

Relative reproductive success of Twisp River hatchery and wild steelhead (*Oncorhynchus mykiss*): Summary report for SNP genotyping of adult collections – Return Year 2012

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Introduction

The Wells HCP requires Douglas County PUD to investigate the relative reproductive success of naturally spawning hatchery-produced steelhead (*Oncorhynchus mykiss*). This project was initiated to fulfill that requirement. Starting in 2011, the Twisp River hatchery program became an integrated program: only naturally produced adults are used as hatchery broodstock and the number of naturally spawning hatchery-produced adults will be controlled (HSRG 2009). Specifically, only wild or naturally-produced adult steelhead from the Twisp River will be used as broodstock to produce offspring to be released in the Twisp River and the number of hatchery-produced adult steelhead allowed to spawn naturally upstream of the Twisp River weir will be limited. All hatchery broodstock and all naturally spawning adults will be sampled for DNA parentage assignment analysis. Their adult offspring will be sampled in later years. The purpose of this report is to summarize the methods and results of genetic analysis of adult steelhead sampling at the Twisp River weir in 2012. Laboratory methods are outlined and the genetic characteristics of the hatchery and natural origin collections are reported.

Methods

Sampling location and methods

Adult hatchery-produced and wild steelhead were collected from a weir located in the Twisp River, a tributary to the Methow River in north central Washington State in 2012. Adult steelhead that entered the trap were sorted by origin (hatchery or wild). A roughly 1:1 ratio of hatchery-produced to natural origin adults were sampled, tagged with a floy or PIT tag and released upstream to spawn naturally. Surplus hatchery-produced fish were killed, and some natural origin fish were retained for hatchery broodstock. Hatchery-produced steelhead were identified by a missing adipose fin (which was removed prior to release from the hatchery as a juvenile), an elastomer mark, a coded-wire tag, or dorsal fin erosion. Individual fish were sampled for body length, sex, arrival date, spawning location and date (when possible), scales were removed for ageing and confirmation of fish origin, and a small fin clip was taken for genetic analysis. Fin clips were preserved in 95% ethanol and stored at room temperature. A few additional adults were captured by the Yakama Tribe in a temporary weir designed to capture kelts on Little Bridge Creek, a tributary to the Twisp River, located upstream of the Twisp weir. These fish were subjected to a similar sampling protocol as those sampled at the Twisp weir.

Genetic sample processing

The panel of WDFW *O. mykiss* single nucleotide polymorphic loci (SNPs) that is used for analysis statewide has been updated and finalized since the completion of the 2012 report. Samples were genotyped using the finalized set of loci. The suite of 192 SNP markers included 189 SNP loci developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Table 1) and three SNP loci developed to distinguish cutthroat trout (*O. clarkii*) from steelhead and rainbow trout (Table 2).

To extract and isolate DNA from fin tissue, Qiagen DNEasy[®] kits (Qiagen Inc., Valencia, CA) were used, following the recommended protocol for animal tissues. SNP genotypes were obtained through PCR and visualization on Fluidigm EP1 integrated fluidic circuits (chips). Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: Samples were pre-amplified by Specific Target Amplification (STA) following Fluidigm's recommended protocol with one modification. The 192 assays were pooled to a concentration of 0.2X and mixed with 2X Qiagen Multiplexing Kit (Qiagen, Inc., Valencia CA), instead of TaqMan PreAmp Master Mix (Applied Biosystems), to a volume of 3.75µl, to which 1.25µl of unquantified sample DNA was added for a total reaction volume of 5µl. Pre-amp PCR was conducted on a MJ Research or Applied Biosystems thermal cycler using the following

profile: 95°C for 15 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 minutes. Post-PCR reactions were diluted with 20µl dH₂O to a final volume of 25µl.

Specific SNP locus PCRs were conducted on the Fluidigm chips. Assay loading mixture contained 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invetrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 2.1 µL template DNA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C.

The SNP assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. To ensure all SNP markers were being scored accurately and consistently, all data were scored by two researchers and scores of each researcher were compared. Disputed scores were called missing data (i.e., no genotype).

Evaluation of loci

To evaluate genetic qualities of loci, we quantified several genetic parameters in the Twisp River adult steelhead collections. To check for systematic scoring issues, we performed a two-tailed exact test of Hardy–Weinberg equilibrium (HWE) for each locus in each collection using the Markov Chain method implemented in Genepop 4.2 (dememorization number 1000, batches 100, 1000 iterations per batch; (Raymond and Rousset 1995; Rousset 2008)). Significance of probability values was adjusted for multiple tests using false discovery rate (Verhoeven et al. 2005). F_{IS} , a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of scoring issues, was calculated according to Weir & Cockerham (1984) using Genepop 4.2. Expected heterozygosity was calculated using GENETIX 4.05 software (Belkhir et al. 2001). Pairwise F_{ST} estimates among collections were calculated and statistical significance was estimated by permutation tests using FSTAT (Goudet 1995) with 1000 permutations.

Results

Samples received

From spring 2012 sampling at the Twisp River weir, 305 tissue samples from hatchery and wild origin steelhead were received by Washington Department of Fish and Wildlife (WDFW) Molecular Genetics Laboratory (MGL; Table 3). Of these, 262 were singled out for genotyping based on whether or not they were allowed upstream to spawn, i.e., all fish euthanized at the weir were not genotyped for this analysis.

Genotyping success

Genotyping success was high, with an average of 98.9% complete (374 alleles out of a total of 378 possible) and a range of 91.0% to 100.0% complete. We had complete genotypes from 10% of samples (28/262) and 99% of samples had between 95% and 100% genotyping success. No adult samples from 2012 had matching genotypes with any other 2012 sample or any sample from previous years. Two wild origin fish appeared to be F1 cutthroat/steelhead hybrids (12DI0088 and 12DI0091). These fish were removed from analysis to evaluate the loci, but will be included in any future parentage assignment analysis.

Evaluation of loci

No loci had significant P values for exact tests of HWE after false discovery rate adjustment for multiple tests (Table 4). In both the wild and hatchery origin fish, several loci were in linkage disequilibrium (LD), after correction for multiple tests. Five loci in particular were in LD in both groups of fish. *AOmy137*, *AOmy152*, and *AOmy214* all showed LD with one another ($P \ll 0.0001$, all six pairwise tests) and *AOmy095* and *AOmy272* were in LD ($P \ll 0.0001$, both tests). The presence of these linked loci will not affect parentage assignment.

The pairwise F_{ST} estimate for 2012 hatchery and wild steelhead was similar to F_{ST} estimates from previous years ($F_{ST} = 0.005$).

Genotypes of all individuals are in Appendix 1.

References cited

- Aguilar, A., and J. C. Garza. 2008. Isolation of 15 single nucleotide polymorphisms from coastal steelhead, *Oncorhynchus mykiss* (Salmonidae). *Molecular Ecology Resources* 8(3):659-662.
- Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2001. GENETIX 4.05, logiciel sous Windows™ pour la genétique des populations.
- Campbell, N. R., and S. R. Narum. 2009. Identification and characterization of heat shock response-related single-nucleotide polymorphisms in *O. mykiss* and *O. tshawytscha*. *Molecular Ecology Resources* 9(6):1460-1466.
- Campbell, N. R., K. E. N. Overturf, and S. R. Narum. 2009. Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout. *Molecular Ecology Resources* 9(1):318-322.
- Finger, A. J., M. R. Stephens, N. W. Clipperton, and B. May. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trouts. *Molecular Ecology Resources* 9(3):759-763.
- Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86:485-486.
- Hansen, M. H. H., and coauthors. 2011. Assembling a dual purpose TaqMan-based panel of single-nucleotide polymorphism markers in rainbow trout and steelhead (*Oncorhynchus mykiss*) for association mapping and population genetics analysis. *Molecular Ecology Resources* 11:67-70.
- HSRG. 2009. Report to Congress on Columbia River Basin hatchery reform. Hatchery Scientific Review Group, http://www.hatcheryreform.us/hrp_downloads/reports/columbia_river/report_to_congress/hsrg_report_12.pdf.
- Limborg, M. T., and coauthors. 2012. Signatures of natural selection among lineages and habitats in *Oncorhynchus mykiss*. *Ecology and Evolution* 2(1):1-18.
- McGlaufflin, M. T., and coauthors. 2010. High-resolution melting analysis for the discovery of novel single-nucleotide polymorphisms in rainbow and cutthroat trout for species identification. *Transactions of the American Fisheries Society* 139(3):676-684.
- Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. *Evolution* 49(6):1280-1283.
- Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8(1):103-106.
- Sánchez, C. C., and coauthors. 2009. Single nucleotide polymorphism discovery in rainbow trout by deep sequencing of a reduced representation library. *BMC Genomics* 10(1):559.
- Sprowles, A. E., M. R. Stephens, N. W. Clipperton, and B. P. May. 2006. Fishing for SNPs: A targeted locus approach for single nucleotide polymorphism discovery in rainbow trout. *Transactions of the American Fisheries Society* 135(6):1698-1721.
- Stephens, M. R., N. W. Clipperton, and B. May. 2009. Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. *Molecular Ecology Resources* 9(1):339-343.
- Verhoeven, K. J. F., K. L. Simonsen, and L. M. McIntyre. 2005. Implementing false discovery rate control: increasing your power. *Oikos* 108(3):643-647.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38(6):1358-1370.

Appendix 1

See attached Appendix 1 (Twisp_2012_adult_genotyping_Appendix_1_2012.xlsx).

Table 1. List of general use, diploid single nucleotide polymorphic (SNP) loci genotyped in Twisp River steelhead

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy005	Omy_aspAT-123	T	C	(Campbell et al. 2009)
AOmy010	Omy_CRB2677.106	G	T	(Sprowles et al. 2006)
AOmy014	Omy_e1-147	G	T	(Sprowles et al. 2006)
AOmy015	Omy_gdh-271	C	T	(Campbell et al. 2009)
AOmy016	Omy_GH1P1_2	C	T	(Aguilar and Garza 2008)
AOmy021	Omy_LDHB-2_e5	T	C	(Aguilar and Garza 2008)
AOmy023	Omy_MYC_2	T	C	(Aguilar and Garza 2008)
AOmy026	Omy_myoD.178	A	C	(Campbell et al. 2009)
AOmy027	Omy_nkef-241	C	A	(Campbell et al. 2009)
AOmy028	Omy_nramp-146	G	A	(Campbell et al. 2009)
AOmy029	Omy_Ogo4.212	T	C	(Campbell et al. 2009)
AOmy042	Omy_BAC-F5.284	C	T	(Limborg et al. 2012)
AOmy047	Omy_u07-79-166	G	T	(Limborg et al. 2012)
AOmy048	Omy_113490-159	C	T	(Abadía-Cardoso et al. 2011)
AOmy049	Omy_114315-438	T	G	(Abadía-Cardoso et al. 2011)
AOmy051	Omy_121713-115	T	A	(Abadía-Cardoso et al. 2011)
AOmy056	Omy_128693-455	T	C	(Abadía-Cardoso et al. 2011)
AOmy058	Omy_130524-160	C	G	(Abadía-Cardoso et al. 2011)
AOmy059	Omy_187760-385	A	T	(Abadía-Cardoso et al. 2011)
AOmy061	Omy_96222-125	T	C	(Abadía-Cardoso et al. 2011)
AOmy062	Omy_97077-73	T	A	(Abadía-Cardoso et al. 2011)
AOmy065	Omy_97954-618	C	T	(Abadía-Cardoso et al. 2011)
AOmy067	Omy_aromat-280	T	C	WSU - J. DeKoning unpubl.
AOmy068	Omy_arp-630	G	A	(Campbell et al. 2009)
AOmy072	Omy_cd59b-112	C	T	WSU - J. DeKoning unpubl.
AOmy073	Omy_colla1-525	C	T	WSU - J. DeKoning unpubl.
AOmy074	Omy_cox2-335	T	G	WSU - J. DeKoning unpubl.
AOmy078	Omy_g1-103	T	C	(Stephens et al. 2009)
AOmy079	Omy_g12-82	T	C	WSU - J. DeKoning unpubl.
AOmy081	Omy_gh-475	C	T	(Campbell et al. 2009)
AOmy082	Omy_gsdf-291	T	C	WSU - J. DeKoning unpubl.
AOmy084	Omy_hsc715-80	C	A	WDFW - S. Young unpubl.
AOmy087	Omy_hsp47-86	T	A	WDFW - S. Young unpubl.
AOmy088	Omy_hsp70aPro-329	A	G	(Campbell and Narum 2009)
AOmy089	Omy_hsp90BA-193	C	T	(Campbell and Narum 2009)
AOmy091	Omy_IL17-185	G	A	WSU - J. DeKoning unpubl.
AOmy092	Omy_IL1b-163	T	G	WSU - J. DeKoning unpubl.
AOmy094	Omy_inos-97	C	A	WSU - J. DeKoning unpubl.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy095	Omy_mapK3-103	A	T	CRITFC - N. Campbell unpubl.
AOmy096	Omy_mcsf-268	T	C	WSU - J. DeKoning unpubl.
AOmy100	Omy_nach-200	A	T	WSU - J. DeKoning unpubl.
AOmy105	Omy_OmyP9-180	C	G	(Sprowles et al. 2006)
AOmy107	Omy_Ots249-227	C	T	(Campbell et al. 2009)
AOmy108	Omy_oxct-85	A	T	WSU - J. DeKoning unpubl.
AOmy110	Omy_star-206	A	G	WSU - J. DeKoning unpubl.
AOmy111	Omy_stat3-273	G	Deletion	WSU - J. DeKoning unpubl.
AOmy113	Omy_tlr3-377	C	T	WSU - J. DeKoning unpubl.
AOmy114	Omy_tlr5-205	T	A	WSU - J. DeKoning unpubl.
AOmy117	Omy_u09-52-284	T	G	(Limborg et al. 2012)
AOmy118	Omy_u09-53-469	T	C	(Limborg et al. 2012)
AOmy120	Omy_u09-54.311	C	T	WDFW - S. Young unpubl.
AOmy123	Omy_u09-55-233	A	G	(Limborg et al. 2012)
AOmy125	Omy_u09-56-119	T	C	(Limborg et al. 2012)
AOmy129	Omy_BAMBI4.238	T	C	WDFW - S. Young unpubl.
AOmy132	Omy_G3PD_2.246	C	T	WDFW - S. Young unpubl.
AOmy134	Omy_II-1b-028	T	C	WDFW - S. Young unpubl.
AOmy137	Omy_u09-61.043	A	T	WDFW - S. Young unpubl.
AOmy144	Omy_UT16_2.173	C	T	WDFW - S. Young unpubl.
AOmy147	Omy_U11_2b.154	T	C	WDFW - S. Young unpubl.
AOmy149	Omy_gluR-79	C	T	CRITFC - unpubl.
AOmy152	Omy_SECC22b-88	T	C	CRITFC - unpubl.
AOmy173	BH2VHSVip10	C	T	Pascal & Hansen unpubl.
AOmy174	OMS00003	T	G	(Sánchez et al. 2009)
AOmy176	OMS00013	A	G	(Sánchez et al. 2009)
AOmy177	OMS00018	T	G	(Sánchez et al. 2009)
AOmy179	OMS00041	G	C	(Sánchez et al. 2009)
AOmy180	OMS00048	T	C	(Sánchez et al. 2009)
AOmy181	OMS00052	T	G	(Sánchez et al. 2009)
AOmy182	OMS00053	T	C	(Sánchez et al. 2009)
AOmy183	OMS00056	T	C	(Sánchez et al. 2009)
AOmy184	OMS00057	T	G	(Sánchez et al. 2009)
AOmy185	OMS00061	T	C	(Sánchez et al. 2009)
AOmy186	OMS00062	T	C	(Sánchez et al. 2009)
AOmy187	OMS00064	T	G	(Sánchez et al. 2009)
AOmy189	OMS00071	A	G	(Sánchez et al. 2009)
AOmy190	OMS00072	A	G	(Sánchez et al. 2009)
AOmy191	OMS00078	T	C	(Sánchez et al. 2009)
AOmy192	OMS00087	A	G	(Sánchez et al. 2009)
AOmy193	OMS00089	A	G	(Sánchez et al. 2009)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy194	OMS00090	T	C	(Sánchez et al. 2009)
AOmy195	OMS00092	A	C	(Sánchez et al. 2009)
AOmy197	OMS00103	A	T	(Sánchez et al. 2009)
AOmy198	OMS00105	T	G	(Sánchez et al. 2009)
AOmy199	OMS00112	A	T	(Sánchez et al. 2009)
AOmy200	OMS00116	T	A	(Sánchez et al. 2009)
AOmy201	OMS00118	T	G	(Sánchez et al. 2009)
AOmy202	OMS00119	A	T	(Sánchez et al. 2009)
AOmy203	OMS00120	A	G	(Sánchez et al. 2009)
AOmy204	OMS00121	T	C	(Sánchez et al. 2009)
AOmy205	OMS00127	T	G	(Sánchez et al. 2009)
AOmy206	OMS00128	T	G	(Sánchez et al. 2009)
AOmy207	OMS00132	A	T	(Sánchez et al. 2009)
AOmy208	OMS00133	A	G	(Sánchez et al. 2009)
AOmy209	OMS00134	A	G	(Sánchez et al. 2009)
AOmy210	OMS00153	T	G	(Sánchez et al. 2009)
AOmy211	OMS00154	A	T	(Sánchez et al. 2009)
AOmy212	OMS00156	A	T	(Sánchez et al. 2009)
AOmy213	OMS00164	T	G	(Sánchez et al. 2009)
AOmy214	OMS00169	A	G	(Sánchez et al. 2009)
AOmy215	OMS00175	T	C	(Sánchez et al. 2009)
AOmy216	OMS00176	T	G	(Sánchez et al. 2009)
AOmy218	OMS00180	T	G	(Sánchez et al. 2009)
AOmy220	Omy_1004	A	T	(Hansen et al. 2011)
AOmy221	Omy_101554-306	T	C	(Abadía-Cardoso et al. 2011)
AOmy222	Omy_101832-195	A	C	(Abadía-Cardoso et al. 2011)
AOmy223	Omy_101993-189	A	T	(Abadía-Cardoso et al. 2011)
AOmy225	Omy_102505-102	A	G	(Abadía-Cardoso et al. 2011)
AOmy226	Omy_102867-443	T	G	(Abadía-Cardoso et al. 2011)
AOmy227	Omy_103705-558	T	C	(Abadía-Cardoso et al. 2011)
AOmy228	Omy_104519-624	T	C	(Abadía-Cardoso et al. 2011)
AOmy229	Omy_104569-114	A	C	(Abadía-Cardoso et al. 2011)
AOmy230	Omy_105075-162	T	G	(Abadía-Cardoso et al. 2011)
AOmy231	Omy_105385-406	T	C	(Abadía-Cardoso et al. 2011)
AOmy232	Omy_105714-265	C	T	(Abadía-Cardoso et al. 2011)
AOmy233	Omy_107031-704	C	T	(Abadía-Cardoso et al. 2011)
AOmy234	Omy_107285-69	C	G	(Abadía-Cardoso et al. 2011)
AOmy235	Omy_107336-170	C	G	(Abadía-Cardoso et al. 2011)
AOmy237	Omy_107806-34	C	T	(Abadía-Cardoso et al. 2011)
AOmy238	Omy_108007-193	A	G	(Abadía-Cardoso et al. 2011)
AOmy239	Omy_109243-222	A	C	(Abadía-Cardoso et al. 2011)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy240	Omy_109525-403	A	G	(Abadía-Cardoso et al. 2011)
AOmy241	Omy_110064-419	T	G	(Abadía-Cardoso et al. 2011)
AOmy242	Omy_110078-294	A	G	(Abadía-Cardoso et al. 2011)
AOmy243	Omy_110362-585	G	A	(Abadía-Cardoso et al. 2011)
AOmy244	Omy_110689-148	A	C	(Abadía-Cardoso et al. 2011)
AOmy246	Omy_111084-526	A	C	(Abadía-Cardoso et al. 2011)
AOmy247	Omy_111383-51	C	T	(Abadía-Cardoso et al. 2011)
AOmy248	Omy_111666-301	T	A	(Abadía-Cardoso et al. 2011)
AOmy249	Omy_112301-202	T	G	(Abadía-Cardoso et al. 2011)
AOmy250	Omy_112820-82	G	A	(Abadía-Cardoso et al. 2011)
AOmy252	Omy_114976-223	T	G	(Abadía-Cardoso et al. 2011)
AOmy253	Omy_116733-349	C	T	(Abadía-Cardoso et al. 2011)
AOmy254	Omy_116938-264	A	G	(Abadía-Cardoso et al. 2011)
AOmy255	Omy_117259-96	T	C	(Abadía-Cardoso et al. 2011)
AOmy256	Omy_117286-374	A	T	(Abadía-Cardoso et al. 2011)
AOmy257	Omy_117370-400	A	G	(Abadía-Cardoso et al. 2011)
AOmy258	Omy_117540-259	T	G	(Abadía-Cardoso et al. 2011)
AOmy260	Omy_117815-81	C	T	(Abadía-Cardoso et al. 2011)
AOmy261	Omy_118175-396	T	A	(Abadía-Cardoso et al. 2011)
AOmy262	Omy_118205-116	A	G	(Abadía-Cardoso et al. 2011)
AOmy263	Omy_118654-91	A	G	(Abadía-Cardoso et al. 2011)
AOmy265	Omy_120255-332	A	T	(Abadía-Cardoso et al. 2011)
AOmy266	Omy_128996-481	T	G	(Abadía-Cardoso et al. 2011)
AOmy267	Omy_129870-756	C	T	(Abadía-Cardoso et al. 2011)
AOmy268	Omy_131460-646	C	T	(Abadía-Cardoso et al. 2011)
AOmy269	Omy_98683-165	A	C	(Abadía-Cardoso et al. 2011)
AOmy270	Omy_cyp17-153	C	T	WSU - J. DeKoning unpubl.
AOmy271	Omy_ftzf1-217	A	T	WSU - J. DeKoning unpubl.
AOmy272	Omy_GHSR-121	T	C	CRITFC - unpubl.
AOmy273	Omy_metA-161	T	G	CRITFC - unpubl.
AOmy274	Omy_UBA3b	A	T	(Hansen et al. 2011)
AOmy275	M09AAC.055	C	T	WDFW - S. Young unpubl.
AOmy276	M09AAE-082	T	G	WDFW - S. Young unpubl.
AOmy277	OMGH1PROM1-SNP1	A	T	(Abadía-Cardoso et al. 2011)
AOmy279	OMS00015	A	T	(Sánchez et al. 2009)
AOmy280	OMS00024	T	G	(Sánchez et al. 2009)
AOmy283	OMS00070	T	C	(Sánchez et al. 2009)
AOmy284	OMS00074	T	G	(Sánchez et al. 2009)
AOmy285	OMS00096	T	G	(Sánchez et al. 2009)
AOmy286	OMS00111	T	C	(Sánchez et al. 2009)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy288	OMS00149	T	G	(Sánchez et al. 2009)
AOmy289	OMS00173	T	C	(Sánchez et al. 2009)
AOmy290	Omy_105105-448	C	T	(Abadía-Cardoso et al. 2011)
AOmy291	Omy_110201-359	T	G	(Abadía-Cardoso et al. 2011)
AOmy292	Omy_128923-433	T	C	(Abadía-Cardoso et al. 2011)
AOmy293	Omy_anp-17	C	A	CRITFC - N. Campbell unpubl.
AOmy294	Omy_bcAKala-380rd	G	A	CRITFC - N. Campbell unpubl.
AOmy295	Omy_cin-172	C	T	CRITFC - N. Campbell unpubl.
AOmy296	Omy_ndk-152	A	G	CRITFC - N. Campbell unpubl.
AOmy297	Omy_nips-299	T	Deletion	CRITFC - N. Campbell unpubl.
AOmy298	Omy_ntl-27	G	A	CRITFC - N. Campbell unpubl.
AOmy299	Omy_rbm4b-203	Deletion	T	CRITFC - N. Campbell unpubl.
AOmy300	Omy_sys1-188	C	A	CRITFC - N. Campbell unpubl.
AOmy301	Omy_txnip-343	T	C	CRITFC - N. Campbell unpubl.
AOmy302	Omy_vamp5-303	A	Deletion	CRITFC - N. Campbell unpubl.
AOmy303	Omy_vatf-406	T	C	CRITFC - N. Campbell unpubl.
AOmy305	OMS00077	C	G	(Sánchez et al. 2009)
AOmy306	OMS00101	A	G	(Sánchez et al. 2009)
AOmy311	Omy_G3PD_2-371	C	A	CRITFC - N. Campbell unpubl.
AOmy320	Omy_redd1-410	C	T	CRITFC - N. Campbell unpubl.
AOmy322	Omy_srp09-37	C	T	CRITFC - N. Campbell unpubl.
AOmy324	Omy1011	C	A	(Hansen et al. 2011)
AOmy326	OMS00068	A	G	(Sánchez et al. 2009)
AOmy327	OMS00079	T	C	(Sánchez et al. 2009)
AOmy328	OMS00106	T	G	(Sánchez et al. 2009)
AOmy329	OMS00179	A	C	(Sánchez et al. 2009)
AOmy331	Omy_114587-480	T	G	(Abadía-Cardoso et al. 2011)
AOmy335	OMS00017	A	G	(Sánchez et al. 2009)
AOmy341	Omy_metB-138	T	A	CRITFC - unpubl.

Primer and probe sequences for unpublished loci available by request.

Table 2. List of species identification single nucleotide polymorphic (SNP) loci genotyped in Twisp River steelhead.

WDFW Name	Locus Name	Expected genotype			Reference
		<i>O. mykiss</i>	<i>O. clarkii clarkii</i>	<i>O. clarkii lewisi</i>	
ASpI001	Ocl_Okerca	T	C	C	(McGlaufflin et al. 2010)
ASpI014	Omy_F5_136	C	G	G	(Finger et al. 2009)
ASpI018	Omy_Omyclmk436-96	A	C	C	CRITFC - S. Narum - unpubl.

Primer and probe sequences for unpublished loci available by request.

Table 3. Twisp River sample inventory.

Collection Description	WDFW Code	# Received	# Genotyped ^a	Notes
Adult Steelhead, Wild origin, Twisp weir	12DI, 12DJ	123	102	
Adult Steelhead, Hatchery origin, Twisp weir	12DI, 12DJ, and 12DL	156	133	
Adult Steelhead Wild origin, Twisp weir, broodstock	12DL	26	24	

^a Only fish that had the possibility of spawning were genotyped.

Table 4. Genetic parameters for all Twisp River steelhead collections at all SNP loci. See attached MS Excel spreadsheet (Twisp_2012_adult_genotyping_Table_4.xlsx).