Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California

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Abstract The effects of landscape features on gene flow in threatened and endangered species play an important role in influencing the genetic structure of populations. We examined genetic variation of trout in the species *Oncorhynchus mykiss* at 22 microsatellite loci from 20 sites in the Russian River basin in central California. We assessed relative patterns of genetic structure and variation in fish from above and below both natural (waterfalls) and man-made (dams) barriers. Additionally, we compared sites sampled in the Russian River with sites from 16 other coastal watersheds in California. Genetic variation among the 20 sites sampled within the Russian River was significantly partitioned into six groups above natural barriers and one group consisting of all below barrier and above dam sites. Although the below-barrier sites showed moderate gene flow, we found some support for sub-population differentiation of individual tributaries in the watershed. Genetic variation at all below-barrier sites was high compared to above-barrier sites. Fish above dams were similar to those from below-barrier sites and had similar levels of genetic diversity, indicating they have not been isolated very long from below-barrier populations. Population samples from above natural barriers were highly divergent, with large F_{st} values, and had significantly lower genetic diversity, indicating relatively small population sizes. The origins of populations above natural barriers could not be ascertained by comparing microsatellite diversity to other California rivers. Finally, below-barrier sites farther inland were more genetically differentiated from other watersheds than below-barrier sites nearer the river's mouth.

Keywords Trout · Microsatellite · Landscape features · Genetic differentiation · Life history types

Introduction

Natural and anthropological landscape features, such as mountain ranges, waterfalls, dams and roads can fragment populations and dramatically affect patterns of migration and differentiation (e.g. Castric et al. 2001; Manel et al. 2003). Evaluation of population genetic structure in widespread species is crucial for the development of conservation and management strategies. Therefore, quantifying patterns of gene flow and genetic drift across both natural and man-made barriers to migration are important for determining their effects on population genetic structure. In this study, we examined within-basin population genetic structure of *Oncorhynchus mykiss*, steelhead or rainbow trout, in the Russian River, California to evaluate the degree of differentiation that occurs on a relatively small scale. We then compared this within-basin genetic structure to that previously described (Garza et al. 2004) for this species throughout the southern part of its native range.

The trout species *O. mykiss* is the most widespread of the Pacific salmonids, with a native range from Russia to southern California and historically to northern Mexico. In addition, *O. mykiss* has been introduced to every continent in the world (except Antarctica) for recreational fisheries and aquaculture and has been planted in nearly every body of water in California (Moyle 2002). Because of this species' broad geographic distribution and the diverse topography in its native range, there are considerable diverse phylogenetic and phylogeographic patterns within the species (e.g. Bagley and Gall 1998). Adding to this complexity is the flexibility in life history strategy, with some populations composed largely of migratory (anadromous) individuals, others of largely non-migratory (resident) individuals and yet others a mixture of the two (Behnke 1972). Even with their complex life history characteristics, individuals with easy ocean access are commonly referred to as steelhead and those without such access as resident rainbow trout.

In spite of their broad geographic distribution and considerable complexity, many populations of steelhead in California and western North America have received protection under the US Endangered Species Act (ESA; Federal Register 1997). In California, five of the six steelhead evolutionarily significant units (ESUs), as described by Busby et al. (1996), are under ESA protection, with the Southern California ESU listed as endangered and the others as threatened. Dams, in particular, have greatly affected migratory fishes in California, as almost all major rivers in the state have been dammed at least once (Reisner 1993). In an attempt to mitigate the loss of spawning habitat for migratory fish because of such dams, many hatcheries have been built to rear and release salmonids. The origin of broodstock and hatchery practices determine whether fish produced at a particular hatchery are included as part of the ESU (Federal Register 1997). In addition, ESA protection has only been extended to fish below barriers to anadromy that are known or expected to be anadromous (Federal Register 1997). However, in practice, this can be nearly impossible to determine, particularly for juvenile fish.

The Russian River is located in central California, with an ocean outfall approximately 90 km north of San Francisco, and is the largest river in the Central California Coast Steelhead ESU. The basin contains many natural waterfalls, as well as two major dams, Warm Springs (built in 1982) and Coyote (built in 1959). These characteristics make the Russian River basin a good system to study the potential influence of both natural and man-made barriers on migration and gene flow on potentially migratory fish.

In this study, we studied population samples of *O. mykiss* at 20 sites within the Russian River by collected genetic data from 22 microsatellite loci and analyzed patterns of genetic diversity and structure. Sampling included 11 sites above putative natural barriers to anadromy (waterfalls), two sites above dams, five sites below any known barriers to anadromy and two samples of offspring of anadromous adults returning to the two dammed tributaries and bred at hatcheries. We then describe the effects of such barriers on local population structure and diversity. We also compare patterns of genetic structure and diversity within the Russian River with those from 16 other coastal watersheds throughout California. Finally, we discuss implications of the results for conservation and management of this ecologically complex species on the small scales at which restoration strategies are frequently devised.

Materials and methods

Sampling

Caudal fin clips were taken, non-lethally, from juvenile trout at five below-barrier sites and from all year classes at 13 above-barrier sites in the Russian River watershed in 2002 (Fig. 1a, Table 1). These sites represent samples from seven tributaries above, between, and below-barriers that were described as impassable to anadromous fish by the California Department of Fish and Game (CDFG 2002). Samples were collected via electrofishing and seining throughout the period prior to the winter run of steelhead (June–October). In addition, the Dry Creek and East Fork Russian River tributaries were studied by analyzing juvenile offspring of anadromous adults that returned to the two dams on these tributaries and were then brought to Warm Springs Hatchery for breeding. Tissue was then collected from their offspring, which were reared separately, before release. Tissue samples were stored in 20% DMSO-saturated NaCl storage buffer and frozen at – 20°C until processed.

DNA extraction and microsatellite analyses

Genomic DNA was isolated from fin tissue using a phenol–chloroform iso-amyl alcohol procedure following Taberlet and Bouvet (1991). DNA was visually inspected for quality and quantity on 1.2% agarose gels. Nine hundred and eight individuals were analyzed with

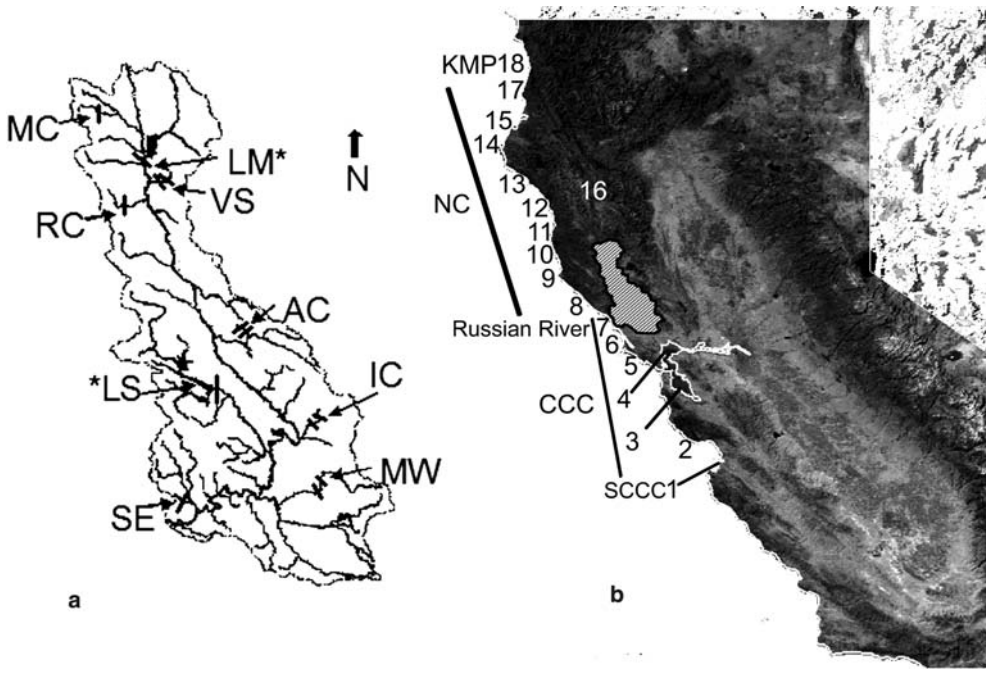

Fig. 1 (**a**) Sites sampled in the Russian River in 2002. Slash marks across waterways indicate barriers, sites were sampled immediately above and below each barrier. *Indicate barriers that are dams, all other barriers are natural, abbreviations as in Table 1. (**b**) Map of California indicating the Russian River watershed (shaded area) in relationship to the other basins evaluated in this study. Numbers 1–18 and abbreviations for ESUs SCCC, CCC, NC, and KMP correspond to Table 2

Table 1 Description of sites and samples

County	Tributary	Classification	Abbreviation	*N*	*D* (m)	Type of barrier
Sonoma	St. Elmo Creek	Below	SEB	39	175	
		Above	SEA	46	> 500	Three 3.0-m waterfalls
Sonoma	Ingalls Creek	Below	ICB	42	102	
		Middle	ICM	48	110	3.7-m boulder fall
		Above	ICA	48	115	21.3-m waterfall
Sonoma	Dry Creek (below Lake Sonoma)	Hatchery	LSB	48	[a]	
	Rancheria Creek (above Lake Sonoma)	Above	LSA	48	110	Dam, built in 1982
Sonoma	Markwest Creek	Below	MWM	48	74	
		Above	MWA	48	> 200	6.1-m waterfall
Sonoma	Alder Creek	Middle	ACM	48	110	
		Above	ACA	48	183	6.7-m waterfall
Mendocino	Robinson Creek	Below	RCB	48	61	
		Above	RCA	48	110	21.3-m steep cascade
Mendocino	Vichy Springs Creek	Below	VSB	16	50	
		Middle	VSM	48	236	6.1-m waterfall
		Above	VSA	48	> 200	12.2-m waterfall/old mining dam above
Mendocino	East fork Russian River (below Lake Mendocino)	Hatchery	LMB	48	[a]	
Mendocino	Busch Creek (above Lake Mendocino)	Above	LMA	48	115	Dam, built in 1959
Mendocino	Mill Creek	Below	MCB	48	100	
		Above	MCA	45	> 500	6.1-m waterfall
			Total	908		

County and tributary sampled from the Russian River Watershed. The site abbreviations used in this study, sample size (*N*) maximum geographic distance between samples (*D*), and the type of barrier

[a] These samples represent fin clips taken from fish held at the Warm Springs Hatchery

22 previously described polymorphic microsatellite loci (Table 3). PCR was performed in 10 μl or 15 μl reactions consisting of Promega reaction buffer or ABI 10X buffer II (Applied Biosystems Inc.), 1.0–3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 μM each primer (one standard primer and fluorescently labeled primer with the ABI buffer and one or two M13-tailed primers with the Promega buffer), 0.1 μM LI-COR IRD700 or IRD800 label (with M13-tailed primers F: CACGACGTTGTAAAACGAC and R: GGATAACAA TTTCACACAGG), 1.25 units *Taq* DNA polymerase and 20–60 ng genomic DNA. PCR was performed with either a single or a variable, two-stage annealing temperature (Table 3). The basic thermocycling regime

Table 2 Description of out of basin sites and samples from Garza et al. (2004) that were used for comparisons among watersheds

ESU	County	River	Tributary	*N*
SCCC	Monterey	Willow Creek (1)		88
CCC	Santa Cruz	San Lorenzo (2)	Zayante	55
CCC	Santa Clara	San Francisco Bay South (3)	Los Trancos	64
CCC	Marin	San Francisco Bay North (4)	Miller Creek	70
CCC	Marin	Lagunitas Creek (5)		61
CCC	Marin	Redwood Creek (6)		73
CCC	Sonoma	Russian[a] (7)	Willow Creek	64
NC	Mendocino	Gualala (8)	Fuller Creek	64
NC	Mendocino	Navarro (9)	Indian Creek	64
NC	Mendocino	Noyo (10)	Little North Fork	64
NC	Mendocino	Ten Mile (11)	Lower South Fork	64
NC	Mendocino	Usal (12)		64
NC	Humboldt	Mattole (13)	South Fork Bear Creek	81
NC	Humboldt	Bear (14)		63
NC	Humboldt	Eel (15)	Lawrence Creek	72
NC	Humboldt	Middle Fork Eel[b] (16)	Plaskett Creek	41
NC	Humboldt	Redwood (17)	Lost Man Creek	56
KMP	Del Norte	Klamath Moutain Province (18)	Wilson Creek	48
			Total	1,156

ESU, County, River and tributary from where samples were collected in 2001. Numbers in parentheses refer to location on map in Fig. 1b and are used for abbreviations on tree figures, followed by sample size (*N*) used for analysis in this study

[a] This is a sample from a tributary on the Russian River sampled in 2001 from Garza et al. (2004)

[b] Above-barrier sample

was 94°C for 3 min; then 9 cycles at 94°C for 30 s, N°C (Table 3) for 2 min, and 72°C for 30 s; followed by 15 cycles at 92°C for 30 s, N°C (Table 3) for 2 min, and 72°C for 30 s, with a final step at 72°C for 10 min. PCR products were electrophoresed in denaturing 6.5% polyacrylamide (Urea concentration 7 M) gels on either a LI-COR Gene ReadIR 4000/4200 or an ABI 377 automated sequencer. Loci genotyped on the LI-COR system had two standards per gel, one a LI-COR size ladder and the other a sequence from a high copy number *E. coli* plasmid cloning vector pUC19 (New England BioLabs Inc). The allele size for each gene copy was determined independently by at least two people, by visual comparison with a pUC19 sequence. The size standard was used to control for consistency between gels. Approximately 5% of the samples were rerun to examine the level of consistency across gels and individual genotype scores. Loci genotyped on an ABI sequencer were analyzed using Genotyper software (Applied Biosystems Inc.). In addition, 10% of the samples were run on both instruments to convert genotypes for six loci (indicated in Table 3) to allow analysis of interbasin variation using a subset of data from Garza et al. (2004).

Intra- and inter-population genetic diversity

Genetic polymorphism within each site, and for all 22 microsatellite loci, was measured as the number of alleles per locus (A), allelic richness (A_r; number of alleles weighted by samples size), observed (H_o) and expected heterozygosity (H_e), and number of private alleles per site (P_a). A, H_o, H_e, and P_a were calculated using GDA V1.1 (Lewis and Zaykin 2001). A_r was calculated using FSTAT V2.9.3.2 (Goudet 2001). A_r was evaluated for significant differences between above and below-barrier sites and between hatchery and naturally spawning trout sites with 1,000 permutations in FSTAT. H_e was also evaluated for significant differences for the same site groupings using a Wilcoxon's signed-ranks test. The Mill Creek above (MCA) site was excluded from inter-site genetic diversity analyses because of known recent stocking with adult steelhead returning to hatcheries (Dan Logan NOAA, pers. comm.).

Equilibrium tests

Tests of Hardy–Weinberg (HWE) and linkage disequilibrium (LD) were performed as implemented in Genepop V3.4 (Raymond and Rousset 1997) using the Monte Carlo method with 1,000 batches and 10,000 iterations per batch. Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique at a significance of 0.05 (Rice 1989).

Watershed population structure and barrier analysis

To assess genetic structure of sites sampled above, between, and below barriers, as well as among all sites,

five approaches were used: (1) Allelic distributions were tested for differentiation across all sites using the genic differentiation test as described by Raymond and Rousset (1995) and implemented with the default values in Genepop V3.4. (2) Weir and Cockerham's (1984) θ estimator of F_{st} was calculated using Arlequin V2.0 (Schneider et al. 2000) for all pairwise comparisons between sites. We refer to the estimator θ as F_{st} throughout this paper. The significance of fixation indices was tested using a non-parametric approach described by Excoffier et al. (1992). Average F_{st} among all sites was estimated using FSTAT. (3) Cavalli-Sforza and Edwards (1967) chord distance (D_{ce}) was calculated for all pairwise comparisons among sites

Table 3 Description of loci used in this study

Locus	Source species	Reference	Sequence	T_a (°C)	N_a	N_g	S_{bp}
Ssa289[a,b]	*S. salar*	McConnell et al. (1995)	F: CTTTACAAATAGACAGACT R: TCATACAGTCACTATCATC	56	12	1,754	121–145
OtsG249[a,b]	*O. tshawytscha*	Williamson et al. (2002)	F: TTCTCAGAGGGTAAAATCTCAGTAAG R: GTACAACCCCTCTCACCTACCC	56	17	1,798	136–204
Ots1b	*O. tshawytscha*	Banks et al. (1999	F: GGAAAGAGCAGATGTTGTTAA R: ATGCTATTTCCAGACGGCA[c]	52.8/60[d]	17	1,754	224–316
OtsG423	*O. tshawytscha*	Williamson et al. (2002)	F: AGGCCTGCCAGGCACTAAAGGTAT R: GCAAGCAAACATGTAGCTTCATGG	52.8/60[d]	27	1,786	101–241
OtsG83b	*O. tshawytscha*	Williamson et al. (2002)	F: TAGCCCTGCACTAAAATACAGTTC R: CATTAATCTAGGCTTGTCAGCAGT	56	26	1,810	104–228
OtsG85[a,b]	*O. tshawytscha*	Williamson et al. (2002)	F: CCATGTCAGCACTGACTTAAT R: GGATGTTGTTCCTAATGTTTT	56	32	1,804	150–298
OtsG3[a,b]	*O. tshawytscha*	Williamson et al. (2002)	F: GGACAGGAGCGTCTGCTAAATGACTG R: GGATGGATTGATGAATGGGTGGG	56	16	1,810	158–258
Omy27	*O. mykiss*	McConnell et al. (1995)	F: TTTATGTCATGTCAGCCAGTG R: TTTATGGCTGGCAACTAATGT	56	8	1,804	138–156
Omy77[a,b]	*O. mykiss*	Morris et al. (1996)	F: CGTTCTCTACTGAGTCAT R: GGGTCTTTAAGGCTTCACTGCA	55/59[d]	27	1,786	105–167
Ssa85[a,b]	*S. salar*	O'Reilly et al. (1996	F: AGGTGGGTCCTCCAAGCTAC R: ACCCGCTCCTCACTTAATC	58/60[d]	21	1,796	136–214
Omm1332	*O. mykiss*	Palti et al. (2002)	F: GCGGAAGTGAAGGTGGTGTAA R: TTGCTGGGGCTCTCATC	52.8	8	1,802	225–253
Omm1329	*O. mykiss*	Palti et al. (2002)	F: GGGAAGTGTTCACCATTACACAAG R: CATCCAGGAACGCACCTTTA	60	26	1,780	181–255
OtsG409[b]	*O. tshawytscha*	Williamson et al. (2002)	F: GTAGCCATTTGTGTCACCATCATT R: CATTCTCCTGCCTCACAGAGTTTA	53/55[d]	4	1,666	84–90
Ots103[b]	*O. tshawytscha*	Small et al. (1998)	F: AGGCTCTGGGTCCGTG R: TGATATGGTGTGATAGCTGG	53/55[d]	8	1,674	54–92
Omy1011[b]	*O. mykiss*	Condrey and Bentzen (1998)	F: AACTTGCTATGTGAATGTGC R: GACAAAAGTGACTGGTTGGT	53/55[d]	18	1,548	132–260
OtsG243[b]	*O. tshawytscha*	Williamson et al. (2002)	F: TTATTAAACTGCACTGTCTAACTACA R: GTATGCAGCAAGCCAGGTG	53/55[d]	10	1,666	97–125
OtsG253b[b]	*O. tshawytscha*	Williamson et al. (2002)	F: CGCTGCAGAAACATTTTCGA[c] R: AATTGGGTCATTAAGGCTCTGTGG	53/55[d]	29	1,462	165–281
OtsG401[b]	*O. tshawytscha*	Williamson et al. (2002)	F: CTGCCCTGAGAAGCTGGAGTGCTC R: TTGCCCCACCCTTGCATCTATCCA	60	26	1,612	165–237
OtsG43[b]	*O. tshawytscha*	Williamson et al. (2002)	F: AACTCCCGTTGACAATTTACTGTTG R: TTTTGGCAAAGTTGGCTACTCTG	55/57[d]	22	1,643	141–205
Oneμ11b[b]	*O. nerka*	Scribner et al. (1996)	F: GTTTGGATGACTCAGATGGGACT R: CCTGCTGCCAACACTGTCAA[c]	53/55[d]	6	1,666	112–124
Oki23[b]	*O. kisutch*	Smith et al. (1998)	F: TGTGCTATAGGGTGAATGTGC R: AACACAGGCATCCCCACTAA	53/55[d]	22	1,572	118–218
Oneμ13b[b]	*O. nerka*	Scribner et al. (1996)	F: TCATACCCCATGCCTCTTCTGTT R: GGGTGGAGAGACAGGTATCTTGTC[c]	53/55[4]	20	1,480	206–254

Annealing temperature (T_a), total alleles observed (N_a), total gene copies sampled (N_g), molecular size range of alleles in base pairs including primer sequence and M13 tail if present (S_{bp})

[a] Loci that were converted for between watershed analyses

[b] Indicate loci used for between watershed analysis

[c] Modified from original publication (Garza et al. 2004)

[d] Two-step annealing temperatures, see PCR protocol in methods

with the GENDIST program in the PHYLIP package V3.57c (Felsenstein 1995). An unrooted neighbor-joining (NJ) tree was constructed using the NEIGHBOR application in PHYLIP (Felsenstein 1995). D_{ce} was chosen since this estimate of genetic distance does not assume population sizes have remained constant and equal (Felsenstein 1995) and has been shown to lead to a higher probability of recovering correct tree topology than other distances (Takezaki and Nei 1996). Trees were visualized with TREEVIEW (Page 1996). To evaluate support for nodes in the NJ tree, 10,000 bootstrapped distance matrices were generated using the SEQBOOT application in PHYLIP, and NJ trees were built with all resulting D_{ce} matrices. A consensus tree of all NJ trees was then built with the CONSENSE application in PHYLIP (Felsenstein 1995). (4) Population assignment tests were performed across all sites using the semi-Bayesian method, which assumes an equal prior probability density of the allelic frequencies for each locus in each population (Rannala and Mountain 1997) and was carried out using the ''leave one out'' procedure in Geneclass V1.0.02 (Cornuet et al. 1999). This procedure does not include the individual who is being assigned when allele frequencies are calculated. (5) An analysis of molecular variance (AMOVA) approach as implemented by Arlequin V2.0 was used to partition variation among groups, among sites within groups, and within sites. The fixation indexes of F_{st} and F_{ct} were calculated as defined by Weir and Cockerham (1984), Excoffier et al. (1992), and Weir and Cockerham (1996), where F_{st} is the total variance in genotype frequencies both among sites and among groups and F_{ct} is the variance in genotype frequencies among groups. Significance of F_{st} and F_{ct} were evaluated as described by Excoffier et al. (1992), using a non-parametric permutation approach and 1,000 permutations were performed. Life history differences, geological patterns among sites and stocking history were used to group sites to test hypotheses of structure. The grouping with the highest significant F_{st} and F_{ct} values were considered the most probable geographic subdivisions (e.g. Girman et al. 2001).

Comparisons with other watersheds

Microsatellite data from 16 loci from all Russian River sites were compared to previously described data from *O. mykiss* in 17 other California watersheds (Garza et al. 2004) that are part of four steelhead ESUs (Fig. 1b and Table 2). F_{st} and significance for all pairwise comparisons of Russian River sites to other watersheds in California were calculated using Arlequin V2.0 (Schneider et al. 2000). Patterns of differentiation (F_{st}) of individual below-barrier sites compared with other watersheds (excluding hatchery sites) were analyzed by performing a Kruskall–Wallace non-parametric ANOVA. This approach is used to test the null hypothesis that all populations have identical levels of differentiation in inter-basin comparisons. Deviations from this expectation were evaluated with a χ^2 test. The geographic distance from the rivermouth to each below-barrier site was used to identify three distance categories within the Russian River (near-mouth, middle and interior). The groupings consisted of St. Elmo Creek samples (14 km from the ocean), Ingalls Creek samples (90 km from the ocean), and samples from Mill Creek, Robinson Creek, and Vichy Springs (187 km, 172 km, and 185 km from the ocean respectively). If significant differences in divergence from other watersheds as measured by F_{st} were detected, pairwise *t*-tests under the protection of the ANOVA were conducted. D_{ce} distances were also generated, an unrooted NJ tree was constructed, and bootstrap analysis was performed as described above.

Results

Population genetic diversity

Basic summary statistics of genetic diversity are presented in Table 4. When sites were grouped by location type (e.g. above vs. below-barrier); genetic variation between groups was significantly different for measured indices of diversity. A_r for fish above natural barriers (4.0) was significantly lower ($P = 0.001$) than for those sampled below-barriers (6.8). Additionally, natural above-barrier sites had significantly lower H_e ($\chi^2 = 9.41$, $P = 0.002$) with a mean difference of 0.16. Estimates of A_r from hatchery-sampled trout representing sites LMB and LSB were not significantly different from naturally spawning populations ($P > 0.05$). However, a comparison of H_e found that it was significantly higher in the naturally spawning below-barrier sites (Table 3; $\chi^2 = 3.96$, $P = 0.047$). Finally, mean A_r estimated from trout above dams (5.9) was not significantly different ($P > 0.05$) from that of hatchery *O. mykiss* (7.2), and their H_e values, which differed by 0.06, were also not significantly different ($\chi^2 = 2.67$, $P > 0.05$). However, comparisons of mean diversity values that involved both hatchery and above-dam sites had a small number of observations. Consequently, there is a chance that the sample design is such that we do not have the power to accurately assess significant differences that involve hatchery and above-dam sites. However, there are only two major

Table 4 Diversity measures calculated from all individuals sampled

Site	N	A	A_r	H_e	H_o	P_a	A_p
SEB	35.0	10.0	7.1	0.72	0.70	3	0.74
SEA	42.5	3.4	2.9	0.48	0.46	0	1.00
ICB	39.8	11.0	7.6	0.74	0.75	10	0.86
ICM	46.1	6.4	4.7	0.61	0.59	3	0.94
ICA	47.3	4.5	3.7	0.54	0.58	1	1.00
LSB	44.7	10.5	7.3	0.75	0.74	6	0.96
LSA	46.1	9.4	6.3	0.69	0.68	6	0.92
MWM	45.6	5.4	3.3	0.55	0.62	1	0.94
MWA	45.1	3.9	3.7	0.53	0.52	3	1.00
ACM	43.3	6.8	5.1	0.67	0.68	1	0.81
ACA	42.1	7.0	5.5	0.69	0.66	2	0.83
RCB	44.2	9.4	6.4	0.71	0.72	1	0.88
RCA	46.7	3.2	2.8	0.46	0.50	1	1.00
VSB	15.8	7.8	7.0	0.74	0.75	2	0.88
VSM	46.3	5.8	4.3	0.60	0.58	6	0.85
VSA	46.0	5.4	4.3	0.59	0.58	3	0.90
LMB	44.0	9.8	7.1	0.75	0.70	4	0.90
LMA	45.8	7.4	5.5	0.68	0.65	1	0.98
MCB	45.0	8.6	6.2	0.72	0.79	2	0.96
MCA	39.8	8.4	6.0	0.69	0.69	5	0.93
Mean	42.6	7.2	5.3	0.64	0.65	3.1	91.63%

N = mean sample size; A = mean number of alleles per locus; A_r = allelic richness across all loci; H_e = expected heterozygosity; H_o = observed heterozygosity; P_a = private alleles; A_p = proportion of individuals assigned correctly

permanent dams and two hatchery broodstock collection sites present in the basin, and these sites, therefore, represent all possible observations.

Equilibrium tests

Tests of HWE found 155 (35% of locus/population combinations) significant departures from equilibrium and tests of LD found 1,149 significant associations (26.6%). After sequential Bonferroni correction only 75 HWE departures were significant (Appendix I, bolded values) which is 17% of the 440 comparisons. LD significant associations were reduced to 157 departures, which is 3.6% of the 4,321 comparisons. There was no consistent pattern of heterozygote deficit or excess observed within and among loci (Appendix I). This suggests that null alleles are not a likely explanation for most of the departures.

Cryptic population structure, inbreeding, admixture and sampling of family groups have previously been suggested as possible factors contributing to departures from equilibrium (summarized in Castric et al. 2002). Given the small spatial scale of the sampling (Table 1) and that most individuals sampled are juveniles, inclusion of related individuals in the study is a likely factor contributing to departures from HWE. If sites were non-randomly sampled and juveniles represent progeny of only a few parents this could substantially affect the results. This possibility led to an exploration of relatedness among individuals within sampling localities with 12 loci (1–12 in Table 3). Matrices of the coefficient of relatedness were constructed using Relatedness V5.0.8 (Goodnight and Queller 2001), pairs of individuals with values consistent with full sib status were identified and one randomly chosen individual of each pair was excluded from the dataset.

The treatment to remove pairs of related individuals resulted in a substantial reduction in significant tests of HWE and LD, with 16.6% (40 out of 240) and 9.0% (94 out of 1,040), respectively. After Bonferroni correction only 12 out of 240 (5.0%) HWE departures were significant and there were no significant LD associations at the 0.05 level. Only slight changes occurred among other statistics related to population structure (data not shown, see Deiner 2004).

However, the removal of related individuals also caused a substantial reduction in sample size, from $N = 908$ to $N = 545$. This caused concern about reduced power for evaluation of significance in population genetic statistics given that approximately one third of the total genotypic data were excluded. To evaluate whether reduced sample size contributed directly to the reduction in departures from equilibria, 25 data sets were generated from the Mill Creek Below (MCB) and Alder Creek Above (ACA) data with the same number of individuals randomly excluded as were excluded on the basis of the relatedness coefficient. HWE and LD were calculated for each, corrected for multiple tests as before, and compared to the data set with all individuals for this site and the data set

with individuals excluded based on relatedness. Analysis of the data sets with individuals randomly excluded revealed similar levels of significant tests for HWE and similar proportions of pairs in LD as when all individuals were included, and did not show the marked decrease in the significant number of departures from equilibrium as observed for the data sets with individuals excluded based on relatedness. These results indicate that the decrease in sample size is not responsible for the decrease in significant departures, at least in MCB and ACA, and lends greater support to the hypothesis that related individuals are causing HWE and LD values to be significantly different from expected proportions.

More importantly, the removal of related individuals did not change the biological interpretation of population structure, as might be expected if these individuals were influencing the analyses. Furthermore, in most cases, samples were collected from sites that would be predicted to have small effective population sizes based on habitat availability (i.e. above waterfalls on small tributaries). For some of these above-barrier sites, almost the entire available stream site was sampled in order to collect desired sample sizes. The majority (74%) of individuals excluded due to relatedness at the full sib or greater level were sampled from these above-barrier sites. Small effective population sizes are expected to result in departures from both HWE (Pudovkin et al. 1996) and linkage equilibrium (Hill 1981) and the departures observed thus likely reflect the biological reality of these populations and not error due to non-random sampling of kin. Finally, since departures from expected proportions were mostly likely due to family structure in the populations that is consistently present, and reducing the sample size by excluding related individuals would inevitably involve some error, we conclude that such removal would likely result in the introduction of error to the parameter estimates of a similar or greater degree than the inclusion of related individuals. Therefore, the reported analyses include data from all individuals genotyped.

Watershed population structure and barrier analysis

Substantial population genetic structure was found among *O. mykiss* sampling sites in the Russian River. Most of this divergence was among populations above natural barriers. We found significant differentiation across all sites using genic differentiation tests of allelic distributions. Mean pairwise F_{st} for intrabasin comparisons was 0.156 (range 0.004–0.385; Table 5). All pairwise F_{st} values were significantly different from 0 ($P < 0.05$) except SEB-LMB ($F_{st} = 0.004$; $P = 0.06$). Mean pairwise F_{st} among above-barrier sites ($F_{st} = 0.237$) was significantly higher for among below-barrier sites ($F_{st} = 0.048$, $P = 0.001$). The mean F_{st} between above-barrier sites and below-barrier sites was 0.158. Pairwise F_{st} values between sites above and in between (middle) barriers on the same tributary were lower than F_{st} values between above- and below-barrier sites on the same tributary (Table 5). There was no significant difference in mean F_{st} among naturally spawning trout below barriers ($F_{st} = 0.048$) and that among naturally spawning trout and hatchery trout ($F_{st} = 0.030$, $P = 0.849$).

The D_{ce} neighbor-joining tree revealed a pattern of differentiation among sites similar to that found with F_{st} estimates (Fig. 2). Specifically, below-barrier sites exhibited short branch lengths and had low bootstrap support, whereas above-barrier sites had long branch lengths. In addition, when multiple above-barrier sites on the same tributary, they clustered together with short branches and high bootstrap support.

Assignment accuracy at each site ranged from 74.4% to 100% (Table 4). A total of 832 of 908 individuals were correctly assigned to site of origin (91.6%). Below-barrier sites tended to have lower assignment accuracy on average than above-barrier sites, with 74.4% and 81.3% accuracy, respectively. Individuals from below-barrier sites that were misassigned were typically assigned to other below-barrier sites, whereas the few individuals from above-barrier sites that were misassigned were generally assigned to one of the above-barrier sites from the same tributary (data not shown).

Of the six different hypotheses tested in the hierarchical analysis of molecular variance, the among-site variance was best explained by a grouping of the natural below-barrier sites, the above-dam sites and the MCA site in one group, versus the above-barrier sites for each tributary in separate groups ($F_{ct} = 0.116$, $P < 0.05$; $F_{st} = 0.173$, $P < 0.05$). However, most of the observed variation (82.7% and 84.7%) in genotype frequencies was due to within-site differences (Table 6).

Russian River compared to other watersheds

Genetic analysis comparing *O. mykiss* from the Russian River to fish from other watersheds in coastal California (Table 2) revealed patterns similar to that of intra-basin comparisons for the Russian River (inter-basin mean pairwise $F_{st} = 0.053$). All pairwise F_{st} values among Russian River sites and those from other

Table 5 Pairwise F_{st} across all populations (matrix) and average F_{st} values (W_a) comparing Russian River sites to *O. mykiss* populations on other watersheds described in Table 2. Abbreviations are listed in Table 1

Sites	SEB	SEA	ICB	ICM	ICA	LSB	LSA	MWM	MWA	ACM	ACA	RCB	RCA	VSB	VSM	VSA	LMB	LMA	MCB	MCA
SEB	0																			
SEA	0.205	0																		
ICB	0.016	0.189	0																	
ICM	0.081	0.245	0.079	0																
ICA	0.124	0.290	0.138	0.074	0															
LSB	0.013	0.188	0.021	0.097	0.149	0														
LSA	0.030	0.189	0.031	0.086	0.135	0.053	0													
MWM	0.111	0.284	0.154	0.222	0.251	0.147	0.119	0												
MWA	0.153	0.345	0.119	0.197	0.239	0.172	0.184	0.213	0											
ACM	0.112	0.269	0.114	0.173	0.194	0.111	0.127	0.227	0.200	0										
ACA	0.084	0.244	0.077	0.142	0.167	0.076	0.087	0.205	0.166	0.029	0									
RCB	0.035	0.218	0.044	0.106	0.154	0.041	0.059	0.151	0.180	0.128	0.089	0								
RCA	0.175	0.385	0.189	0.260	0.292	0.209	0.212	0.305	0.331	0.263	0.238	0.217	0							
VSB	0.035	0.237	0.034	0.120	0.169	0.047	0.068	0.141	0.131	0.119	0.073	0.053	0.228	0						
VSM	0.132	0.316	0.139	0.204	0.238	0.137	0.162	0.250	0.276	0.207	0.174	0.163	0.285	0.151	0					
VSA	0.130	0.309	0.140	0.210	0.244	0.138	0.165	0.247	0.281	0.210	0.176	0.166	0.292	0.161	0.035	0				
LMB	0.004*	0.184	0.012	0.089	0.146	0.025	0.044	0.145	0.141	0.106	0.068	0.028	0.202	0.026	0.132	0.138	0			
LMA	0.070	0.252	0.065	0.141	0.183	0.068	0.089	0.195	0.169	0.142	0.103	0.091	0.259	0.074	0.174	0.185	0.060	0		
MCB	0.037	0.210	0.037	0.103	0.143	0.047	0.057	0.133	0.204	0.111	0.080	0.063	0.217	0.069	0.142	0.140	0.031	0.085	0	
MCA	0.037	0.233	0.043	0.104	0.134	0.047	0.043	0.112	0.176	0.121	0.082	0.055	0.234	0.048	0.168	0.171	0.035	0.077	0.052	0
W_a	0.024	0.215	0.041	0.109	0.153	0.057	0.065	0.141	0.180	0.124	0.088	0.059	0.216	0.063	0.156	0.161	0.046	0.085	0.061	0.068

All pair-wise F_{st} values are significant *$P < 0.05$, except SEB to LMB ($P = 0.06$)

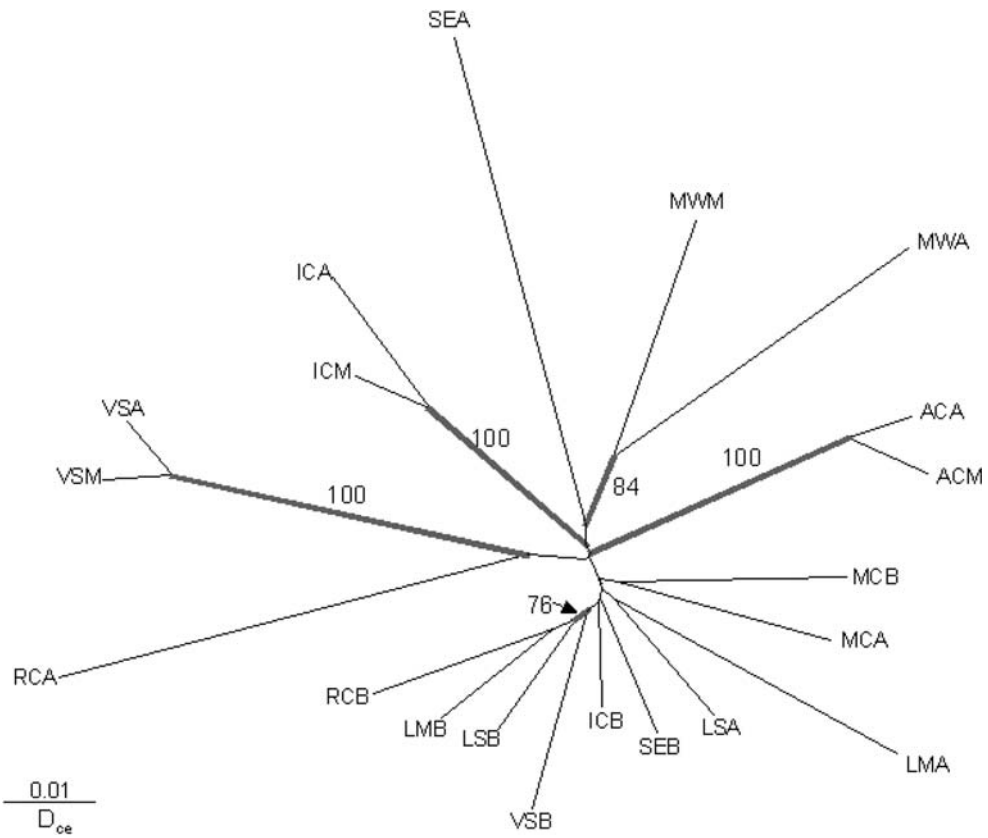

Fig. 2 Neighbor joining tree constructed from chord distances (D_{ce}) using allele frequencies from the 22 microsatellite loci listed in Table 2. Bootstrap support above 65% (10,000 replicates) is indicated by gray branches. Abbreviations as in Table 2

basins were significant ($P < 0.05$). Moreover, a significant pattern of increasing differentiation of below-barrier sites (excluding the hatchery samples) to other watersheds was detected with increasing distance from the mouth of river ($\chi^2 = 38.05$, $P < 0.001$). For example, SEB, near the mouth (14 km from the ocean)

Table 6 Support for groupings of populations estimated using an analysis of molecular variance based on microsatellite allele frequencies across 22 loci

Description of groups	Specific sites in groups (indicated by [])	F_{st}	F_{ct}	Percent variation explained	
				Among sites within groups	Within sites
Above vs. Below	[ACA, ACM, ICM, ICA, LSA, MWA, MWM, LMA, SEA, RCA, VSM, VSA, MCA] [ICB, LMB, LSB, SEB, RCB, VSB, MCB]	0.154	-0.00032^{NS}	15.40	84.63
Aboves as individual populations by tributary vs. Below[a] vs. Above dams as single population	[ACA, ACM] [ICM, ICA] [MWA, MWB] [SEA] [RCA] [VSM, VSA] [ICB, LMB, LMA, LSB, LSA, SEB, RCB, VSB, MCB, MCA]	0.173	0.116	5.56	82.71
Aboves as individual populations by tributary vs. Belows vs. Above dams	[ACA, ACM] [ICM, ICA] [MWA, MWB] [SEA] [RCA] [VSM, VSA] [ICB, LMB, LSB, SEB, RCB, VSB, MCB, MCA] [LMA, LSA]	0.163	0.11	5.73	83.72
Aboves as individual populations by tributary vs. recently hatchery stocked Belows vs. non-recently stocked Belows	[ACA, ACM] [ICM, ICA] [MWA, MWB] [SEA] [RCA] [VSM, VSA] [ICB, SEB, RCB, VSB] [LSA, LMA LMB, LSB, MCB, MCA]	0.161	0.102	5.90	83.92
Aboves as individual populations by tributary and all Belows as individual populations	[ACA, ACM] [ICM, ICA] [MWA, MWB] [SEA] [RCA] [VSM, VSA] [LSA] [LMB] [MCA] [ICB] [SEB] [RCB] [VSB] [LMB] [LSB] [MCB]	0.151	0.072	7.89	84.90
By Tributary	[ACA, ACM] [ICB, ICM, ICA] [MWB, MWA] [SEB, SEA] [RCB, RCA] [VSB, VSM, VSA] [LSB, LSA] [LMB, LMA] [MCB, MCA]	0.152	0.047	10.49	84.78

Statistical significance of the fixation indices (F_{st}, F_{ct}) were based on probabilities derived from 1,023 permutations; NS denotes $P > 0.05$

[a] Below group includes above barrier population known to be recently stocked with hatchery propagated trout

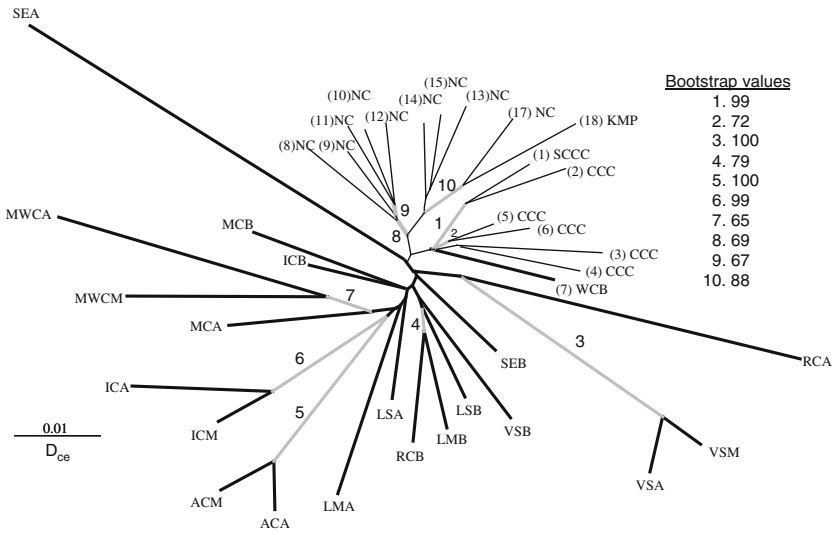

Fig. 3 Neighbor joining tree constructed from chord distances (D_{ce}) using allele frequencies from 16 microsatellite loci listed in Table 3. Bootstrap support above 65% (10,000 replicates) is indicated by gray branches. Russian River sites which are also in the Central California Coast ESU are indicated by bolded branches. Abbreviations for Russian River sites and numbers corresponding to other rivers are found in Tables 1 and 2, respectively

had an average pairwise F_{st} of 0.024, while ICB near the middle of the watershed (90 km from the ocean) had an average pairwise F_{st} of 0.041, and the three interior sites (average distance from the ocean of 183.7 km) had an average pairwise F_{st} of 0.061. Pairwise *t*-tests under the protection of the ANOVA were significant for all comparisons among geographic categories (near mouth, middle, interior) based on distance from the ocean. Pairwise F_{st} values measured between Russian River above-barrier sites to below-barrier sites from other watersheds was comparable in degree (mean pairwise F_{st} = 0.159) to the differentiation estimated between above-barrier and below-barrier sites within the Russian River. The D_{ce} NJ tree found the above-barrier sites to be highly differentiated from below-barrier sites in other watersheds with bootstrap values on branches leading to above-barrier populations similar to those of the within-basin tree (Figs. 2, 3).

Discussion

Genetic analysis of population structure for the trout species *O. mykiss* in the Russian River revealed contrasting results for sites below barriers to anadromy and those found above man-made (dams) and natural (waterfalls) barriers. The seven samples from sites with easy ocean access had similar genetic composition and limited genetic differentiation, indicating recent gene flow among them. Additionally, the two samples from above dams were similar to those from below-barrier sites, both in terms of genetic composition and diversity, indicating they are not highly divergent from below-barrier sampling localities. In contrast, most of the samples from above natural barriers were highly divergent and had evidence of small population sizes.

Below-barrier sites

Comparison of below-barrier sites, which are presumed to contain mainly anadromous fish, revealed significantly less genetic structure than sites above barriers. Results of the AMOVA suggest that the best way to account for the variation in the data is to treat the below-barrier sites as a single population. However, the relationships among below-barrier sites are complex. The assignment tests performed across all sites assigned most individuals to their site of collection (91%), yet the misassignments were almost entirely among below-barrier sites, supporting a hypothesis of moderate gene flow among below-barrier sites and that each site may retain some level of genetic distinction, likely due to tributary-level homing ability. Comparisons to recent studies of within-watershed analyses suggest that the levels of divergence found among below-barrier sites in the Russian River are either similar or relatively low (Carlsson and Nilsson 2001; Spidle et al. 2001; Wenberg and Bentzen 2001; Olsen et al. 2003; Poissant et al. 2005; Crispo et al. 2006). However, a study of temporal variation should be undertaken to determine whether different tributaries support differentiated populations or whether the significant F_{st} values and assignment results are due to transient, kin-based correlations within sites due to sampling juveniles.

Juvenile hatchery trout were not highly differentiated from the juveniles of naturally spawning adults below barriers and thus do not appear to be highly divergent stocks. A review of stocking history of

steelhead trout over the last century, summarized by Steiner Environmental Consulting (1996), revealed that during the period of 1911–1995 an estimated 29.9 million fish from the species of *O. mykiss* were released in the Russian River and that 49% were from Russian River broodstock. Other known sources of broodstock during this period include 16% from the Eel River, 4% from a combination of California rivers including Mad River, Prairie Creek, San Lorenzo Creek, and Scott Creek, and 1% came from Washougal, WA. Broodstock for 30% of these fish were of unknown origin. Despite the considerable number of out-of-basin broodstock sources, results from this study and Garza et al. (2004) suggest that the Russian River population has not been dramatically altered by hatchery releases.

When below-barrier sites on the Russian River were compared to the 16 other coastal watersheds, genetic differentiation was significantly greater among comparisons to interior sites than to those sampled closer to the river mouth. It may be the case that tributaries closer to the mouth of rivers experience higher gene flow from other watersheds than do interior tributaries. The different phylogeographic patterns observed for downstream and interior sites highlight the importance of gene flow from neighboring basins in shaping within-basin population structure. Additional study is needed to determine what effect intrabasin differentiation has on interbasin comparisons.

There were no substantial differences in genetic diversity among below-barrier sites and those of fish propagated from adult steelhead returning to the two dams on the Russian River. There is a large body of research demonstrating that hatchery-propagated salmonids can be less fit than naturally spawned ones (Healey 1991; Heard 1991; Quinn 1993; Moran et al. 1994; Fleming et al. 1996), although this has generally been studied in situations where the hatchery fish are from a distinct stock propagated mainly from hatchery adults, unlike the hatchery protocol followed here, in which mainly non-hatchery produced fish are used as broodstock. However, microsatellite loci generally measure neutral genetic variation and are not a direct estimate of adaptive variation. Even so, the retention of genetic variation probably means that the populations also retain much of their adaptive potential (Sherwin and Moritz 2000).

Above-dam sites

Trout sampled above the two dams were not highly differentiated from those sampled at below-barrier sites, with F_{st} values similar to comparisons among below-barrier sites. In addition, misassignments were typically to below-barrier sites, a pattern rarely found with sites above natural barriers (data not shown). The AMOVA results suggest that the variance in genotype frequencies found in the two sites above dams are best explained by grouping these sites with below-barrier sites (Table 6).

Resident rainbow trout stocked above dams in the Russian River are the product of CDFG broodstock programs at various hatcheries around the state. Records indicate that approximately five different strains of rainbow trout were planted, but none have been planted below barriers since 1958 (SEC 1996). Historically, approximately 21,000 resident rainbow trout were planted annually in the East Fork Russian River above Coyote Dam and Lake Mendocino (SEC 1996). Given the extensive stocking history above this lake, it is perhaps surprising that a greater amount of differentiation of the LMA site from below-barrier samples was not found. However, it may be the case that the stocked trout have not widely affected the genetic composition of fish spawning in tributaries that feed into the lake.

Sites sampled above the two dams possessed levels of heterozygosity and allelic richness that were not significantly different from those of the hatcheries below, suggesting that trout sampled above the two dams have not lost substantial diversity since they were isolated from trout sampled below. At least one study suggests that isolation by a dam can cause a loss of genetic variation in trout populations that are trapped above (Nielsen et al. 1997). The higher levels of genetic diversity in this study can possibly be explained by the fact that the dams are recent (25- and 45-years old) and genetic drift may not have had enough time to erode ancestral genetic variation (Srikwian and Woodruff 2000), but it is likely also due to the subsequent movement of adult steelhead above the dams (Brett Wilson, CDFG, pers. comm.). Another possible factor contributing to the higher levels of genetic diversity measured in the above-dam sites compared to the sites above natural barriers is that there is still sufficient habitat above the dams to support larger populations. There are numerous tributaries that feed into the reservoirs (Lakes Sonoma and Mendecino), and it is likely that there is migration among them. Lake-dwelling rainbow trout typically migrate from lakes up into tributaries to spawn (Northcote 1969), as do some other closely related inland trout populations (Neraas and Spruell 2001). However, the extent of gene flow between different tributaries feeding into the reservoirs should be further evaluated and continued monitoring of genetic diversity in these above-dam sites

should be done to determine the potential future effects that dams may have on the loss of genetic diversity.

Above natural barrier sites

Most of the observed genetic structure in the Russian River was due to trout sampled from above natural barriers to anadromy in the different tributaries. These trout populations (excluding MCA) were characterized by large pairwise F_{st} values and long branches on the D_{ce} tree to most other populations in the study. Comparisons among above-barrier sites from different tributaries of the Russian River had the largest F_{st} values in the study. Such comparisons involve populations that are geographically distant and physically separated by multiple barriers to migration. Additionally, these populations almost certainly contain smaller numbers of individuals than those from below barriers because of the limited amount of habitat available (Hilderbrand and Kershner 2000). Small population size in above-barrier populations was observed during sampling. For example, the RCA and SEA sites were exhaustively sampled from barrier to headwaters and less than 60 fish were found in each stream, whereas at the below-barrier sites, a similar number were captured in a small fraction of the stream (Table 1).

The high levels of genetic differentiation found across the barriers were largely due to differences in the frequencies of shared alleles rather than differences in allele presence or absence. Comparisons between two sites on the same tributary that were both above a barrier to anadromy, but also separated by another natural barrier (i.e. comparisons of above and middle sites), were characterized by low F_{st} values and lower assignment accuracies. Additionally, they always grouped together with short branch lengths and very high bootstrap support on the D_{ce} tree. This characteristic pattern between above and middle sites on the same tributary was observed to a lesser degree on Mark West Creek, possibly because of the recent removal of the barrier below both sites and subsequent immigration of steelhead into the MWM site. The close relationships of multiple above-barrier sites on the same tributary suggests that these populations have had levels of gene flow across the waterfall that is similar in magnitude to the amount of gene flow among trout sites below barriers in the Russian River or that they are both the result of stocking from the same source population.

Ubiquitous stocking of hatchery rainbow trout in most bodies of water in California for the last century has likely also contributed to the high differentiation of these sites. While much movement and stocking of rainbow trout is either unrecorded or using fish of unknown origin, a summary of the recent history of fish movement and plantings in the Russian River (SEC 1996) suggests that no organized stocking effort of hatchery rainbow trout has occurred above natural barriers since 1958. In addition, several studies have demonstrated a lack of significant genetic introgression of hatchery stocks into wild populations (Wishard et al. 1984; Currens et al. 1990; Waples 1991; Williams et al. 1997; LeClair et al. 1999). However, complicating matters is that many of the hatchery stocks used for planting in California appear to have originated from coastal trout of the same subspecies (*O. mykiss irideus*), and specifically from stocks in the San Francisco Bay area (Benhke 2002), which is in the same ESU as the Russian River. Unfortunately, we did not have appropriate reference samples from hatchery rainbow trout stocks to ascertain whether the above natural barrier populations studied here originated from vicariant events, stocking with hatchery rainbow trout or some combination of the two.

The amount of genetic variation in the sites above natural barriers was significantly lower than that of below-barrier sites for most measures of diversity (A_r and H_e). This finding was congruent with those of two other studies that used either elevation as a surrogate for above-barrier sites (brook charr; Castric et al. 2001) or examined known landlocked sites (Atlantic salmon; King et al. 2001). Given the degree of isolation, limited habitat, unidirectional migration over waterfalls, and consequeny smaller population sizes of these natural above-barrier populations, genetic drift is likely the major force causing the lower genetic variation, as well as at least partially causing the large genetic distances (Nei et al. 1975; Hedrick and Gilpin 1997).

The MCA site was the only site that did not fit the general pattern of other above natural barrier sites. Pairwise F_{st} values for MCA to below-barrier sites were low and the D_{ce} tree topology indicated that MCA is more closely related to below-barrier sites. The MCA site also had genetic diversity greater than all other above natural barrier sites. However, this site is known to have been recently stocked with steelhead adults returning to both dams (Dan Logan, NOAA; pers. comm.). MCA thus provided a good comparison to other above-barrier sites, indicating that they have likely not been similarly affected by recent stocking from local anadromous fish.

Conservation implications

The results of this study indicate that *O. mykiss* below barriers in the Russian River are best treated as a

single, large, genetically interacting population with moderate sub-population structure. Since genetic connectivity is indicative of demographic connectivity, below-barrier sites should be treated as a single population in population viability analyses. Additionally, the finding that trout samples in tributaries above the two major dams do not show high levels of genetic distinction from trout sampled below barriers and do not appear to be inbred, indicates that they may be suitable for use in restoration and recovery activities. The substantial differences between populations above and below natural barriers on the same tributary indicate that they are not interacting substantially, although it is possible that some gene flow occurs from above to below barriers on some tributaries.

The results presented here suggest that analyses of population structure and diversity within watersheds, in addition to consideration of among-basin and ESU level structure, provides important insights for management of *O. mykiss* populations. Currently, *O. mykiss* above and below putative barriers to anadromy are typically regarded as separate populations, have different levels of conservation protection, and are managed separately. However, as stated in the status review for this species that resulted in the ESA listings, managers often remain uncertain regarding the genetic connectivity between putative resident and anadromous fish (Busby 1996). In this context, our results make clear that specific above-barrier sites are, genetically, very distinct populations from those with access to the ocean, whereas other above-barrier sites are not. Therefore, all putative above-barrier sites may not warrant the same management approach. This and other recent studies (e.g. Poissant et al. 2005; Crispo et al. 2006), demonstrate how an evaluation of landscape features and their impact on gene flow can be a valuable tool, among many, to accurately assess population structure and help managers in development of conservation plans for species protected under the US Endangered Species Act.

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Appendix

Appendix I Sample sizes (N), number of alleles (A) and observed and expected heterozygosities (Ho and He) at 22 microsatellite loci with all individuals for the twenty sites sampled on the Russian River watershed. Abbreviations listed in Table 1 and loci described in Table 3

Locus		ACA	ACM	ICB	ICM	ICA	LMB	LMA	MWM	MWA	SEB	SEA	RCB	RCA	LSB	LSA	VSM	VSA	VSB	MCB	MCA
Ssa289	N	47	48	39	48	48	48	48	48	48	39	46	48	48	47	47	46	44	16	39	35
	A	5	5	7	7	6	6	6	5	7	8	4	6	5	8	7	7	6	5	9	8
	H_o	0.74	0.52	0.72	0.60	**0.96**	0.71	0.63	0.75	0.81	**0.92**	0.63	**0.58**	0.77	**0.49**	**0.70**	0.78	0.73	0.69	0.85	0.49
	H_e	0.76	0.64	0.82	0.72	0.79	0.69	0.69	0.68	0.75	0.77	0.60	0.60	0.71	0.77	0.73	0.79	0.80	0.70	0.77	0.52
OtsG249	N	47	48	40	48	48	48	48	48	48	39	46	48	48	47	47	46	48	16	46	45
	A	9	9	17	6	5	14	10	5	4	11	4	10	3	14	12	5	5	10	10	11
	H_o	0.85	0.88	0.80	0.65	0.75	0.94	0.79	0.79	0.40	0.92	**0.67**	**0.94**	0.48	0.98	0.89	0.59	0.63	0.81	**0.96**	0.91
	H_e	0.86	0.87	0.89	0.68	0.69	0.91	0.83	0.67	0.38	0.88	0.57	0.83	0.43	0.89	0.87	0.67	0.55	0.90	0.87	0.85
Ots1	N	48	48	34	44	47	48	46	48	48	39	44	48	48	48	48	48	48	16	45	34
	A	5	4	11	3	1	9	10	3	3	6	3	7	2	7	6	6	5	8	8	8
	H_o	0.46	0.65	0.82	**0.05**	–	0.83	**0.57**	0.65	0.58	**0.44**	0.52	**0.44**	0.35	0.63	0.71	0.69	0.75	0.81	0.84	0.74
	H_e	0.66	0.62	0.82	0.39	–	0.79	0.85	0.65	0.58	0.69	0.64	0.65	0.37	0.79	0.74	0.74	0.76	0.86	0.73	0.74
OtsG423	N	48	48	42	47	48	48	48	48	48	39	46	48	48	48	47	47	48	16	47	34
	A	11	11	17	11	8	16	10	10	4	17	4	9	3	14	22	6	6	14	12	11
	H_o	0.75	0.96	0.90	0.91	0.81	0.79	0.91	0.85	0.75	**0.79**	0.83	**0.73**	**0.77**	1.00	**0.83**	0.77	0.77	1.00	**0.94**	**0.97**
	H_e	0.80	0.79	0.91	0.85	0.78	0.85	0.85	0.74	0.71	0.89	0.63	0.80	0.54	0.91	0.94	0.78	0.75	0.92	0.87	0.88
OtsG83b	N	48	48	42	48	48	48	48	48	48	39	46	48	48	48	48	48	48	16	46	44
	A	8	9	20	9	5	14	9	9	5	17	3	15	4	20	19	8	8	12	14	15
	H_o	0.81	0.88	0.90	0.90	0.63	0.79	0.75	0.58	0.63	**0.87**	0.59	**0.77**	0.52	0.88	0.92	0.83	0.85	0.88	**0.93**	**0.77**
	H_e	0.80	0.84	0.94	0.82	0.73	0.86	0.74	0.61	0.68	0.91	0.63	0.89	0.64	0.91	0.92	0.81	0.84	0.94	0.89	0.89
OtsG85	N	48	48	41	48	47	48	47	47	48	39	46	48	48	48	48	48	48	16	47	44
	A	11	11	22	8	7	13	14	8	7	20	4	15	4	16	15	8	7	13	13	18
	H_o	0.79	0.35	**0.98**	0.79	0.68	0.92	**0.81**	**0.83**	**0.63**	**0.77**	**0.54**	**0.98**	0.67	0.94	0.85	0.58	0.77	1.00	**0.98**	**0.93**
	H_e	0.77	0.45	0.94	0.73	0.62	0.91	0.79	0.73	0.75	0.92	0.62	0.88	0.54	0.91	0.80	0.71	0.77	0.91	0.85	0.90
OtsG3	N	48	48	42	48	48	48	48	48	48	39	46	48	48	48	47	48	48	16	47	44
	A	4	4	9	3	3	5	4	3	2	5	1	6	2	6	4	4	5	4	5	5
	H_o	0.63	0.73	0.55	0.25	0.17	0.27	0.48	0.40	0.10	0.56	–	0.50	0.58	0.38	0.36	0.71	0.71	0.69	0.53	0.14
	H_e	0.62	0.67	0.53	0.24	0.18	0.33	0.49	0.34	0.12	0.49	–	0.46	0.50	0.36	0.35	0.67	0.65	0.56	0.54	0.15
Omy27	N	48	48	41	48	48	48	48	48	48	39	46	48	48	47	48	48	48	16	47	42
	A	5	4	4	3	3	5	4	2	2	4	2	4	3	5	4	6	4	4	4	4
	H_o	0.65	0.77	**0.61**	0.65	0.77	0.69	**0.52**	0.35	0.38	0.62	0.26	0.79	0.79	0.74	0.52	0.69	0.58	0.88	0.87	0.62
	H_e	0.71	0.66	0.71	0.59	0.65	0.69	0.69	0.34	0.43	0.62	0.31	0.60	0.63	0.74	0.65	0.66	0.70	0.68	0.67	0.68
Omy77	N	46	48	42	48	48	47	48	48	48	39	38	48	48	47	48	48	48	16	46	44
	A	7	7	15	9	4	14	9	6	4	13	3	12	4	19	10	6	6	9	16	9
	H_o	0.65	0.67	0.86	0.81	0.75	**0.81**	**0.77**	0.54	0.71	0.85	0.74	**0.81**	0.54	**0.64**	0.63	0.58	**0.48**	0.63	0.80	0.52
	H_e	0.62	0.67	0.88	0.81	0.65	0.90	0.84	0.55	0.57	0.90	0.64	0.84	0.50	0.94	0.74	0.71	0.76	0.70	0.83	0.57
Ssa85	N	47	48	42	48	48	48	48	48	48	39	45	48	48	48	48	48	48	16	42	43
	A	3	3	8	3	4	7	6	2	1	6	4	9	1	10	4	6	3	6	5	7
	H_o	0.34	**0.35**	0.45	0.27	0.23	0.60	0.38	0.40	–	0.21	0.40	0.65	–	0.67	**0.25**	0.13	0.29	0.44	0.64	0.44
	H_e	0.54	0.45	0.39	0.26	0.26	0.64	0.41	0.34	–	0.22	0.44	0.59	–	0.62	0.39	0.18	0.27	0.39	0.65	0.45
Omm1332	N	47	48	42	48	48	48	47	48	48	39	45	48	48	47	48	47	48	16	47	44
	A	5	7	7	4	3	6	5	5	3	6	6	6	3	5	6	5	4	6	6	6
	H_o	0.72	0.92	0.86	0.60	**0.88**	0.73	0.77	0.71	0.71	0.74	0.60	0.79	0.79	0.77	0.83	0.53	0.56	0.88	0.68	0.86
	H_e	0.73	0.74	0.78	0.57	0.62	0.77	0.74	0.66	0.55	0.74	0.66	0.73	0.60	0.72	0.80	0.66	0.52	0.83	0.70	0.80

Appendix I continued

Locus		ACA	ACM	ICB	ICM	ICA	LMB	LMA	MWM	MWA	SEB	SEA	RCB	RCA	LSB	LSA	VSM	VSA	VSB	MCB	MCA
Omm1329	N	47	48	42	48	48	47	46	48	48	39	44	48	48	46	45	46	48	16	47	41
	A	6	9	13	10	7	14	10	8	4	15	2	16	3	14	11	9	9	10	9	15
	H_o	0.62	0.73	0.90	0.71	0.85	0.81	0.78	0.65	0.44	0.92	0.41	**0.85**	0.54	0.96	0.82	**0.63**	0.81	0.69	0.94	0.93
	H_e	0.74	0.82	0.91	0.76	0.80	0.90	0.82	0.64	0.56	0.90	0.36	0.91	0.55	0.89	0.82	0.82	0.78	0.87	0.87	0.90
Omy1011	N	46	46	40	47	45	14	45	26	47	36	45	38	47	25	33	48	46	14	47	39
	A	8	7	12	8	5	10	9	4	6	11	4	10	3	13	10	5	5	9	7	10
	H_o	0.70	0.61	0.83	0.70	0.81	0.71	0.78	0.35	**0.62**	0.72	0.58	0.82	0.34	0.80	0.67	0.44	0.54	1.00	0.89	0.85
	H_e	0.79	0.59	0.81	0.76	0.78	0.91	0.78	0.48	0.70	0.76	0.56	0.84	0.44	0.91	0.71	0.42	0.49	0.88	0.77	0.88
OtsG103	N	47	36	40	46	47	48	47	42	48	25	40	43	46	47	48	45	35	16	48	43
	A	1	1	2	4	2	2	1	1	1	3	2	3	2	5	3	2	1	2	3	4
	H_o	–	–	0.08	0.22	0.13	0.10	–	–	–	0.28	0.00	0.05	0.22	0.36	0.08	0.02	–	0.25	0.10	**0.16**
	H_e	–	–	0.10	0.29	0.14	0.16	–	–	–	0.25	0.07	0.07	0.22	0.38	0.10	0.04	–	0.28	0.10	0.26
OtsG243	N	46	44	40	47	48	24	45	48	48	32	45	30	47	47	48	48	44	15	47	40
	A	3	3	4	3	3	8	3	4	3	5	4	4	3	4	5	3	3	4	4	5
	H_o	0.59	0.64	0.80	0.53	0.58	0.75	0.42	0.48	0.48	0.66	0.58	0.70	0.28	0.83	0.73	0.54	0.41	0.47	0.66	**0.88**
	H_e	0.55	0.54	0.66	0.50	0.50	0.83	0.45	0.56	0.65	0.73	0.54	0.73	0.33	0.71	0.68	0.57	0.39	0.58	0.67	0.57
OtsG253b	N	25	33	39	43	48	31	42	41	30	32	43	37	41	27	47	46	46	16	36	28
	A	12	9	17	10	5	16	11	9	4	15	3	16	4	14	14	8	9	8	14	9
	H_o	0.88	0.88	0.95	**0.65**	**0.77**	0.90	0.76	**1.00**	0.80	0.94	0.44	**0.70**	0.41	0.81	0.91	0.63	0.57	0.75	**0.97**	0.86
	H_e	0.88	0.84	0.93	0.85	0.73	0.93	0.81	0.78	0.76	0.92	0.46	0.89	0.35	0.87	0.89	0.67	0.61	0.86	0.88	0.86
OtsG401	N	39	45	38	42	47	45	43	48	47	28	21	37	45	47	45	39	46	16	46	42
	A	15	12	14	8	5	15	9	6	6	13	8	15	5	14	13	12	8	11	10	6
	H_o	**0.87**	**0.91**	**1.00**	0.86	0.79	0.93	0.81	**0.94**	0.81	0.93	**0.67**	**0.92**	0.71	0.89	0.98	0.90	0.80	0.94	**0.93**	0.60
	H_e	0.87	0.82	0.92	0.80	0.75	0.90	0.82	0.65	0.71	0.89	0.75	0.91	0.69	0.89	0.86	0.82	0.82	0.89	0.82	0.78
Oki23a	N	29	35	35	41	45	48	48	44	41	28	41	37	42	44	46	45	45	0	43	33
	A	9	9	15	11	6	12	13	8	4	14	4	13	3	13	16	6	6	0	12	10
	H_o	0.83	0.89	0.97	0.83	**0.60**	0.77	0.81	**1.00**	0.73	0.93	**0.51**	**0.86**	0.40	0.86	0.91	0.62	0.56	–	**0.91**	**0.79**
	H_e	0.83	0.82	0.93	0.83	0.79	0.89	0.84	0.78	0.64	0.87	0.69	0.86	0.52	0.83	0.87	0.64	0.49	–	0.87	0.83
One13b	N	21	29	34	41	45	47	44	38	39	11	35	42	46	41	44	45	44	15	45	34
	A	13	10	15	8	7	12	9	9	6	10	2	12	6	12	10	6	8	7	12	8
	H_o	1.00	0.90	0.79	0.76	0.67	0.91	0.84	**0.97**	0.62	0.73	0.37	0.79	0.78	1.00	0.86	0.82	0.66	1.00	0.93	0.88
	H_e	0.92	0.85	0.88	0.71	0.68	0.88	0.85	0.81	0.54	0.93	0.47	0.79	0.70	0.81	0.85	0.69	0.74	0.83	0.88	0.84
One11b	N	34	33	40	46	47	43	46	44	44	38	41	46	47	46	43	48	46	16	44	41
	A	4	5	3	2	3	4	2	3	4	4	2	4	2	4	2	2	2	3	4	4
	H_o	0.59	0.52	0.68	0.37	0.17	**0.28**	0.43	0.50	**0.43**	0.42	**0.07**	**0.67**	0.40	**0.46**	0.44	0.54	0.37	0.44	0.80	0.56
	H_e	0.72	0.67	0.53	0.49	0.25	0.50	0.47	0.48	0.56	0.56	0.51	0.56	0.46	0.48	0.47	0.50	0.52	0.57	0.60	0.55
OtsG409	N	37	38	40	46	46	47	33	44	43	37	46	44	45	46	45	45	46	16	45	44
	A	4	3	2	1	1	3	2	2	2	2	1	3	1	3	2	2	2	2	2	2
	H_o	0.57	0.45	0.20	–	–	0.51	0.58	0.07	0.16	0.22	–	0.52	–	0.26	0.20	0.13	0.24	0.31	0.24	0.41
	H_e	0.54	0.54	0.20	–	–	0.51	0.46	0.09	0.34	0.26	–	0.55	–	0.30	0.24	0.18	0.28	0.50	0.24	0.40
OtsG43	N	34	37	40	44	48	48	45	43	41	34	41	43	46	45	46	42	42	16	44	38
	A	7	7	8	9	7	10	7	7	4	14	5	11	5	11	11	5	7	8	10	10
	H_o	0.53	**0.54**	0.85	0.80	**0.77**	0.71	0.69	**0.77**	0.61	0.88	**0.73**	0.88	0.63	**0.89**	0.85	0.55	0.74	0.94	**0.89**	0.89
	H_e	0.71	0.67	0.84	0.79	0.67	0.86	0.75	0.67	0.70	0.88	0.67	0.86	0.59	0.88	0.80	0.55	0.69	0.88	0.86	0.86

Bold and underlined H_o values are significant ($P < 0.05$) from the H_e after sequential Bonferroni corrections

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