/fall-creek-dam-fish-fancy-future-facility/
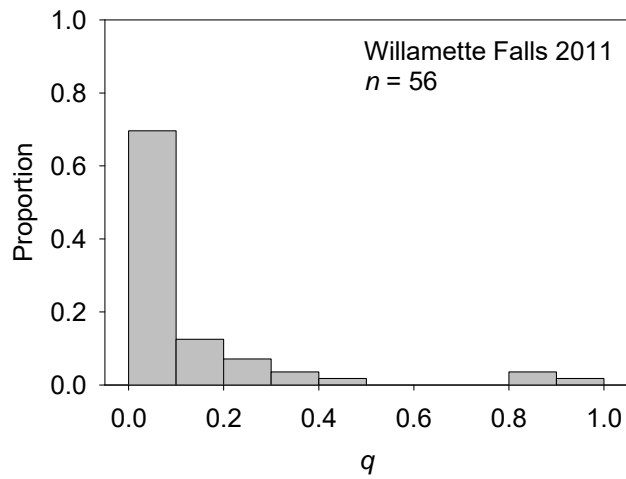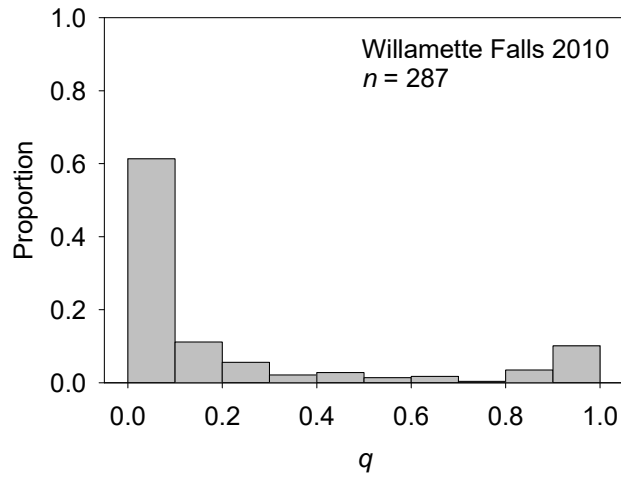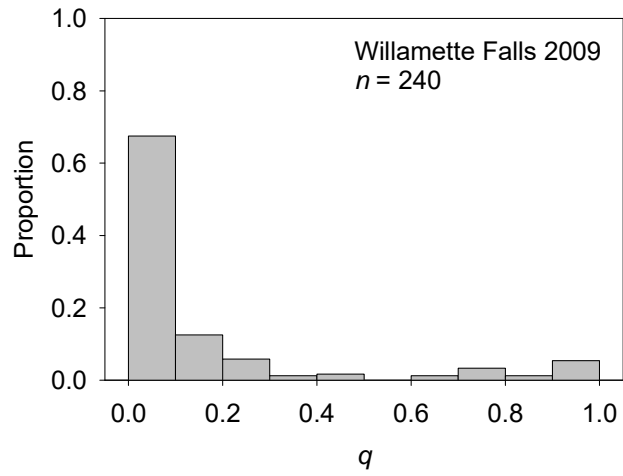
Figure 2. The proportion of unmarked, juvenile *O. mykiss* captured at Willamette Falls in 2009, 2010 and 2011, binned according to increasing (left to right) summer steelhead genetic influence (*q*).

Table 3. The number of *O. mykiss* captured from major UWR tributaries in 2014 and classified as pure or hybrids of the following genetic lineages: S = hatchery summer steelhead, NW = native winter steelhead, BC = Big Creek stock winter steelhead, RB = native rainbow trout. Classifications were based on STRUCTURE *q* value criteria described in the text.

UWR Tributary	S	NW	RB	BC	S×NW	S×RB	S×BC	NW×RB	NW×BC	RB×BC	3×	Subtotal
Calapooia	0	21	0	0	1	0	0	0	1	0	0	23
McKenzie												
above dams	0	0	18	0	0	0	0	1	0	0	0	19
below dams	9	1	15	0	0	3	0	5	0	1	1	35
Middle Fork Willamette												
above dams[2]	0	12	38	0	0	0	0	11	2	2	1	66
below dams	13	4	0	0	3	0	1	0	0	0	2	23
Molalla	3	41	1	1	10	0	1	0	12	3	5	77
North Santiam												
above dams	0	5	0	0	0	0	0	0	0	1	0	6
below dams	2	5	0	2	2	2	1	0	10	0	2	26
South Santiam												
above dams	0	56	0	0	1	0	0	7	1	1	0	66
below dams	3	35	0	0	8	0	0	5	6	0	1	58
Tualatin	0	0	0	10	0	0	0	0	0	0	0	10
Yamhill	0	3	0	9	0	0	1	0	6	0	1	20
Total	30	183	72	22	25	5	4	29	38	8	13	429
Percent of Total	7.0	42.7	16.8	5.1	5.8	1.2	0.9	6.8	8.9	1.9	3.0	100

[2] Pure and hybrid native winter steelhead (NW) were found above Fall Creek Dam, but not above Lookout Point Dam. See text for details.

Our CPUE was highest in the Molalla River (Table 4), 0.42 fish * min^{-1}, suggesting that juvenile *O. mykiss* were most abundant – or at least most detectable – in this system. Interestingly, genetic analyses of these fish revealed a highly mixed stock, consisting primarily of native winter steelhead (53%; 41/77), but also including summer steelhead (4%; 3/77), native rainbow trout (1%; 1/77), Big Creek stock steelhead (1%; 1/77) and hybrids representing several combinations of these lineages (Table 3). In fact, 40% (10/25) of the native winter × summer steelhead hybrids identified in our 2014 sample were from the Molalla River.

Hybrid class assignments

We used the software NEWHYBRIDS (Anderson 2008) to probabilistically assign individuals to hybrid classes, then used the software HYBRIDDETECTIVE (Wringe et al. 2017) to assess the accuracy of assignments made with variably stringent posterior probability cutoff values. We restricted these analyses to only those individuals that appeared to represent hatchery summer steelhead, native winter steelhead, or a mixture thereof (see Methods). Of 496 juvenile *O. mykiss* analyzed with NEWHYBRIDS, 176 assigned to the pure native winter class with a posterior probability, $P > 0.90$. Twenty-four assigned to the pure summer steelhead class at $P > 0.90$ and none assigned to the F1 hybrid class with $P > 0.10$. We also found no compelling evidence for F2s in our sample, though some individuals assigned to the backcross 1 class ($n = 10$) at $P > 0.80$. Only five individuals assigned to the backcross 2 class at $P > 0.70$. The proportions of the total sample assigning to each pure and hybrid class at the full range of P values (0-1.0) are represented in Figure 3.

Table 4. The catch per unit effort (CPUE) for steelhead captured with backpack electrofisher in major UWR tributaries, 2014.

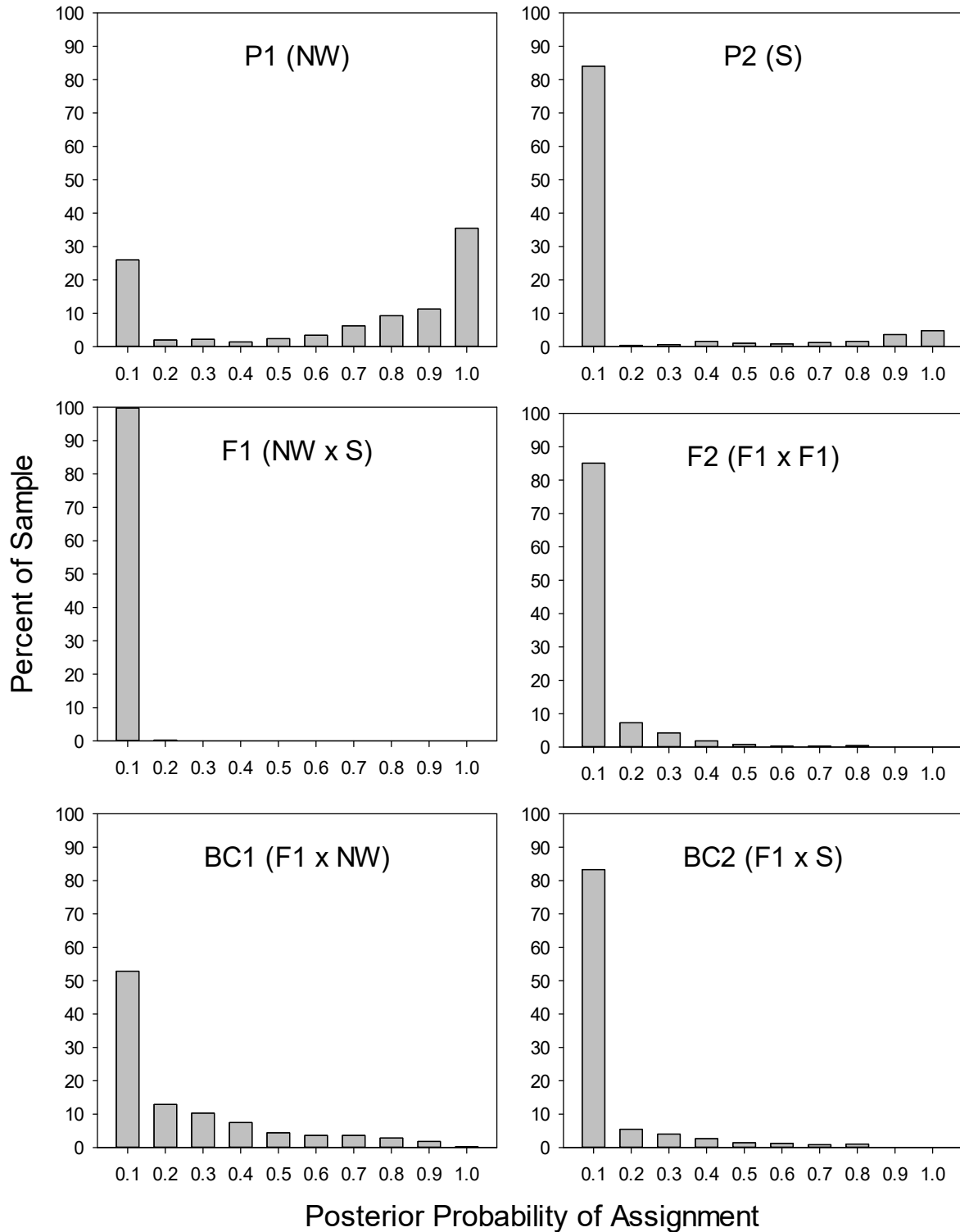| UWR tributary	N Sites	Effort (secs)	Total catch	CPUE (fish*min^{-1})
Middle Fork Willamette	48	38,412	96	0.15
McKenzie	43	32,564	71	0.13
North Santiam	26	23,512	41	0.10
South Santiam	29	22,947	117	0.31
Yamhill	7	4,205	20	0.29
Tualatin	10	1,919	10	0.31
Molalla	13	11,243	78	0.42
Pudding	4	3,255	0	0.00
Calapooia	16	7,532	31	0.25
Total	196	145,589	464	0.19

Figure 3. The percent of the unmarked juvenile *O. mykiss* sample (*n* = 496) that received posterior probabilities of assignment, *P* (*x* axis), to the following pure and hybrid classes: P1 (NW = native winter winter steelhead); P2 (S = summer steelhead); F1, F2, BC1 and BC2 (BC = backcross). Hybrid class assignments were performed with NEWHYBRIDS (Anderson 2008). See text for details.

We found that the accuracy of class assignments, defined as the number of individuals assigned to a given class, divided by the number known to exist for that class (Vähä and Primmer 2006), declined steadily for most classes below $P = 0.80$. At this P-value cutoff, assignment accuracy to the F1 and both pure classes exceeded 80%, but was significantly lower for F2 and backcross classes (Figure 4). Accuracy of assignment to backcross classes appeared low (<75%) or unreliable across the full range of critical posterior probabilities. Taken in context of our NEWHYBRIDS results, which revealed that 43% of the 496 juvenile fish examined presented posterior probabilities of assignment $P < 0.80$ for all classes, we conclude that our current microsatellite data cannot be used to confidently resolve hybrid class.

DISCUSSION

Willamette Falls stands 12 m high and historically represented a formidable barrier to anadromous salmonids that might ascend the river to spawn in the upper basin. This geographic feature likely prevented summer, fall and early winter run types of steelhead and salmon from colonizing the UWR, due to seasonal flow conditions, until a fish ladder was constructed at the falls in the 1880s. Relatively low, but reliable, late-winter flows at Willamette Falls likely selected for the run timing of native UWR steelhead, which typically arrive and spawn later in the season than most lower Columbia River winter-run steelhead. Native steelhead primarily spawn in the eastern tributaries of the UWR, where volitional access to high quality habitats of upper reaches is now impeded by numerous high-head dams. Impacts from these decades-old dams, as well as from other threats, such as recently intensifying predation by pinnipeds (Wright et al. 2017), have undoubtedly contributed to a severe decline in abundance of UWR winter steelhead (Figure 5).

A long-held perception of under-utilization of potential spawning habitat (Williams 1983) and mitigation accords for dam construction motivated fisheries managers to introduce a variety of anadromous salmonids to the UWR during the past century, including summer- and early winter-run steelhead. Hatchery programs supporting these efforts have provided recreational anglers with enhanced harvest opportunities, but may also pose ecological and genetic risks to wild populations through effects from competition, disease transmission, interbreeding and consequent impacts on fitness and productivity (Araki et al. 2009; Chilcote et al. 2011; Christie et al. 2014).

Our results clearly indicate that summer steelhead, introduced to the UWR through hatchery programs, reproduce in the wild and have to some extent genetically introgressed with native UWR winter steelhead. We also found evidence of winter steelhead closely related to the Big Creek hatchery stock among outmigrants sampled at Willamette Falls and in western UWR tributaries. Although these winter steelhead could be descendants of lower Columbia River migrants that simply used improved access at Willamette Falls to volitionally colonize the Yamhill and Tualatin rivers, the genetic proximity and protracted stocking history of Big Creek hatchery steelhead strongly suggests that this introduced stock has naturalized and persisted in the UWR beyond the duration of hatchery programs. Regardless of the manner by which late winter-run steelhead arrived, natural production and introgression from this stock should be carefully considered by managers, as it could mask the true abundance and influence the genetic integrity of native UWR steelhead.

Averaged across three years of sampling, we found that only little over half (51.3%) of the unmarked juvenile *O. mykiss* captured at Willamette Falls were of pure, native ancestry. The remainder of our sample was comprised primarily of Big Creek stock winter- and Skamania stock summer-run steelhead, and hybrids produced through matings of these with the native stock (Table 2). We found relatively little
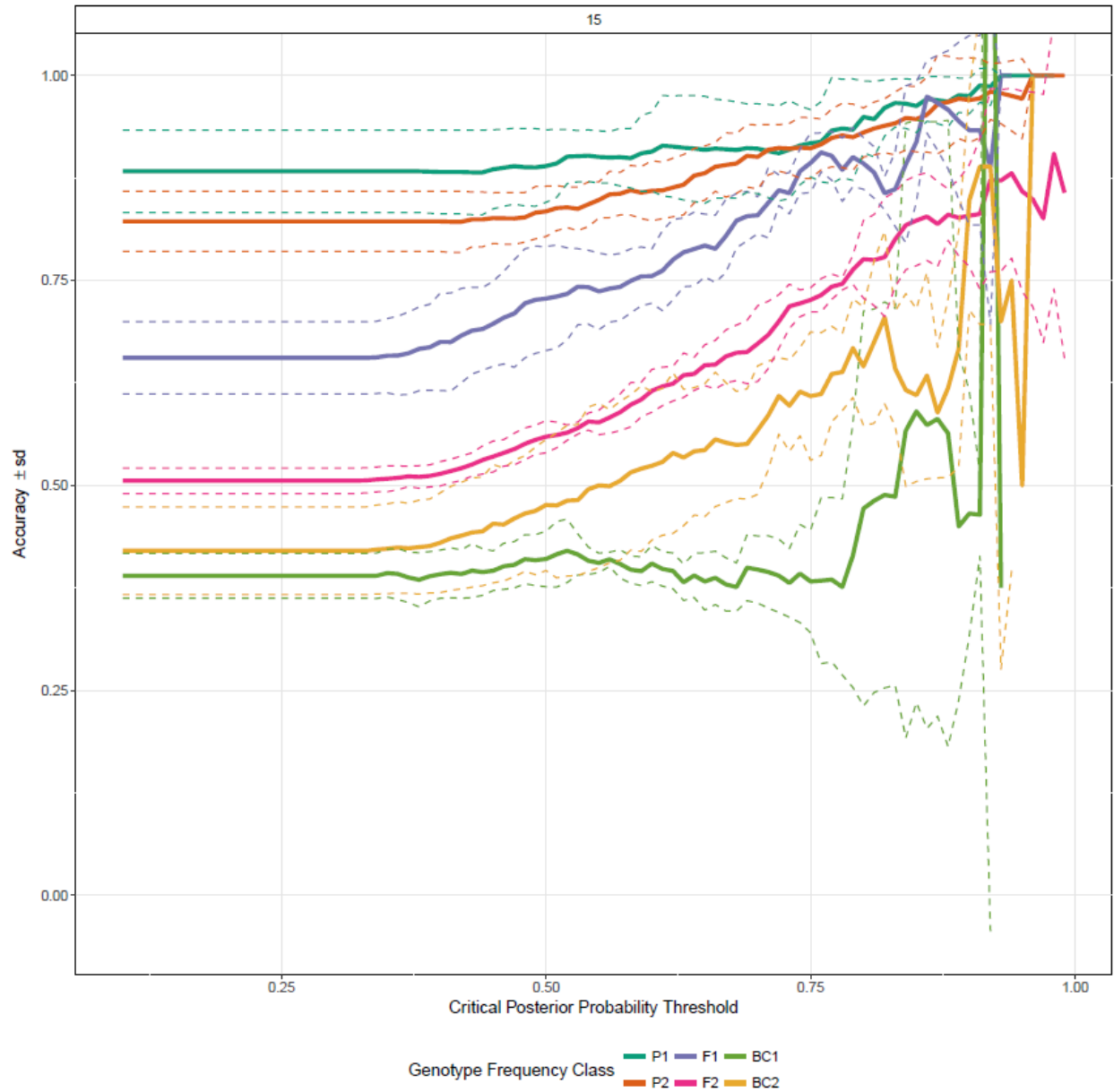
Figure 4. The accuracy of assignment to hybrid classes P1, P2, F1, F2, Backcross 1, and Backcross 2, as predicted by HYBRIDDETECTIVE (Wringe et al. 2017) through replicate analyses of UWR steelhead microsatellite data with the software NEWHYBRIDS (Anderson 2008). Solid colored lines represent mean accuracy and dashed lines are the standard deviation from the mean, as calculated from three independently simulated datasets, each analyzed in triplicate. See text for additional details.
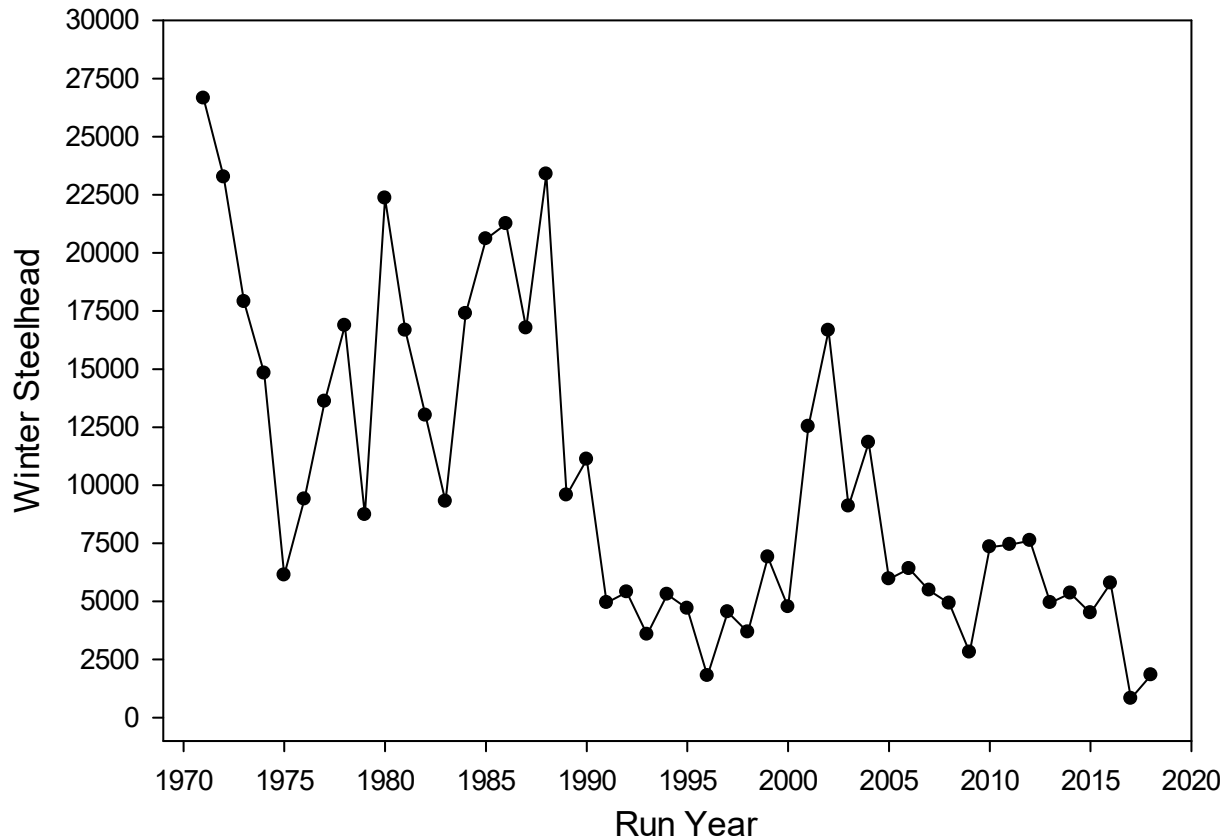
Figure 5. Winter steelhead counts at Willamette Falls through the 2017-2018 run year. The count for the 1998-1999 run year (1 November 1998 to 31 May 1999) is incomplete due to the performance of fishway repairs. Data are from ODFW (2018).

evidence for hybridization between introduced steelhead and native resident rainbow trout, though hybridization between resident rainbow trout and UWR steelhead appears to occur in the Middle Fork Willamette River, where the anadromous form is now being trapped, transported and released above Fall Creek Dam, into habitats outside its historic range.

Trapping, transport and upstream release of unmarked winter steelhead also occurs at Foster Dam, on the South Santiam River (Mapes et al. 2017). The area above this dam is currently managed as a wild steelhead sanctuary, and our results provide evidence that this management strategy effectively excludes non-native *O. mykiss*, which are present in both pure and hybrid forms below the dam (Table 3). In recent years, hatchery summer steelhead have been used as "surrogates" for winter steelhead in fish passage evaluations at Foster Dam. Potential risks from such actions, such as residualism above the dam, should be carefully considered. Given the extremely depressed state of UWR winter steelhead, we recommend that all reasonable measures be taken to preserve the genetic integrity of native UWR *O. mykiss* in stronghold areas such as the upper South Santiam.

Interestingly, our small sample from above dams in the North Santiam River suggested that *O. mykiss* in these areas are of native steelhead ancestry (Table 3). An early hatchery program on the upper North Santiam (Marion Forks) used native UWR winter steelhead as broodstock, and may have contributed to the population that currently exists above Detroit Dam. Otherwise, or in addition to this possibility, progenitors of these fish were likely isolated in headwater habitats at the time of dam construction (1960s) without genetic influx from anadromous migrants since that time. This population, therefore, represents a genetic legacy of native North Santiam River winter steelhead and an interesting study group for the selective effects of dam construction on anadromy.

A high CPUE and noteworthy genetic integrity of *O. mykiss* from the Calapooia River suggest that this watershed supports another stronghold population of native UWR steelhead with intrinsic conservation value. However, Calapooia winter steelhead are estimated to be at greater risk of extinction than other major populations of the eastern UWR. As of 2011, extinction risks for UWR steelhead were classified as "low" for the Molalla, North Santiam, and South Santiam populations, and "moderate" for the Calapooia population (ODFW and NMFS 2011), though this assessment occurred prior to the sharp increase in steelhead predation by pinnipeds at Willamette Falls (Wright et al. 2017). Nevertheless, a recent population viability analysis conducted by ODFW (Falcy 2018) concluded that the probability of quasi-extinction (<100 individuals returning over four consecutive years) was 99% in the Calapooia basin, regardless of the level of pinniped predation.

A relatively high percentage of our Molalla River sample classified as native winter × summer steelhead (13%; 10/77), even though summer steelhead have not been released into this watershed for decades. This finding further suggests that hatchery steelhead can reproduce in the wild and found naturalized populations, but underscores the importance of determining the hybrid class of individual fish. If hybrids in the Molalla could be identified as F1s, this result would suggest a process of ongoing hybridization between native winter and hatchery summer steelhead, perhaps mediated through straying behavior of the latter. However, if F1s were not present among samples, then one could conclude that the hybrid signal likely represents a legacy of past hatchery practices, which included the release of summer steelhead in the Molalla River.

Unfortunately, the extent to which hybridization between introduced hatchery and native steelhead currently occurs in the UWR remains unknown. Although we found evidence of introgression from the ongoing summer (and likely descendants of the discontinued winter) steelhead hatchery program, our NEWHYBRIDS analysis did not identify any F1 offspring from summer and native winter steelhead matings (Figure 3). However, a relatively high posterior probability would be required for confident assignment to this hybrid class (Figure 4), which we did not observe from our results. This finding could reflect a current reality of low or non-existent hatchery-wild hybridization, or arise from a lack of statistical power to detect F1s with our microsatellite data. Nevertheless, the preponderance of putative Big Creek stock steelhead in western UWR tributaries, as well as the occurrence of summer steelhead in the Molalla, all suggest that introduced hatchery stock steelhead can reproduce, naturalize and persist in the UWR beyond the period of stocking activities, and hybrid signals could similarly persist in absence of strong selection.

The impact that genetic introgression from introduced stocks has on the fitness, productivity and viability of wild UWR steelhead is also unknown. However, reductions in fitness of wild steelhead and salmon have been widely documented as result of interbreeding with hatchery fish (Christie et al. 2014) and these reductions may limit wild population productivity (Chilcote et al. 2011). Fisheries managers have

recently taken measures to reduce genetic risk from hatchery steelhead in the UWR, including reductions in hatchery program size, increased removal rates at fish traps (i.e. reduced "recycling") and establishment of wild fish sanctuaries above dams. We encourage managers to both monitor the effectiveness of these management strategies and explore new options to further reduce genetic risk from hatchery steelhead.

To improve our understanding of genetic influence from hatchery stocks in the UWR, we recommend conducting additional, directed field sampling similar to our 2014 effort. Periodic yet systematic genetic analyses would serve to inform status reviews and conservation and recovery efforts. Anticipating this need, ODFW staff began collecting samples from juvenile *O. mykiss* in the Molalla, Calapooia, North Santiam, and South Santiam rivers in April 2018. We also recommend investigations into the feasibility and analytical advantages that might be gained through recently developed genetic techniques, such as GT-seq (Campbell et al. 2015), which is used to genotype hundreds of single nucleotide polymorphism (SNP) loci and could potentially improve our accuracy to assign individual steelhead to hybrid classes.

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REFERENCES

Anderson, E. C. 2008. Bayesian inference of species hybrids using multilocus dominant genetic markers. Philosophical Transactions of the Royal Society of London B: Biological Sciences 363(1505):2841–2850.

Araki, H., B. Cooper, and M. S. Blouin. 2009. Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. Biology Letters 5(5):621–4.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tschawytscha*). Journal of Heredity 90(2):281–288.

Buchholz, W., S. J. Miller, and W. J. Spearman. 1999. Summary of PCR primers for salmonid genetic studies. Fish Genetics Laboratory, US Fish and Wildlife Service.

Burgarella, C., Z. Lorenzo, R. Jabbour-Zahab, R. Lumaret, E. Guichoux, R. J. Petit, A. Soto, and L. Gil. 2009. Detection of hybrids in nature: application to oaks (*Quercus suber and Q. ilex*). Heredity 102(5):442.

Cairney, M., J. B. Taggart, and B. Høyheim. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Molecular Ecology 9(12):2175–2178.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15(4):855–867.

Chilcote, M. W., K. W. Goodson, and M. R. Falcy. 2011. Reduced recruitment performance in natural populations of anadromous salmonids associated with hatchery-reared fish. Canadian Journal of Fisheries and Aquatic Sciences 68(3):511–522.

Christie, M. R., M. J. Ford, and M. S. Blouin. 2014. On the reproductive success of early-generation hatchery fish in the wild. Evolutionary Applications 7(8):883–896.

Condrey, M. J., and P. Bentzen. 1998. Characterization of coastal cutthroat trout (*Oncorhynchus clarki clarki*) microsatellites and their conservation in other salmonids. Molecular Ecology 7(6):787-789.

ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Falcy, M. 2017. Population viability of Willamette River steelhead: an assessment of the effect of sea lions at Willamette Falls. Unpublished report, Oregon Department of Fish and Wildlife, Salem. Available at: https://falcy.weebly.com/pva.html. (April 2018)

Ivanova, N. V, J. R. Dewaard, and P. D. N. Hebert. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Resources 6(4):998–1002.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806.

Johnson, M. A., T. A. Friesen, D. J. Teel, and D. M. Van Doornik. 2013. Genetic stock identification and relative natural production of Willamette River steelhead. Final Report to the U.S. Army Corps of Engineers, Portland District. Task Order number W9127N-10-2-0008-0015. Available at: https://nrimp.dfw.state.or.us/re/pictures/1362_3_ODFW%20Willamette%20Steelhead%20Genetics%20Synthesis%20FINAL2.1.pdf (May 2018).

Kalinowski, S. T., K. R. Manlove, and M. L. Taper. 2007. ONCOR a computer program for genetic stock identification. Montana State University, Department of Ecology, Bozeman.

Mapes, R.L., C.S. Sharpe, and T.A. Friesen. 2017. Evaluation of the trap and transport of adult steelhead above USACE Project dams in the upper Willamette basin. Final report to the University of Idaho and U.S Army Corps of Engineers, project code APH-09-01-FOS. Oregon Department of Fish and Wildlife (ODFW), Salem. Available at: https://odfw.forestry.oregonstate.edu/willamettesalmonidrme/sites/default/files/2016_odfw_uwr_winter_steelhead_-_final_-_sept_2017.pdf (May 2018)

McConnell, S., L. Hamilton, D. Morris, D. Cook, D. Paquet, P. Bentzen, and J. Wright. 1995. Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. Aquaculture 137(1):19–30.

Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53(1):120–126.

Nelson, R. J., and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics 30(3):228–229.

NMFS (National Marine Fisheries Service). 2016. 5-Year review: summary & evaluation of Upper Willamette River steelhead & Chinook. NMFS, Northwest Region Portland, Oregon. Available at: http://www.westcoast.fisheries.noaa.gov/publications/status_reviews/salmon_steelhead/multiple_species/5-yr-uwr.pdf (May 2018)

NMFS. 2008. 2008-2023 Willamette River Basin Project Biological Opinion. F/NWR/2000/02117. NOAA - National Marine Fisheries Service, Northwest Region, Seattle, WA. Available at: http://www.westcoast.fisheries.noaa.gov/fish_passage/willamette_opinion/ (May 2018)

ODFW (Oregon Department of Fish and Wildlife). 2018. Lower Willamette fisheries and Willamette Falls fish counts. Available at: www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp. (May 2018)

ODFW. 1992. Middle Fork Willamette Subbasin Fish Management Plan. ODFW, Salem, OR.

ODFW and NMFS Northwest Region. 2011. Upper Willamette River Conservation and Recovery Plan for Chinook Salmon and Steelhead. ODFW, Salem. Available at: http://www.nmfs.noaa.gov/pr/pdfs/recovery/chinook_steelhead_upperwillametteriver.pdf (May 2018)

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1083-1090.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945–59.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53(4):833–841.

Smith, C. T., B. F. Koop, and R. J. Nelson. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Molecular Ecology 7(11):1614–1616.

Spies, I. B., D. J. Brasier, P. T. L. O'Reilly, T. R. Seamons, and P. Bentzen. 2005. Development and characterization of novel tetra□, tri□, and dinucleotide microsatellite markers in rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Resources 5(2):278–281.

Stephenson, J. J., M. R. Campbell, J. E. Hess, C. Kozfkay, A. P. Matala, M. V McPhee, P. Moran, S. R. Narum, M. M. Paquin, and O. Schlei. 2009. A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. Conservation Genetics 10(4):1145–1149.

Vähä, J., and C. R. Primmer. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Molecular Ecology 15(1):63–72.

Van Doornik, D. M., M. A. Hess, M. A. Johnson, D. J. Teel, T. A. Friesen, and J. M. Myers. 2015. Genetic population structure of Willamette River steelhead and the influence of introduced stocks. Transactions of the American Fisheries Society 144(1):150–162.

Williams, R. 1983. Coho salmon in the upper Willamette River, Oregon: a history of establishing a run of non-indigenous salmon and prospects for renewed efforts to increase the run. Oregon Department of Fish and Wildlife report. Available at https://digital.osl.state.or.us/islandora/object/osl%3A96183/datastream/OBJ/view (May 2018)

Wright, B., T. Murtagh, R. Brown, and S. Riemer. 2017. Willamette Falls Pinniped Monitoring Project, 2017. ODFW, Salem. Available at https://www.dfw.state.or.us/fish/SeaLion/docs/Willamette_Falls_2017_sea_lion_report.pdf (May 2018)

Wringe, B. F., R. R. E. Stanley, N. W. Jeffery, E. C. Anderson, and I. R. Bradbury. 2017. Hybriddetective: a workflow and package to facilitate the detection of hybridization using genomic data in R. Molecular Ecology Resources 17(6):275-284.

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