

Genetic Population Structure of Olympic Peninsula Bull Trout Populations and Implications for Elwha Dam Removal

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Abstract

In the Elwha River, two hydroelectric dams constructed nearly a century ago fragment previously continuous habitat and isolate migratory bull trout. Removal of the dams is scheduled to begin in 2011, and represents an opportunity to help recover this threatened species. Large-scale disturbance is expected when accumulated sediments behind the dams are released downstream, which may initially negatively affect bull trout. To inform restoration planning, we investigated levels of genetic variation within and among bull trout populations from six Olympic Peninsula watersheds with an emphasis on the Elwha River. We determined genetic relationships among Elwha bull trout from four distinct river sections and performed population assignments for fish collected from the lower Elwha and Dungeness rivers. There were greater levels of variation and gene flow in coastal watersheds (Hoh, South Fork Hoh, Kalaloch) compared to populations isolated by dams (Elwha, North Fork Skokomish). Elwha bull trout represented an independent spawning population and were highly differentiated from other populations. Bull trout from the Elwha ($n = 21$) and Dungeness ($n = 18$) estuaries all assigned to the river they were collected from. Despite long-term fragmentation, there was no significant genetic variation among Elwha bull trout separated by the dams, although fish from the Elwha headwaters were genetically distinct. Results suggest that bull trout still migrate downstream through both Elwha River dams and that anadromous bull trout will likely help to recolonize the Elwha River following dam removal. Baseline data from this study will be useful for monitoring bull trout recovery following dam removal.

Introduction

Fragmentation as a result of dam construction has affected riverine systems worldwide. The effects of fragmentation on fish species have been well documented and include alteration of life history patterns (Morita et al. 2000, Morita et al. 2009), loss of migratory corridors (Neraas and Spruell 2001, Schmetterling 2003), reduced genetic variation in populations isolated above dams (Yamamoto et al. 2004, Wofford et al. 2005, Reid et al. 2008), and increased genetic differentiation among populations separated by dams (Yamamoto et al. 2004, Reid et al. 2008).

As the effects of fragmentation on fish species and the aquatic ecosystem due to dam construction become more evident, dam removal is becoming an increasingly popular option for the restoration of aquatic ecosystems, particularly as many dams age and the need for maintenance and repairs

increases. Although studies that examine the effects of dam removal are currently limited, initial results suggest that dam removal often benefits fish species (Hart et al. 2002).

Bull trout (*Salvelinus confluentus*), a federally threatened species, are especially susceptible to the effects of fragmentation caused by dams. Bull trout are native to northwestern North America and exhibit diverse life history strategies that include small resident fish that spend their entire lives in their natal tributaries, and larger migratory fish that move downstream to larger rivers, lakes, and reservoirs for growth and rearing and then return to their natal tributaries to spawn (Rieman and McIntyre 1993, Northcote 1997). In river systems of coastal Washington, many bull trout are anadromous and utilize the marine environment for feeding and as a migratory corridor during interbasin migrations (Brenkman and Corbett 2005, Brenkman et al. 2007). The presence of migratory life history type bull trout such as those found in coastal Washington river systems is important for the continued persistence of many populations

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(Rieman and Dunham 2000). Migratory individuals can reduce the effects of inbreeding by providing a means for genetic exchange among populations (Rieman and Allendorf 2001), migratory fish can colonize newly available habitats following stochastic events and the removal of barriers (Northcote 1997), and large migratory adults can contribute higher numbers of gametes than smaller resident fish during reproduction (Fraley and Shepard 1989, Rieman and McIntyre 1993). Because of the importance of migratory adults to the persistence of many bull trout populations, dams that fragment migration corridors utilized by bull trout are recognized as a major threat to the species (Rieman et al. 1997).

Genetic analyses have proven useful for documenting the effects of dams and migratory barriers on bull trout populations. Populations isolated above barriers often show reduced levels of genetic variation (Whiteley et al. 2006, DeHaan et al. 2007) and migratory barriers can have a significant influence on how variation is partitioned among populations (Costello et al. 2003, Meeuwig et al. 2010). Genetic studies have also been used to document fragmentation of important migratory corridors for bull trout following dam construction (Neraas and Spruell 2001).

Currently two dams that lack fish passage facilities prevent upstream migrations of Pacific salmonids in the Elwha River, Washington, USA. The Elwha River originates in Washington's Olympic Mountain Range and flows northward into the Strait of Juan de Fuca (Figure 1). Elwha Dam was completed in 1913 and limits Pacific salmonids to 7.9 km of habitat below the dam. The second dam, Glines Canyon Dam, is located at river kilometer 21.6 and was completed in 1927. Presently bull trout are found in the Elwha River from the river mouth to the headwaters (Brenkman et al. 2008). The majority of bull trout upstream of the two dams exhibit an adfluvial life history form where individuals migrate between the Elwha River and Lake Aldwell and Lake Mills (Brenkman et al. 2008). Recent surveys found that the abundance of bull trout in the Elwha River was relatively low and that the highest densities of bull trout occur immediately above and below Glines Canyon Dam (Brenkman et al. 2008). It is unknown if bull trout in the sections of the Elwha River separated by the dams represent genetically distinct spawning groups or if one-way (downstream) gene flow occurs among these groups. Recent radio tracking data has indicated that bull trout do move downstream through the

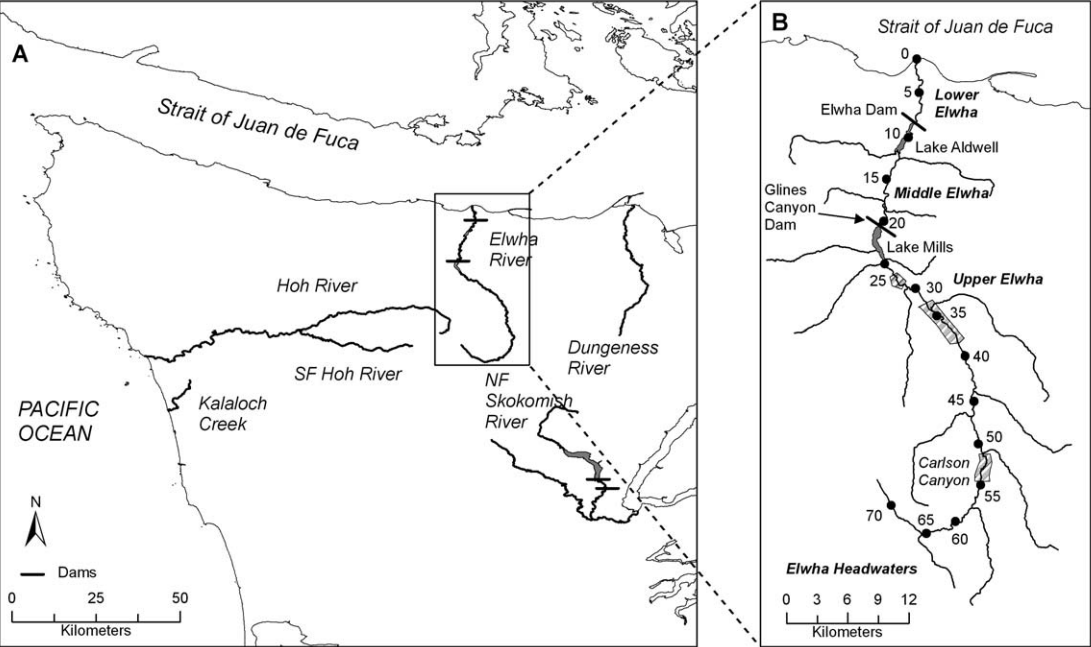


Figure 1. Study location, the Olympic Peninsula, WA, USA. (A) shows the location of all six watersheds where bull trout were collected; (B) shows the Elwha River Basin. Hatched areas in (B) represent potential seasonal velocity barriers and numbers in (B) represent river kilometers. (Figure 1B was adopted from Brenkman et al. 2008).

dams (Corbett and Brenkman, *in press*), and it is unknown if anadromous bull trout below Elwha Dam represent fish that originated upstream of the dam(s) or if spawning occurs in the lower Elwha River below Elwha dam. Given that Olympic Peninsula bull trout migrate large distances in the marine environment, these fish could also be migrants from other nearby populations (e.g., Dungeness River).

Removal of the two Elwha Dams is scheduled to begin in 2011 and represents the largest dam removal project to date in the United States (Duda et al. 2008). Removal of the dams will benefit several species of anadromous fishes, including bull trout, by providing increased access to spawning and juvenile rearing habitat and increased connectivity among populations. Despite the long-term benefits, large amounts of sediment that have accumulated behind the dams will be released downstream and may negatively impact fish populations and fish habitat. A restoration plan was developed that outlines strategies to mitigate the effects of dam removal for native Elwha fishes (Ward et al. 2008). The restoration plan for Elwha bull trout calls for natural recolonization of the system (Ward et al. 2008) which will presumably occur from upstream and downstream individuals (Brenkman et al. 2008).

Genetic information will be important for guiding and evaluating restoration efforts following dam removal. Previous research has demonstrated that Elwha bull trout are genetically distinct from other Olympic Peninsula populations (Winans et al. 2008); therefore restoration activities should focus on preserving the unique genetic variation found in this population. Information on the origins of bull trout below Elwha Dam and the relationship among bull trout separated by the dams will also be important for assessing the role that anadromous individuals will play in recolonization and for informing potential relocation of individuals collected in areas impacted by sedimentation.

In this study, we investigated levels of genetic variation within and among bull trout from six Olympic Peninsula watersheds and determined genetic relationships among bull trout collected from four distinct sections of the Elwha River. Our specific objectives were to: 1) examine the degree of genetic variation within and among bull trout in Olympic Peninsula river systems; 2) use genetic assignment techniques to identify the population-of-origin of bull trout collected in the

lower Elwha and lower Dungeness Rivers; and 3) examine the degree of genetic variation among bull trout from different sections of the Elwha River separated by the dams (lower, middle, upper) to determine if multiple distinct populations exist within the Elwha system. This information will be useful for planning restoration activities and for monitoring bull trout recovery during and after dam removal.

Methods

Sample Collection

We collected bull trout from six different watersheds on Washington's Olympic Peninsula: Hoh River ($n = 59$), South Fork (SF) Hoh River ($n = 21$), Kalaloch Creek ($n = 22$), Elwha River ($n = 98$), Dungeness River ($n = 36$), and North Fork (NF) Skokomish River ($n = 24$) (Figure 1). We collected bull trout from each river or creek by angling, electrofishing, seining (lower Elwha and lower Dungeness), or trapping (Dungeness River) and fish ranged in size from approximately 200 mm total length to over 850 mm total length. In the Elwha River, we collected bull trout from four distinct river sections: below Elwha Dam (lower Elwha; $n = 21$); between Elwha Dam and Glines Canyon Dam (middle Elwha $n = 35$); the area above Glines Canyon Dam upstream to approximately river kilometer 44 (upper Elwha $n = 25$); and near the Elwha headwaters from approximately river kilometer 58 to river kilometer 65 (Elwha headwaters $n = 17$; Figure 1). In the Dungeness River, we also collected bull trout from the lower part of the river and the estuary below river kilometer 5 ($n = 18$) whereas all other Dungeness samples were collected at approximately river kilometer 17 or further upstream. Tissue samples were taken from all bull trout captured and stored in 100% non-denatured ethanol.

Laboratory Analysis

We extracted DNA from all tissue samples using Qiagen DNeasy 96 extraction kits (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. All individuals were genotyped at a suite of 16 microsatellite loci: *Omm1128*, *Omm1130* (Rexroad et al. 2001), *Sco102*, *Sco105*, *Sco106*, *Sco107*, *Sco109*, (Ken Warheit and Sewall Young, Washington Dept. of Fish and Wildlife, *unpublished*), *Sco200*, *Sco202*, *Sco212*, *Sco215*, *Sco216*, *Sco218*, *Sco220* (DeHaan and Ardren 2005), *Sfo18*

(Angers et al. 1995) and *Smm22* (Crane et al. 2004). We conducted polymerase chain reactions (PCR) in 10 μ L volumes containing 2 μ L of template DNA, 5 μ L of 2X Qiagen Multiplex PCR Master Mix (final concentration of 3mM $MgCl_2$), and 0.2 μ L of oligonucleotide PCR primer mix. Primer mix concentrations and annealing temperatures for each multiplex are given in Appendix I. PCR conditions were as follows: initial denaturation at 95 °C for 15 min., then 29 cycles of 95 °C for 30 sec., 90 sec. at the multiplex specific annealing temperature, and 60 sec. primer extension at 72 °C, followed by a final extension at 60 °C for 20 min. Following PCR, capillary electrophoresis was carried out on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. The G5 filter set was used to produce electropherograms, and electrophoresis data was analyzed using the program GeneMapper v4.0 (Applied Biosystems Inc.).

Statistical Analysis

We initially grouped individuals into six putative populations for statistical analysis based on the river or creek that they were collected from. Population groupings were as follows: Hoh River, SF Hoh River, Kalaloch Creek, Elwha River, Dungeness River, and NF Skokomish River. Bull trout from the Elwha headwaters, upper Elwha, and middle Elwha were initially combined for statistical analyses. Bull trout often migrate large distances between spawning periods and the origin of bull trout collected in the lower Elwha and lower Dungeness was uncertain. Because of this, we omitted bull trout collected in the lower rivers from initial analyses and treated these fish as unknown origin individuals to be genetically assigned to a population of origin.

The six putative populations were tested for conformance to Hardy-Weinberg equilibrium (HWE) using exact tests implemented in the program GENEPOP v4.0.7 (Raymond and Rousset 1995). GENEPOP was also used to test each population for evidence of linkage disequilibrium. Significance values for HWE and linkage disequilibrium tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). We used the program GDA (Lewis and Zaykin 2001) to estimate measures of genetic variation within each population including the mean number of alleles per locus (A), expected heterozygosity (H_{exp}),

and observed heterozygosity (H_{obs}). We also used the program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness (A_R) for each population based on a minimum sample size of 38 genes (two times the minimum sample size). This program provides estimates of allelic richness corrected for differences in sample size among populations.

We used the program FSTAT v2.9.3.2 (Goudet 2001) to estimate the overall level of genetic variation among the six putative populations (F_{ST} ; Weir and Cockerham 1984) and the associated 95% confidence level based on 1000 bootstrap replicates. We also used FSTAT to estimate the level of genetic variation between each pair of populations (pairwise F_{ST}). Using GENEPOP, we performed Fisher's exact tests to determine if there were significant differences in allele frequencies among the different spawning tributaries. P-values were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989) as well as the B-Y FDR correction described in Narum (2006). We performed a multidimensional factorial correspondence analysis (FCA) using the program GENETIX (Belkhir et al. 2004) to examine the relationship among the different sampling locations. This method makes no *a priori* assumptions about an individual's population of origin and provides an unbiased graphical approach for viewing the data where individuals that are more genetically similar cluster together on the graph.

We used genetic assignment tests to identify the most likely population of origin for individuals collected from the lower Elwha and Dungeness rivers. In order to determine the ability of our baseline dataset to accurately assign individuals to their location of collection, we performed a leave-one-out test of our baseline using the program ONCOR (Kalinowski et al. 2008). In this analysis, fish are removed from the baseline one at a time and treated as unknown origin individuals, the baseline allele frequencies are then recalculated without that individual, and then the unknown individual is assigned to its most likely population of origin. The proportion of individuals assigned to the location they were collected from provides a means of assessing the accuracy of the genetic baseline for assigning individuals. Following the leave-one-out analysis, we used ONCOR to assign individuals from the lower Elwha and Dungeness Rivers to their most likely population of origin using our genetic baseline.

Following analyses of the different river basins, samples from the Elwha River were divided into four groups based on sampling location (headwaters, upper, middle, lower) in order to determine if there were differences in Elwha bull trout collections separated by the two dams and to determine the relationship between the headwater fish and the other locations. Lower Elwha fish were included in these analyses based on the results of genetic assignment tests (see below). We tested the four groups for conformance to HWE and for evidence of linkage disequilibrium using the methods described above.

Several methods exist for determining the number of distinct populations present in a system (Waples and Gaggiotti 2006). We performed a variety of analyses to examine the number of bull trout populations present within the Elwha River system. We first examined the multi-dimensional genetic relationship among the four sampling locations by performing a factorial correspondence analysis (FCA) using GENETIX. We then used the Bayesian clustering method of STRUCTURE v2.3 (Pritchard et al. 2000) to provide information on the number of distinct populations. This program uses a model-based clustering approach to determine the number of populations or clusters (K) that are present. STRUCTURE also gives the estimated proportional membership of each individual in each of the K clusters. We performed 10 replicate unsupervised STRUCTURE runs for each K from 1-6. All runs consisted of 30,000 preliminary iterations followed by 100,000 data collection iterations. Pritchard et al. (2000) showed that the value of K with the highest posterior probability can be used to infer the number of distinct populations present. Alternatively, Evanno et al. (2005) suggested that this method often leads to an over estimate of K and recommended using the second order rate of change between K and $K+1$ clusters, ΔK , as a more effective identifier of the correct K for the dataset. We compared both methods to determine the most likely value of K . We also estimated the pairwise level of genetic variation among the four sampling locations (F_{ST}) using FSTAT. Finally, we used GENEPOP to perform a Fisher's exact test to determine if there were significant differences in allele frequencies among the different sampling locations. P-values were adjusted for multiple comparisons using a sequential Bonferroni correction (Rice 1989) as well as the B-Y FDR correction described in Narum (2006).

Results

Genetic Variation Within and Among Olympic Peninsula Bull Trout Populations

We investigated levels of genetic variation within and among Olympic Peninsula bull trout populations using 16 microsatellite loci. Two of the sixteen loci we analyzed (*Sco215* and *Sfo18*) were fixed for a single allele (i.e., no variation was observed) in all six populations. Additionally, *Sco105* was fixed in the Dungeness population and *Sco202* was fixed in the Hoh, SF Hoh, and Kalaloch populations. All variable loci conformed to HWE in all populations except *Sco109* which deviated from HWE in the Dungeness and Elwha samples due to a heterozygote deficiency and *Sco212* which deviated from HWE in the Kalaloch sample also due to a heterozygote deficiency. A total of eight out of 494 pairs of loci showed evidence of linkage. Evidence of linkage was observed in Dungeness (three pairs of loci) and Elwha (five pairs of loci) only. Since few loci showed evidence of departure from HWE and evidence of linkage, no loci were removed from the analysis.

The mean number of alleles per locus, allelic richness, and expected heterozygosity were all lowest in the NF Skokomish River ($A = 3.563$, $A_R = 3.472$, $H_{exp} = 0.496$) and observed heterozygosity was lowest in the Dungeness River ($H_{obs} = 0.467$) (Table 1). The mean number of alleles was greatest in the Hoh River ($A = 7.125$) and all other measures of genetic variation were greatest in the SF Hoh River ($A_R = 5.952$, $H_{exp} = 0.590$, $H_{obs} = 0.572$) (Table 1).

TABLE 1. Sample sizes (n) and estimates of genetic variation (based on 16 microsatellite loci) including mean number alleles per locus (A), allelic richness (A_R), expected heterozygosity (H_{exp}) and observed heterozygosity (H_{obs}) for bull trout collected from six Olympic Peninsula Rivers.

Population	n	A	A_R	H_{exp}	H_{obs}
Dungeness River	36	5.188	4.810	0.523	0.467
Elwha River	77	4.625	4.181	0.503	0.472
Hoh River	59	7.125	5.723	0.575	0.559
Kalaloch Creek	22	5.813	5.699	0.574	0.533
NF Skokomish River	24	3.563	3.472	0.496	0.516
SF Hoh River	21	6.063	5.952	0.590	0.572
Mean		5.396	4.973	0.544	0.520

TABLE 2. Pairwise estimates of genetic variation (F_{ST}) among six Olympic Peninsula bull trout populations below the diagonal and P -values for exact tests of differentiation above the diagonal.

	Dungeness	Elwha	Hoh	Kalaloch	NF Skokomish	SF Hoh
Dungeness River	---	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Elwha River	0.259	---	<0.001*	<0.001*	<0.001*	<0.001*
Hoh River	0.280	0.202	---	0.045	<0.001*	<0.001*
Kalaloch Creek	0.289	0.193	0.003	---	<0.001*	0.044
NF Skokomish River	0.241	0.257	0.275	0.279	---	<0.001*
SF Hoh River	0.274	0.198	0.008	0.003	0.271	---

*denotes significance at the level $\alpha = 0.05$ following Bonferroni and B-Y FDR corrections.

The overall level of genetic variation among populations was 0.241 and was significantly different from zero (95% C.I. = 0.167-0.262). Pairwise estimates of genetic variation ranged from 0.003 for the comparisons between Hoh River and Kalaloch Creek and SF Hoh and Kalaloch Creek to 0.289 between Dungeness River and Kalaloch Creek (Table 2). In general, pairwise estimates of F_{ST} were lowest for the comparisons among the Hoh, SF Hoh, and Kalaloch collections and all other comparisons among river systems were substantially greater (Table 2). Following Bonferroni and BY-FDR correction, Fisher's exact tests indicated significant differences in allele frequencies among all population pairs except for Hoh River and Kalaloch Creek ($P = 0.045$) and SF Hoh River and Kalaloch Creek ($P = 0.044$). The FCA analysis showed three separate clusters of individuals corresponding to the collections from the Elwha, Dungeness, and NF Skokomish rivers and a fourth cluster of individuals corresponding to the Hoh River, SF Hoh River, and Kalaloch Creek collections (Figure 2).

Population Assignments of Elwha and Dungeness Bull Trout

Assignment success was generally low for the Hoh, SF Hoh and Kalaloch Creek during leave-one-out assignment tests (range 0.429 to 0.596; Table 3). All misassigned individuals from these three locations were assigned among these three locations (e.g., all misassigned Hoh River fish were either assigned to SF Hoh or Kalaloch Creek). All of the individuals collected from the Elwha River, Dungeness River, and NF Skokomish River were assigned to the location they were collected from in the leave-one-out analysis (Table 3). When we performed population assignments for bull trout collected in the lower Elwha and Dungeness rivers,

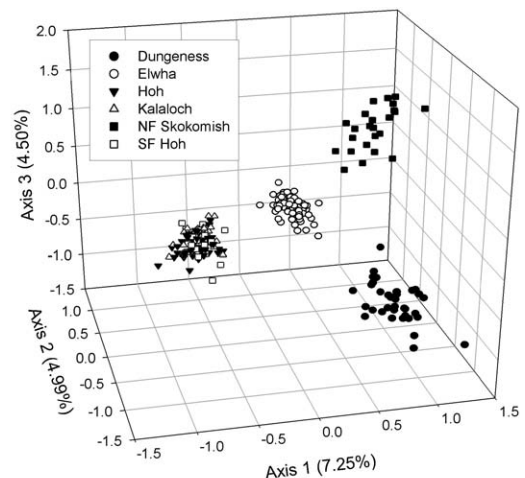


Figure 2. Correspondence analysis (FCA) of Olympic Peninsula bull trout. Each point on the graph represents an individual bull trout in the analysis. Points that cluster closer together on the graph are more genetically similar. Numbers in parentheses represent the percent of the variation observed accounted for by each axis.

all unknown origin bull trout collected in the lower Elwha River were assigned to the Elwha River with 100% probability and all bull trout collected in the lower Dungeness River were assigned to the Dungeness River with 100% probability.

Analyses of Elwha River Bull Trout

We examined the degree of genetic variation among bull trout from different sections (lower, middle, upper, headwaters) of the Elwha River to determine if multiple distinct populations exist within the Elwha system. When individuals collected from the Elwha River were split into four groups based on sampling location, all four groups conformed to HWE at all loci except for

TABLE 3. Proportions of individuals in the baseline dataset assigned to each sampling location during leave-one-out analysis. Values in bold represent the proportions of individuals assigned to the location they were collected from.

Collected from	Assigned to					
	Dungeness	Elwha	Hoh	Kalaloch	SF Hoh	NFSkokomish
Dungeness River	1.000	0.000	0.000	0.000	0.000	0.000
Elwha River	0.000	1.000	0.000	0.000	0.000	0.000
Hoh River	0.000	0.000	0.596	0.154	0.250	0.000
Kalaloch Creek	0.000	0.000	0.500	0.429	0.071	0.000
SF Hoh River	0.000	0.000	0.235	0.176	0.588	0.000
NF Skokomish River	0.000	0.000	0.000	0.000	0.000	1.000

the lower Elwha which deviated from HWE at the locus *Sco105* due to a heterozygote deficiency. One pair of loci (*Sco105* and *Sco220*) showed

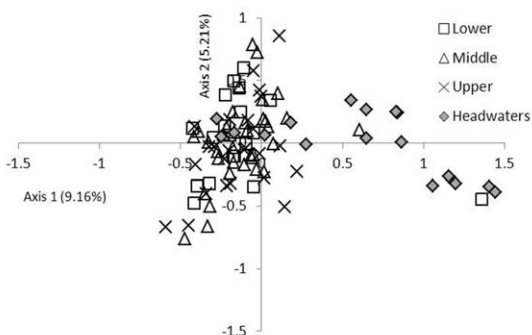


Figure 3. Correspondence analysis (FCA) of Elwha River bull trout. Each point on the graph represents an individual bull trout in the analysis. Points that cluster closer together on the graph are more genetically similar. Numbers in parentheses represent the percent of the variation observed accounted for by each axis.

evidence of linkage in the headwaters and in the middle Elwha collections. The FCA plot of the four Elwha sampling locations showed that the majority of the individuals collected from the lower, middle, and upper Elwha clustered together, and the individuals from the Elwha headwaters clustered separately (Figure 3). A few individuals from the upper, middle, and lower Elwha did cluster with the headwater fish and a few headwater fish did cluster with the upper, middle, and lower Elwha individuals (Figure 3).

STRUCTURE analysis indicated that the most likely number of populations in the Elwha dataset was four (mean likelihood over 10 runs = -2910.16). Examination of the graphical results for the $K = 4$ STRUCTURE analysis indicate that the majority of the Elwha headwaters fish belonged to a distinct cluster and the fish from the upper, middle, and lower Elwha were distributed among three clusters that did not necessarily correspond to sampling locations (Figure 4). When

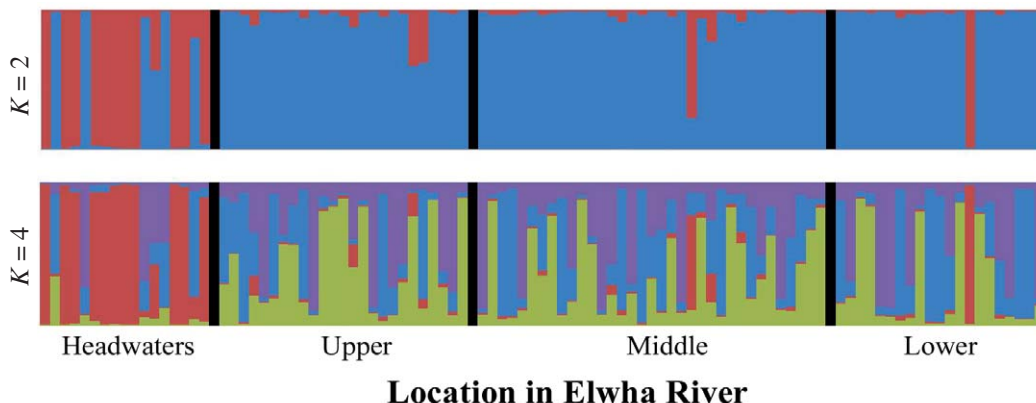


Figure 4. Output from the program STRUCTURE assuming two ($K = 2$; upper plot) and four ($K = 4$; lower plot) populations of bull trout in the Elwha River. Each vertical bar on the graph represents an individual in the analysis and the different colored regions represent the proportional membership in each population. Individuals are grouped by sampling locations with dark bars separating the four different sampling locations.

TABLE 4. Pairwise estimates of genetic variation (F_{ST}) among Elwha River bull trout sampling locations below the diagonal and P values for exact tests of differentiation above the diagonal.

	Headwaters	Lower	Middle	Upper
Headwaters	---	<0.001*	<0.001*	<0.001*
Lower	0.072	---	0.345	0.599
Middle	0.088	0.004	---	0.027
Upper	0.072	0.004	0.007	---

*denotes significance at the level $\alpha = 0.05$ following Bonferroni and B-Y FDR corrections.

we examined the results of the ΔK analysis, a K of 2 had the highest ΔK . Graphical results of the $K = 2$ STRUCTURE analysis also showed that the majority of the Elwha headwaters fish formed one genetic cluster and fish from the other sampling locations formed a second genetic cluster (Figure 4).

Pairwise estimates of F_{ST} among Elwha sampling locations ranged from 0.004 for the comparisons between the lower Elwha and the middle and upper Elwha to 0.088 for the comparison between the Elwha headwaters and the middle Elwha (Table 4). The levels of variation observed between the Elwha headwaters and the other Elwha locations were an order of magnitude greater than comparisons among the upper, middle, and lower Elwha. Fisher's exact tests indicated that there were significant differences in allele frequencies between the Elwha headwaters and all other locations but no significant differences were observed between the upper, middle, and lower Elwha collections.

Discussion

Genetic Variation Within and Among Olympic Peninsula Bull Trout Populations

Understanding the level of genetic variation within other Olympic Peninsula populations where bull trout are more abundant will be important for evaluating the recovery of Elwha bull trout following dam removal. In general, estimates of genetic variation were greatest within western Olympic Peninsula Rivers that drain directly to the Pacific Ocean (Hoh, SF Hoh, Kalaloch). One key difference between bull trout populations in these watersheds and the other populations (e.g., NF Skokomish and Elwha) in our study is the degree of isolation among populations. No

barriers exist among western Olympic Peninsula coastal bull trout populations and radio telemetry has documented extensive movement of bull trout among these rivers (Brenkman and Corbett 2005, Brenkman et al. 2007). Conversely bull trout in the Elwha and NF Skokomish Rivers have been isolated above dams for nearly a century. Data presented in this study along with previous analyses indicate that genetic variation is reduced in bull trout populations isolated above dams and other barriers (Whiteley et al. 2006, DeHaan et al. 2007); a pattern that has been observed in other species of salmonids as well (Wofford et al. 2005; Neville et al. 2006, 2009). Although bull trout in the Dungeness River can migrate downstream to the marine environment, dams in the Elwha River (the nearest spawning population) prevent genetic exchange with Elwha fish located above the dams. Isolation from other nearby populations may explain why estimates of variation within the Dungeness River were similar to those in the Elwha and NF Skokomish.

Previous studies have demonstrated a relatively high degree of genetic variation among bull trout populations and indicate that genetically distinct local populations often exist within individual tributaries (Costello et al. 2003, Spruell et al. 2003, Taylor and Costello 2006, Whiteley et al. 2006). Radio tracking data has documented that Olympic Peninsula bull trout frequently migrate among marine and freshwater environments and also migrate among different river systems (Brenkman and Corbett 2005, Brenkman et al. 2007). Based on these data we might expect to see reduced levels of variation among populations; however, we still observed a relatively high degree of genetic variation among populations (global $F_{ST} = 0.241$). This is likely due in some part to isolation among populations separated by dams. Levels of genetic variation among Olympic Peninsula bull trout populations in this study were consistent with the high degree of genetic variation among coastal bull trout populations in British Columbia (Taylor and Costello 2006).

Gene flow among rivers on the western Olympic Peninsula (Hoh, SF Hoh, Kalaloch) appears to be much higher than gene flow among the rivers that drain into the Strait of Juan de Fuca and Hood Canal (Elwha, Dungeness, NF Skokomish). As mentioned above, bull trout have been observed to migrate extensively among west side Olympic Peninsula tributaries whereas dams restrict the

migration of bull trout among other Olympic Peninsula Rivers. Furthermore, there are fewer bull trout populations and greater geographic distance between populations in rivers that drain into the Strait of Juan de Fuca and Hood Canal and presumably less migration among rivers as a result. Increased connectivity among western Olympic Peninsula tributaries appears to facilitate genetic exchange among these populations. The absence of significant genetic variation between the Hoh River and Kalaloch Creek and between the SF Hoh and Kalaloch Creek suggests that Kalaloch Creek does not contain a distinct spawning population. Brenkman and Corbett (2005) suggest that small coastal watersheds such as Kalaloch Creek serve as important foraging habitat and provide refuge from high winter flows for anadromous bull trout that originate from larger river systems such as the Hoh River. We cannot rule out the possibility that reduced genetic variation among these rivers may be due in part to the inclusion of SF Hoh River fish with the Hoh River collection and vice versa. Not all bull trout spawn annually (Fraley and Shepard 1989), and the possibility exists that these fish were migrants sampled between spawning periods.

Understanding the genetic relationship between the Elwha River and other Olympic Peninsula watersheds is also important for bull trout recovery following dam removal. Previously, Winans et al. (2008) found that bull trout in the Elwha River were genetically distinct from other populations. Data presented in our study provide further evidence that bull trout in the Elwha represent an independent spawning population. The Elwha River clustered separately from the Dungeness River (the nearest spawning tributary to the Elwha) on the FCA plot (Figure 2) and we observed a high degree of pairwise variation between these two rivers (pairwise $F_{ST} = 0.259$). Leave-one-out assignment tests provided further evidence that these populations were genetically distinct; all Dungeness River individuals were assigned to the Dungeness and all Elwha fish were assigned to the Elwha. Although the Elwha dams prevent Dungeness bull trout from accessing spawning areas above Elwha Dam, anadromous fish from the Elwha which are unable to access their natal spawning habitat could certainly access spawning areas in the Dungeness River. Preservation and restoration efforts during and following dam removal should focus on preserving the unique genetic variation observed in the Elwha River.

Analysis of Elwha River Bull Trout

Following dam removal, anadromous bull trout will have access to spawning habitat in the Elwha River and these fish will be important to the recovery process due to their increased fecundity and potential for genetic exchange with other populations. Although bull trout collected from the Elwha River estuary are presumably anadromous, little information exists regarding the extent of anadromy in Elwha River bull trout, and previously it was unclear if these were Elwha origin fish or migrants from other watersheds. In this study, unknown origin bull trout collected in the lower Elwha and Dungeness Rivers were genetically assigned to spawning populations in the rivers they were collected from. The majority of the fish collected in the lower Elwha and Dungeness Rivers were sub-adults (135-330 mm), although some larger adult fish were collected. Data from the Hoh River system revealed that most anadromous bull trout migrate to the marine environment at age three or four and anadromous fish averaged larger sizes than individuals sampled in the lower Elwha and Dungeness Rivers (Brenkman et al. 2007). The lower Elwha and Dungeness River estuaries may provide important rearing habitat for juvenile and sub-adult bull trout before they migrate to the marine environment. Downstream erosion of sediment following dam removal may alter this habitat and could force pre-migratory juvenile fish in these areas to migrate to the marine environment prematurely.

Fragmentation due to the construction of dams and other barriers has led to the evolution of significant genetic differences among salmonid populations separated by barriers (Taylor et al. 2003, Yamamoto et al. 2004, Neville et al. 2006); however, our results do not support the notion that fragmentation has led to the evolution of genetically distinct spawning populations in the different segments of the Elwha River. The STRUCTURE analysis of $K = 4$ (the solution with the highest likelihood) showed that only fish collected in the Elwha headwaters formed a distinct cluster and there was no clear relationship between collection location and genetic clustering for the remaining sampling locations (Figure 4). Alternatively, the ΔK method identified only two distinct populations in the Elwha; one primarily consisting of the Elwha headwaters fish and one consisting of fish from all other sampling locations. The one

consistent result between the two analyses was the presence of a distinct group of fish in the Elwha headwaters. Pairwise estimates of variation and Fisher's exact tests also indicated that there was no significant genetic difference among bull trout collections separated by the dams (Table 4). This lack of differentiation is presumably due to downstream movement through the dams. Recent radio telemetry work in the Elwha River documented that 24% (23 out of 96) of radio tagged bull trout moved downstream over the dams (Corbett and Brenkman, *in press*). Other studies reported similar downstream movements by bull trout over dams. Eight percent of radio tagged bull trout that originated in the Blackfoot River, MT, swam downstream over Milltown Dam (Swanberg 1997) and 18% of bull trout tagged in the Boise River system passed downstream over Arrowrock Dam (Flatter 1998).

One interesting finding was the presence of a genetically distinct group of bull trout in the Elwha headwaters. Several suspected seasonal velocity barriers exist within the Elwha River above Lake Mills (Figure 1) and the Elwha headwaters fish are separated from the other Elwha collections by one of these barriers, Carlson Canyon. Radio telemetry studies found that neither rainbow trout (*Oncorhynchus mykiss*; Wampler 1984) nor bull trout (Corbett and Brenkman, *in press*) moved upstream through Carlson Canyon. Our data suggest limited gene flow among bull trout above and below Carlson Canyon; however, we did observe fish on the FCA plot and on the STRUCTURE plots that were collected in the Elwha headwaters but were genetically more similar to fish below the barrier (and vice versa) suggesting that the canyon does not represent a complete barrier to migration. Genetic differences may also be the result of behavioral adaptations by the two populations (i.e., resident vs. migratory life history). Regardless, restoration activities should take this information into account. For instance, if most fish from the headwaters exhibit a resident life history, they may not contribute substantially to re-colonization of downstream habitat. Furthermore, restoration activities associated with other salmonid populations in the Elwha watershed should carefully evaluate potential effects on the headwater bull trout population, due to its isolation within a very limited portion of the watershed.

Implications for Elwha Dam Removal

The construction of dams and other migratory barriers has been linked to changes in the migratory behavior of salmonids including the reduction and disappearance of migratory life history types (Rieman et al. 1997, Morita et al. 2000, Morita et al. 2009). Data from this study indicate that bull trout still migrate downstream in the Elwha River and an anadromous life history likely persists. Although the extent of anadromy in Elwha River bull trout remains unknown, the number of anadromous fish will presumably increase once migratory corridors are re-established following dam removal. The potential increase in anadromous life history type bull trout has important genetic implications for this population. The downstream erosion of sediment following dam removal may result in the initial loss of juvenile bull trout within the Elwha River. Anadromous migratory fish from the Elwha River will help maintain the unique genetic variation in the Elwha system assuming that these fish return to the Elwha to spawn. Anadromous migratory fish could also provide a means of genetic exchange among the Elwha River and other Olympic Peninsula bull trout populations including the Dungeness River. The Elwha and Dungeness Rivers showed reduced levels of within population genetic variation when compared to larger bull trout populations in the Hoh River system. Previous studies have shown that genetic exchange among isolated populations has led to increased genetic variation over a relatively short period of time (Madsen et al. 1999, Yamamoto et al. 2006, Bouzat et al. 2009).

Several dams that limit the migration of bull trout and other Pacific Northwest salmonids have recently been removed (e.g., Mill Town Dam on the Clark Fork River, MT) or are scheduled for removal in the near future (e.g., Condit Dam on the White Salmon River, WA). Collection of baseline genetic information prior to dam removal is important for recovery planning for threatened and endangered fish species following dam removal (Winans et al. 2008). Genetic data from populations that have not been fragmented can be used to set recovery goals and monitor the recovery process following dam removal. For example, comparisons between the Elwha River and other Olympic Peninsula bull trout populations can help to determine if increased connectivity between the Elwha River and other

watersheds results in levels of genetic variation similar to those observed in populations where bull trout are more abundant (e.g., Hoh River). Incorporating genetic data into monitoring plans following dam removal will be useful for evaluating both short term effects (e.g., colonization of newly available habitat; Perrier et al. 2010) as well as monitoring long term evolutionary processes (e.g. gene flow among populations; Palstra and Ruzzante 2010).

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Appendix I.

Bull trout PCR multiplex primer concentrations and annealing temperatures.

Multiplex Set 1		T _A = 54°C
Locus Name	Dye	Final Concentration
Sfo18	6FAM	0.3μM
Sco212	VIC	1.0μM
Sco220	NED	3.3μM
Sco216	PET	4.0μM
Sco109	6FAM	6.6μM
Multiplex Set 2		T _A = 59°C
Locus Name	Dye	Final Concentration
Sco202	6FAM	0.6μM
Sco102	PET	1.0μM
Sco215	PET	1.3μM
Sco200	VIC	2.0μM
Omm1128	VIC	2.0μM
Sco105	NED	1.3μM
Smm22	6FAM	4.6μM
Multiplex Set 3		T _A = 56°C
Locus Name	Dye	Final Concentration
Sco106	6FAM	1.0μM
Sco107	VIC	2.6μM
Omm1130	NED	5.3μM
Sco218	PET	3.3μM

T_A = Annealing temperature