

Genetic profile for Makah NFH Winter Steelhead

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Prepared by

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National Fish Hatchery Broodstock Genetic Profile

- Facility:** Makah National Fish Hatchery
- Stock:** Winter Steelhead (*Oncorhynchus mykiss*)
- Parental stock:** Eggs transferred from Quinault NFH ^{Ref 1}.
- Year founded:** 1982. Additional eggs imported from Quinault NFH in two years in the late 1980s when the local steelhead (including natural-origin juveniles in the Tsoo-Yess River) were eliminated as a measure to control VHS ^{Ref 1}.
- Generation time:** 3 years (90%) or 4 years (10%) ^{Ref 2}.

Segregation / Integration history: Segregated. The Quinault NFH stock was selectively bred and managed to return earlier than the natural-origin run to support harvest ^{Ref 1}. MNFH has preserved this earlier run timing through timing of broodstock collection. Presently, the electric weir is turned off on March 1 and it is assumed that only natural-origin fish are returning and spawning after that time.

Table 1 - Broodstock samples analyzed:

Description	Year	n	Life stage	Data source
Makah NFH	1997	94	returning adult	AFTC
Makah NFH	2008	94	returning adult	AFTC
Makah NFH	2009	68	returning adult	AFTC
Makah NFH	2011	94	returning adult	AFTC

Table 2 – Steelhead samples analyzed for comparison:

Description	H/W	Year	n	Life stage	Data source*
Tsoo-Yess River trap	W	2012	49	smolt	AFTC
Quinault NFH	H	2000	66	returning adult	AFTC
Quinault NFH	H	2008	145	returning adult	WDFW
Quinault NFH	H	2010	95	returning adult	AFTC
Quinault NFH	H	2011	90	returning adult	AFTC
Lake Quinault Net Pens	H	2010	149	returning adult	AFTC
Lake Quinault Net Pens	H	2011	94	returning adult	AFTC
Salmon River Hatchery	H	2008	134	returning adult	WDFW
Bogachiel Hatchery	H	2008	144	returning adult	WDFW
S. F. Hoh River	W	2008	19	returning adult	WDFW
upper Hoh River	W	2009	45	returning adult	WDFW
upper Hoh River	W	2010	44	returning adult	WDFW
Bogachiel River	W	2009	13	returning adult	WDFW
Bogachiel River	W	2010	12	returning adult	WDFW
Calawah River	W	2009	17	returning adult	WDFW
Calawah River	W	2010	19	returning adult	WDFW
Solduc River	W	2009	114	returning adult	WDFW
Solduc River	W	2010	67	returning adult	WDFW
Queets River	W	2010	27	returning adult	WDFW
Salmon River	W	2010	19	returning adult	WDFW

*Data provided by WDFW were originally described in Ref 3.

Genetic markers analyzed:

SPAN microsatellites^{Ref 4} (11 / 13 loci). A complete list of the markers analyzed is provided in Appendix 1. Genotyping success rates are presented in Appendix 2.

Table 3 - Diversity within samples. Expected (H_e) and observed (H_o) heterozygosity, allelic richness (AR), number of loci exhibiting departures from Hardy-Weinberg Equilibrium (HWE), F_{IS} (a measure of departure from random mating), percent of pairwise tests for linkage disequilibrium yielding significant results (LD), and effective population size (N_e) based on LD are listed for each sample. Samples in which we observed no evidence for any disequilibrium caused by genetic drift due to a finite number of parents (i.e. those with an estimated N_e of infinity) have N_e marked by “-“.

No	Description	He	Ho	AR	HWE	FIS	LD (%)	Ne
1	Makah NFH 1997	0.77	0.78	6.68	0	-0.01	21.8%	137 (91-244)
2	Makah NFH 2008	0.76	0.72	6.49	6	0.06	67.3%	38 (29-51.2)
3	Makah NFH 2009	0.74	0.75	6.25	0	-0.02	14.5%	110 (73-201.6)
4	Makah NFH 2011	0.76	0.76	6.54	1	0.00	49.1%	111 (79-174.5)
5	Tsoo-Yess River 2012	0.81	0.79	7.61	0	0.02	3.6%	-
6	Quinault NFH 2000	0.77	0.76	6.56	0	0.01	5.5%	799 (201-Infinite)
7	Quinault NFH 2008	0.77	0.78	6.50	0	-0.01	27.3%	119 (93-157.1)
8	Quinault NFH 2010	0.78	0.78	6.53	3	0.00	10.9%	104 (72-169.7)
9	Quinault NFH 2011	0.76	0.75	6.40	2	0.01	9.1%	161 (101-337)
10	Lake Quinault Net Pens 2010	0.75	0.76	6.04	8	-0.01	89.1%	27 (23-30.5)
11	Lake Quinault Net Pens 2011	0.77	0.78	6.17	2	0.00	30.9%	46 (36-59.4)
12	Salmon River Hatchery 2008	0.77	0.78	6.51	1	-0.01	34.5%	90 (69-120)
13	Bogachiel Hatchery 2008	0.80	0.80	7.07	0	-0.01	25.5%	137 (98-207.6)
14	S. F. Hoh River 2008	0.82	0.79	7.70	0	0.04	3.6%	275 (58-Infinite)
15	upper Hoh River 2009	0.83	0.82	8.24	0	0.01	1.8%	-
16	upper Hoh River 2010	0.79	0.79	7.70	0	0.01	5.5%	-
17	Bogachiel River 2009	0.79	0.75	7.64	0	0.05	0.0%	-
18	Bogachiel River 2010	0.79	0.85	7.74	0	-0.07	0.0%	1454 (38-Infinite)
19	Calawah River 2009	0.81	0.79	7.39	1	0.02	0.0%	-
20	Calawah River 2010	0.82	0.81	8.02	0	0.02	0.0%	-
21	Solduc River 2009	0.80	0.78	7.55	1	0.02	1.8%	779 (234-Infinite)
22	Solduc River 2010	0.78	0.80	7.04	6	-0.03	60.0%	27 (22-33.7)
23	Queets River 2010	0.76	0.73	6.54	1	0.05	5.5%	99 (43-Infinite)
24	Salmon River 2010	0.76	0.74	6.68	1	0.03	3.6%	-

Figure 1 – Correspondence Analysis (CA) of allele frequencies observed in samples from Makah NFH and other Olympic Peninsula winter steelhead populations. Sample numbers are those listed in Table 3. Axis 1 and 2 accounted for 19.9%, and 14.3% of the variance, respectively.

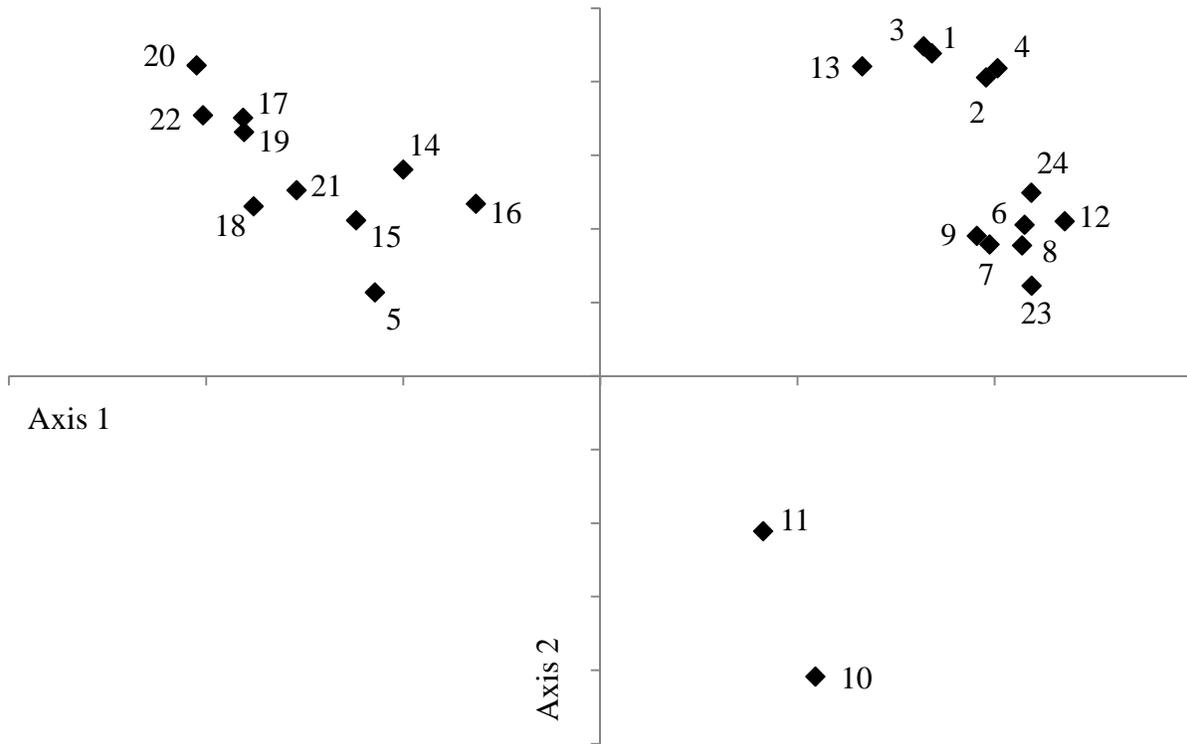
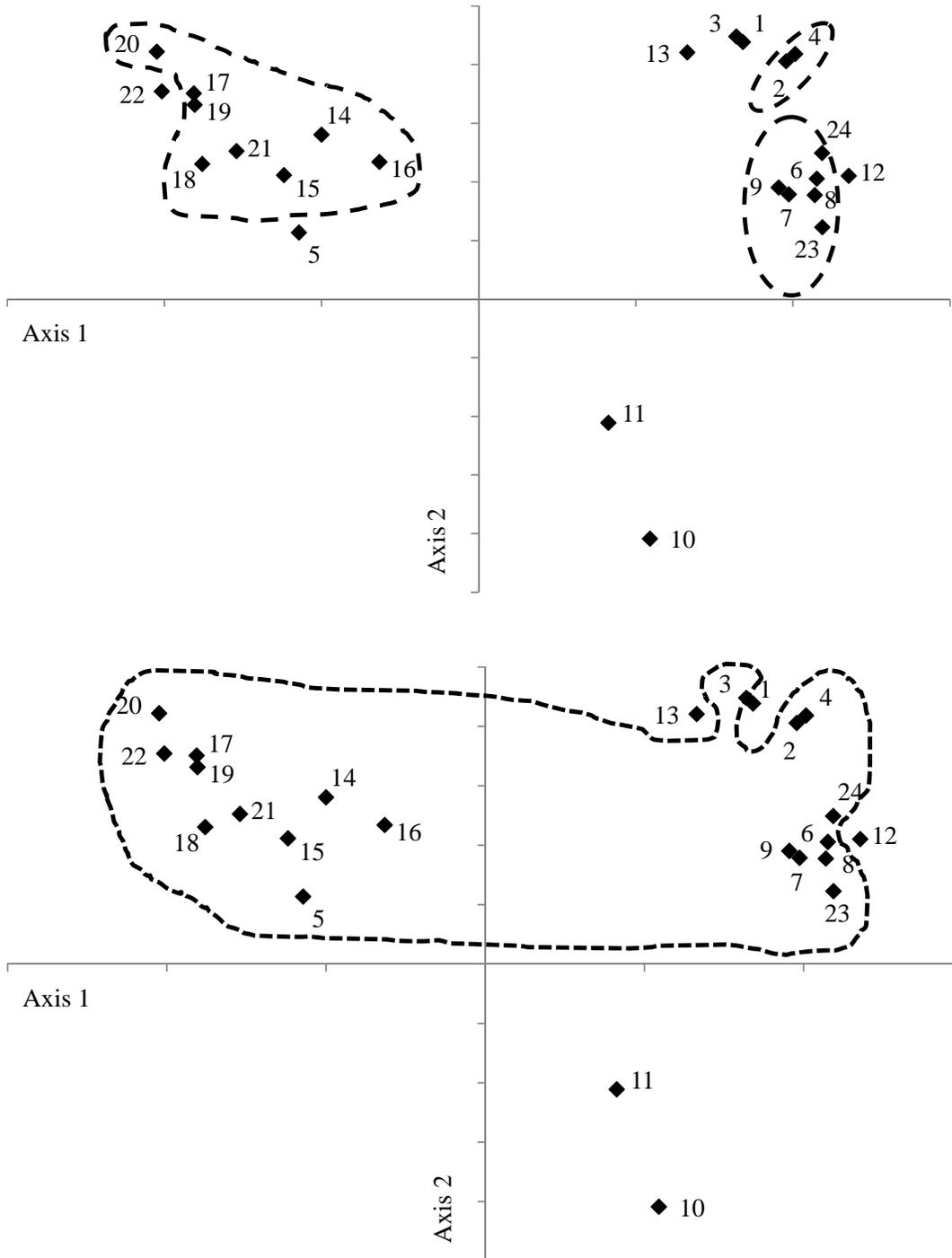


Figure 2 - Statistical tests of divergence among samples from Makah NFH and other Olympic Peninsula winter steelhead populations. Sample numbers are those listed in Table 3. Dashed lines indicate groups of samples lacking statistically different allele frequencies (top) and statistically significant F_{ST} values (bottom).



Comments

- The goals of this report series are 1) to summarize available genetic information for NFH broodstocks and make that information available to hatchery managers, and 2) to make sure that data for the NFH broodstocks are available for internal hatchery reviews and HET meetings, as well as to our partners.
- Measures of genetic diversity (H_e , H_o , AR) were generally slightly lower in hatchery populations (including Makah NFH) than in wild populations, and LD was generally greater in hatchery populations (although Solduc River 2010 is one example of an exception).
- The Tsoo-Yess River collection exhibited greater genetic diversity and effective size (N_e), and lower LD than any of the Makah NFH samples.
- The correspondence analysis plot was concordant with previous work (Ref 3) in identifying two divergent groups of winter steelhead on the Olympic Peninsula; a hatchery group and a wild group. Samples from Makah NFH were relatively similar to each other (with some statistically significant differences among years), and clustered with the hatchery group. The Tsoo-Yess River collection, in contrast, clustered with the wild group. Note that F_{ST} between one collection which clustered with hatchery populations (24) and one that clustered with the wild populations (14) was not significant, but this result should be interpreted with caution given the small sample sizes involved.
- Our results provide no evidence of gene flow between the Makah NFH stock and the naturally spawning population of winter steelhead in the Tsoo-Yess River. Return timing differences between natural-origin and hatchery-origin populations may be a key factor keeping the two populations separate. Hatchery spawning protocols which prevent integration of wild individuals into the broodstock might help to reinforce this difference.
- It should be noted that our results for the Tsoo-Yess River are based on a single sample of juvenile fish. Incorporation of samples collected in one or two more years would improve our confidence in these results.

Sources cited

1. USFWS Columbia Basin Hatchery Review Team. 2009. Quilcene, Quinault and Makah National Fish Hatcheries: Assessments and Recommendations. Final Report, May 9, 2009. Hatchery Review Team, Pacific Region. U.S. Fish and Wildlife Service, Portland, Oregon. Available:
http://www.fws.gov/pacific/Fisheries/Hatcheryreview/Reports/olypen/Olympic_PeninsulaNFHReview_FinalReport_May2009_FINAL.pdf
2. Based on tag recoveries in return year 2004-2005. Data provided by WWFRO.
3. Kassler, T.W., Gilbertson, J., Brenkman, S., Gross, M., Low, D., and Spidle, A. 2010. Genetic Relationships among hatchery- and natural-origin winter steelhead (*Oncorhynchus mykiss*) from Washington's Olympic Peninsula with an Emphasis on the Hoh River. Washington Department of Fish and Wildlife Final Report: 37 pp.
4. Stephenson JJ, Campbell MR, Hess JE, et al. (2009) A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. *Conservation Genetics* 10, 1145-1149.

Availability

Genotype data and allele frequencies for NFH stocks are available from Abernathy Fish Technology Center upon request.

Disclaimer

The findings and conclusions in this report are those of the author and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Appendix 1. Eleven microsatellite markers used to analyze Makah NFH winter steelhead.

Microsatellite markers	
1	Ogo4
2	Oke4
3	Omy1001
4	Omy1011
5	Omy7i
6	One14
7	Ots100
8	Ots3
9	Ots4
10	Ssa407
11	Ssa408

Appendix 2. Genotype call rates (completeness of data). Shaded cells indicate data that are <90% complete.

Description	Year	Microsatellite call rate
Makah NFH	1997	0.997
Makah NFH	2008	0.996
Makah NFH	2009	0.996
Makah NFH	2011	0.995
Tsoo-Yess River	2012	1.000
Quinault NFH	2000	0.986
Quinault NFH	2008	0.996
Quinault NFH	2010	0.981
Quinault NFH	2011	0.970
Lake Quinault Net Pens	2010	0.996
Lake Quinault Net Pens	2011	0.997
Salmon River Hatchery	2008	0.989
Bogachiel Hatchery	2008	0.993
S. F. Hoh River	2008	0.990
upper Hoh River	2009	0.992
upper Hoh River	2010	0.977
Bogachiel River	2009	0.965
Bogachiel River	2010	0.985
Calawah River	2009	1.000
Calawah River	2010	0.990
Solduc River	2009	0.990
Solduc River	2010	0.991
Queets River	2010	0.997
Salmon River	2010	1.000

Appendix 3. Pairwise F_{ST} values between collections of steelhead. Sample numbers are those listed in Table 3. Shaded cells indicate non-significant results (pairwise F_{ST} values not $>95\%$ of a null distribution in which individuals were permuted among samples).

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	0.006	0.007	0.008	0.021	0.015	0.018	0.016	0.015	0.042	0.036	0.022	0.017	0.017	0.017	0.018	0.028	0.019	0.026	0.028	0.025	0.027	0.016	0.010
2	-	0.002	0.000	0.023	0.015	0.016	0.019	0.015	0.037	0.040	0.026	0.021	0.013	0.021	0.016	0.028	0.025	0.029	0.031	0.024	0.030	0.017	0.003
3		-	0.001	0.022	0.017	0.017	0.022	0.017	0.038	0.042	0.030	0.025	0.012	0.020	0.011	0.030	0.026	0.028	0.030	0.022	0.027	0.020	0.006
4			-	0.019	0.015	0.016	0.017	0.016	0.037	0.037	0.026	0.021	0.012	0.022	0.017	0.028	0.028	0.031	0.030	0.024	0.031	0.020	0.005
5				-	0.018	0.019	0.019	0.019	0.025	0.028	0.030	0.021	0.003	0.008	0.013	0.008	0.010	0.015	0.019	0.012	0.026	0.019	0.019
6					-	-0.002	0.001	-0.001	0.027	0.027	0.011	0.018	0.015	0.019	0.015	0.023	0.021	0.025	0.023	0.019	0.032	0.003	0.002
7						-	0.002	0.000	0.026	0.027	0.013	0.020	0.015	0.019	0.015	0.025	0.019	0.024	0.024	0.018	0.030	0.002	0.001
8							-	0.004	0.029	0.030	0.010	0.019	0.017	0.021	0.017	0.023	0.025	0.028	0.029	0.021	0.033	0.003	0.002
9								-	0.027	0.024	0.015	0.018	0.013	0.017	0.014	0.026	0.016	0.020	0.023	0.018	0.025	0.001	0.001
10									-	0.033	0.043	0.045	0.027	0.030	0.026	0.047	0.034	0.034	0.044	0.032	0.045	0.016	0.028
11										-	0.042	0.035	0.023	0.023	0.030	0.036	0.019	0.028	0.035	0.029	0.034	0.029	0.031
12											-	0.024	0.027	0.027	0.027	0.036	0.034	0.038	0.037	0.034	0.047	0.012	0.012
13												-	0.019	0.025	0.022	0.026	0.024	0.025	0.025	0.025	0.035	0.020	0.014
14													-	-0.002	-0.001	0.002	0.007	-0.004	0.008	0.002	0.007	0.016	0.009
15														-	0.003	0.011	0.000	0.001	0.005	0.004	0.009	0.021	0.018
16															-	0.017	0.010	0.008	0.007	0.008	0.012	0.015	0.006
17																-	0.008	-0.003	0.012	0.000	0.012	0.030	0.025
18																	-	0.003	0.007	0.001	0.006	0.018	0.022
19																		-	0.002	-0.001	0.001	0.021	0.021
20																			-	0.006	0.014	0.033	0.024
21																				-	0.010	0.022	0.019
22																					-	0.033	0.026
23																						-	-0.004

Appendix 4. Glossary

Allele – A unique genetic character state. Each locus has two alleles.

Allelic richness – The number of alleles observed in a sample of individuals, corrected for unequal sample sizes by rarefaction.

Effective population size (N_e) – The number of individuals in a model population which would lose genetic variation at the same rate as an observed population. Deviations from model behavior in real populations (e.g. unequal sex ratios, some individuals reproducing more than others, etc...) tend to make N_e lower than census size (N).

F_{IS} – Correlation of alleles in an individual relative to the subpopulation in which it occurs. Commonly used as a measure of departure from random mating within a subpopulation.

F_{ST} – Correlation of alleles within the same subpopulation relative to the entire population. Commonly used as a measure of divergence between subpopulations.

Gene flow – Movement of genetic material from one population to another. Implies both physical movement and successful integration into the recipient population.

Genetic Drift – Process of genetic divergence between populations based on random sampling of alleles each generation.

Heterozygosity – Proportion of individuals in a population that are heterozygotes (i.e. do not have two identical alleles at a locus).

Hardy-Weinberg Equilibrium (HWE) – Genotype ratios expected under a random mating model.

Linkage Disequilibrium (LD) – A measure of departure from independence of alleles in a pair of loci.

Locus – A physical location on the DNA of an organism. The term “locus” is often used synonymously with “marker” or with any type of marker (e.g., “SNP” or “microsatellite”).