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Assessment of bull trout genetic diversity, population connectivity, and genetic introgression in the Upper Willamette Basin, OR

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By: Justin Bohling

Abernathy Fish Technology Center
Longview, Washington

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David Andres, ODFW

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Prepared by:

Justin Bohling

Abernathy Fish Technology Center
US Fish and Wildlife Service
1440 Abernathy Creek Rd.
Longview, WA 98626
360-425-6072 x311
Justin_bohling@fws.gov

Submitted to:

Upper Willamette Bull trout Working Group

Contact: Nik Zymonas
Oregon Department of Fish and Wildlife
Corvallis Research Lab
28655 Highway 34
Corvallis, Oregon 97333
(541) 757-5100
Nik.Zymonas@oregonstate.edu

Summary

The Upper Willamette River Basin of Oregon has been identified as a core area for bull trout population recovery. From 2005 to 2014 the Upper Willamette Bull Trout Working Group collected genetic samples from adult bull trout captured at spawning locations and fish passage facilities. We performed genetic analyses of these samples to identify trends in genetic diversity and dispersal over time. Using 16 microsatellite loci, we estimated heterozygosity, allelic richness, and effective population size for each spawning population. We also evaluated population structure and movement of bull trout among spawning locations using assignment tests. Our microsatellite panel included several loci for which brook trout have unique alleles compared to bull trout, allowing us to identify potential hybrids. Results align closely with a earlier analysis of these populations: many were similar genetically to Anderson Creek, which served as source for translocations. The most unique population was Roaring River, which appears to have been genetically isolated for a considerable time period and has not received migrants from any other populations. Upper McKenzie was also distinct, but its reduced genetic diversity and effective size suggests it has been recently isolated and its distinctiveness is driven by small population size. Migrants from other populations appear to be present in the Upper McKenzie spawning location, which may alleviate the current low levels of genetic diversity. Brook/Bull trout hybrids composed only 1.4% of the sampled individuals but appear to be concentrated in certain areas, such as the Upper McKenzie spawning location.

Introduction

The bull trout (*Salvelinus confluentus*) is a species of high management concern in the Pacific Northwest. It is listed as threatened under the Endangered Species Act across its entire distribution, which includes several small populations in the Upper Willamette River Basin in central Oregon. These populations form a Core Area for bull trout recovery, which itself is part of the larger Coastal Recovery Unit. The primary threats to these populations include habitat loss, reduced connectivity due to hydropower development, and competition with non-native brook trout.

In identifying relevant conservation needs outlined by a U.S. Fish and Wildlife Service (USFWS) 2008 Biological Opinion and Upper Willamette Bull Trout Working Group, the U.S. Army Corps of Engineers (COE) contracted with USFWS Abernathy Fish Technology Center (AFTC) to describe the genetic characteristics of these populations and develop a genetic monitoring plan for the upper Willamette River Basin. Within the core area there are four recognized local bull trout populations: the upper McKenzie River above Trail Bridge Dam, the upper McKenzie River below Trail Bridge Dam, the South Fork McKenzie River, and an introduced population in the Middle Fork Willamette River. These populations are separated from one another by one or more dams. An initial analysis (DeHaan and Diggs 2009) examined genetic diversity within and between these populations, along with comparing them to other populations from the Columbia River Basin. Overall they had levels of genetic variation slightly lower than those observed in other bull trout populations. The study also suggested the Upper Willamette populations can be grouped into three main genetic groups: upper McKenzie River above Trail Bridge Dam, South Fork McKenzie (aka Roaring River), and a third group consisting of Anderson Creek (a tributary of the upper McKenzie River below Trail Bridge Dam) and other tributaries that had been supplemented with fry and juvenile bull trout that originated

in Anderson Creek. This third group included the Middle Fork Willamette River, a spatially separated reintroduced population founded with Anderson Creek-origin bull trout (Figure 1). Gene flow among these populations appeared to be low, although assignment tests identified individuals from multiple distinct populations captured at the same dams.

Based on these analyses, AFTC collaborated with the COE and the Upper Willamette Bull Trout Working Group to develop a genetic monitoring plan for upper Willamette core area (DeHaan 2010). This was incorporated in the 2014 Upper Willamette Bull Trout Action Plan. The goal of this plan was to use genetic information to track population status and the response of populations to management actions in the system. For this study, Oregon Department of Fish and Wildlife (ODFW) collected additional bull trout from the Upper Willamette populations to be analyzed at the same suite of genetic markers as in the initial genetic report (DeHaan and Diggs 2009). To assess any changes in the characteristics of these populations, we combined these genotypes with the previous data to examine bull trout collected for the time period from 2005 to 2014. Specific goals included:

1) Assess genetic variation of bull trout in each spawning tributary. Spawning streams include the upper McKenzie River and its tributaries (Sweetwater Creek, Anderson Creek, Olallie Creek), the South Fork of the McKenzie (Roaring River), and the upper Middle Fork Willamette River.
2) Conduct population assignments for bull trout captured at downstream dam locations to assess distribution, connectivity, and potential for genetic exchange. Samples were collected at Trail Bridge Dam (including Carmen-Smith spawning channel and Trail Bridge Reservoir), Cougar Dam, Leaburg Dam, and Hills Creek Dam.
3) Determine species and degree of hybridization for samples preliminarily identified as bull trout, brook trout, and potential hybrids.

Completing these analyses will fulfill the needs expressed in the Upper Willamette Bull Trout Action Plan and provide data that will be used to assess the impact of current management efforts and guide future activities to restore bull trout.

Methods

ODFW and members of the Upper Willamette Bull Trout Working Group collected genetic samples from 2005 to 2014. Fin clips were taken from adult fish captured at spawning locations or at fish passage sites at dams. These samples were stored in 100% non-denatured ethanol and then transported to AFTC. DNA was extracted using Qiagen Tissue Kits (Qiagen, Valencia, CA) following the manufacturer's protocol. We generated genotypes for individuals collected at the spawning sites and the tailraces of all four dams (Table 1). These individuals were genotyped with a panel of 16 microsatellite loci that were previously applied to this system (DeHaan and Diggs 2009). Conditions for the PCR were the same as described in DeHaan and Diggs (2009) and PCR products were separated using capillary electrophoresis carried out on an ABI 3730xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). Microsatellite genotypes were scored using Genemapper v4.0 software (Applied Biosystems Inc.).

We grouped samples by the spawning location or fish passage facility from which they were collected. Some individuals were captured at a fish passage facility and later at a spawning location: we only included their genotypes within the spawning groups. A concern with multi-year datasets is that temporal shifts in allele frequencies due to genetic drift can create genetic heterogeneity within a population. Since several spawning locations were sampled in multiple

years, we performed a multilocus analysis of molecular variance (AMOVA) on the spawning sites using Arlequin 3.5 (Excoffier and Lischer 2010). Individuals were grouped by year of capture and then years grouped by spawning site. The purpose of this analysis was to determine where it was appropriate to group individuals captured in different years together for population-level analyses. Some individuals were sampled over multiple years. For most of these fish we placed the genotype in the group corresponding with the first year of capture. We did this to avoid violating some assumptions in the genetic analyses that overlapping generations are not present in the dataset (i.e. parents not in the same dataset with their offspring).

Combining individual genotypes across years, we estimated deviations from Hardy-Weinberg Proportions (HWP) for each spawning location at each locus using the R package *pegas* (Paradis 2010). HWP refers to the relationship between allele and genotype frequencies observed in a population. We measured observed and expected heterozygosity (H_O and H_E, respectively), allelic richness (A_R), and heterozygote deficit (F_{IS}) for each spawning population. H_O and H_E are the proportion of heterozygotes in a population and common measures of genetic diversity. A_R is a measure of the average number of alleles observed at a locus standardized by sample size. F_{IS} is a measure of deviation from neutral HWP: positive values indicate a heterozygote deficiency and negative values a heterozygote excess. Ninety-five percent confidence intervals for A_R and F_{IS} were estimated using a bootstrapping procedure with 1000 replicates. Along with a combined value for each spawning site, we calculated genetic diversity on a per year basis to evaluate changes over time. Annual values were only conducted for years in which seven or more bull trout were sampled to ensure adequate estimation of allele frequencies. H, A_R, and F_{IS} were calculated using the R package *diveRsity* (Keenan et al. 2013)

To estimate the degree of genetic differentiation between spawning populations, we estimated pairwise F_{ST} using the R package *diveRsity* (Keenan et al. 2013). F_{ST} is a pairwise comparison and estimates the degree to which genetic variation is shared between two populations. Potential values range between 0 and 1: values close to 0 indicate the populations have similar allele frequencies, whereas values close to 1 indicate higher differentiation. We also conducted a Principal Components Analysis (PCA) based on allele frequencies for individuals captured at the spawning locations to discern population structure. Individuals closer in principal component space have similar allele frequencies.

One of the main goals of this project was to determine the origin of bull trout captured at passage sites (i.e. dams) to assess the potential for migration between the various spawning locations. We performed genetic assignment tests using the maximum-likelihood method implemented in the program ONCOR (Kalinowski et al. 2007). This method identifies the most likely population of origin and provides the probability of originating from that population. Along with assessing the probability of assignment for individuals captured at dams to the spawning locations, we estimated the self-assignment rate of individuals from the spawning location themselves with the leave-one-out test.

Assignment tests such as ONCOR rely on *a priori* grouping of individuals into reference populations to serve as baselines for assignment. However, cryptic population structure can bias assignment tests if the true underlying structure does not correspond to the reference groupings. To provide an alternative perspective on population structure and assignment, we used the Bayesian clustering framework implemented in the program STRUCTURE (Pritchard et al. 2000, Falush et al. 2003). This program simultaneously estimates the optimal pattern of genetic clustering based on HWP expectations and ancestry proportions for individuals based on their genotypes. For this analysis we used only the individuals captured at spawning locations. We

used the correlated allele frequency model with admixture and no prior population information. We began with an initial burn-in of 50,000 repetitions followed by 500,000 MCMC reps. The number of clusters (*K*) was set to vary from 1-10 with five iterations at each *K* value.

Bull trout typically form small local spawning aggregations with limited gene flow between populations. For each spawning location we estimated effective population size (N_E) using the linkage disequilibrium method implemented in NeEstimator 2.01 (Do et al. 2014). There were two issues with this dataset: one is that bull trout have long generation periods (5-7 years), meaning that calculating N_E for consecutive years violates assumptions of overlapping generations. Second, for many years the number of sampled fish was low; small sample sizes reduce the accuracy of single-sample estimators. Therefore, we calculated N_E for only two years for each spawning location, using only samples separated by at least six years. Also, we only estimated N_E when the sample size was greater than seven individuals.

At ten of the loci we examined there are private alleles that are found only in brook trout (*S. fontinalis*), which are non-native to the Willamette basin and hybridize with bull trout. We assessed the extent of hybridization by calculating the number of brook trout-specific alleles that were observed among the captured bull trout. We considered any individual with two or more brook trout alleles to be a hybrid. These individuals were excluded from any of the population genetic analyses.

Results

A total of 722 genotypes were generated from the pool of collected genetic samples. Several of these were duplicate genotypes, most of which were the result of sampling the same individual fish twice in the field. After removing duplicates, there were 706 unique genotypes. We removed those possessing more than 10 loci with missing data and those with no data on year or origin of capture. Ten individuals had two or more brook trout-specific alleles and were removed from the dataset for analyses of population structure. The final dataset had 687 unique bull trout genotypes with known year and location of capture.

Genotypes were grouped by year and spawning location (n=491) to calculate variance across collection years with an AMOVA (Table 2). Only years with >10 individuals were included. The resulting model revealed that 19.8% of the variation in the dataset was among groups (i.e. spawning sites), whereas only 0.7% of the variance was among populations within groups (i.e. collection years within the same spawning site). This suggests there was little variance in allele frequencies across collection years at the same spawning locations, giving us confidence in combining samples across years.

Patterns of deviations from HWP were variable across spawning sites (Table 3). Some sites (e.g. Anderson and Olallie Creeks) had few deviations over the suite of loci. Others (e.g. Roaring River and Upper McKenzie) displayed deviations at over half the loci. Most populations produced low point values of F_{IS} with 95% confidence intervals that overlapped zero. The exceptions were Sweetwater Creek, which had a significant heterozygote excess, and Upper McKenzie, which had a significant heterozygote deficit.

Although there was variation in genetic diversity across spawning locations, these differences were not substantial (Figure 2). Anderson Creek had among the highest levels of H_O and A_R, but Sweetwater Creek and Middle Fork Willamette produced similar values. The lowest values were recorded at Roaring River and Olallie Creek. There were no strong temporal trends in either A_R or H_O at the spawning sites (Figure 3).

Results from the PCA revealed that the Roaring River spawning population formed a distinct cluster highly diverged from other populations (Figure 4). Individuals from the other spawning locations highly overlapped, forming no distinct clusters except for some of the Upper McKenzie bull trout that formed an indistinct group. The distinctiveness of Roaring River bull trout was further reflected in the measures of F_{ST} (Figure 5). All of the highest pairwise comparisons involved the Roaring River population. The next highest values involved the Upper McKenzie population. All pairwise comparisons produced 95% confidence intervals that did not overlap zero.

These results mirrored the results of the leave-one-out self-assignment test performed with ONCOR. All individuals sampled at Roaring River were assigned back to that population with 100% probability (Table 4). The next highest self-assignments were Upper McKenzie (75%) and Olallie Creek (73%). The lowest self-assignment was Anderson Creek (55.4%). No individuals were incorrectly assigned to the Roaring River population. Patterns of population of origin for bull trout captured at fish passage sites depended on the specific site (Table 5). Bull trout captured at Cougar Dam, on the South Fork McKenzie River, were predominantly from Roaring River, but several individuals were from other spawning locations. Leaburg Dam on the lower McKenzie River contained bull trout assigned to all the populations from the upper McKenzie River and potentially the Middle Fork Willamette. Only bull trout from the Middle Fork Willamette were captured at the Hills Creek Dam.

Results from STRUCTURE were also similar to the previous analyses. K=2 appeared to be the optimal clustering pattern based on likelihood values and the ΔK statistic. This partitioning split the dataset into a cluster containing only the Roaring River population and another containing all other populations. No individual bull trout had ancestry proportions less than 0.9 for either cluster. Other levels of K provided insight into additional patterns of genetic structure. At K=3 the Upper McKenzie spawning location formed a distinct cluster separate from the Roaring River and Anderson Creek-origin populations. However, individuals assigned to the Upper McKenzie cluster were observed in spawning locations below Trail Bride Dam and individuals from the Anderson Creek cluster were found in the Upper McKenzie spawning area. At K=4 Ollalie Creek formed a distinct cluster. The Middle Fork Willamette population contained individuals with ancestry assigned to the Anderson Creek, Upper McKenzie, and Ollalie populations.

All point estimates of N_E were below 50, except for the Middle Fork Willamette estimate from 2014 (Table 6). Aside from that one estimate, the highest estimates of N_E were from the Roaring River. The lowest estimates by far were from the Upper McKenzie spawning location. For populations with two temporal estimates, all but one population (Roaring River) saw an increase in N_E over time.

Ten individuals across the dataset had brook trout-specific alleles (Table 7). Most (n=6) were captured at Trail Bridge Dam. Three fish were captured at the Upper McKenzie spawning location and another one at the Middle Fork Willamette spawning location.

Conclusions

The patterns of genetic structure we observed align with the findings of DeHaan and Diggs (2009). A majority of the populations share genetic ancestry with Anderson Creek, which served as a source for restoration efforts within this region. This complicates assessments of population assignment since many populations have shared ancestry. Values of F_{ST} between

Anderson Creek and Sweetwater Creek and Middle Fork Willamette River (both Anderson Creek recipients) were among the lowest observed. These three populations along with Olallie Creek substantially overlapped in the PCA plot, indicative of high genetic similarity. This was further reflected in the STRUCTURE analysis. Most of the out-of-tributary assignments estimated by ONCOR involved the four populations of Anderson Creek origin, opposed to the more distinctive populations of the Upper McKenzie and Roaring River. Therefore, interpretations should be made with caution concerning movement between these Anderson Creek-derived spawning populations and the ancestry of bull trout captured at fish passage facilities. For example, when a probability of assignment threshold of 90% is applied to the ONCOR results, the number of individuals captured at fish passage facilities that could be assigned to spawning populations decreased 24% (Table 5).

One clear pattern, though, is the distinctiveness of the Roaring River and Upper McKenzie populations, matching the findings of DeHaan and Diggs (2009). The Roaring River population is by far the most genetically distinct population, likely a consequence of limited gene flow. DeHaan and Diggs (2009) speculated this distinctiveness was the result of historic isolation due to the number of private alleles unique to the population that were observed and lack of a genetic signature of a bottleneck. With a larger dataset, this observation was further confirmed in this study (data not shown). No Roaring River-origin fish were detected at any other spawning location or fish passage site except for Cougar Dam, indicating limited movement of these bull trout across the system. Three non-Roaring River origin fish were captured at Cougar Dam. Genetic assignments suggest they originated from an Anderson Creek-ancestry population and the Upper McKenzie River. Although the presence of out-of-basin fish at Cougar Dam suggests the potential for gene flow, these individuals were just a small portion (3 out of 24) of the bull trout collected at the dam and there was no indication any out-of-tributary individuals eventually made it to the spawning grounds. PIT tag monitoring also confirms that one of these out of basin individuals later returned to the Upper McKenzie watershed (N. Zymonas, personal comm.).

Roaring River likely contains a relic native population that has managed to persist in the face of isolation and anthropogenic perturbations. The samples analyzed here exhibited no evidence of introgression from brook trout. Values of effective population size were not dramatically low, but still below thresholds often considered viable by conservation geneticists. Genetic diversity was also low, likely a product of isolation and limited population size. Improving conditions to facilitate the movement of migrants into the population would likely have long-term genetic benefits. However, of any population in this system, Roaring River has the highest likelihood of local adaptation to its environment. The high fidelity of individuals to the spawning location further supports this hypothesis. Thus, any attempts to introduce external genetic variation to this population should be done carefully to avoid genetic swamping that would reduce the uniqueness of the population.

The Upper McKenzie River spawning population was also distinct genetically, although it is clearly much more closely related to Anderson Creek than Roaring River is with either. The distinctiveness of this population has likely been influenced by contemporary forces: in terms of river distance this population in only several kilometers from other spawning location. Trail Bridge Dam has formed a major barrier limiting gene flow and facilitating a low effective population size has likely resulted in rapid genetic drift. This spawning population has the lowest genetic diversity and would benefit from gene flow. Already this appears to be happening: both the results of the PCA and the ONCOR assignments suggest that bull trout from Anderson Creek-ancestry populations are present in the Upper McKenzie. Half (29/60) of the bull trout

captured at Trail Bridge Dam were not of Upper McKenzie origin. This was further confirmed by the STRUCTURE analysis. These individuals are either bull trout of Sweetwater Creek origin attempting to return to the areas above the dam or dispersing from populations below the dam. Either scenario has positive implications for the Upper McKenzie River, assuming these individuals disperse to and reproduce in this river. Although slight, the increases in genetic diversity and effective size in the Upper McKenzie observed from 2007 to 2014 are likely due to at least the presence of migrants. Interesting, the proportion of "Anderson Creek" ancestry observed in Upper McKenzie bull trout increased over time: in 2005 ~10% of the ancestry was assigned to the "Anderson Creek" cluster in STRUCTURE (K=3). In 2013 and 2014, that proportion had grown to 32% and 38%, respectively. Additional monitoring will be necessary to determine whether gene flow is impacting the genetic characteristics of the Upper McKenzie spawning population or if the increases we observed were simply due to sampling variability.

Based on the leave-one-out test, PCA, and STRUCTURE, some bull trout captured in Anderson Creek and the Middle Fork Willamette were similar to the Upper McKenzie population. Dispersal is one explanation, especially for those captured in Anderson Creek, but it seems unlikely bull trout from the Upper McKenzie have navigated multiple dams to reach the Middle Fork Willamette. A more likely explanation is that some of bull trout used to found this population were originally from the Upper McKenzie. The correspondence analysis conducted by DeHaan and Diggs (2009) suggest some of the captive individuals were similar genetically to the Upper McKenzie population. Our results further suggest that although the fry transfers used to found Middle Fork Willamette population were from Anderson Creek, this was actually a mixed stock containing individuals of Anderson Creek, Upper McKenzie, and even potentially Ollalie Creek origin. Our results further confirm the findings of DeHaan and Diggs (2009): the contemporary population appears completely derived from these Anderson Creek transfers with no ancestry from potential relic populations.

The Middle Fork Willamette has retained levels of genetic diversity comparable with its source. It also displayed many of the characteristics we expect with a classic randomly mating population: there were few loci out of HWP, F_{IS} was close to zero, and levels of genetic diversity were consistent over time. This suggests the population has not experienced significant drift or inbreeding. Estimates of effective population size were also among the highest values observed across the entire system. The very high value of N_E observed in 2014 was unusual: it was three times higher than any other observed value. A jump from 11 to 144 from 2007 to 2014 was unexpected. A potential explanation is that the adult fish captured in 2007 were from the initial fry transfers. In 2007 fry raised in ODFW Leaburg Fish Hatchery were released into the Middle Fork Willamette. Releasing large numbers of fish propagated in captivity potentially reduced the variance in family size and increased the number of contributing parents compared to wild populations. This could have provided an apparent boost in N_E, especially since the method we used (linkage disequilibrium) is sensitive to these factors. Also, the presence of bull trout from multiple populations (i.e. Upper McKenzie) would further elevate linkage disequilibrium. In other words, the large N_E observed in 2014 was most likely artificially inflated due to factors that would have confounded the analysis.

The short-term outlook of the Middle Fork Willamette population from a genetic perspective appears positive. Considering the spatial distance of this population from other spawning locations and the number of barriers in the basin, the Middle Fork Willamette seems unlikely to be the recipient of gene flow from other populations. A large, robust population of mixed ancestry could be resistant to declines in genetic diversity and effective size. However,

one of the goals in the Upper Willamette Action Plan is to ensure that populations are connected via genetic exchange. Few bull trout were captured at Hills Creek Dam and those that were assigned to the Middle Fork Willamette population. Again, given the shared ancestry we must be careful about drawing conclusions regarding movements between this population and others. Long-term monitoring will determine whether bull trout from other populations disperse into the Middle Fork Willamette and if declines in genetic diversity are substantial enough to warrant management action.

Individuals with brook trout alleles composed ~1.4% of the sampled individuals. The nature of the data does not allow us to quantitatively evaluate whether these individual are F1 hybrids or backcrosses. In general, however, a low number of brook trout specific alleles tends to be observed in later generational backcrosses between hybrids and bull trout. Most potential hybrids were captured at Trail Bridge Dam, which means they could have been removed from the population prior to reaching the spawning grounds. The fact that three hybrids were found in the Upper McKenzie spawning location is a concern: the reduced effective size of the population suggests this is not a particularly large or robust population that can resist introgression.

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References

DeHaan, P, and M. Diggs. 2009. Analysis of genetic variation in Upper Willamette River bull trout populations. US Fish and Wildlife Service, AFTC Final Report: 66.

DeHaan, P. 2010. Genetic Monitoring and Evaluation Plan for Bull Trout in the Upper Willamette River, Oregon. US Fish and Wildlife Service.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14: 209–214.

Excoffier, L., and H. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567–87.

Kalinowski, S.T., Manlove, K.R., and Taper, M.L. 2007. ONCOR: software for genetic stock identification. Montana State University, Bozeman, Mont.

Keenan, K., P. McGinnity, T. F. Cross, W. W. Crozier, and P. A. Prodöhl. 2013. diveRsity : An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4: 782–788.

Paradis, E. 2010. Pegas: An R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419–420.

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

U.S. Fish and Wildlife Service. 2008. Biological Opinion on the continued operation and Maintenance of the Willamette River Basin Project and Effects to Oregon Chub, Bull Trout and Bull Trout Critical Habitat Designated Under the Endangered Species Act. Portland, Oregon.

Table 1: Distribution of 687 genotyped individual bull trout according to capture location. Note that this does not include ten individuals that were identified as having brook trout alleles or individuals that were captured in multiple locations. For individuals captured at dams and then later at spawning locations, they were assigned to the spawning location.

Tributary	Capture location	Location type	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
Lower McKenzie River	Leaburg Dam	Passage					11				8	5	24
McKenzie River	Anderson Creek	Spawning			50						8	19	77
	Sweetwater Creek	Spawning				21					5	11	37
	Upper McKenzie	Spawning	24								18	31	73
	Olallie Creek	Spawning				38							38
	Carmen-Smith Spawning Channel	Passage						1					1
	Trail Bridge Dam Tailrace	Passage			9		16	9	3	5	6	12	60
	Trail Bridge Reservoir	Passage	41									1	2
Middle Fork Willamette River	Forest Rd 2143	Spawning		7	20	5		3	3	9	2	16	65
	Hills Cr Dam Tailrace	Passage							1			1	2
South Fork McKenzie River	Cougar Dam Tailrace	Passage					1	1	5	10	4	3	24
	Roaring River	Spawning	17	12	56						24	92	201

Table 2: Results from the multilocus AMOVA. Individuals were grouped by collection year and spawning site. Variation among spawning sites indicates the amount of genetic variation that was partitioned among spawning location when individuals from multiple years were grouped together. Variation among collection years within spawning sites is the portion of genetic variation partitioned among collection years within the same sample sites. Variation among individuals within collection years refers to the proportion of genetic variation partitioned by collection year.

Source of variation	Sum of squares	Variance components	Percentage variation
Among spawning sites	516.47	0.87	19.80
Among collection years within spawning sites	47.89	0.03	0.71
Among individuals within collection years	1433.80	0.09	2.11
Within individuals	1407.5	3.42	77.38
Total	3405.66	4.41	

Table 3: List of *p*-values generated for each microsatellite locus by an exact test of Hardy-Weinberg Proportions (HWP). Individuals were grouped by spawning location. Lower *p*-values indicate departures from neutral HWP.

Locus	Anderson Creek	Mid Fork Willamette	Olallie Creek	Roaring River	Sweetwater Creek	Upper McKenzie
Sco109	0.522	0.043	0.774	0.019	0.009	0
Sfo18	1	0.015	1	1	0.001	0.01
Sco216	0.27	0.456	0.532	0.001	0.241	0.388
Sco212	0.706	0.778	0.552	0.078	0.762	0.267
Sco220	0.396	0.088	1	0.007	0.901	0.014
Sco215	1	1	1	0.004	1	1
Sco105	1	0.391	0.454	0.441	0.579	0.085
Sco200	0.561	0.113	0.244	0.441	0.691	0
Smm22	0.309	0.18	0.206	0	0.693	0.008
Sco202	1	1	1	1	1	1
Omm1128	0.001	0	0.052	0	0.047	0
Sco102	1	0.011	1	1	1	1
Sco106	0.557	0.744	0.021	0.001	0.501	0.072
Sco107	0.94	0.041	0.01	0	0.186	0
Omm1130	0.521	0.755	0.134	0.442	0.039	0.022
Sco218	0.519	0.032	0.634	0.011	0.003	0

Table 4: Results from the leave-one-out assignment tests of collections from Upper Willamette bull trout spawning locations. Values indicate the number of individuals with rows reflecting the population of origin and columns reflecting the assigned population. Values along the diagonal (in bold) indicate assignment to the population of origin.

		Population assigned by ONCOR					
		Anderson Creek	Mid Fork Willamette	Olallie Creek	Roaring River	Sweetwater Creek	Upper McKenzie
Population of origin	Anderson Creek	**41**	10	0	0	12	11
	Mid Fork Willamette	13	**39**	3	0	3	4
	Olallie Creek	3	5	**27**	0	2	0
	Roaring River	0	0	0	**183**	0	0
	Sweetwater Creek	6	5	0	0	**24**	0
	Upper McKenzie	2	4	0	0	11	**51**

Table 5: Number of bull trout captured at fish passage facilities assigned to spawning populations in the Upper Willamette River basin by ONCOR. A.) Assignments based solely on most likely population of origin, regardless of actual probability. B.) Assignments for which individuals were assigned to the most likely population of origin with >90% probability.

A.

	Spawning location					
Passage location	Anderson Creek	Mid Fork Willamette	Olallie Creek	Roaring River	Sweetwater Creek	Upper McKenzie
Carmen-Smith by-pass						1
Cougar Dam	1	1		21		1
Hills Creek Dam		2				
Leaburg Dam	2	4	1		6	11
Trail Bridge Dam	2	13			14	31
Trail Bridge Reservoir	1	3			5	33

B.

	Spawning location					
Passage location	Anderson Creek	Mid Fork Willamette	Olallie Creek	Roaring River	Sweetwater Creek	Upper McKenzie
Carmen-Smith by-pass						1
Cougar Dam				21		1
Hills Creek Dam		2				
Leaburg Dam		2	1		3	9
Trail Bridge Dam		2			12	27
Trail Bridge Reservoir		2			1	32

Table 6: Estimates of effective population size (N_E) produced with the single sample linkage disequilibrium method. Estimates are for bull trout collected from specific years for the six spawning sites. Included are the lower and upper 95% confidence intervals bounding the point estimates. Note: both Anderson Creek 2007 and Olallie Creek collections were composed of juvenile fish.

Population	Year	N_E	Lower CI	Upper CI
Anderson Creek	2007	13.8	11.1	17.2
Anderson Creek	2014	23.5	15.9	39.3
Mid Fork Wil	2007	11.1	8.3	14.9
Mid Fork Wil	2014	143.6	39.1	Infinite
Olallie Creek	2008	9.5	6.6	13.2
Roaring River	2007	35.8	24.3	57.2
Roaring River	2014	29.5	20.5	43.7
Sweetwater Creek	2008	15.9	10.8	25.1
Sweetwater Creek	2014	47.2	16.4	Infinite
Upper McKenzie	2005	1.7	1.3	2.2
Upper McKenzie	2014	3.8	3.2	6

Table 7: Individuals collected during bull trout surveys that possessed brook trout-specific alleles. Location indicates the spawning area or fish passage facility where the individual was captured. Numbers of brook trout alleles (out of a potential maximum of 20) exhibited by each of the individuals are listed.

Individual	Location	Year	Number of alleles
1544-015	Mid Fork Willamette	2010	2
2375-022	Trail Bridge Dam	2012	3
2375-021	Trail Bridge Dam	2012	4
1533-082	Trail Bridge Dam	2009	5
2021-016	Trail Bridge Dam	2011	5
1552-083	Trail Bridge Dam	2010	13
2375-028	Trail Bridge Dam	2012	13
2318-087	Upper McKenzie	2012	4
2022-006	Upper McKenzie	2014	6
2022-083	Upper McKenzie	2014	6

Figure 1: Map of the McKenzie and Willamette River basins with relevant hydropower structures and bull trout spawning location in purple. The inset is a more detailed map of the tributaries and spawning locations around Trail Bridge Dam.

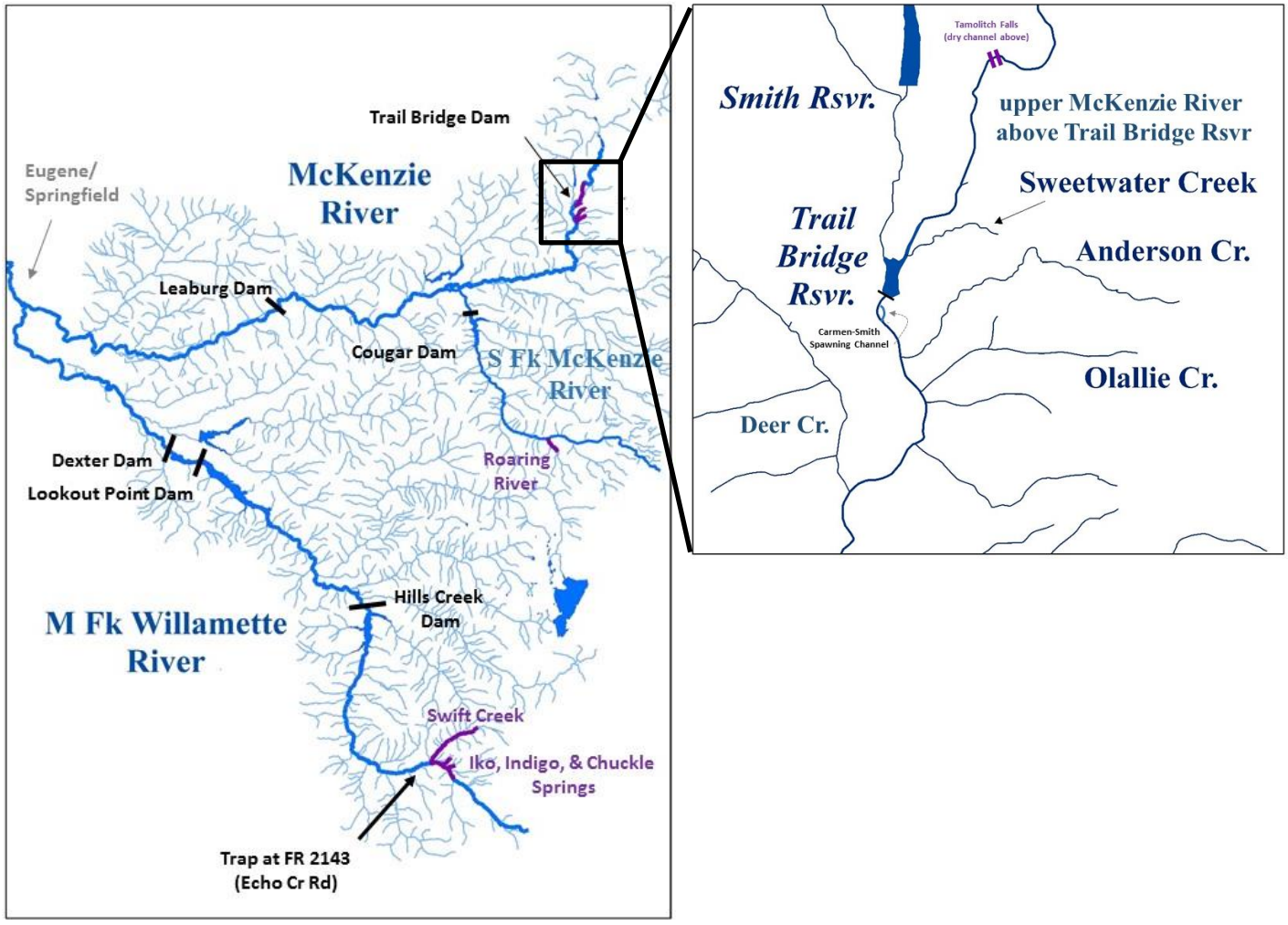


Figure 2: Plots of genetic diversity for bull trout captured at spawning locations in the Upper Willamette River basin. Individuals were aggregated across years. The metrics displayed are observed and expected heterozygosity (H_O and H_E, respectively), allelic richness (A_R), and F_{IS}. These values were averaged across loci.

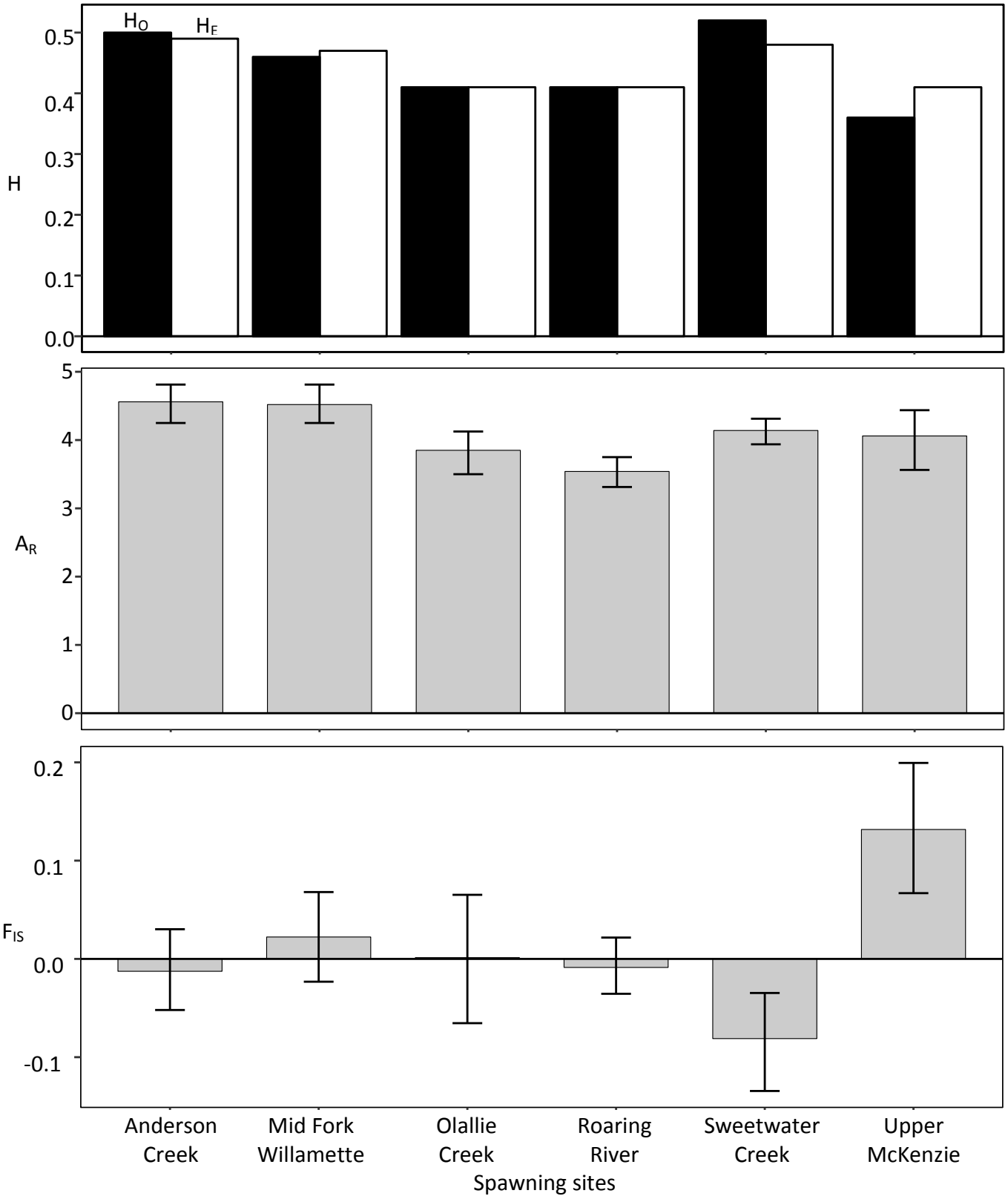
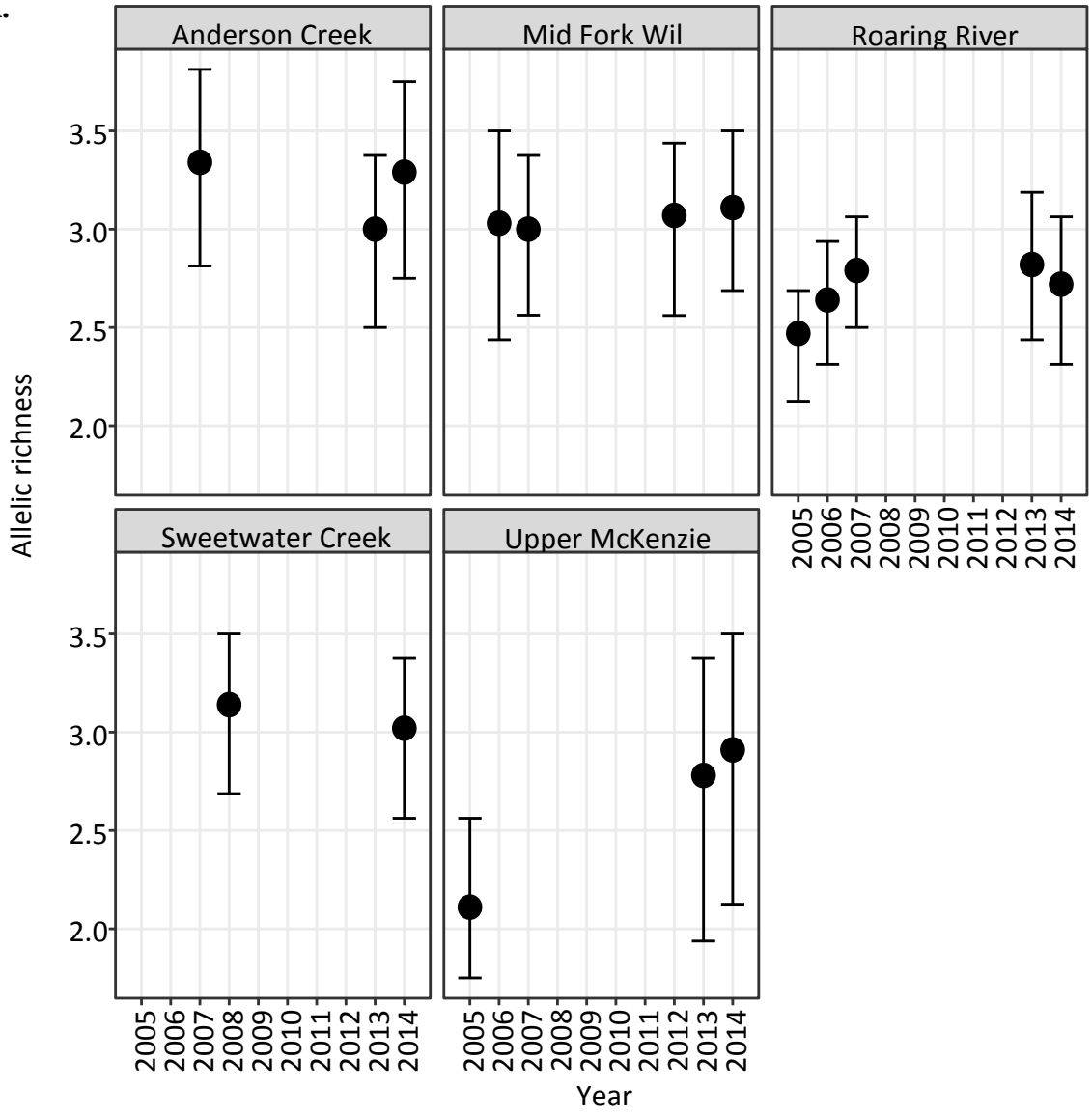


Figure 3: Estimates of A.) allelic richness (A_R) and B.) observed heterozygosity (H_O) across years for bull trout spawning sites in the Upper Willamette River. Only data from years in which seven or more individuals were genotyped are included. Ollalie Creek is excluded because collection occurred only during one year. Allelic richness includes 95% confidence intervals around each point estimate.

A.



B.

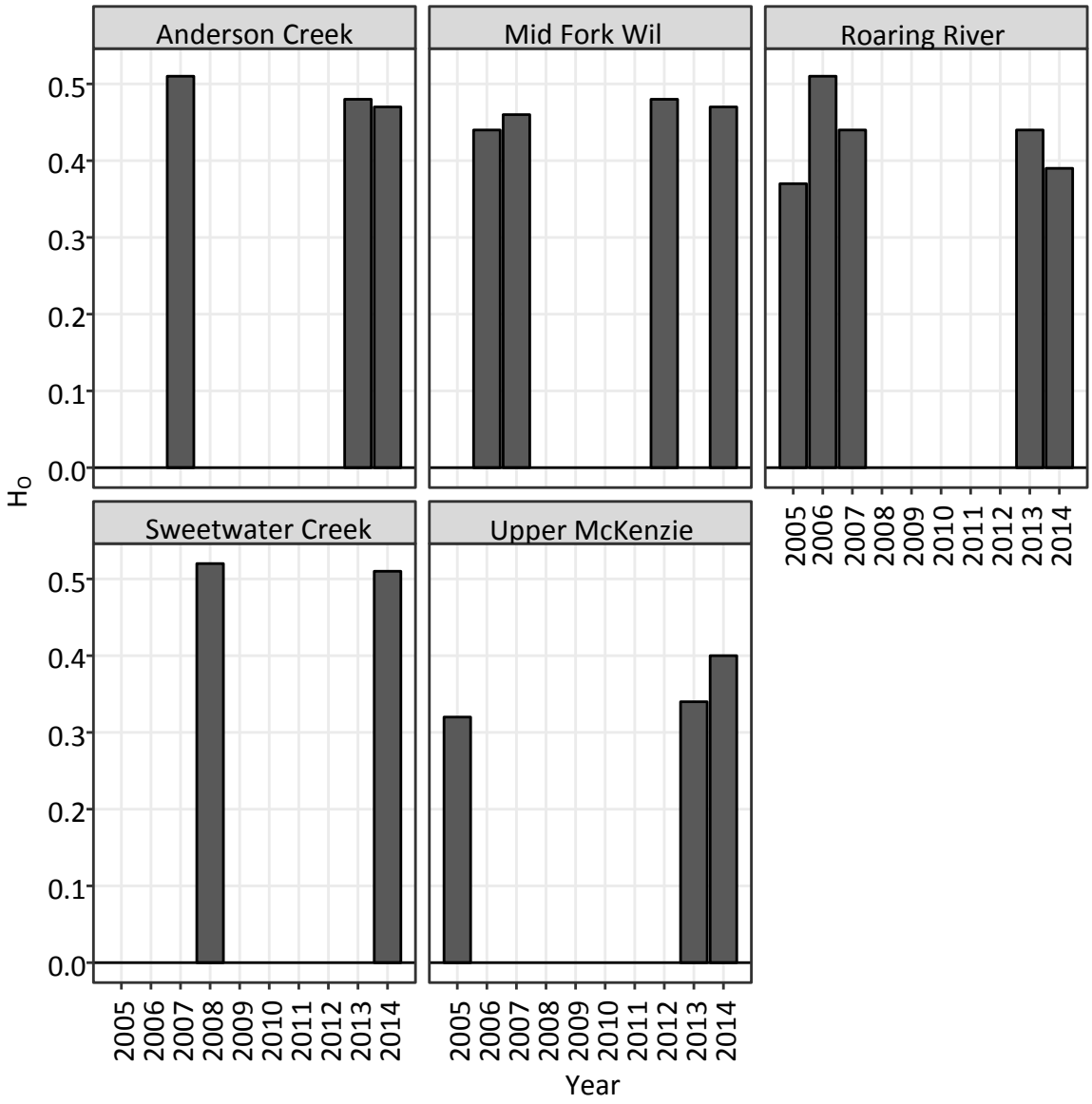

Anderson Creek
Mid Fork Wil
Roaring River
Sweetwater Creek
Upper McKenzie
H_O
0.0
0.1
0.2
0.3
0.4
0.5
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Year

Figure 4: Principal component analysis based on allele frequencies for all bull trout captured at spawning locations in the Upper Willamette River basin. The percentages along the axes indicate how much variation in allele frequencies is encapsulated by the first two principal components. Color codes: Anderson Creek=black, Middle Fork Willamette=orange, Olallie Creek=red, Roaring River=gray, Sweetwater Creek=blue, Upper McKenzie=green.

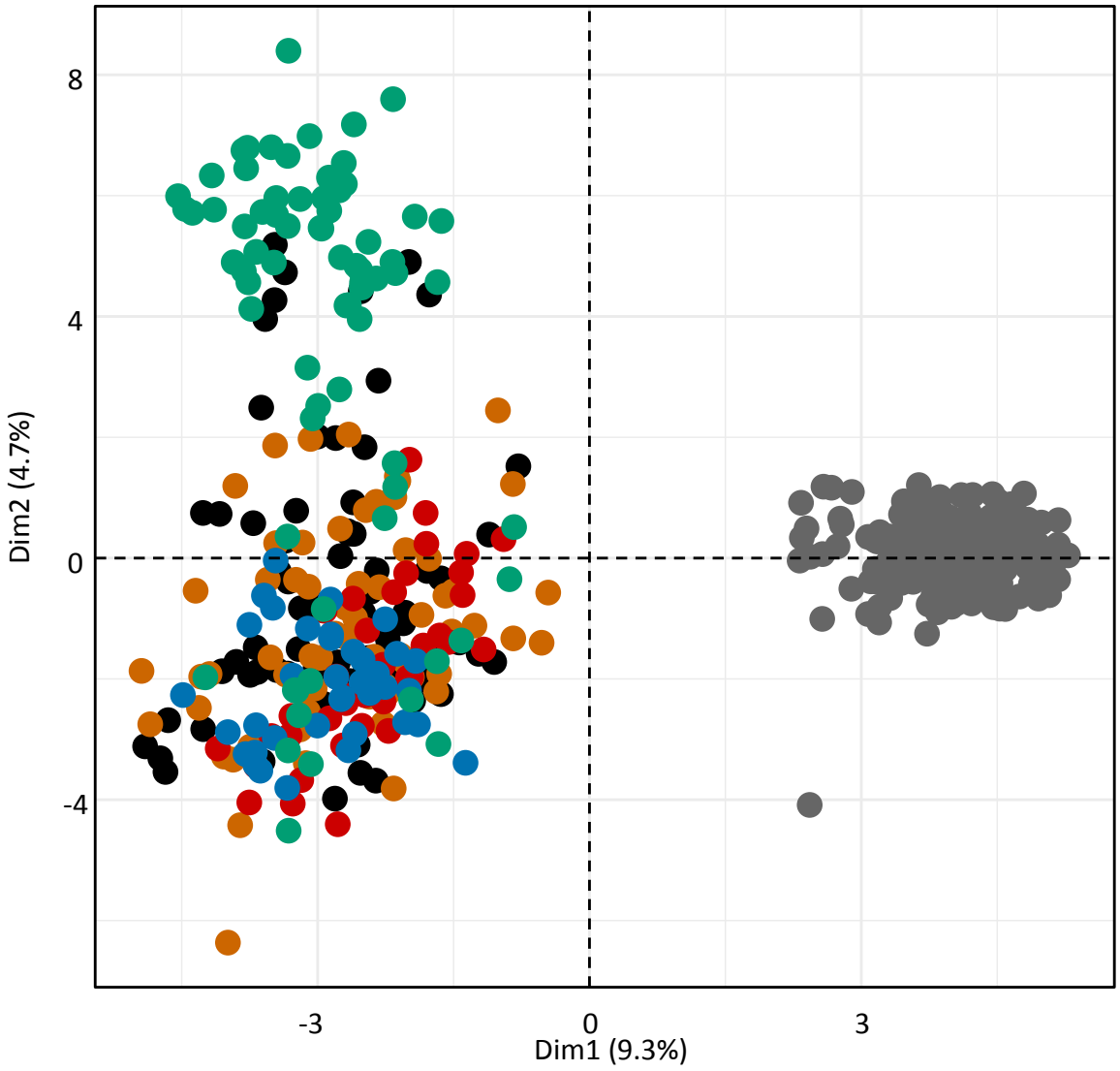


Figure 5: Pairwise estimates of F_{ST} between bull trout spawning populations in the Upper Willamette River basin. The F_{ST} point values are bounded by 95% confidence intervals. Codes for the spawning locations: AC=Anderson Creek, MFW=Middle Fork Willamette, OC=Olallie Creek, SC=Sweetwater Creek, RR=Roaring River, UFM= Upper Fork McKenzie.

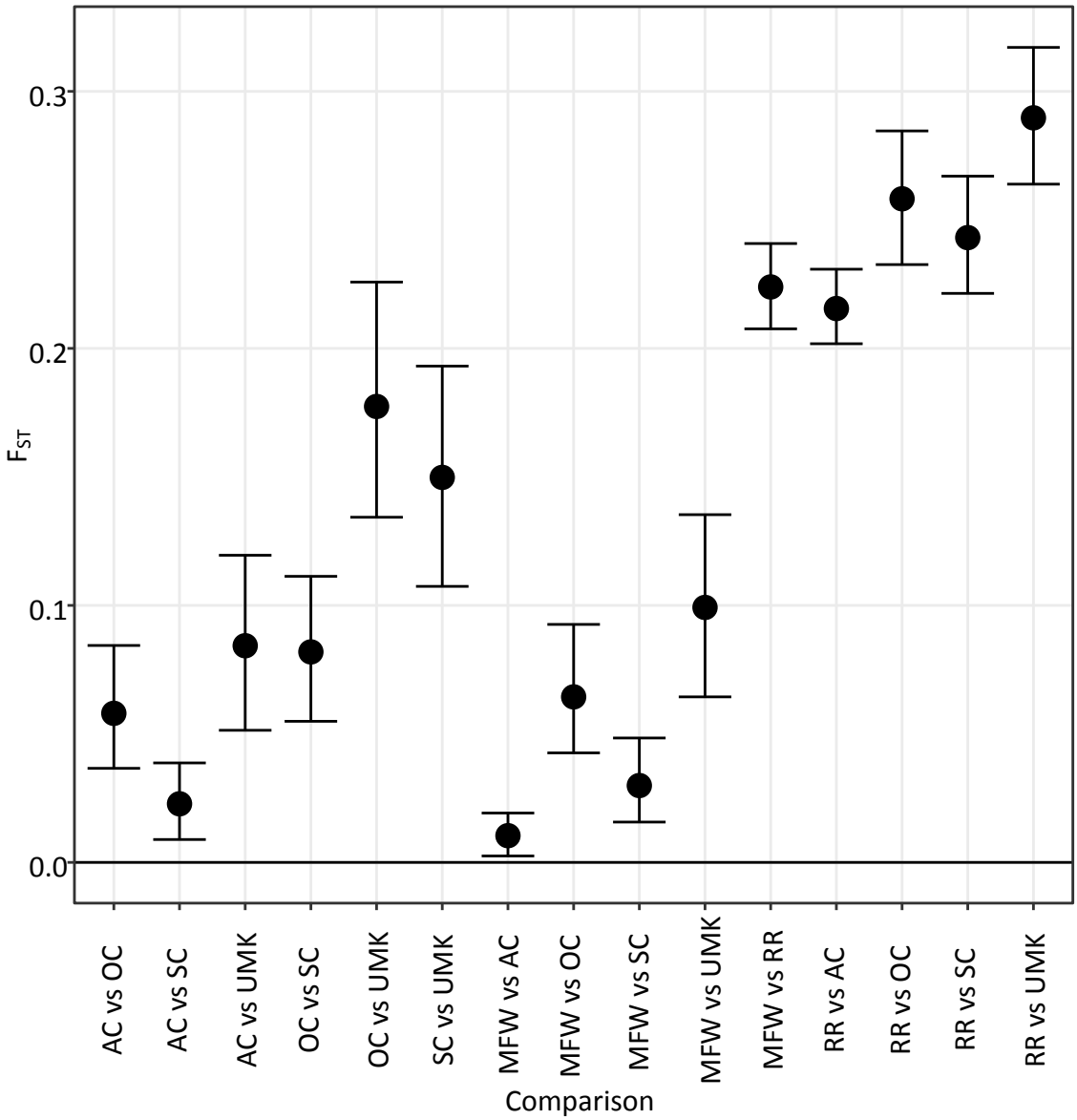


Figure 6: Plots of A.) mean log-likelihood and B.) Δ*K* produced by the STRUCTURE analysis of genotypes captured at spawning locations. The mean log-likelihood is based on the average likelihood from ten iterations of STRUCTURE. Error bars indicate the standard deviation of the log-likelihood. Δ*K* is estimated based on the Evanno et al. (2005) method. Values were estimated for *K* ranging from 1-10.

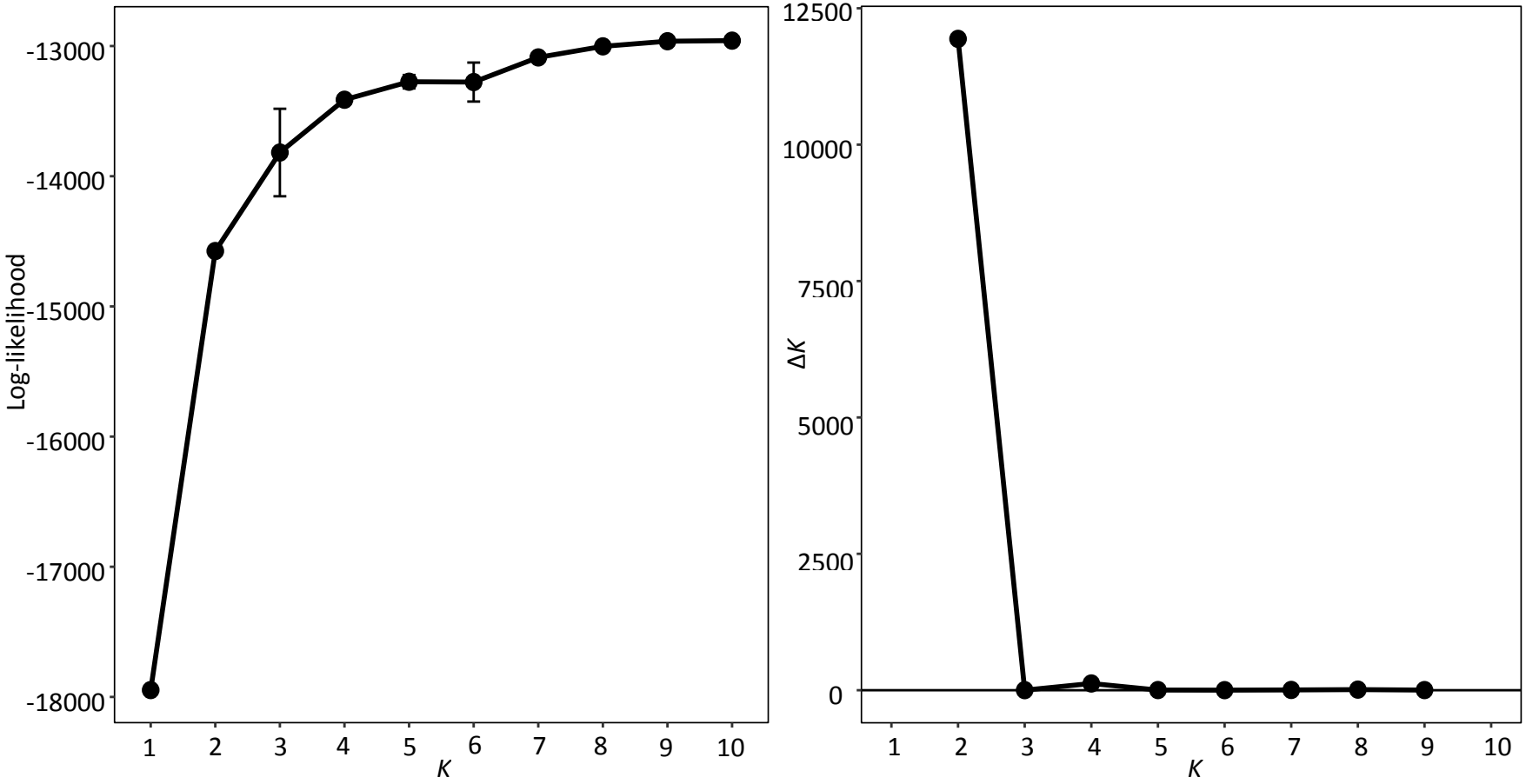
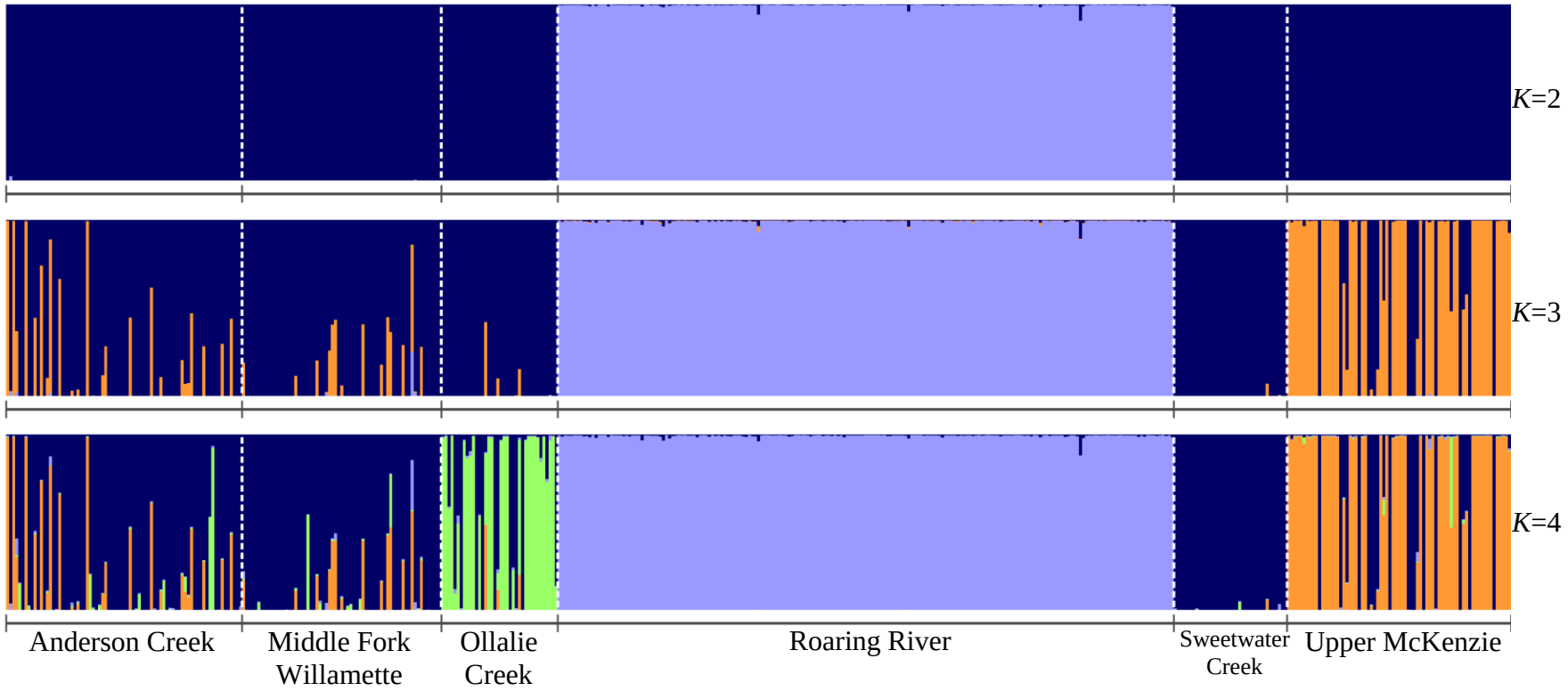


Figure 7: Barplots of ancestry proportions estimated by STRUCTURE for genotypes captured at spawning locations. Results are presented for values of *K* ranging from 2-4. Each individual is represented by a single vertical bar and the colors correspond to the proportion on that individual's ancestry assigned to a specific cluster. The clusters identified by STRUCTURE at each value of *K* are denoted by unique colors. Individuals are grouped according to the spawning location where they were sampled.



U.S. Fish & Wildlife Service
Abernathy Fish Technology Center
Longview, Washington, 98632
Phone:360-425-6072
http://www.fws.gov/aftc/index.html