AN ABSTRACT OF THE THESIS OF

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Title: Factors Influencing Within-Watershed Genetic Variation of Coastal Cutthroat Trout in Camp Creek, Oregon.

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Abstract approved: _____

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Because human land use activities often result in increased fragmentation of headwater stream habitats, a better understanding of the effects of fragmentation on the genetic heterogeneity of stream salmonids is useful for effective management. We used eight microsatellites to examine the genetic structure of potamodromous coastal cutthroat trout (*Oncorhynchus clarki clarki*) in Camp Creek, an isolated headwater stream in western Oregon. Our objectives were to determine if coastal cutthroat trout were genetically structured at fine spatial scales and to assess the effects of natural and anthropogenic barriers on coastal cutthroat trout genetic variation. Fish sampling occurred at 10 locations, and allele frequencies differed significantly among all sampling sections. Dispersal barriers strongly influenced coastal cutthroat trout genetic structure and were associated with reduced genetic

diversity and increased genetic differentiation. Results indicate that Camp Creek coastal cutthroat trout exist as many small, partially independent populations connected by low to moderate levels of gene flow. In headwater streams, increased habitat fragmentation can result in genetic and demographic isolation leading to reduced coastal cutthroat trout genetic diversity and compromising long-term population persistence.

Factors Influencing Within-Watershed Genetic Variation of

Coastal Cutthroat Trout in Camp Creek, Oregon

By

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
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John E.B. Wofford, Author

ACKNOWLEDGEMENTS

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CHAPTER 1: INTRODUCTION

Proper conservation and management of aquatic species require accurate descriptions of genetic structure and an understanding of the processes that produce genetic heterogeneity. Investigations of genetic structure can provide insight into large-scale historical and evolutionary phenomena (e.g. post-Pleistocene dispersal, adaptive radiation) as well as recent population events (e.g. human induced population bottlenecks). In salmonid species, studies have revealed complex hierarchical or mosaic patterns of population organization at a variety of spatial scales (Waples et al. 2001). These patterns are created by the interaction of genetic drift, selection, inbreeding, and mutation, with the factors that influence gene flow, such as landscape structure and dispersal behavior.

Lotic fish biologists have recently gained a greater appreciation of the importance of dispersal in shaping genetic, demographic, and community structure (Schlosser and Angermeier 1995, Maret et al. 1997). In salmonids, dispersal between populations can provide demographic connections that can be critical for population persistence (Morita and Yamamoto 2001) and recolonization following extirpation (Hansen and Mensberg 1996, Gresswell 1999). In addition, dispersal provides genetic linkages important for maintaining genetic variation that is critical to preserving long-term adaptive potential of populations and species (Allendorf 1986).

From the perspective of a fish, headwater streams of western Oregon are frequently fragmented by both natural (e.g. waterfalls and cascades) and

anthropogenic (e.g. culverts) barriers to dispersal. Many of these headwater streams are inhabited by potamodromous coastal cutthroat trout (*Oncorhynchus clarki clarki*) (Hooton 1997). Compared to other Pacific salmonids, potamodromous coastal cutthroat trout are relatively sedentary, often exhibiting every life stage in a small portion of a headwater stream (Trotter 1989). Although prior studies of anadromous coastal cutthroat trout have revealed significant genetic heterogeneity among streams (Wenburg and Bentzen 2001), little work has been done to quantify potamodromous coastal cutthroat trout population structure or to address the effects of human activities on coastal cutthroat trout genetic variation.

Headwater streams can be negatively affected by upslope land uses, such as road building and logging (Meehan 1991), and these actions can produce varying effects on coastal cutthroat trout demographics (Murphy et al. 1986, Connolly and Hall 1999). In order to further evaluate coastal cutthroat trout population responses to habitat alterations and as a means to develop conservation goals, an improved understanding of coastal cutthroat trout genetic structure is needed. Stream fish conservation measures often fail because ecologists neglect to conduct research at spatial scales that are appropriate for the organism of study and that are relevant to human management of watersheds (Fausch et al. 2002). In an attempt to address this problem, this study investigates genetic structure at an intermediate spatial scale, where land use activities and coastal cutthroat trout populations occur in concert. We used eight microsatellites to provide an assessment of coastal cutthroat trout genetic differentiation within an isolated watershed. Our objective was to gain inference on

the conditions that influence coastal cutthroat trout population structure in stream

networks and to discuss the implications of these factors for management of coastal

cutthroat trout.

CHAPTER 2: MATERIALS AND METHODS

<u>Study Site and Sampling Procedures</u>

Camp Creek, a third-order stream in the Umpqua river basin of western

Oregon, was chosen for this study (Figure 1, Figure 2). The Camp Creek watershed

has a drainage area of 2200 hectares and is primarily composed of sedimentary rock

with riparian vegetation consisting of red alder (*Alnus rubra*), vine maple (*Acer*

circinatum), and big-leaf maple (*Acer macrophyllum*) (BLM 1995). Old-growth

Douglass-fir (*Pseudotsuga menziesii*) and red cedar (*Thuja plicata*) are present

throughout the riparian corridor. Although extensive logging has occurred on

tributaries and ridge tops, the watershed is currently managed as a late-successional

reserve (BLM 1995). The study sections of Camp Creek are isolated from migratory

fish by a 15-m waterfall (barrier 1, Figure 1). Coastal cutthroat trout, reticulate

sculpin (*Cottus perplexus*), and longnosed dace (*Rhinichthys cataractae*) are the only

fish species present, and there are no records of fish stocking in the Camp Creek

basin.

During the summer of 2002, the watershed was surveyed in order to identify

geomorphic or anthropogenic barriers to fish passage. Two culvert barriers and four

geomorphic (waterfalls and bedrock cascades) barriers were identified by visual

assessments. Culverts were verified as fish passage barriers using FishXing v. 2.2

software (http://stream.fs.fed.us/fishxing/). An examination of historical aerial

photographs indicated that the two culvert barriers were installed in the mid to late

1950s. We were unable to date geomorphic barriers.
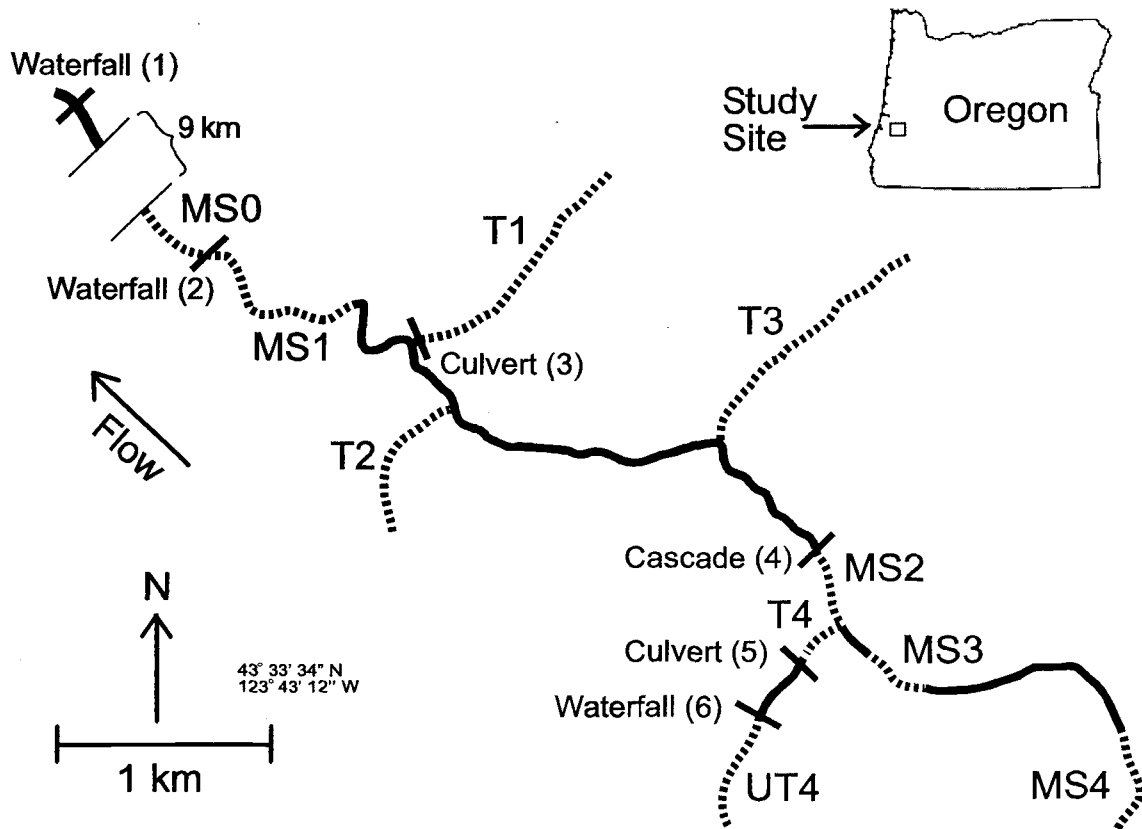
Figure 1. Map of coastal cutthroat trout distribution and sampling sections in the Camp Creek study area. Sampling sections are identified by dashed lines. Solid bars indicate barriers to fish passage. Captions associated with bars specify barrier types and numbers in parentheses identify barriers. MS = mainstem, T = tributary, UT = upper tributary.

Figure 2. Color shaded relief map of coastal cutthroat trout distribution and sampling
sections in the Camp Creek study area. Sampling sections are identified by colored
lines. Solid bars indicate barriers to fish passage. MS = mainstem, T = tributary,
UT = upper tributary.

Culvert barrier

Geomorphic barrier

MS4

MS3

UT4

MS2

T4

T3

T2

T1

MS1

MS0

43° 33' 34" N
123° 43' 12'' W

N

1 km

WASHINGTON

OREGON
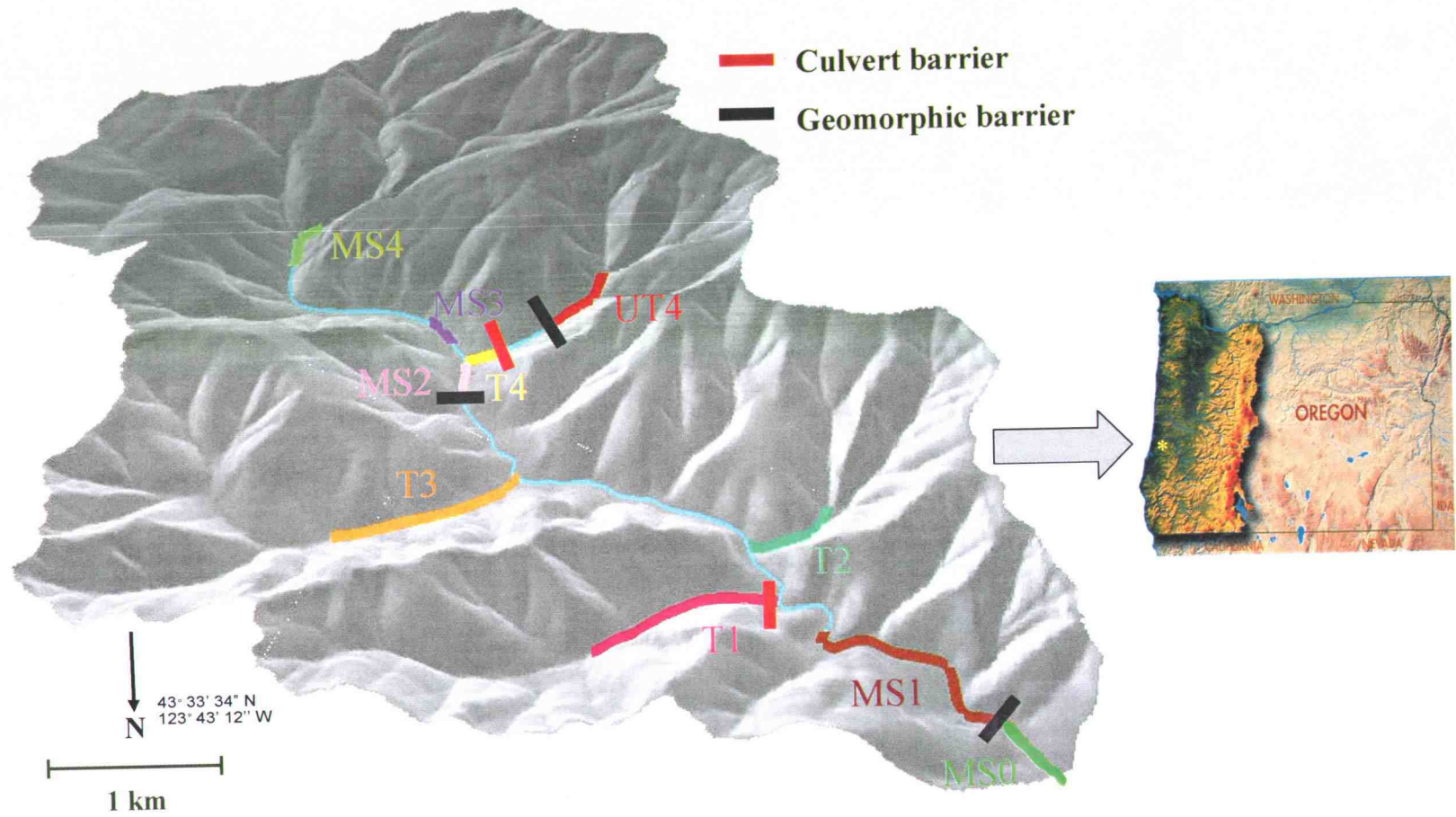
Figure 2.

All genetic sampling locations were determined by tributary junctions or fish passage barriers, excluding sites MS3 and MS4 (Figure 1, Figure 2). Sites MS3 and MS4 were selected because it was important to sample the entire watershed, and no tributaries or passage barriers occurred in the relatively extensive upper portions of Camp Creek. In all, genetic sampling occurred at ten sites in the Camp Creek watershed.

In August 2002, electrofishing crews began sampling at barrier 2 (Figure 1) and proceeded upstream, sampling every pool and cascade in the fish-bearing portions of the watershed using single-pass electrofishing without blocknets. For estimates of adult maximum abundance (see below) we were primarily interested in capturing age1+ coastal cutthroat trout, so riffles were not sampled in the lower portions of the basin because previous research has revealed that age-1+ coastal cutthroat trout are almost exclusively found in pools, particularly during summer months in watersheds dominated by sedimentary rock (Connolly 1996, Hicks and Hall 2003). Due to additional research questions not addressed in this study, upstream of barrier 3 (Figure 1), riffle habitats were also sampled.

Prior to release, trout were measured (nearest mm, fork length) and weighed (nearest 0.1 g), and a small portion of caudal fin tissue (1.5 mm^2) was collected. In sections MS1, T1, T2, and T3, up to ten fin clips were taken from trout (>50 mm in length) in each 10 mm size class until 100 samples were collected or until sampling crews reached the end of trout distribution. At site MS0 and at all sites above barrier 3, fin clips were obtained from every captured trout. Fin tissue was stored in 2 ml

vials with a calcium sulfate desiccant (Indicating Drierite®). If needed, trout were anesthetized in a solution of water and clove oil (Taylor and Roberts 1999). During May 2003, we returned to the basin, and additional samples were collected at site MS1 using a hook and line: fish were also collected at site MS0 for the first time. Thus, nine sites were sampled solely in 2002, one site in 2003 (MS0), and one site in both years (MS1). Length-frequency histograms were used to identify trout age groups.

Microsatellite Typing

Genomic DNA was extracted from small portions of tissue (0.5 mm^2) in 200 μL of 5% Chelex®100 (Biorad) in 96-well PCR trays (0.2 μL) using a MJ research PT-100 thermocycler. Tissue extracts were heated at 65°C for 3 hrs, boiled at 103°C for 10 min, and stored at 4°C. Eight microsatellite loci were used to characterize coastal cutthroat trout genetic variability in the Camp Creek watershed. All forward primers were labeled with fluorescent phosphoamidite (HEX, TET, or FAM). We developed two multiplex sets: set A (*Ots-209* and *Ots-212* (Greig et al. 2003)) and set B (*One-102*, *One-103*, and *One-108* (Olsen et al. 2000)). *Ots-9,-10* (Banks et al. 1999) and *Omy-1046* (Rexroad et al. 2002) were amplified individually in separate PCR reactions. Thermocycler profiles and PCR reactions are listed in Appendix Table A.1. DNA fragments were fractioned by size on a 5% acrylamide gel and visualized using a MJ Research Base-Station DNA fragment analyzer. Gels were manually scored using MJ Bioworks Cartographer version 1.2.3sg software. In order

to maximize sample sizes, we made second attempts at PCR reactions that failed to produce scoreable products during initial processing.

Genetic and Statistical Analysis

Allele frequencies, number of alleles per locus, allelic richness, and estimates of genetic distance (F_{st}) (Wright 1951), in the form of Weir and Cockerham (1984), were calculated using FSTAT software (Goudet 1995). Significance of probability values was evaluated by permutation procedures as implemented in FSTAT. Allelic richness values were standardized by sample sizes using a rarefaction technique (El Mousadik and Petit 1996, Petit et al. 1998). To test whether genetic divergence was related to the geographic distance between populations, a Mantel test using 2000 randomizations was employed using FSTAT. Observed heterozygosity and gene diversity (expected heterozygosity) were calculated using GENEPOP version 3.3 (Raymond and Rousset 1995). Tests for genotypic differentiation between all locus-population combinations were accomplished using exact tests and Marcov chain methods as implemented in GENEPOP. Marcov chain methods from GENEPOP were used to assess deviations from Hardy-Weinberg expectations and to evaluate genotypic linkage disequilibrium between loci. Parameters for all Marcov chain iterations included: dememorization number of 1000, 200 batches, and 1000 iterations. When appropriate, Bonferroni adjusted P-values were used for evaluating statistical significance (Rice 1989).

Genetic distance measures, phylograms, and phylogram bootstrap values (1,000 replicates) were generated using SEQBOOT, GENEDIST, NEIGHBOR, and

CONSENSE computer programs, respectively, as implemented in the PHYLIP software package (Felsenstein 1991). Trees were edited using TREEVIEW (Page 1996).

When large numbers of juveniles were present in a sample, or when sampling occurred over consecutive years, we tested for non-random sampling of family groups (Hansen et al. 1997) and temporal stability of allele frequencies by examining genotypic distributions, deviations from Hardy-Weinberg expectations, and genotypic linkage disequilibrium between age groups/sample years within sampling locations. If multilocus genotypic differences were encountered across age groups/sample years at a sample location, the respective subsamples were considered to be separate populations. Within age-0 samples, deviations from Hardy-Weinberg expectations or multiple failures of tests for genotypic linkage disequilibrium (>10% failures per population (Banks et al. 2000)) were viewed as evidence of family sampling.

Three-dimensional visualizations of each population were accomplished using factorial correspondence analysis (FCA) as performed by GENETIX v 4.04. Missing genotypes can impair the interpretation of FCA results, so prior to this analysis, genotype data from each population was resampled in order to produce 500 new individuals per population with complete genotypes using WHICHLOCI software (Banks et al. 2003). This program generates new populations of individuals with complete genotypes whose allele frequencies are concordant with original populations.

Spatially explicit depictions of coastal cutthroat trout genotypes were produced using ArcScene software with techniques described in Torgersen, et al. (In press.). Using WHICHLOCI software, genotypes were simulated for all captured coastal cutthroat trout in frequencies that were concordant with observed allele frequencies. Relative abundance data were not available for the MS0 population, so this population was excluded from Figures 4 and 5.

Genetic simulations were executed using EASYPOP 1.7 (Balloux 2001) and were performed under the following conditions: random mating, free recombination between loci, a single step mutation model (rate of 0.001), eight loci with a maximum of eight alleles (average number of alleles per locus in Camp Creek basin). Variance estimates were calculated from 200 replicates of each simulation.

Age-1+ Maximum Abundance and N_e Estimates

Coastal cutthroat trout relative abundance data allowed us to estimate maximum abundance for age-1+ trout in 9 of the 10 sampling locations. Age-1+ maximum abundance was estimated by dividing the number of age-1+ fish captured by a capture probability of 0.5. Although electrofishing capture probabilities in headwater streams of western Oregon approach 0.9 (Bateman D.B., unpublished data), a capture probability of 0.5 was used as a conservative measure.

These maximum abundance values were used to estimate coastal cutthroat trout effective population sizes (N_e). Allendorf et al. (1997) suggested $N_e/N = 0.2$ as an approximation for wild populations of Pacific salmon, where N represents the total population size per generation, estimated by multiplying the number of spawning

adults by mean generation time. The $N_e/N = 0.2$ approximation has been validated for a potamodromous salmonid species (Rieman and Allendorf 2001). The N_e of each coastal cutthroat trout population was estimated with this approximation, using age-1+ maximum abundance as a conservative measure of spawner abundance and assuming a mean generation time of two years (Nicholas 1978).

Using aerial photography, it was possible to estimate when the T1 population was isolated from the Camp Creek stream network, and thus, we were able to evaluate N_e of T1 using genetic techniques employing moment and likelihood methods of MLNE software (Wang and Whitlock 2003). We assumed that prior to culvert installation the T1 population had allele frequencies that were similar to the current MS1 population. The MS1 population appears to be the largest population (above barrier 2) in Camp Creek and thus is expected to have changed the least in the last 40 years. Although it is unlikely that allele frequencies in the T1 population were identical to those in the MS1 population, the small F_{st} values between MS1 and nearby T2 and T3 suggest that allele frequencies were probably similar and appropriate for a coarse estimate of N_e.

CHAPTER 3: RESULTS

Loci Diagnostics

Sample sizes for individual populations ranged from 31 (*One-102* in T2) to 118 (*Omy-1046* in MS3) and averaged 68 (Appendix Table A.2). All eight of the microsatellite loci analyzed were polymorphic in Camp Creek coastal cutthroat trout. Across all populations, the number of alleles per locus ranged from 3 (*Ots-209*) to 11 (*One-102*) with an average of 8.0 alleles per locus, although upstream of barrier 2, the average number of alleles per locus dropped to 4.1. A total of 20 private alleles (rare alleles observed in a single population) were documented, at least one private allele (mean frequency = 0.038) occurred at each locus. Seventeen of these private alleles were found in the MS0 population; upstream of barrier 2, three private alleles (average frequency 0.013) occurred in two loci (*One-102* and *One-108*) with three populations (T3, T4, and MS3) each containing one private allele.

Deviations from Hardy-Weinberg equilibrium were observed in 9 of 82 (10.9 %) possible tests within loci across populations ($\alpha = 0.05/11 = 0.0045$) and in 10 of 82 (12.2 %) possible tests across loci within populations ($\alpha = 0.05/8 = 0.00625$). Heterozygote deficits were spread among six populations and five loci with no more than three deficits at any one locus or population. A total of 15 of 267 (5.6%) possible loci combinations failed tests for genotypic linkage equilibrium ($\alpha = 0.05/28 = 0.0018$). These failures occurred across 10 loci combinations with no more than 3 at any one loci pair.

Temporal Stability and Family Sampling

We were able to test for temporal stability of allele frequencies and sampling of related individuals because of two sampling issues: 1) The MS1 sample location was sampled during consecutive years, and (2) length-frequency histograms indicated that several samples contained enough age-0 trout for reasonable sub-sampling (>10 age-0 trout at sites MS2, T4, UT4, MS3, and MS4). Tests for genotypic differentiation between sample years in the MS1 location revealed no significant temporal changes in allele frequencies ($\alpha = 0.05/8 = 0.0063$). Therefore, the MS1 samples from 2002 and 2003 were pooled into one sample. Comparisons across age groups at other sample locations indicated that three populations (MS2, MS4, and T4) contained genotypic differences between age-0 and age-1+ trout. In the MS2 and MS4 populations, age groups differed at a single locus (*Omy-1046 and Ots-10*, respectively), but in the T4 location, age groups differed at five of seven possible loci comparisons (*Ots-212, Omy-1046, Ots-10, One-102, One-103*) ($\alpha = 0.05/8 = 0.0063$). Because of the significant multilocus differences between age groups, samples from the T4 location were split into two samples (one containing age-0 trout, the other composed of age-1+ trout).

Tests for genotypic linkage disequilibrium and Hardy-Weinberg expectations within age groups indicated potential sampling of many highly related individuals in the MS4 age-0 population. In the MS4 age-0 samples, 5 of 21 (24%) possible loci comparisons failed tests for genotypic equilibrium ($\alpha = 0.05/28 = 0.0018$). In addition, three of seven polymorphic loci in the MS4 age-0 population differed

significantly from random mating proportions ($\alpha = 0.05/8 = 0.00625$). Poor

discriminatory power among loci precluded the correction of age-0 MS4 sample for

relatedness (Banks et al. 2000), therefore, all age-0 fish were removed from the MS4

sample before further analysis. None of the other age-class subsamples revealed

genotypic linkage disequilibrium at greater than 10% of possible comparisons, and

only one population showed evidence for deviation from Hardy-Weinberg

expectations. This deviation occurred at a single locus (*Omy-1046*) in the UT4 age1+

population ($\alpha = 0.05/28 = 0.0018$ and $\alpha = 0.05/8 = 0.00625$ respectively).

Final data adjustments, therefore, included pooling the 2002 and 2003 samples

from the MS1 location, splitting the T4 sample into two samples of age-0 and age-1+

trout (resulting in samples T4-0 and T4-1), and removing all age-0 fish from the MS4

sample (resulting in sample MS4-1). Following these data adjustments, heterozygote

deficits decreased within loci across populations (10.9% to 6.1%) and within

populations across loci (12.2% to 7.3%), and total genotypic linkage disequilibrium

decreased from 5.6% to 4.9%.

Gene Diversity and Differentiation

Mean within-population gene diversity was 0.50, and mean allelic richness

was 3.96. Gene diversity and allelic richness tended to decrease with increasing

genetic isolation (Figure 3, Appendix Table A.2). Tributaries that were connected

with mainstem habitats tended to have relatively high levels of allelic richness and

gene diversity, and low values were associated with barriers to gene flow (Figure 4,

Figure 5, Appendix Table A.2). Genetic drift dramatically impacted the isolated

tributaries, resulting in occasional increased frequencies of rare genotypes (associated

with basin-wide rare alleles) (Figure 4) and a loss of genetic diversity (Figure 5).

Simulations reveal the strong influence of genetic drift on the loss of alleles in small

populations (Figure 6).
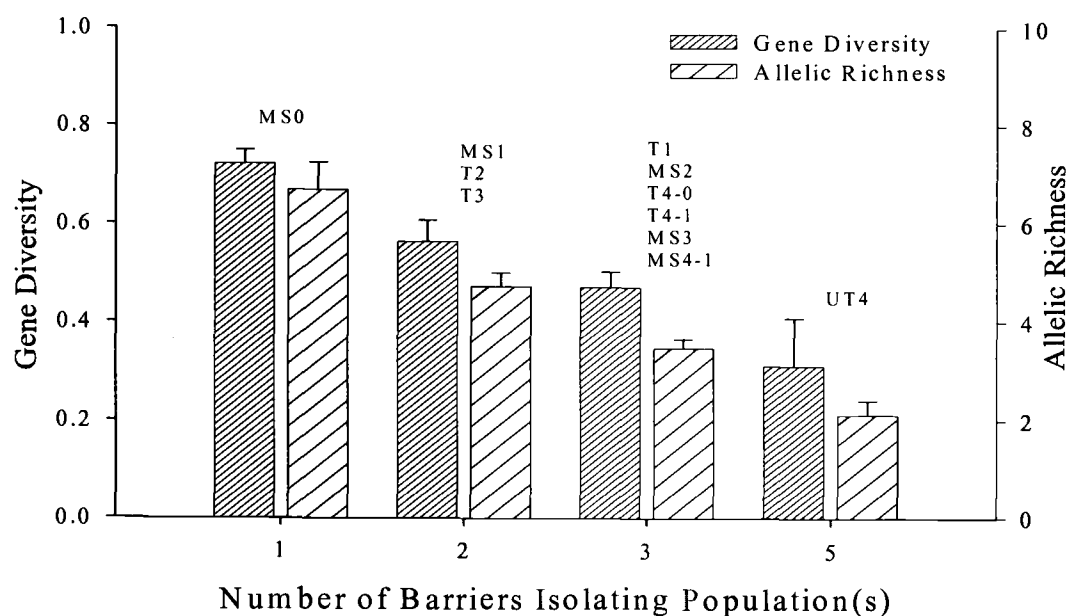
Figure 3. Mean gene diversity and allelic richness of 11 Camp Creek populations in relation to the number of barriers (anthropogenic and geomorphic) located downstream of the respective population. None of the populations in Camp Creek are isolated by four barriers. Bars show ± 1 standard error.

Figure 4. Spatially explicit color depiction of *Ots-209* genotype distributions in Camp Creek. Heights of bars represent the abundance of coastal cutthroat trout captured at a location and color codes indicate the probable genotypes of captured trout. Note the unusual distribution of genotypes associated with the isolated tributaries.
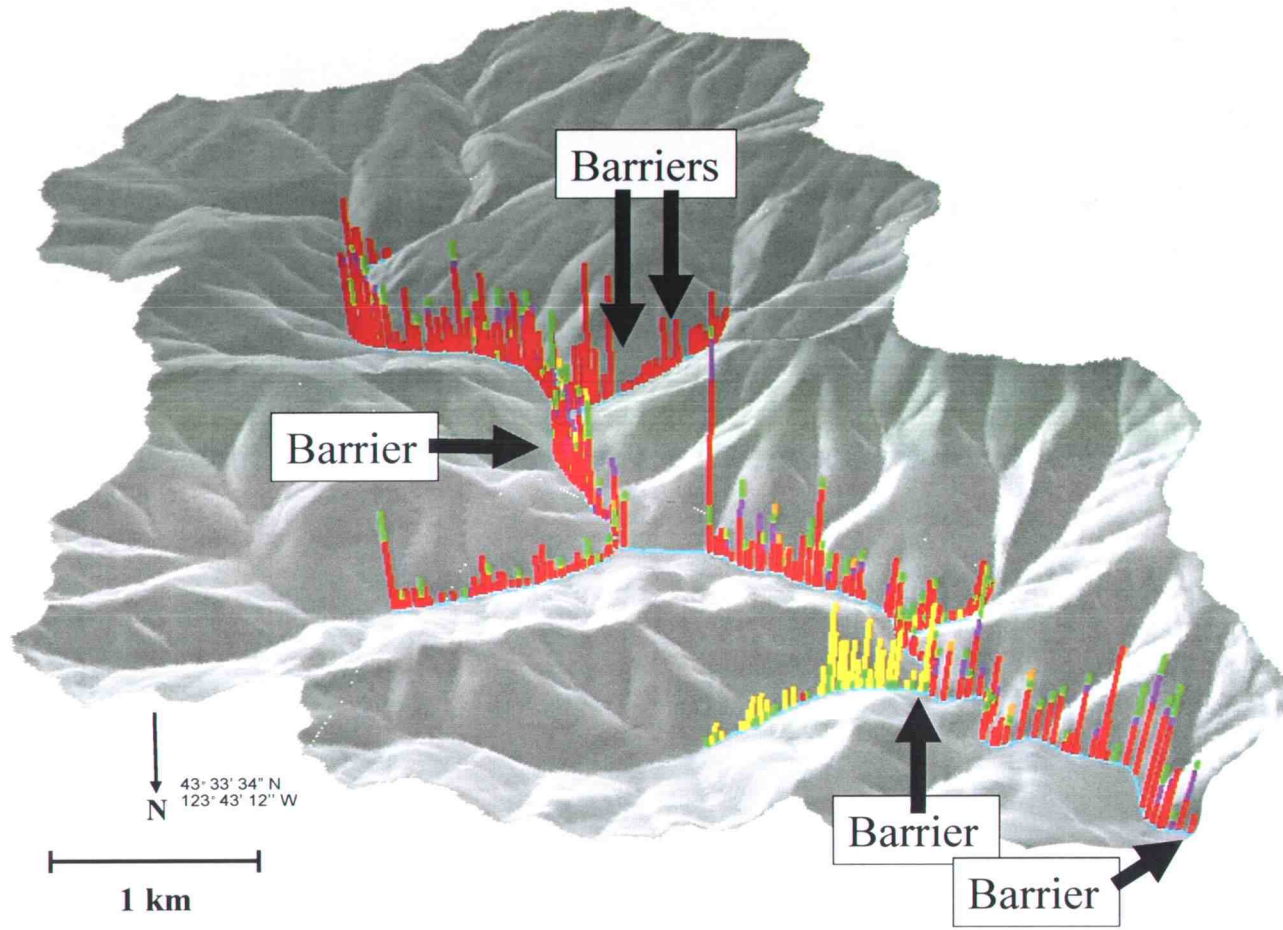
Figure 4.

Figure 5. Spatially explicit color depiction of *Ots-9* genotype distributions in Camp Creek. Heights of bars represent the abundance of coastal cutthroat trout captured at a location and color codes indicate the probable genotypes of captured trout. Note the reduced number of genotypes (associated with a reduced number of alleles) in the isolated tributaries.

Barriers

Barrier

Barrier

Barrier

43° 33' 34" N
123° 43' 12" W

N

1 km
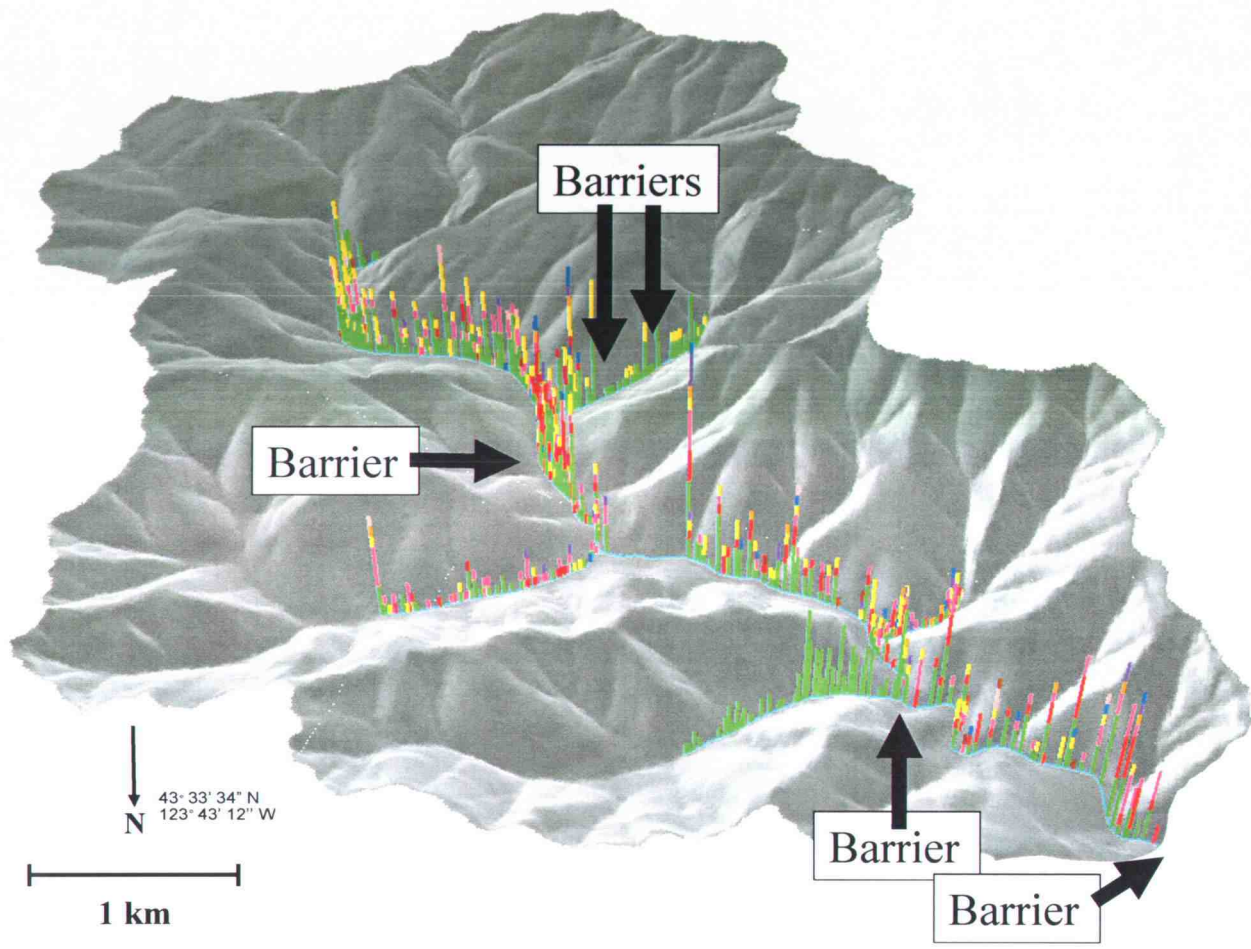
Figure 5.

Figure 6. Simulation revealing the effect of small population size on the rate of allele loss due to genetic drift. At generation 0, the simulated isolated population contained 8 alleles at maximum variability. Bars show ± 1 standard deviation.

When comparing each population-pair across loci, significant genotypic differences were detected in 369 of 433 (85.2%) possible tests ($\alpha = 0.05/8 = 0.00625$). Genotype distributions differed between population-pairs at a majority of loci (mean = 6.7 loci; range = 2 loci - 8 loci) (Table 1). Genetic differences between populations were also evident from pairwise F_{st} estimates (mean = 0.124; range = 0.014 to 0.393) (Table 1). The largest F_{st} values were associated with the two fully

isolated populations (T1, UT4). The mean pairwise F_{st} value for populations that were not separated by barriers (0.062) was significantly different from the mean F_{st} between barrier-separated populations (0.144) (Mann Whitney, $P < 0.01$). This result was related to the effects of the tributaries that were completely isolated from the remainder of the stream network (sites T1 and UT4); excluding these populations, differences in mean pairwise F_{st} values between separated (0.085) and connected (0.062) populations were not significant (two sample T-test, $P > 0.05$). No significant relationship was observed between genetic distance and geographic distance and therefore migration rates were estimated assuming an island model. According to Wright's island model [$F_{st} \approx 1/(4Nm + 1)$, where Nm represents the effective number of migrants between populations per generation] (1931), a mean F_{st} of 0.124 translates into a migration rate between populations of approximately 2 effective migrants per generation. After excluding populations T1 and UT4, the estimated migration rate between populations was approximately 4 effective migrants per generation (range = < 1 - 12 effective migrants).

Despite significant differences in allele frequencies among populations, factorial correspondence analysis revealed extensive overlap of populations. This overlap was not a result of compression of the FCA due to outlying populations, because after removing the three most differentiated populations (T1, UT4, and MS4-1), the overlapping patterns remained. A neighbor-joining phylogram of Cavalli-Sforza and Edwards chord distance illustrates the influence of fish passage barriers on coastal cutthroat trout genetic structure in Camp Creek (Figure 7). Phylogram

coastal cutthroat trout genetic structure in Camp Creek (Figure 7). Phylogram

organization is related to the spatial location of mainstem barriers, dividing the

phylogram into the lower (MS0), middle (MS1, T1, T2, T3) and upper watershed

(MS2, MS3, T4-0, T4-1, UT4, MS4). The large divergence of tributary populations

T1 and UT4 is associated with passage barriers.

Table 1. Population structure and genotypic differentiation of coastal cutthroat trout in Camp Creek. Values above the diagonal represent pairwise F_{st} values, and numbers below the diagonal represent the number of loci (out of 8) that revealed significant genotypic differentiation betweem populations ($\alpha = 0.05/8 = 0.00625$). Following Bonferroni adjustments, all F_{st} values were significant at P<0.001 excluding the T3/ MS1 comparison (significant at P<0.01).

Sample Section

Sample Section	MS0	MS1	T1	T2	T3	MS2	T4-0	T4-1	UT4	MS3	MS4-1
MS0	-	0.04	0.19	0.06	0.03	0.07	0.10	0.10	0.19	0.06	0.09
MS1	8	-	0.22	0.03	0.01	0.04	0.08	0.08	0.19	0.05	0.09
T1	8	8	-	0.25	0.24	0.26	0.37	0.33	0.39	0.23	0.32
T2	8	6	7	-	0.04	0.08	0.11	0.10	0.21	0.04	0.10
T3	6	2	7	4	-	0.05	0.09	0.09	0.21	0.04	0.06
MS2	8	5	8	7	5	-	0.05	0.03	0.15	0.03	0.06
T4-0	8	8	8	7	7	4	-	0.12	0.20	0.09	0.15
T4-1	7	6	8	8	6	5	5	-	0.20	0.07	0.06
UT4	8	8	6	8	8	8	7	7	-	0.12	0.17
MS3	8	7	6	8	4	5	7	7	8	-	0.06
MS4-1	8	7	8	8	7	5	4	4	7	7	-
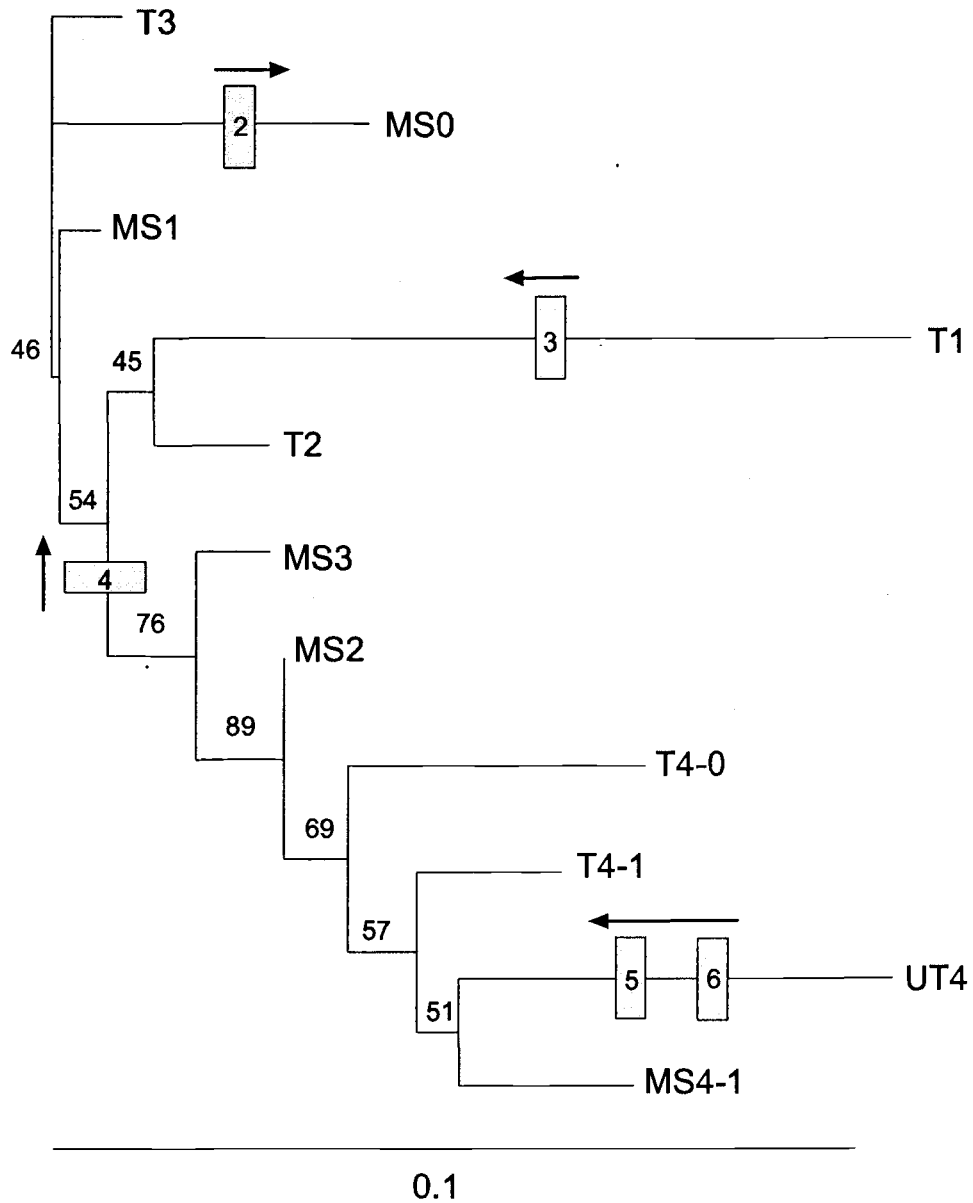
Figure 7. Neighbor-joining phylogram of 11 Camp Creek coastal cutthroat trout populations using Cavalli-Sforza and Edwards chord distance for 8 microsatellite loci. Numbers at nodes represent the percentage of bootstrap simulations that support the associated groups (1000 replicates). Gray bars represent fish passage barriers, numbers in bars identify barriers (as depicted in Figure 1), and arrows indicate potential downstream directional gene flow.

Age-1+ Maximum Abundance and N_e Estimates

Maximum abundance estimates for age-1+ coastal cutthroat trout revealed

small populations within sampling locations (Table 2). Fewer than 4% of the

captured age-1+ trout were found in riffle habitats, supporting the assumption that the

majority of age-1+ trout would be found in pools and cascades. The N_e estimate for

the T1 population using genetic data was 27 (95% C.I. = 19-40), lower than the

approximation derived from the relative abundance data ($N_e = 65$). The use of a

conservative capture probability could lead to overestimation of maximum spawner

abundance (and thus an overestimate of N_e) and might explain the difference between

the genetic and maximum abundance estimates of N_e in the T1 population.

Table 2. Age-1+ Camp Creek coastal cutthroat trout N_e estimates. Maximum
abundance data was not estimated for the MS0 population.

[a]Population estimate using genetic data.

Sampling Section	Age-1+ maximum abundance	N_e estimate
MS0	NA	NA
MS1	222	89
T1	162	65 (27[a])
T2	72	29
T3	80	32
MS2	72	29
T4	68	27
UT4	68	27
MS3	102	40
MS4	70	28

CHAPTER 4: DISCUSSION

This study provides the first attempt at describing fine-scale within-watershed population structure of potamodromous coastal cutthroat trout. All of the examined populations were genetically distinct, despite small distances separating populations. High F_{st} values, reduced heterozygosity, and a loss of alleles associated with barriers to fish passage demonstrated the strong genetic effects of population isolation. However, moderate genetic structure was also observed in continuous sections of stream at micro-scales (< 200 m). It appears that coastal cutthroat trout in Camp Creek are structured as partially independent populations that are connected with low to moderate amounts of gene flow. Where dispersal is possible, this gene flow is adequate for preserving genetic diversity (at least over short time periods), but gene flow is not sufficient to maintain genetic homogeneity throughout the watershed. Small effective population sizes, in conjunction with population isolation related to fish passage barriers, creates a situation where random genetic drift strongly impacts genetic heterogeneity.

In salmonid genetic studies, collecting samples (particularly from juveniles) over a short section of stream can lead to the collection of many highly related individuals, creating biased results and increasing deviations from Hardy-Weinberg expectations (Allendorf and Phelps 1981, Hansen et al. 1997). This has proved to be a problem in prior genetic studies of coastal cutthroat trout (Wenburg et al. 1998). If a large number of juveniles are sampled, it is advisable to identify and selectively

remove related individuals prior to data analysis (Banks et al. 2000). However, in populations with low polymorphism, or if few loci are examined, it can be difficult to acquire the necessary statistical power for an analysis of relatedness. In this study, we assessed departures from Hardy-Weinberg equilibrium and linkage disequilibrium within age groups as a means of addressing the potential sampling of related individuals. Although this technique is not as effective as a relatedness analysis, relatively few loci are required and the information content of the loci is essentially irrelevant. Subsequent data adjustments allowed us to substantially reduce departures from HWE and improved the validity of our results.

Fine-scale population structure in streams has been noted in other salmonid species including bull trout (*Salvelinus confluentus*) (Spruell et al. 1999, Neraas and Spruell 2001), brook trout (*Salvelinus fontinalis*) (Herbert et al. 2000), and brown trout (*Salmo trutta*) in Europe (Carlsson et al. 1999, Carlsson and Nilsson 2000, 2001). Reproductive isolation, due to precise natal homing or barriers to fish movement, is frequently cited as the cause of fine-scale genetic heterogeneity in trout populations. However, significant allelic divergence can occur even with considerable gene flow between populations (Allendorf and Phelps 1981). This fact is accentuated in small populations because divergence owing to genetic drift occurs at a rate that is inversely proportional to population size (Allendorf and Phelps 1981). Thus, estimates of effective population size can help provide a context for interpreting genetic distance measures (Moritz et al. 1995). In small populations, short term variations in dispersal rates coupled with overriding forcing by genetic

drift can lead to significant genetic divergence even if large amounts of genetic exchange occurs over longer time scales.

Dispersal rates in salmonids can be highly variable (Rieman and Dunham 2000), and this variation can increase temporal instability of allele frequencies in trout populations (Spruell et al. 1999). Even so, because different age classes within sampling sites were most often homogenous, much of the genetic structure observed in Camp Creek appears to be stable (at least over a few generations). Nevertheless, the T4 population had substantial evidence for temporal instability. Pairwise comparisons indicated that fry in the T4 location were not genetically similar to any other population in Camp Creek, and considering the extensive sampling that occurred in the watershed, it is unlikely that the fry were offspring from some unknown population. In addition, the age-0 T4 population met Hardy-Weinberg expectations, and there was no evidence for linkage among loci, thus ruling out a strong family sampling effect. Although it is possible that genetic drift contributed to the heterogeneity between age groups in T4, the large F_{st} value observed between the age groups, in conjunction with the relatively small F_{st} value between the age-0 T4 population and the MS2 population, indicates that the temporal variation in the T4 cutthroat trout likely occurred as a result of recent gene flow into T4 from the nearby MS2 population and not as a result of genetic drift.

The unusual distribution of private alleles as well as the extensive population overlap in the FCA provides additional evidence for demographic connections among the Camp Creek populations. Very few private alleles were documented above

barrier 2, indicating repeated founder events or extensive gene flow in the upper portions of the watershed (Slatkin 1985). Based on F_{st} values, dispersal rates between populations ranged from low to moderately high. However, because of the strong effect of genetic drift on the small Camp Creek populations, inferring dispersal rates from F_{st} values might lead to underestimation of movement rates. Even so, these estimates of dispersal and genetic divergence apply only to recent time periods. Fluctuations in habitat quality and population densities along with the formation or elimination of dispersal barriers can alter salmonid migration rates (Rieman and Dunham 2000), and genetic divergence or convergence can occur over extremely short time scales (one or two generations, in this case, two to four years) with small effective population sizes.

Recent studies of watershed-scale movement of coastal cutthroat trout in Camp Creek also suggest that gene flow may be limited. Hendricks (2002) reported that most coastal cutthroat trout in Camp Creek moved at the local spatial scale (10 m); however, some individuals moved from 100 to 1000m, distances greater than those separating many populations in this study. Although it is impossible to determine the genetic contribution of individual fish from mark-recapture data, trout movement in Camp Creek was most frequent during spawning seasons, suggesting that movement was reproductively motivated. Such highly mobile individuals may succeed in partially counteracting the effects of genetic drift and inbreeding in these populations.

Current levels of gene flow in Camp Creek do not appear to be adequate for maintaining homogeneity in allele frequencies, but where migration was possible, persistence of alleles and maintenance of relatively high levels of gene diversity were noted. In contrast, reduced genetic diversity and allelic richness were directly related to the degree of habitat fragmentation and ensuing genetic isolation. Genetic theory predicts that when migration between small populations is restricted by dispersal barriers, the subsequent loss of genetic diversity can occur rapidly (Hartl and Clark 1997). Indeed, the T1 population, which has only been isolated for 45 years (as of 2003), had roughly 50% fewer alleles than nearby tributaries with mainstem connections. Populations in isolated tributaries T1 and UT4 underscored the importance gene flow from either upstream or downstream; with no potential immigration, coastal cutthroat trout in these tributaries exhibited the lowest gene diversity, lowest allelic richness, and the highest degree of genetic divergence. The rapid loss of alleles and increased genetic differentiation in these isolated populations is likely an effect of genetic drift and inbreeding, speeded by small effective population sizes and short generation times of coastal cutthroat trout.

The effect of barriers on the genetic heterogeneity of coastal cutthroat trout in Camp Creek appears to be largely dependent upon the extent of isolation caused by the respective barrier. Just as the spatial organization of critical habitats affects the distribution of populations, the spatial location of dispersal barriers influences the genetic consequences of isolation. When barriers are located on small tributaries, individual populations are isolated. In contrast, barriers in the mainstem tend to

isolate many populations. Gene flow among multiple populations increases long term effective population sizes thus reducing the effects of genetic drift and inbreeding. This might explain the relatively low F_{st} values that occurred among most mainstem populations and the large F_{st} values associated with the isolated tributaries. Although mainstem barriers were related to decreased genetic diversity, the fact that they did not have a major effect on allele frequencies suggests that some downstream gene flow is occurring in the mainstem of Camp Creek.

Certainly, divergence between below- and above-barrier populations will be dependent not only upon the amount of downstream dispersal, but also on the time since isolation and the respective demographic histories of the individual populations. Considering the small effective population sizes in Camp Creek, even if mainstem barriers were formed relatively recently, with little gene flow, greater divergence than was observed would be expected (Allendorf and Phelps 1981). Other genetic studies suggest that the degree of divergence among coastal cutthroat trout populations separated by natural barriers is variable (Griswold et al. 1997). It has been noted that downstream movement of salmonids in isolated headwaters would be limited by strong genetic selection against downstream dispersal. Nevertheless, downstream movement of above-falls individuals has been documented (Johnston 1982, Northcote and Hartmann 1988, Northcote 1992, Hendricks 2002). In the headwaters of Camp Creek, these emigrants appear to be contributing genetically to downstream populations. Because our sampling was limited above a barrier to anadromous fishes,

this study does not necessarily apply to genetic interactions between anadromous and potamodromous coastal cutthroat trout.

Partially independent populations that exist in patchy, dynamic habitats can be loosely characterized as metapopulations (Hanski and Simberloff 1996). Metapopulation theory conceptualizes the dynamics of population extirpation and recolonization, and substantial attention has been focused on integrating metapopulation concepts with lotic fish biology (Schlosser and Angermeier 1995). Although there is little support for a simple, generalizable model of metapopulations for salmonids, evidence does suggest that metapopulation processes do play a role in regulating salmonid populations (Rieman and Dunham 2000). We did not document an extirpation and recolonization event, but based on evidence of intermittent gene flow among populations, metapopulation structure is likely in some portions of Camp Creek.

Considering the small spatial scale of the watershed and the relative success of coastal cutthroat trout in headwater habitats, it appears that following an extirpation event available habit would be quickly recolonized. Rapid recolonization of accessible habitat is common in salmonid population (Gresswell 1999). The reduced heterozygosity and low allelic richness observed in the upper portions of Camp Creek (MS4-1), despite the absence of definitive fish barriers, may be an indication that the population was recently founded by individuals from downstream populations. However, high gradient cascades in this portion of the basin could limit upstream gene flow that would be necessary for maintaining genetic variation even without a

bottleneck or extirpation event. Indeed, there are many geomorphic structures, such as cascades, that could function as dispersal filters, restricting but not eliminating gene flow among populations (Kocik and Ferreri 1998). Following floods or other disturbance events, new filters or barriers may be created, or existing structures may be altered, thus affecting future dispersal rates. Habitat changes can also influence dispersal through indirect processes related to density dependent movements or fluctuations in critical habitat availability (Rieman and Dunham 2000). In this way, genetic variability reflects environmental variability and future population structure is linked with past alterations of habitat.

Coastal cutthroat trout in the Pacific Northwest occupy headwater streams that are frequently fragmented by geomorphic dispersal barriers. Although the spatial distribution and characteristics of these features have not been adequately quantified, field observations of over 50 watersheds in western Oregon indicate that natural barriers are rarely found at tributary junctions (Gresswell R.E., unpublished data). Tributaries provide a major function as sediment delivery systems and the deposition of alluvium at tributary junctions likely reduces the probability of barrier formation as well as the likelihood that a barrier could persist over time. However, road construction along the narrow terraces of headwater streams is frequently associated with culvert installations that impede fish movements into small tributaries. The watershed scale demographic effects of this fragmentation are unknown; however, in Camp Creek, isolation of small tributaries quickly resulted in a loss of upstream

genetic variation, thus reducing the spatial distribution of individual alleles in the stream network and restricting more of the genetic diversity to mainstem habitats.

The spatial distribution of alleles in Camp Creek is especially important because the watershed is small and stochastic disturbances, such as fires or debris flows, could negatively affect a significant portion of the fish-bearing habitats. When allelic spatial distributions are reduced, the probability of a permanent loss of genetic diversity and associated phenotypic diversity following a disturbance, increases. A loss of genetic variation can negatively affect the ability of a population to adapt and persist in the face of environmental change (Allendorf et al. 1987), and without a better understanding of the relationship between genetic diversity and fitness, maintaining genetic diversity should be a driving objective for salmonid conservation (Wang et al. 2002).

In the absence of extirpations, fragmented populations can actually retain higher genetic diversity than a single population of the same total size (Kimura and Crow 1963). As a result, zoo managers often intentionally fragment captive populations of endangered species (Margan et al. 1998, Woodward et al. 2002). This technique can work well when individual populations have low extirpation risks (as in many captive populations), but in natural situations with small populations, habitat fragmentation often increases extirpation risks (Morita and Yamamoto 2001) and usually results in genetic (Maruyama and Kimura 1980), demographic (Pulliam and Dunning 1997), and ecological degradation (Noss and Csuti 1997). From a genetic

perspective, intentional fragmentation of wild populations has little conservation value and should not be advocated (Chambers 1995, Kruse et al. 2001).

Nevertheless, the existence of more than 250 coastal cutthroat trout populations above natural barriers in western Oregon (Gresswell et al. 2003) suggests that coastal cutthroat trout are at least partially adapted to fragmented stream habitats. At a range-wide spatial scale, this fragmentation potentially contributes to coastal cutthroat trout genetic diversity. However, at small spatial scales, fragmentation will generally have long-term negative consequences on the genetic variation of individual assemblages of coastal cutthroat trout. Despite the risks involved with genetic isolation, when introduced species threaten native salmonid populations with hybridization and competitive exclusion, intentional isolation is increasingly viewed as an appropriate measure for conservation (Kruse et al. 2001, Novinger and Rahel 2003). Evidence suggests that managers should consider intentional isolation only when other conservation strategies have been unsuccessful, and it is important to evaluate trout population sizes, local disturbance regimes, and habitat connectivity in conjunction with population genetic characteristics when determining the potential effects of isolation (Hilderbrand and Kruse 2000, Novinger and Rahel 2003).

Substantial genetic structure has been observed at multiple levels of coastal cutthroat trout spatial distributions: range-wide (Williams, T.H. 2003, personal communication), among watersheds (Wenburg et al. 1998, Wenburg and Bentzen 2001), large watersheds (Zimmerman 1996, Wenburg and Bentzen 2001), and small watersheds (Griswold et al. 1997). In resource management, genetic structure is often

used to determine the appropriate spatial scale for management actions (Allendorf et al. 1987). Independent management of genetically distinct populations is predicated on the assumption that genetic structure represents demographic independence and the potential for local adaptations (Carvalho 1993, Moritz et al. 1995). Although significant genetic structure was observed in the Camp Creek basin, much of this fine-scale heterogeneity is likely derived from the effects of genetic drift and is not a result of natural selection and complete reproductive isolation. Although salmonids can develop local adaptations at small spatial scales (Gresswell et al. 1997, Olsen and Vollestad 2001, Koskinen et al. 2002, Olsen and Vollestad 2003), the apparent gene flow and metapopulation structure among populations in Camp Creek suggests that management should be focused at larger spatial scales. For proper conservation of salmonid metapopulations, managers must focus not only upon individual populations and critical habitat areas, but also, and perhaps more importantly, on reestablishing linkages among tributary and mainstem populations, thereby obtaining the demographic and genetic benefits of population connectivity (Gresswell 1997).

Although many populations in Camp Creek have relatively low genetic diversity, these data do not directly address the probability of future persistence, nor do they suggest that coastal cutthroat trout are resilient to the negative effects of genetic homogeneity. The fact that there are many isolated streams with adequate habitat that do not support fish suggests that at some temporal scale, isolation leads to extirpation, and this may be related to the process of genetic degradation. Furthermore, in fragmented habitats, demographic and environmental stochasticity

alone can lead to extirpation of salmonid populations (Morita and Yokota 2002).

Indeed, at short temporal scales, demographic and environmental variability may be

more important to population persistence than genetic heterogeneity.

CHAPTER 5: CONCLUSION

Significant fine-scale genetic structure was noted in coastal cutthroat trout within a 3rd order headwater stream of western Oregon. Available evidence suggests that this structure was a result of the combined effects of genetic drift and population isolation. When passage barriers were present, gene flow was reduced and the effects of random drift and inbreeding resulted in a loss of genetic variation and increased population differentiation. At landscape scales, barriers may have a potential role in maintaining coastal cutthroat trout genetic diversity, and it is not recommended that natural barriers be modified for fish passage. However, isolation of small tributaries as a result of land management activities has few beneficial consequences for coastal cutthroat trout because of negative genetic, and likely demographic, effects at all spatial scales. These data suggest that habitat fragmentation in headwater streams can lead to a rapid reduction of genetic diversity that is detrimental to coastal cutthroat trout population persistence and long term conservation planning. For successful conservation of coastal cutthroat trout, managers should be mindful of the importance of habitat connectivity and strive to maintain or restore trout dispersal pathways in streams.

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APPENDICES

Table A.1. Thermocycler profiles and PCR Reactions.

Multiplex	Loci	Thermocycler Steps			PCR Reagents[a]				
		1	2	3	Primer (mM)	MgCl$_2$ (mM)	dNTPs (mM)	Promega® PCR buffer (µL)	Tris/KCl buffer (µL)
A	Ots-209 Ots-212	1 cycle @ (94°C for 3 min)	32 cycles @ (94°C for 30s + 63°C for 20s + 72°C for 30s)	1 cycle @ 72°C for 10 min	0.075 0.5	2.0	0.125	0.5	0
B	One-102 One-103 One-108		see Olsen et al. 2000[b]		0.025 0.025 0.025	2.0	0.200	0	0.5
NA	Omy-1046	1 cycle @ (94°C for 3 min)	29 cycles @ (94°C for 30s + 55°C for 20s + 72°C for 30s)	1 cycle @ 72°C for 10 min	0.2	1.5	0.200	0.5	0
NA	Ots-9	1 cycle @ (94°C for 3 min)	32 cycles @ (94°C for 30s + 63°C for 20s + 72°C for 30s)	1 cycle @ 72°C for 10 min	0.2	2.0	0.125	0.5	0
NA	Ots-10	1 cycle @ (94°C for 3 min)	32 cycles @ (94°C for 30s + 63°C for 20s + 72°C for 30s)	1 cycle @ 72°C for 10 min	0.2	2.0	0.125	0.5	0

[a]All PCR reactions were conducted in 5 µL volumes using 1 µL genomic DNA template, 0.25 µL BSA PCR enhancer, 0.025 U Taq DNA polymerase
[b]Olsen, J.B., S.L. Wilson, E.J. Kretschmer, K.C. Jones, and J.E. Seeb. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Molecular Ecology 9:2185-2187

Table A.2. Locus summary for Camp Creek coastal cutthroat trout populations (including subsamples from age groups and sample years). A = number of alleles with allelic richness in parentheses, R = allele size range, S = most common allele, F = frequency of most common allele, G_d = gene diversity (expected heterozygosity), H_o = observed heterozygosity, N = sample size (number of successfully genotyped individuals).

[a] Indicates populations that were used in the final analysis.

Sample Section		Ots-9	Ots-209	Ots-212	Omy-1046	Ots-10	One-102	One-108	One-103	Mean
MS0[a]	A	7 (5.4)	5 (4.67)	6 (5.56)	8 (6.09)	6 (5.01)	10 (8.74)	10 (7.88)	8 (6.14)	7.63 (6.19)
	R	107-125	139-159	107-155	95-147	172-198	200-256	155-251	114-154	
	S	113	147	107	111	190	240	0.559	114	
	F	0.553	0.558	0.355	0.309	0.339	0.261	163	0.512	
	G_d	0.644	0.63	0.76	0.782	0.738	0.865	0.666	0.687	0.72
	H_o	0.56	0.597	0.711	0.787	0.729	0.783	0.5	0.6	
	N	75	77	83	89	59	69	76	85	77
MS1 (2001, 2002)[a]	A	4 (3.68)	3 (2.94)	5 (4.31)	6 (4.75)	7 (4.65)	8 (6.79)	7 (4.31)	6 (3.58)	5.75 (4.38)
	R	113-119	147-155	107-155	95-131	180-198	200-252	155-251	114-150	
	S	113	147	107	115	190	240	163	114	
	F	0.688	0.784	0.494	0.399	0.376	0.354	0.796	0.811	
	G_d	0.487	0.362	0.657	0.724	0.668	0.779	0.357	0.33	0.55
	H_o	0.468	0.329	0.702	0.724	0.584	0.799	0.308	0.343	
	N	141	167	178	173	137	144	159	172	159
MS1 (2002)	A	4 (3.78)	3 (2.95)	5 (4.40)	5 (4.44)	6 (4.68)	7 (6.41)	6 (4.51)	5 (3.44)	5.13 (4.32)
	R	113-119	147-155	107-155	95-119	180-198	200-252	155-251	114-150	
	S	113	147	107	115	190	240	163	114	
	F	0.673	0.789	0.429	0.44	0.461	0.368	0.733	0.815	
	G_d	0.508	0.357	0.684	0.697	0.651	0.769	0.445	0.322	0.55
	H_o	0.524	0.344	0.761	0.758	0.605	0.842	0.419	0.326	
	N	84	90	92	90	76	76	86	89	85
MS1 (2003)	A	4 (3.56)	3 (2.95)	5 (4.22)	6 (5.01)	6 (4.69)	8 (7.14)	7 (3.77)	5 (3.55)	5.50 (4.36)
	R	113-119	147-155	107-155	95-131	180-198	200-252	155-251	114-150	
	S	113	113	107	115	188	240	163	114	
	F	0.711	0.779	0.564	0.355	0.393	0.338	0.87	0.807	
	G_d	0.46	0.37	0.616	0.75	0.688	0.794	0.241	0.335	0.53
	H_o	0.386	0.312	0.64	0.687	0.557	0.75	0.178	0.361	
	N	57	77	86	83	61	68	73	83	74
T1[a]	A	1 (1.00)	2 (1.96)	3 (3.00)	3 (3.00)	5 (4.58)	4 (3.94)	2 (2.00)	1 (1.00)	2.63 (2.56)
	R	113	147-155	107-135	95-115	180-198	200-248	159-163	114	
	S	113	155	119	111	188, 190	204	163	114	
	F	1	0.913	0.604	0.364	0.302	0.605	0.576	1	
	G_d	0	0.161	0.56	0.667	0.752	0.583	0.492	0	0.40
	H_o	0	0.143	0.514	0.6	0.698	0.439	0.424	0	
	N	53	63	72	70	43	57	66	72	62
T2[a]	A	4 (3.93)	2 (2.00)	5 (4.36)	6 (5.32)	5 (4.40)	7 (6.38)	5 (4.13)	4 (2.75)	4.75 (4.16)

		Locus								
Sample Section		*Ots-9*	*Ots-209*	*Ots-212*	*Omy-1046*	*Ots-10*	*One-102*	*One-108*	*One-103*	Mean
	R	113-119	147-155	107-155	95-131	180-198	200-252	155-251	114-146	
	S	113	147	107	103	188	240	163	114	
	F	0.514	0.863	0.31	0.338	0.486	0.306	0.757	0.925	
	G_d	0.634	0.24	0.755	0.751	0.672	0.788	0.412	0.144	0.55
	H_o	0.722	0.175	0.857	0.838	0.743	0.871	0.378	0.15	
	N	36	40	42	37	35	31	37	40	37
T3[a]	A	4 (3.91)	3 (2.30)	5 (4.42)	5 (4.93)	6 (5.36)	8 (7.14)	6 (4.31)	5 (4.40)	5.25 (4.60)
	R	113-119	147-155	107-155	95-119	180-198	200-252	155-251	114-150	
	S	113	147	115	111	190	204, 240	163	114	
	F	0.605	0.875	0.398	0.367	0.409	0.236	0.807	0.787	
	G_d	0.576	0.223	0.7	0.753	0.725	0.834	0.342	0.372	0.57
	H_o	0.535	0.25	0.694	0.778	0.614	0.917	0.273	0.383	
	N	43	48	49	45	44	36	44	47	45
MS2[a]	A	4 (3.75)	3 (2.61)	4 (3.92)	5 (4.78)	5 (4.33)	8 (5.50)	6 (4.26)	4 (3.56)	4.88 (4.09)
	R	113-119	147-155	107-135	95-131	180-198	200-252	155-251	114-150	
	S	113	147	107	95	190	240	163	114	
	F	0.71	0.888	0.576	0.406	0.435	0.527	0.659	0.799	
	G_d	0.467	0.205	0.606	0.691	0.626	0.653	0.513	0.349	0.51
	H_o	0.449	0.211	0.595	0.696	0.613	0.732	0.381	0.364	
	N	69	76	79	69	62	56	63	77	69
MS2-0 (age-0)	A	4 (3.61)	3 (2.83)	4 (3.98)	5 (4.32)	5 (4.17)	6 (5.33)	6 (4.46)	4 (3.58)	4.63 (4.03)
	R	113-119	147-155	107-135	95-131	180-198	200-252	155-251	114-150	
	S	113	147	107	111	190	240	163	114	
	F	0.7	0.821	0.616	0.388	0.486	0.529	0.686	0.826	
	G_d	0.478	0.31	0.577	0.69	0.612	0.664	0.488	0.311	0.52
	H_o	0.45	0.357	0.558	0.725	0.676	0.735	0.4	0.349	
	N	40	42	43	40	37	34	35	43	39
MS2-1 (age-1+)	A	4 (3.93)	3 (1.88)	4 (3.66)	5 (3.99)	5 (4.52)	6 (5.24)	5 (4.05)	4 (3.67)	4.38 (3.87)
	R	113-119	147-155	107-135	95-131	180-198	200-252	155-251	114-150	
	S	113	147	107	95	188	240	163	114	
	F	0.724	0.971	0.528	0.448	0.48	0.523	0.625	0.765	
	G_d	0.457	0.058	0.627	0.678	0.642	0.632	0.548	0.362	0.51
	H_o	0.448	0.029	0.639	0.655	0.52	0.727	0.357	0.429	
	N	29	34	36	29	25	22	28	34	30
T4	A	4 (3.47)	1 (1.00)	4 (3.77)	5 (4.81)	5 (4.14)	5 (3.97)	4 (2.75)	3 (2.95)	3.88 (3.36)
	R	113-119	147	107-135	95-131	180-194	200-240	155-239	114-150	
	S	113	147	107	95	188	204	163	114	
	F	0.645	1	0.588	0.343	0.568	0.57	0.765	0.75	
	G_d	0.549	0	0.572	0.737	0.585	0.573	0.397	0.406	0.48
	H_o	0.431	0	0.537	0.714	0.627	0.52	0.246	0.471	
	N	51	66	67	63	59	50	61	63	67
T4-0 (age-0)[a]	A	3 (3.00)	1 (1.00)	4 (3.45)	4 (3.98)	3 (2.99)	3 (2.88)	2 (2.00)	3 (2.48)	2.88 (2.72)
	R	113-119	147	107-135	95-131	188-194	200-240	163-239	114-150	
	S	113	147	107	111	188	240	163	114	
	F	0.558	1	0.5	0.516	0.519	0.886	0.776	0.766	
	G_d	0.601	0	0.634	0.649	0.582	0.212	0.354	0.371	0.42
	H_o	0.5	0	0.697	0.656	0.741	0.136	0.172	0.469	
	N	26	33	33	32	27	22	29	31	29
T4-1 (age-1+)[a]	A	4 (3.72)	1 (1.00)	4 (3.54)	5 (4.39)	5 (4.33)	5 (3.86)	4 (3.16)	3 (2.39)	3.88 (3.30)
	R	113-119	147	107-135	95-131	180-194	200-240	155-239	114-150	
	S	113	147	107	95	188	240	163	114	

					Locus					
Sample Section		*Ots-9*	*Ots-209*	*Ots-212*	*Omy-1046*	*Ots-10*	*One-102*	*One-108*	*One-103*	Mean
	F	0.70	1	0.676	0.565	0.625	0.393	0.719	0.705	
	G_d	0.479	0	0.489	0.632	0.565	0.694	0.439	0.429	0.46
	H_o	0.36	0	0.382	0.774	0.531	0.821	0.313	0.538	
	N	25	33	34	31	32	28	32	32	38
UT4[a]	A	2 (1.74)	1 (1.00)	3 (2.96)	3 (3.00)	2 (2.00)	2 (2.00)	3 (3.00)	1 (1.00)	2.13 (2.09)
	R	113-119	147	107-119	95-111	188-194	204-240	155-239	114	
	S	113	147	115	111	188	240	239	114	
	F	0.964	1	0.5	0.48	0.865	0.798	0.422	1	
	G_d	0.07	0	0.578	0.615	0.237	0.326	0.658	0	0.31
	H_o	0.071	0	0.686	0.725	0.271	0.404	0.49	0	
	N	42	52	51	51	48	47	51	52	49
UT4-0 (age-0)	A	2 (2.00)	1 (1.00)	3 (3.00)	3 (3.00)	2 (2.00)	2 (2.00)	3 (3.00)	1 (1.00)	2.13 (2.12)
	R	113-119	147	107-119	95-111	188-194	204-240	155-239	114	
	S	113	147	115	111	188	240	239	114	
	F	0.967	1	0.471	0.647	0.912	0.781	0.559	1	
	G_d	0.067	0	0.613	0.528	0.166	0.353	0.572	0	0.29
	H_o	0.067	0	0.882	0.647	0.176	0.438	0.588	0	
	N	15	18	17	17	17	16	17	18	17
UT4-1 (age-1+)	A	2 (1.81)	1 (1.00)	3 (2.95)	3 (3.00)	2 (2.00)	2 (2.00)	3 (3.00)	1 (1.00)	2.13 (2.09)
	R	113-119	147	107-119	95-111	188-194	204-240	155-239	114	
	S	113	147	115	95	188	240	239	114	
	F	0.963	1	0.515	0.441	0.839	0.806	0.353	1	
	G_d	0.073	0	0.568	0.631	0.275	0.317	0.676	0	0.32
	H_o	0.074	0	0.588	0.765	0.323	0.387	0.441	0	
	N	27	34	34	34	31	31	34	34	32
MS3[a]	A	4 (3.59)	3 (2.67)	4 (3.91)	6 (4.19)	5 (4.58)	7 (5.27)	7 (5.60)	5 (3.46)	5.13 (4.16)
	R	113-119	147-155	107-135	95-131	180-198	200-252	155-255	114-150	
	S	113	147	107	115	188	240	163	114	
	F	0.755	0.891	0.395	0.326	0.358	0.403	0.377	0.782	
	G_d	0.406	0.201	0.696	0.704	0.718	0.742	0.734	0.37	0.57
	H_o	0.445	0.2	0.618	0.669	0.585	0.833	0.71	0.397	
	N	110	110	110	118	106	72	69	78	97
MS3-0 (age-0)	A	4 (3.62)	3 (2.72)	4 (3.96)	4 (3.72)	5 (4.42)	5 (4.37)	7 (5.55)	4 (3.24)	4.88 (4.36)
	R	113-119	147-155	107-135	95-115	180-198	200-252	155-255	114-150	
	S	113	147	107	115	188	240	163	114	
	F	0.754	0.871	0.355	0.373	0.385	0.39	0.372	0.811	
	G_d	0.408	0.234	0.717	0.684	0.726	0.736	0.72	0.329	0.57
	H_o	0.462	0.226	0.597	0.701	0.574	0.854	0.744	0.333	
	N	65	62	62	67	61	41	39	45	55
MS3-1 (age-1+)	A	4 (3.68)	3 (2.25)	4 (3.85)	6 (4.77)	5 (4.74)	7 (6.15)	6 (5.77)	5 (3.68)	3.25 (3.03)
	R	113-119	147-155	107-135	95-131	180-198	200-252	155-255	114-150	
	S	113	147	107	95	190	240	163	114	
	F	0.756	0.917	0.448	0.402	0.444	0.419	0.383	0.742	
	G_d	0.408	0.156	0.661	0.708	0.687	0.757	0.758	0.428	0.57
	H_o	0.422	0.167	0.646	0.627	0.6	0.806	0.667	0.485	
	N	45	48	48	51	45	31	30	33	41
MS4	A	2 (2.00)	1 (1.00)	3 (3.00)	5 (4.84)	4 (3.33)	6 (5.20)	2 (2.00)	3 (2.90)	3.25 (3.02)
	R	113-119	147	107-119	95-119	188-198	200-248	163-239	114-150	
	S	113	147	115	95	188	204	163	114	
	F	0.841	1	0.5	0.412	0.682	0.406	0.692	0.828	
	G_d	0.269	0	0.619	0.725	0.477	0.724	0.429	0.301	0.44

					Locus					
Sample Section		*Ots-9*	*Ots-209*	*Ots-212*	*Omy-1046*	*Ots-10*	*One-102*	*One-108*	*One-103*	Mean
	H_o	0.273	0	0.585	0.846	0.494	0.713	0.36	0.269	
	N	88	94	94	91	85	80	86	93	89
MS4-0 (age-0)	A	2 (1.99)	1 (1.00)	3 (3.00)	5 (4.87)	4 (3.24)	6 (5.19)	2 (2.00)	3 (2.87)	3.13 (3.00)
	R	113-119	147	107-119	95-119	188-198	200-248	163-239	114-150	
	S	113	147	115	95	188	204	163	114	
	F	0.882	1	0.458	0.988	0.788	0.404	0.735	0.845	
	G_d	0.21	0	0.644	0.738	0.356	0.725	0.393	0.276	0.42
	H_o	0.164	0	0.542	0.897	0.385	0.617	0.333	0.207	
	N	55	59	59	58	52	47	51	58	55
MS4-1 (age-1+)[a]	A	2 (2.00)	1 (1.00)	3 (2.99)	5 (4.70)	4 (3.44)	5 (4.88)	2 (2.00)	3 (2.96)	
	R	113-119	147	107-119	95-119	188-198	200-248	163-239	114-150	
	S	113	147	115	95	188	204	163	114	
	F	0.773	1	0.571	0.455	0.515	0.409	0.629	0.8	
	G_d	0.357	0	0.57	0.703	0.6	0.731	0.474	0.345	0.47
	H_o	0.455	0	0.657	0.758	0.667	0.848	0.4	0.371	
	N	33	35	35	33	33	33	35	35	34