

Supplementary Materials

Table S1. Summary alignment and quality filtering statistics for read pairs across the 1,123 samples retained for downstream analyses from the original RADsequencing of 1,334 samples.

	Population	Sample Size	Sum of Quality Filtered Reads	Sum of Clone Filtered Read Pairs	Sum of Mapped Read Pairs	Average Percent Reads Mapped
Pre-Dam Removal	Above the Dams (AD)	208	214853211	74787568	146324196	97.48
	In Between the Dams (ID)	169	194678581	70081716	137204353	97.46
	South Branch of the Little River (SBLR)	86	89071386	34666100	67962209	97.39
	Below the Dams (BD)	104	128606386	37176209	72791991	97.41
Post-Dam Removal	Above the Dams (AD)	3	6201694	4174262	8142605	97.15
	In Between the Dams (ID)	304	480555579	317623165	621223732	97.15
	Below the Dams (BD)	249	597275595	323771647	633175507	97.35

Table S2. Mean population genetics statistics calculated in VCFtools (π , Tajima's D, A_{jk}), NeEstimator v2 (N_e), and the inbreeding coefficient (F_{IS}) temporally, across sampling location, among life history forms, and across life history cohorts. Confidence intervals represent non-parametric bootstrapped 95% confidence intervals for all estimates except for N_e which represent internally calculated 95% credible intervals.

		Nucleotide Diversity (π)	Tajima's D	Relatedness (A_{jk})	Inbreeding Coefficient (F_{IS})	Effective Population Size (N_e)	Total non-polymorphic loci	Number of Individuals
Prior to dam removal	All	0.056 + 0.078	-0.42 + 0.58	0.0017 + 0.072	0.030 + 0.10	80.3.1 (66.6-97.0)	1,230	567
		0.056 + 0.080	-0.41 + 0.518	0.0021 + 0.081	0.032 + 0.10	58.4 (49.7-68.5)	1,617	463
	Unknown	0.053 + 0.081	-0.60 + 0.553	0.0090 + 0.140	-0.0060 + 0.10	141.3 (102.8-213.8)	7,152	104
		0.053 + 0.083	-0.46 + 0.578	0.0047 + 0.11	0.027 + 0.11	120.9 (97.1-154.6)	7,563	208
	AD	0.061 + 0.085	-0.449 + 0.562	0.0057 + 0.11	0.028 + 0.11	150.7	4,127	169
	ID							

	SBLR	0.044 + 0.11	-0.082 + 0.899	0.018 + 0.16	0.011 + 0.12	(109.4-114.7) 38.3 (26.5-58.3)	28,727	86
	BD	0.053 + 0.081	-0.60 + 0.556	0.0090 + 0.14	-0.038 + 0.16	141.3 (102.8-213.8)	7,144	104
Post dam removal	All (Steelhead)	0.058 + 0.083	-0.361 + 0.572	0.002 + 0.066	0.011 + 0.078	139.7 (112.5-171.8)	3,786	556
	Adults Pre 2015	0.054 + 0.086	-0.624 + 0.59	0.017 + 0.19	0.0079 + 0.11	159.6 (99.3-356.4)	10,159	57
	Adults 2015	0.057 + 0.090	-0.437 + 0.620	0.006 + 0.12	0.011 + 0.11	63.2 (52.6-76.9)	5,913	172
	Adults 2016	0.060 + 0.10	-0.645 + 0.65	0.0047 + 0.11	0.0065 + 0.17	132.1 (80.6-332.1)	16,008	24
	Adults 2017	0.061 + 0.10	-0.674 + 0.665	0.051 + 0.32	-0.002 + 0.167	44 (21-406.8)	17,949	18
	Juveniles 2016	0.058 + 0.082	-0.511 + 0.552	0.0059 + 0.11	0.0067 + 0.10	264.9 (194.3-311.8)	2,176	166
	Juveniles 2017	0.057 + 0.083	-0.56 + 0.554	0.0081 + 0.13	0.003 + 0.12	341.2 (323.2-347.4)	3,570	119

Table S3. The results from simulations performed on all 479 individuals included in the reference collections reflected mixed results for accuracy of assignment of individual fish back to their respected reference collections, or sampling sites, based on their genotypes. The probabilities for self-assignment of individuals back to their sampling site were calculated by taking the mean of the scaled likelihoods. The scaled likelihoods reflect the probability of assigning an individual fish to the inferred collection.

Sampling Site	Inferred Sampling Site													
	Chicago Camp	Wilder	Hayes	Elkhorn	Geyser	Cat Creek	Altaire	Campground Creek	Madison Creek	Little River	Indian Creek	Aldwell	South Branch Little River	Elwha River
Chicago Camp	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Wilder	0.000	0.246	0.156	0.442	0.043	0.114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hayes	0.000	0.272	0.215	0.384	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Elkhorn	0.000	0.032	0.078	0.823	0.066	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Geyser	0.000	0.027	0.007	0.319	0.542	0.105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cat Creek	0.000	0.000	0.000	0.015	0.985	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Altaire	0.000	0.000	0.000	0.000	0.000	0.004	0.314	0.341	0.074	0.132	0.000	0.136	0.000	0.000
Campground Creek	0.000	0.000	0.000	0.000	0.000	0.105	0.239	0.233	0.102	0.193	0.000	0.128	0.000	0.000
Madison Creek	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.033	0.581	0.259	0.000	0.081	0.000	0.000
Little River	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.917	0.000	0.023	0.000	0.000
Indian Creek	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
Aldwell	0.000	0.000	0.000	0.000	0.000	0.000	0.255	0.274	0.125	0.250	0.000	0.096	0.000	0.000
South Branch Little River	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.976	0.012
Elwha River	0.000	0.000	0.000	0.000	0.000	0.148	0.006	0.001	0.011	0.001	0.000	0.000	0.000	0.833

Table S4. Inferred reporting units for the five individuals that had low (<0.9) posterior probabilities of membership to their inferred collection. .

Life History Cohort	Sample ID	Inferred Population	Inferred Sample Site	Posterior Probability of Membership	Log Likelihood
Pre Dam Removal	34188_008	ID	Altaire	0.551	-191.159
Juvenile 2016	51659_M_042816_smo_2	AD	Elkhorn	0.888	-234.457
Pre Dam Removal	33649_39	ID	Aldwell	0.660	-231.978
Adult Pre 2015	51029_E13_004b	AD	Cat Creek	0.720	-247.024
Juvenile 2017	51786_M_050217_28	AD	Cat Creek	0.882	-233.127

Table S5. The mean posterior probability of membership to reference collections for the 86 individuals not included in the reference sampling set due to incongruent sampling location and DAPC population assignments..

Sampling Sites	Sample ID	Above the Dams (AD)						In Between the Dams (ID)						South Branch Little River (SBLR)	Below the Dams (BD)
		Chicag o Camp	Wilder	Hayes	Elkhorn	Geyser	Cat Creek	Altaire	Campgrou nd Creek	Madiso n Creek	Little River	Indian Creek	Aldwel l		
Elkhorn	33696_011	0	0	0.01	0	0.988	0.002	0	0	0	0	0	0	0	0
Geyser	34186_032	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Geyser	34186_041	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_001	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_002	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_003	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Whiskey Bend	34185_004	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Whiskey Bend	34185_005	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_006	0	0	0	0	0	0	0.219	0.781	0	0	0	0	0	0
Whiskey Bend	34185_007	0	0	0	0	0	0	0.775	0.004	0.214	0.006	0	0	0	0
Whiskey Bend	34185_008	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Whiskey Bend	34185_009	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_010	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Sampling Sites	Sample ID	Above the Dams (AD)					In Between the Dams (ID)							South Branch Little River (SBLR)	Below the Dams (BD)
		Chicag o Camp	Wilder	Hayes	Elkhorn	Geyser	Cat Creek	Altaire	Campgrou nd Creek	Madiso n Creek	Little River	Indian Creek	Aldwel l		
Whiskey Bend	34185_011	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_012	0	0	0	0	0	0	0.801	0.003	0.195	0	0	0.001	0	0
Whiskey Bend	34185_013	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Whiskey Bend	34185_014	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_015	0	0	0	0	0	0	0.001	0	0	0.999	0	0	0	0
Whiskey Bend	34185_016	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_017	0	0	0	0	0	0	0.149	0.003	0.849	0	0	0	0	0
Whiskey Bend	34185_018	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_019	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Whiskey Bend	34185_020	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_021	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Whiskey Bend	34185_022	0	0	0	0	0	0	0.026	0	0	0.974	0	0	0	0
Whiskey Bend	34185_023	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Whiskey Bend	34185_024	0	0	0	0	0	0	0.002	0	0	0.997	0	0	0	0
Whiskey Bend	34185_025	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Sampling Sites	Sample ID	Above the Dams (AD)					In Between the Dams (ID)							South Branch Little River (SBLR)	Below the Dams (BD)
		Chicago Camp	Wilder	Hayes	Elkhorn	Geyser	Cat Creek	Altaire	Campground Creek	Madison Creek	Little River	Indian Creek	Aldwell		
Cat Creek	34188_001	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cat Creek	34188_003	0	0	0	0	0	0.948	0	0	0	0.052	0	0	0	0
Cat Creek	34188_004	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Cat Creek	34188_005	0	0	0	0.781	0	0	0	0	0	0	0	0	0	0.22
Cat Creek	34188_006	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cat Creek	34188_007	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cat Creek	34188_009	0	0	0	0	0	0	0.073	0.927	0	0	0	0	0	0
Cat Creek	34188_010	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cat Creek	34188_012	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Cat Creek	34188_013	0	0	0	0	0	0	0.999	0	0	0.001	0	0	0	0
Cat Creek	34188_014	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cat Creek	34188_015	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cat Creek	34188_016	0	0	0	0	0	0	0.001	0.991	0	0	0	0.008	0	0
Cat Creek	34188_019	0	0	0	0	0	0	0	0	0.002	0.998	0	0	0	0
Cat Creek	34188_021	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Sampling Sites	Sample ID	Above the Dams (AD)					In Between the Dams (ID)							South Branch Little River (SBLR)	Below the Dams (BD)
		Chicago Camp	Wilder	Hayes	Elkhorn	Geyser	Cat Creek	Altaire	Campground Creek	Madison Creek	Little River	Indian Creek	Aldwell		
Cat Creek	34188_022	0	0	0	0	0	0	0	0	0.129	0.871	0	0	0	0
Cat Creek	34188_011	0	0	0	0	0	0.932	0.067	0	0	0	0	0	0	0
Altaire	33694_027	0	0	0	0	0	0	0	0.027	0.965	0.004	0	0.005	0	0
Altaire	33694_058	0	0	0	0	0	0	0.995	0.003	0	0.002	0	0	0	0
Altaire	33694_095	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Altaire	33694_097	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Altaire	34189_003	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Campground Creek	34190_001	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Campground Creek	34190_005	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Campground Creek	34190_008	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Campground Creek	34190_012	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Campground Creek	34190_017	0	0	0	0	0	0	0.002	0.998	0	0	0	0	0	0
Campground Creek	34190_019	0	0	0	0	0	0	0.965	0.035	0	0	0	0	0	0
Campground Creek	34190_020	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Campground Creek	34190_024	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Figures

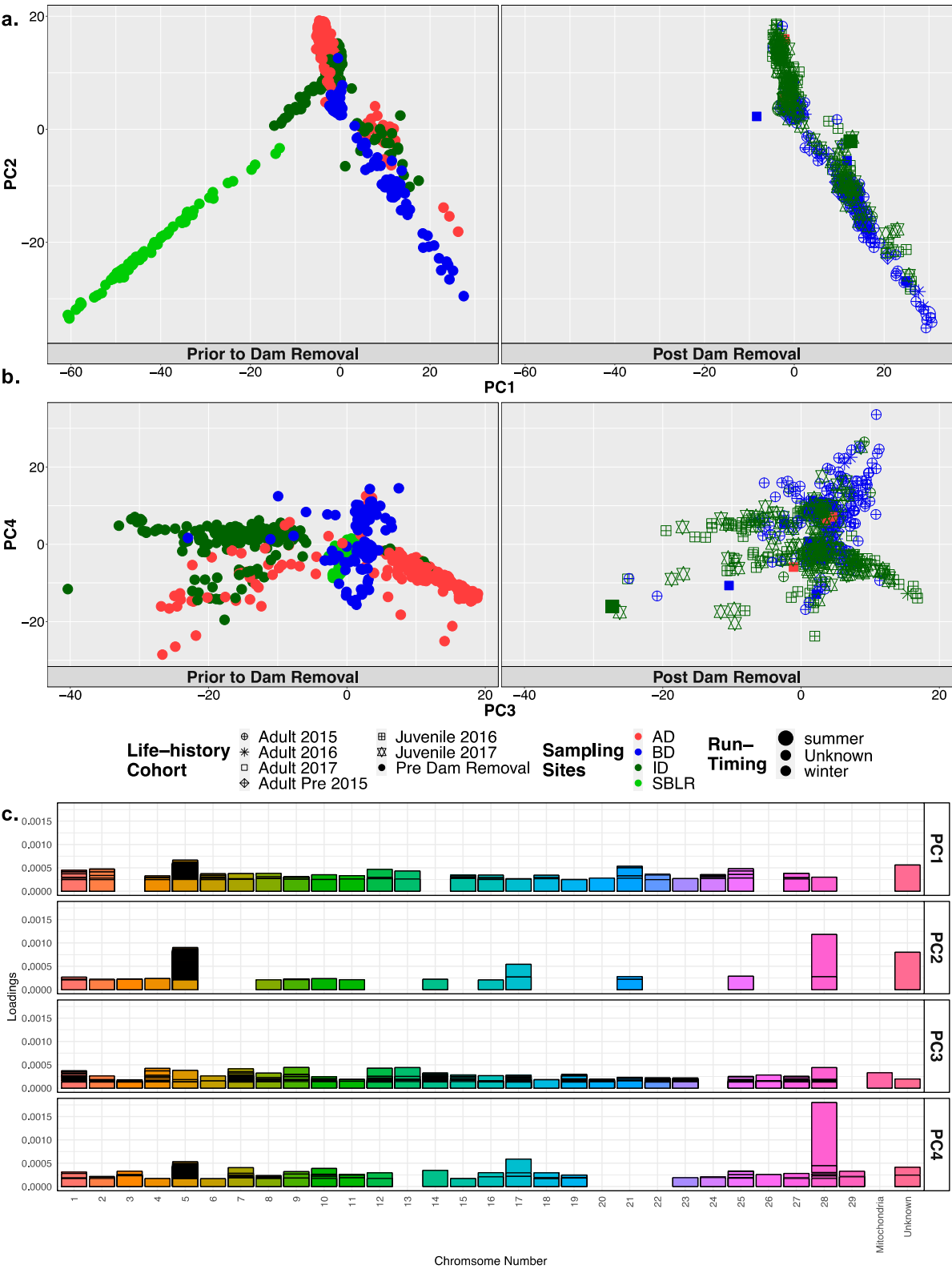


Figure S1. There were some discernible clustering patterns in the PCA conducted among all 71,320 SNPs used in this study among the samples collected prior to or following dam removal. Specifically, the South Branch of the Little River appeared to split from most of the AD, ID and BD populations along principal component one (proportion of variance explained = 2.87%) and from the remainder of the ID population along principal component two (2.12%) (a). There were no distinct clustering patterns in the PCA for principal components three (1.22%) and four (0.83%) (b). Points were colored based on the sampling site location relative to anadromous barrier. Point shapes were reflective of life history cohort and point size was indicative of known migratory life history phenotype. A large number of loci on Chromosome 5 (Omy5) loaded on the first, second, and fourth principal components and a small number of loci on Chromosome 28 (Omy28) with large loadings loaded on the second and fourth principal components (c). Each bar includes the top 1% SNPs across chromosomes loading on individual PCs. Black boxes within each bar represent a single SNP and the width of the black box is proportional to the cumulative proportion of the loadings explained by each SNP.

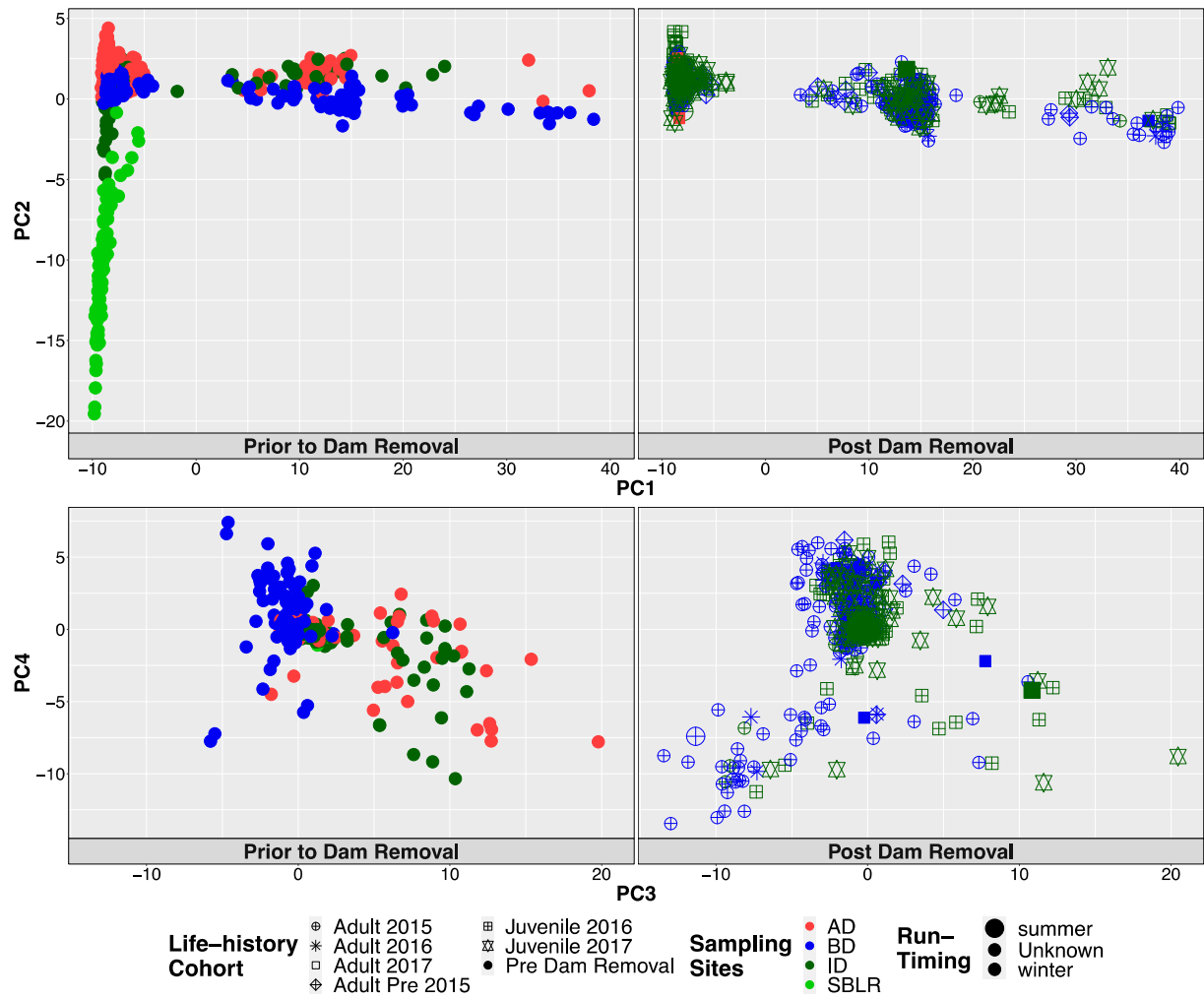


Figure S2. There were three distinct clusters in the PCA conducted among all 2,552 SNPs called on Omy5 among the samples collected prior to or following dam removal among principal components one (proportion of variance explained = 27.16%) and two (2.08%) (a), or three (1.59%) and four (1.28%) (b). Points were colored based on the sampling site location relative to anadromous barrier. Point shapes were reflective of life history cohort and point size was indicative of known migratory life history phenotype.

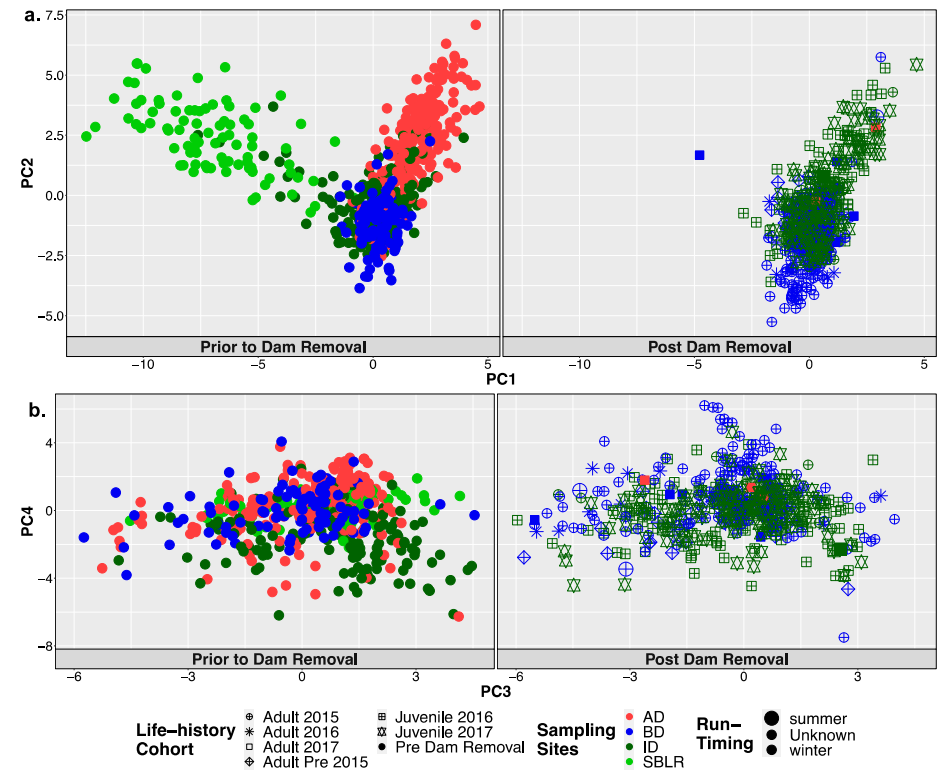


Figure S3. There were no discernible clustering patterns in the PCA conducted among the 202 SNPs called on Omy28 among the samples collected prior to or following dam removal among principal components one (proportion of variance explained = 3.18%) and two (2.37%) (a), or three (1.63%) and four (1.46%) (b). Points were colored based on the sampling site location relative to anadromous barrier. Point shapes were reflective of life history cohort and point size was indicative of known migratory life history phenotype.

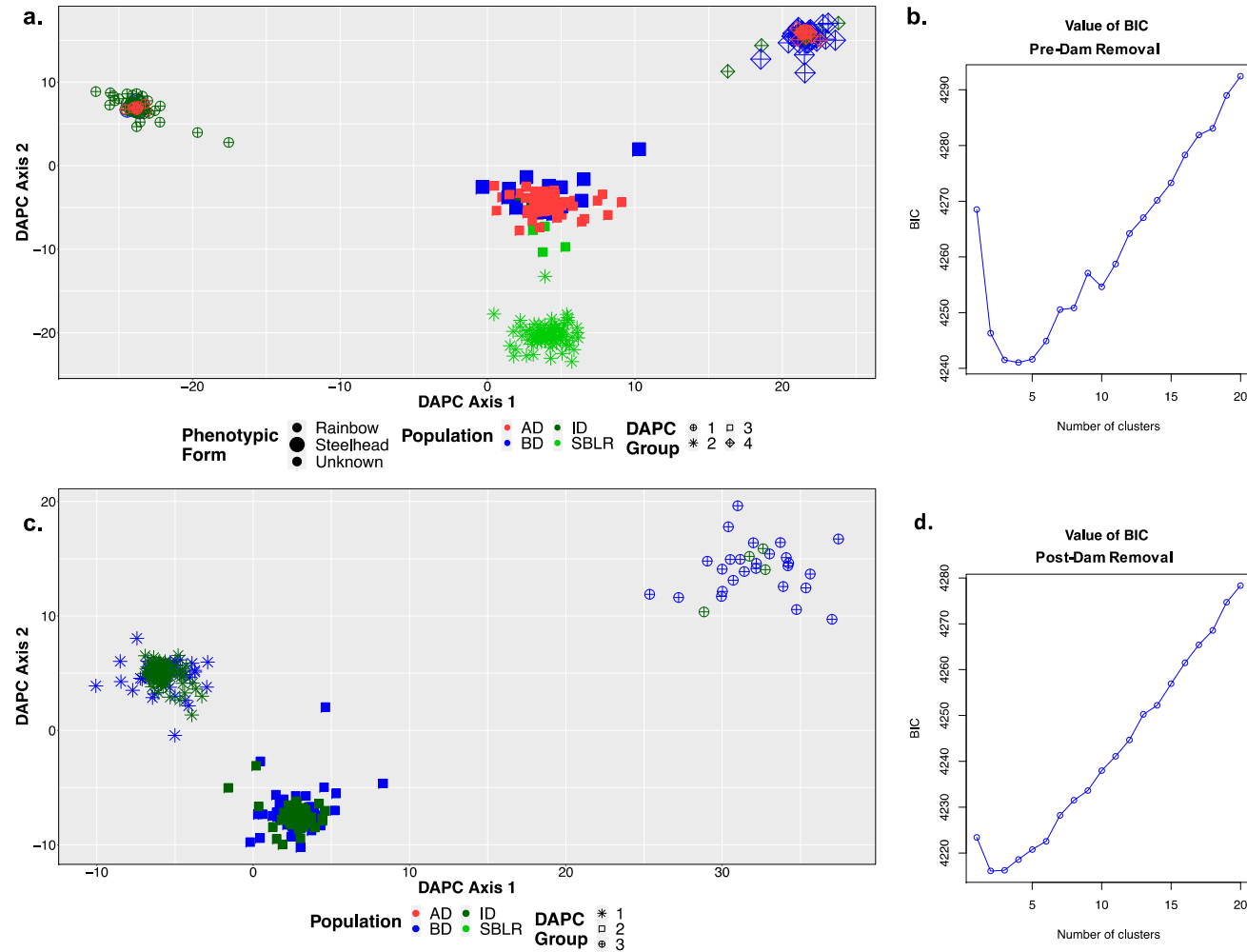


Figure S4. (a) DAPC results for the second-best K value (K=4) prior to dam removal and the (b) BIC plot. (c) The second-best supported structure model in DAPC post-dam removal was K=3 genetic clusters, (d) as shown by the elbow in the BIC plot. Shapes were indicative of population assignment by DAPC and color was based on sampling site location relative to the dams. Larger points in (a, b) were indicative of the Steelhead life history phenotypic form while smaller points were indicative of the resident Rainbow Trout form or fish that could not be categorized in either form. The elbow for BIC plots generated in DAPC genetic

structure analysis of RAD-Sequenced samples supported (a) $K=3$ genetic clusters pre-dam removal (b) and $K = 2$ genetic clusters post-dam removal shown in Figure 4.

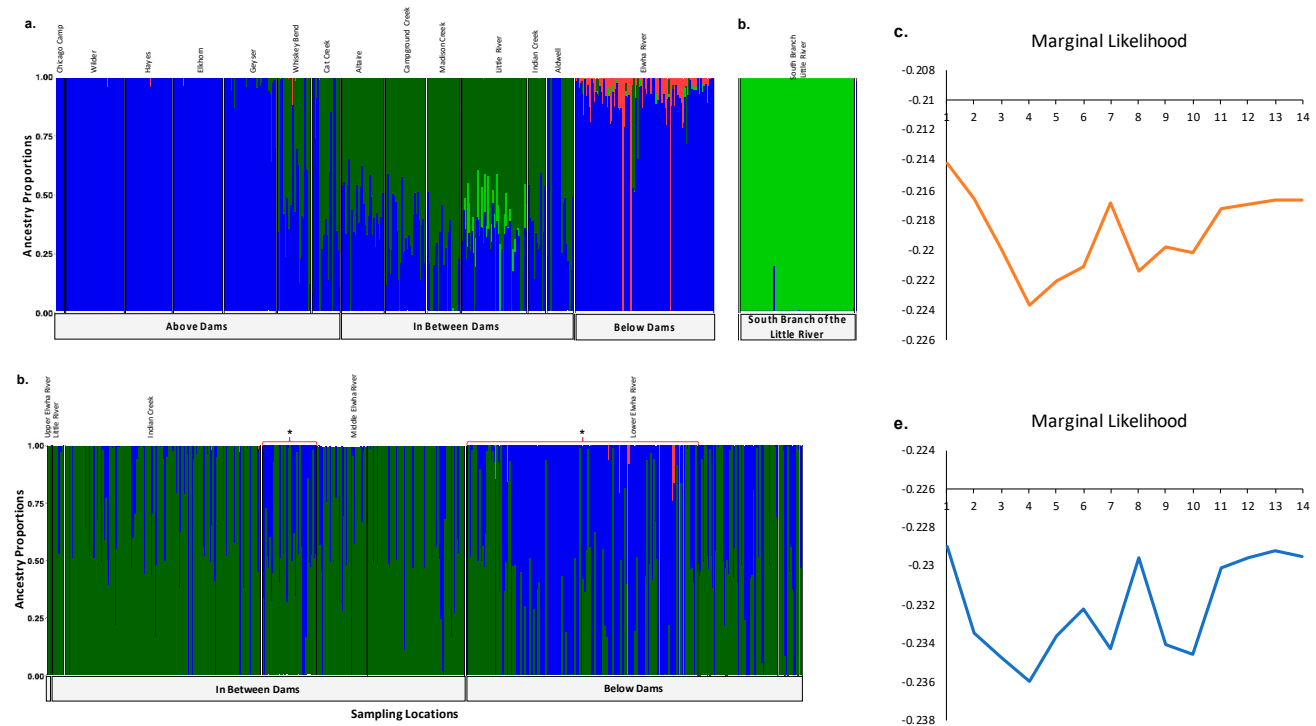
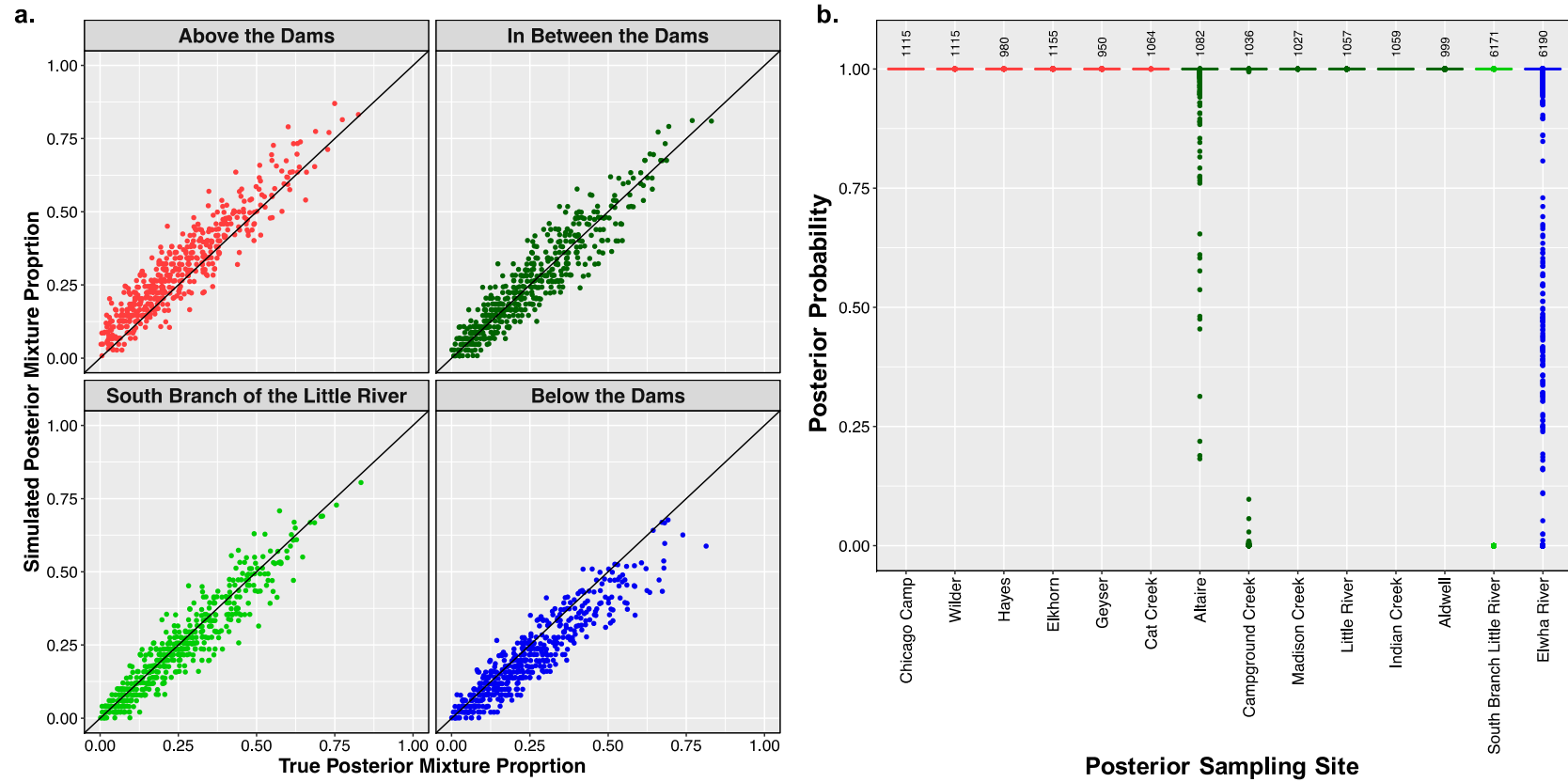


Figure S5. FastSTRUCTURE population assignments for samples collected (a-b) prior to dam removal and supported four distinct genetic clusters ($K=4$). (a) High levels of genetic structure were observed among sampling locations split by dams (b) and natural barriers including South Branch Falls. (c) Marginal likelihood values were lowest at $K=4$ prior to dam removal, but model components that explained structure support a K -value of 3 (Figure 4). (c) Post dam removal, we detected three genetic clusters respectively. (e) Marginal likelihood values were lowest at $K=3$ post-dam removal, but model components that explained structure support $K=2$ (Figure 4). Each vertical bar represents on fastSTRUCTURE plots (a-b,d) represented a single individual sampled at one of the sampling sites labelled across the top x-axis which are organized from up-river to down river and divided by anadromous barrier location which are labelled on the bottom x-axis. The left-most samples in the plot, denoted by an empty gray box that is unlabeled, include sampled from above the dams, furthest up-river. Each color represents a distinct genetic cluster. The red brackets and asterisks represent the individuals sampled post-dam removal that were identified as hatchery-origin fish.



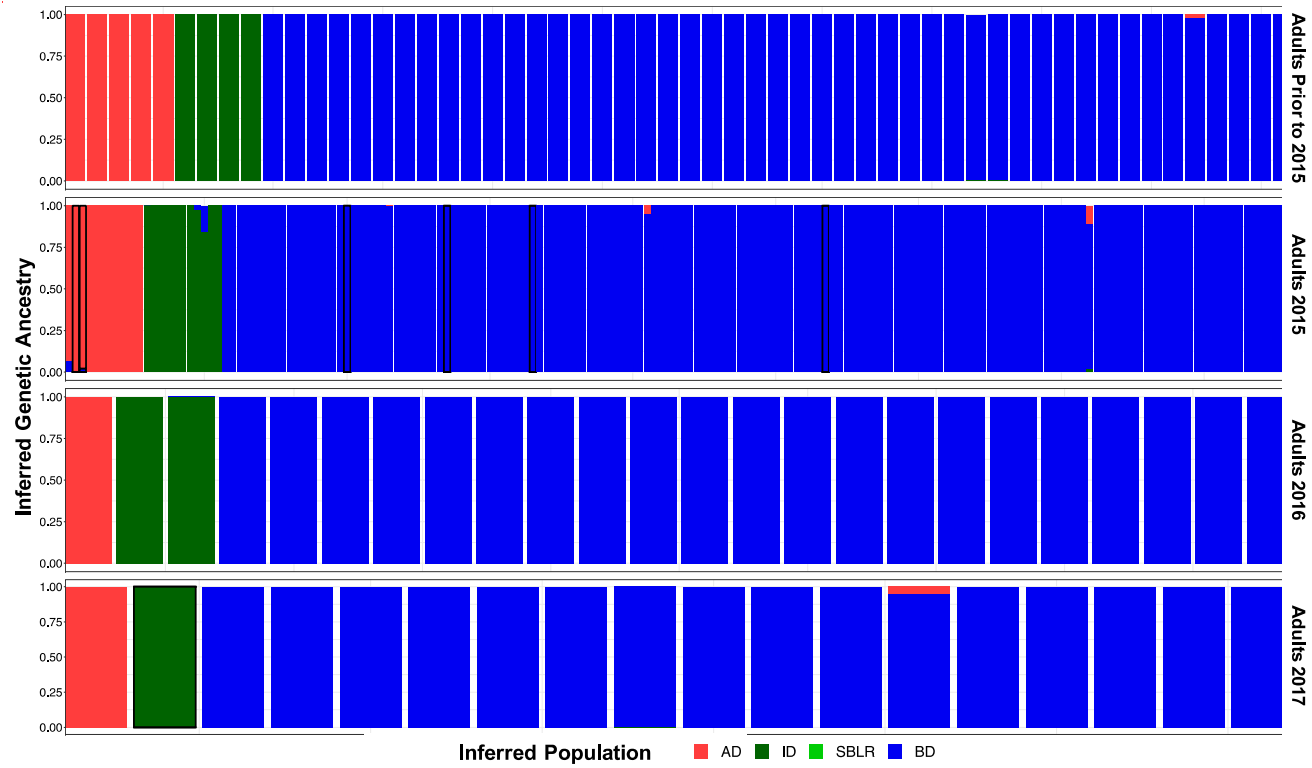


Figure S7. The majority of GSI assignments computed by RUBIAS for adult Steelhead collected post dam removal were to BD reference collections and no individuals were assigned back to the SBLR. Each vertical bar represents a single individual from one adult cohort labelled along the right y-axis. Colors represented the reference reporting unit or sampling location on the Elwha River. Adult individuals that were known to be summer Steelhead have a black outline.

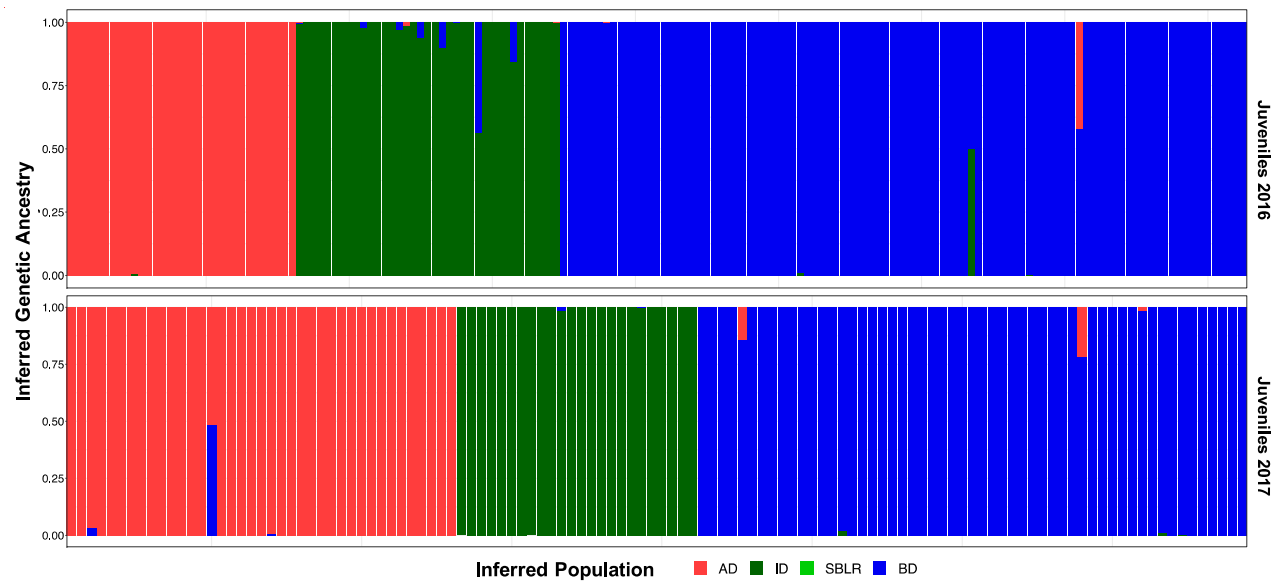


Figure S8. The majority of GSI assignments computed by RUBIAS for juvenile smolts collected post dam removal were to BD ID or AD reference collections. No individuals were assigned back to the SBLR. Each vertical bar represents a single individual from one adult cohort labelled along the right y-axis. Colors represented the reference reporting unit or sampling location on the Elwha River.

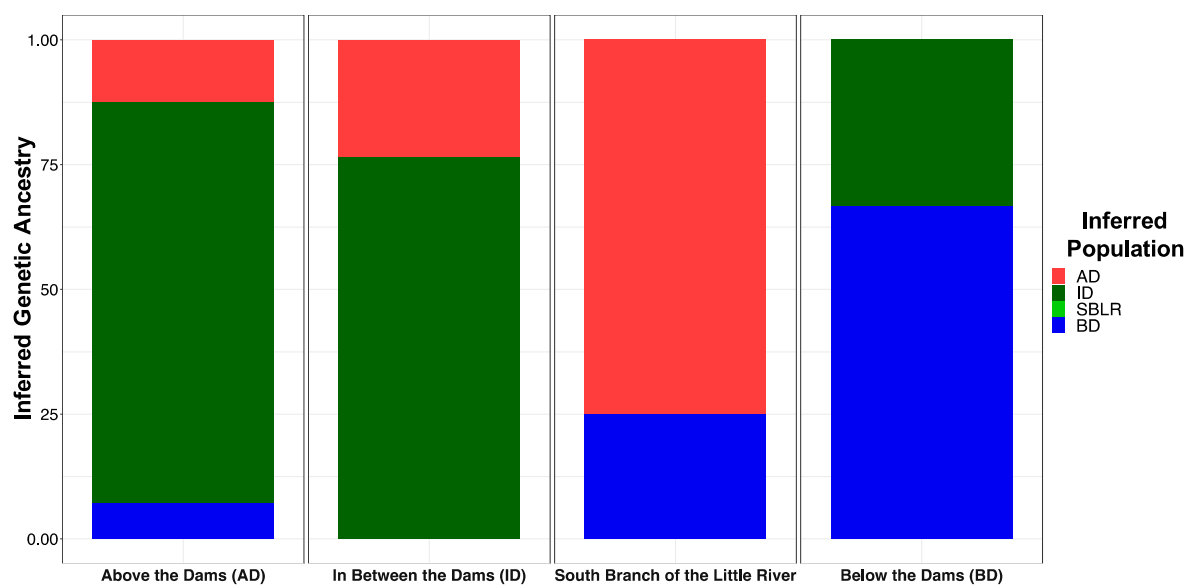


Figure S9. Inferred proportions of genetic ancestry from each of the four reference reporting units assigned to the 85 individuals sampled prior to dam removal with discordant DAPC population and sampling location assignments. These samples were assigned to the “prior to dam removal” mixture collection in our GSI analysis of sample set one. Colors are representative of the inferred reporting unit. The bottom x-axis divides sampling locations by relative anadromous barrier location.