Transactions of the American Fisheries Society 139:201–213, 2010

DOI: 10.1577/T08-245.1

[Article]

Introgressive Hybridization of Redband Trout in the Upper McCloud River Watershed

RACHEL E. SIMMONS,* PHIL LAVRETSKY, AND BERNIE MAY

Department of Animal Science, University of California–Davis, Meyer Hall, One Shields Avenue, Davis, California 95616, USA

Abstract.—Introgressive hybridization is an obstacle to the conservation of many species and subspecies. Diagnostic genetic markers or markers with high allele frequency differentials, such as single-nucleotide polymorphisms (SNPs), are becoming increasingly useful for detecting introgression between species or subspecies, such as subspecies of rainbow trout *Oncorhynchus mykiss* known as redband trout in the upper McCloud River watershed. Using a diagnostic mitochondrial SNP as well as nine nuclear SNPs, we quantified introgression levels between native redband trout and nonnative rainbow trout at 14 locations in the upper McCloud watershed and two locations in the lower McCloud region. Our analyses suggest that Sheepheaven, Edson, Moosehead, and Swamp creeks contain a large portion of the nonintrogressed redband trout individuals remaining in the upper McCloud watershed, implying a dramatic loss of populations free from introgressive hybridization. The results of this study have key management implications, such as the need to erect migration barriers and cease all stocking in the area. However, for managers to make fully informed decisions, additional research is needed on the population genetic structure and phylogenetics of these populations to clarify the issues of low genetic variation, inbreeding, or both and to define an appropriate management unit.

Hybridization is a complex and critical issue for the conservation of many species. Hybridized populations resulting from the introduction of nonnative species can be detrimental to already imperiled species, producing effects such as outbreeding depression, extinction through hybridization, and questions regarding the appropriateness of legal protection of certain populations (Allendorf et al. 2001). Although rules such as the intercross policy have been proposed (USFWS 1996), there is no consistent overall policy on how to treat introgressed populations under the Federal Endangered Species Act (Allendorf et al. 2004). However, evaluating individual populations and assessing hybridization levels relative to the entire species in order to create case-specific critical thresholds may be beneficial in determining how much introgression is acceptable for a population to still be conserved as a part of an endangered species complex (Sanz et al. 2009). For this reason, it is imperative to accurately assess levels of introgression in populations of conservation concern.

Issues of introgression are of great conservation interest not only for species but also for subspecies (e.g., O'Brien and Mayr 1991). Intraspecific hybridization can have the negative consequences that apply to interspecific hybridization, such as loss of genetic identity and local adaptations (Wolf et al. 2001), though the lower degree of genetic differentiation found among subspecies makes introgression more difficult to detect. Many studies have attempted to combat this power issue with diagnostic markers (e.g., Young et al. 2001; Aparicio et al. 2005; Cordes et al. 2006). This approach is now facilitated by the advent of single nucleotide polymorphisms (SNPs) as a viable molecular marker choice in nonmodel organisms (Morin et al. 2004). Due to the high density of SNPs in most genomes, it is often easier to find diagnostic SNPs than to find diagnostic microsatellite alleles (Sprowles et al. 2006; Stephens et al. 2009). Though the biallelic status of SNPs theoretically makes them a less effective or less powerful marker choice, it also allows for a simpler assessment of diagnostic power (e.g., Smith et al. 2001), and smaller panels of diagnostic SNPs can carry the same or even greater diagnostic power than a suite of microsatellites (Shriver et al. 1997; Rosenberg et al. 2003; Wang 2003; Yang et al. 2005).

One case study of introgressive hybridization at a subspecific level is the redband trout (subspecies of rainbow trout *Oncorhynchus mykiss*) in tributary streams to the upper McCloud River in California (Figure 1). Redband trout in the McCloud basin were proposed as a candidate species for federal endangered status (USFWS 1994) and designated by the California Department of Fish and Game (CDFG) as a state species of special concern (Moyle et al. 1995). The candidate status was subsequently removed following a

* Corresponding author: resimmons@ucdavis.edu

Received December 17, 2008; accepted August 17, 2009
Published online December 1, 2009

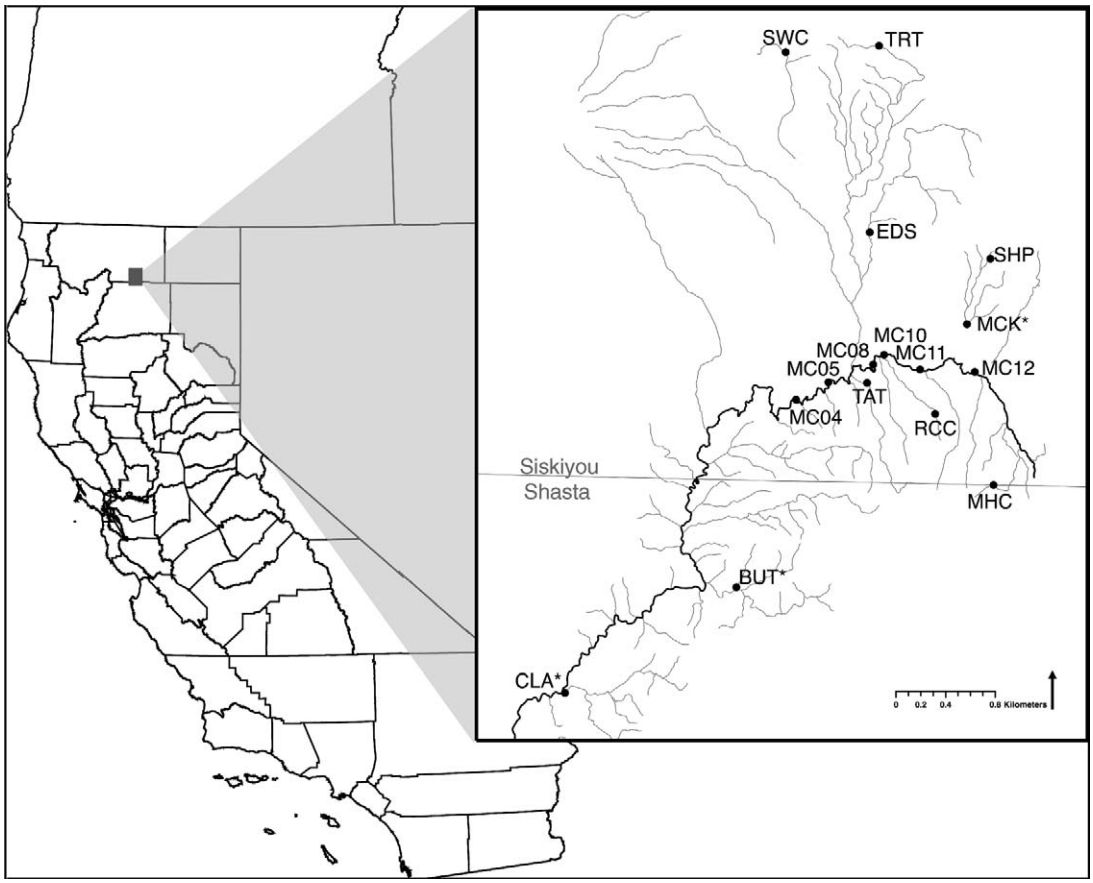

FIGURE 1.—Map of redband trout sampling locations in the McCloud River watershed. Sites with asterisks are approximate locations. Location codes are defined in Table 1.

signing of a conservation agreement in 1998 between federal and state agencies, private industries, and private citizens (USFWS 2000). Some of the most serious threats to the persistence of these populations include habitat loss or degradation and introgressive hybridization with nonnative rainbow trout *O. mykiss* and derived or mixed subspecies (Moyle 1996). As a result of the conservation agreement, several conservation actions have been taken, including restoring habitat, creating refugial pools, and transplanting individuals from Sheepheaven Creek (the population believed to be least introgressed) to Swamp and Trout creeks. However, the efficacy of these measures in preserving nonintrogressed populations of redband trout has not previously been tested.

The McCloud River watershed (Figure 1) was historically heavily planted with several trout species. Brown trout *Salmo trutta* were planted above Big Springs (Wales 1939) and brook trout *Salvelinus fontinalis* were planted past Upper Falls (Nielsen et al. 1999), although records of exact planting events are scarce. Both brown and brook trout were observed above Tate Creek, the site of a former trout hatchery in 1979 (Bacon et al. 1980) and in 2007 (R. E. Simmons, personal observation; B. Jong, personal communication). Both invasive trout species can outcompete and displace redband trout during mild temperature and water flow conditions (Bacon et al. 1980; Moyle et al. 1995). In addition, there is a long and extensive history of planting rainbow trout (most likely Mount Shasta strain or Pit strain hatchery trout) throughout the public areas of the McCloud River, including the upper McCloud, or the McCloud River above Upper Falls, which is a series of waterfalls that provide a natural effective fish barrier (Moyle et al. 1995 and references therein; CDFG 2008). Hatchery rainbow trout continued to be stocked at Fowler's Camp near Big Springs as late as 2008 (CDFG 2008), though there is some evidence that little to none survive to reproduce in that area (Moyle et al. 1995 and references therein).

TABLE 1.—Sources of the samples used in this study of introgression of redband trout and rainbow trout. Redband trout were obtained from 16 sampling locations in the upper and lower McCloud River watershed; the rainbow trout represented seven different strains from various locations.

Site or strain (code)	Year	*N*
Redband trout		
Upper McCloud River		
Edson Creek (EDS)	2003	29
McKay Creek (MCK)	2007	30
Moosehead Creek (MHC)	2007	30
Raccoon Creek (RCC)	2007	24
Sheepheaven Creek (SHP)	2002	34
Swamp Creek (SWC)	2007	22
Tate Creek (TAT)	2005	29
Trout Creek (TRT)	2007	30
McCloud River, station 4 (MC04)	2007	7
McCloud River, station 5 (MC05)	2007	13
McCloud River, station 8 (MC08)	2007	13
McCloud River, station 10 (MC10)	2007	15
McCloud River, station 11 (MC11)	2007	6
McCloud River, station 12 (MC12)	2007	22
Lower McCloud River		
Butcherknife Creek (BUT)	2006	40
Claiborne Creek (CLA)	2006	32
Rainbow trout		
Coleman strain, Crystal Hatchery (CS)	2004	26
Pit strain, Crystal Hatchery (PS)	2004	26
Mount Shasta strain, Mount Shasta Hatchery (MSS)	2002	30
Mount Whitney strain, Mount Whitney Hatchery (MWS)	2002	30
Eagle Lake, Darrah Springs Hatchery (EL)	2004	30
North Fork American River, Mumford bar (NFAR)	2000	20
Navarro River near coast (NF)	2000	31

Redband trout in the upper McCloud watershed are a distinctive form of rainbow trout with a complex systematic history. Morphologically, they have similarities to California golden trout *O. m. aguabonita* but also have a vestigial cutthroat slash (Behnke 1992; 2002). To date, conflicting phylogenetic patterns have been seen in various studies based on different marker types such as karyotyping (Gold 1977), allozymes (Berg 1987), mitochondrial DNA and single copy nuclear DNA, (Bagley and Gall 1998), and microsatellites (Nielsen et al. 1999). However, morphological analyses (Behnke 1992, 2002), and an amplified fragment length polymorphism study (Stephens 2007) show Sheepheaven Creek (the type specimen) to be sufficiently different that the upper McCloud River redband trout may merit its own subspecific status.

The unique signal from Sheepheaven Creek as described above leaves unanswered questions about other redband trout in the upper McCloud River. Is the Sheepheaven Creek population, and possibly other isolated streams in the area, representative of redband trout common to the upper McCloud River prior to the stocking of hatchery rainbow trout and subsequent introgression? Alternatively, has the long history of isolation of Sheepheaven Creek led to its existing as a unique lineage among populations in the watershed?

In order to begin answering these questions, it is necessary to determine whether the distinctiveness of Sheepheaven Creek is representative of redband trout differentiation in the upper McCloud River and, if so, whether introgressive hybridization with hatchery rainbow trout has genetically homogenized other populations such that they are no longer a distinct form. In the current study, we use previously discovered SNP markers shown to have high allelic frequency differentials between Sheepheaven Creek and other rainbow trout in a small discovery panel (Sprowles et al. 2006). These markers have been used to genotype larger sample sizes from Sheepheaven Creek, rainbow trout, and other creeks containing redband trout with lesser-known demographic histories. Important questions to ask about these markers are (1) whether or not the allelic frequency differentials found in the discovery panel remain high when larger sample sizes are genotyped, and (2) if those alleles are common to upper McCloud redband trout populations other than Sheepheaven Creek. Once these questions have been addressed, we will attempt to detect introgression and describe the penetration into the upper McCloud watershed of alleles specific to or more common in rainbow trout.

Methods

Sample collection and storage.—Redband trout samples were collected from locations in the McCloud drainage (Figure 1; Table 1) using backpack electrofishing equipment between 2002 and 2007. Tissue samples were collected from at least 24 and at most 40 individuals. Effort was made to represent as equally as possible all size-classes from each of the sampled locations. A total of seven reference samples of rainbow trout were collected from two inland rainbow trout populations (North Fork American River and Eagle Lake), a coastal population of steelhead *O. mykiss* (Navarro River), and four hatchery strains (Coleman, Pit, Mount Shasta, and Mount Whitney). These samples were included in all analyses as a presumed approximation of fish that might have been used to stock the area. A fin clip of at least 5 mm^2 was obtained from the caudal fin of each fish. Tissue samples were placed inside a folded piece of blotter paper (Whatman brand or equivalent), air-dried, and placed in coin envelopes stored at room temperature.

Molecular methods.—Genomic DNA was extracted from fin clips using the Puregene extraction kit (Gentra Systems). For high-throughput genotyping of SNPs for this study, published sequences flanking previously discovered SNPs were provided to Assays By Design

TABLE 2.—TaqMan forward (F) and reverse (R) primer and 6-FAM and VIC-labeled probe sets used for real-time polymerase chain reaction genotyping, annealing temperature (T_a), amount of assay mix per reaction, and reagents. Bolded nucleotides in the probes are the polymorphism. The numbers in parentheses in the locus column are the assay identification numbers from dbSNP (www.ncbi.nlm.nih.gov/projects/SNP).

Locus	Oligonucleotide sequence (5′–3′)	T_a	Assay mix[a] (μL)	Reagents[b]
B9 90		58	0.1	MM
(ss52084305)	F: CCATAATTCCTATCAAGACTAGTATAAGTGGTT,			
	R: GTGTTCTGTGCATGAAAATGTTTAAA,			
	VIC: TGTATTTTGAC**G**TTTGC, and FAM: TGTATTTTGAC**A**TTTGC			
CHIT 80		60	0.1	MM
(ss38350751)	F: GGCCTTATCAATTATGTCACGTGGAT,			
	R: CCCTTTTCTCTCACAGTAACTTTCCA,			
	VIC: CACCCTT**G**AATAACA, and FAM; CACCCTT**C**AATAACA			
CRB-2677 106		58	0.075	MM
(ss46566266)	F: GCTCAAAAAGATTCTGCCAAATTCACA,			
	R: ATTACAATGAAAGTACTTGAGTGTTTATGCAAA,			
	VIC: TTGCAATG**C**GTCTTT, and FAM: TTGCAATG**A**GTCTTT			
CRB-2677 117		60	0.1	MM
(ss46566267)	F: TCTGCCAAATTCACATGACAAAAGAC,			
	R: ATTACAATGAAAGTACTTGAGTGTTTATGCAAA,			
	VIC: CATTGCAACA**T**AGGGTTG, and FAM: TGCAACA**G**AGGGTTG			
CTSD 33		60	0.1	MM
(ss46566519)	F: CGTCCTCATTCTCCACTATCCATCA,			
	R: ACAAGTAGTGTGCAGAACAAGTGTA,			
	VIC: ACTTCAGGAA**A**AGGGTAG, and FAM: ACTTCAGGAA**G**AGGGTAG			
E1 147		60	0.125	Promega
(ss52084332)	F: GCACTGACTGTTACCAGGAAAGAG,			3.5 mM
	R: GTACTGCAGTGTTGAGGCTATATCA,			MgCl 1 M
	VIC: CCATCCTGAAT**C**TGATTAA, and FAM: CCATCCTGAAT**A**TGATTAA			betaine
FGG 259		60	0.1	MM
(ss46566536)	F: CCACACACACAAACACACATACAC,			
	R: CAAGCATTCTTCTGTTAAATGTGGTCTA,			
	VIC: CACACACA**A**ACAGCA, and FAM: ACACACACA**C**ACAGCA			
G6PD 103		61.5	0.1	MM
(ss38350764)	F: CTCAGCAAAAAAGAAACGTCCCTTT,			
	R: AGTCGTGACAATGAGAAACAGTGTT,			
	VIC: CCTTTTACA**A**TGAAGATC, and FAM: CTTTTACA**G**TGAAGATC			
GH2C 501		57	0.1	MM
(ss46566276)	F: CACACACAGGTCCTGAAGCT,			
	R: GCTAGGGTACTCCCAGGATTCA,			
	VIC: CATATCTCTTTCC**G**CCTGAT, and FAM: CATATCTCTTTCC**A**CCTGAT			
ID1C 160		60	0.1	MM
(ss46566546)	F: GCATACAGTATAAATAATATGGAGAAGTCTTGAGT,			
	R: CTAGTCTGTTTGACAATTCAGCACAT,			
	VIC: ATTGTCAATATCC**G**TCACGAC, and FAM: TTGTCAATATCC**A**TCACGAC			
LDH 156		62	0.075	Promega
(ss46565746)	F: GTTTTGAAACCAGTTTAAGGTTGATTGC,			5 mM MgCl
	R: ACGGCATAGTCTGGACAGAGAT,			
	VIC: CCATTTAGA**C**GTTTTTT, and FAM: CCATTTAGA**T**GTTTTTT			
LDH 201		62, 45 cycles	0.125	MM
(ss46565747)	F: CCCCTGCTAAATGGGAAAAGTCT,			
	R: ACGGCATAGTCTGGACAGAGAT,			
	VIC: CGTAATTCAAT**G**GCTCTT, and FAM: ACGTAATTCAAT**T**GCTCTT			
RAPD 132		60	0.1	MM
(ss38508087)	F: ATCATTACCACGCCCAACGTTA,			
	R: AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT,			
	VIC: CATGTTGGGA**T**ATATGA, and FAM: ATGTTGGGA**A**ATATGA			
RAPD 167		55	0.075	MM
(ss38508088)	F: CCCAACATGCTCTATTGCAGCTA,			
	R: AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT,			
	VIC: ATTAAAACAATCCC**C**CCCAAAA, and FAM: TTAAAACAATCCC**A**CCCAAAA			
D-loop 164[c]		60	0.075	MM
	F: CCCTTAACTCCCAAAGCTAAGATTCT,			
	R: GTAAAGACGGAGCCCGTGTTA,			
	VIC: ACAGCTATGTA**C**AACTGT, and FAM: CAGCTATGTA**A**AACTGT			

[a] Amount of assay mix used in 5-μL total volume reaction.

[b] Reagents were ABI 2× TaqMan Universal Master Mix (MM) or Promega (20 μ/mL Taq polymerase, 0.2 mM dNTP, 1× Mg-free buffer, and MgCl, as specified).

[c] From Bagley and Gall (1998).

(Applied Biosystems) in order to develop TaqMan assays with internal primers and probes specific to each SNP variant (Table 2). We developed a total of 15 TaqMan assays for 14 nuclear SNPs (discovered by Sprowles et al. 2006) and the D-loop 164 locus of Bagley and Gall (1998). Sprowles et al. (2006) used a small number of samples to examine sequences for fixed SNPs. The authors found that the nuclear SNPs were fixed between Sheepheaven Creek redband trout and both the Mount Shasta strain and North Fork American River rainbow trout in the discovery panel of limited sample size (one to four samples per population). Loci were amplified using real-time polymerase chain reactions (PCR) with TaqMan probes. Most assays used 2× TaqMan Universal Master Mix (Applied Biosystems). A subset of loci required Promega reagents for a reaction of 20u/mL *Taq* polymerase, 0.2 mM of each deoxynucleotide triphosphate, and 1× $MgCl_2$-free buffer (see Table 2 for protocols specific to each locus). Polymerase chain reaction was performed on a Chromo4 real-time PCR detector (MJ Research–BioRad Laboratories, Inc.) with a general thermal cycling protocol of initial denaturation at 94°C for 5 min, then 40 cycles of 92°C for 15 s, annealing at 55–62°C for 1 min, then a plate scan for fluorescence in each well (see Table 2 for specific annealing temperatures). At least three controls were present on all plates: a known homozygote of each type and a negative control. MJ Option Monitor analysis software (version 3.1; Bio-Rad Laboratories, Inc.) was used to score genotypes based on endpoint fluorescence and each plate's controls. Individuals that repeatedly failed to amplify for the mitochondrial locus were sequenced using the primers and reaction conditions from Bagley and Gall (1998). Individuals in which less than 70% of loci were successfully amplified after repeated genotyping attempts were removed from further analyses (adjusted numbers are as in Table 1). Tests for linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were conducted with Genepop 3.4 (Raymond and Rousset 1995). Linkage disequilibrium was tested by population for each locus pair; HWE was tested by population for each locus and over all loci. For pairs of SNP loci in LD, the most diagnostic SNP (highest allele frequency differential between Sheepheaven Creek and rainbow populations) was retained.

Introgression estimates.—The frequencies of diagnostic SNPs (including the mitochondrial SNP) were calculated for all locations. We then used a Bayesian clustering algorithm in STRUCTURE, version 2.2 (Pritchard et al. 2000), using nuclear SNPs to describe the highest order of clustering in the data set. STRUCTURE's admixture model was used to verify a division between upper McCloud redband and rainbow trout, and to estimate introgression levels in each of the other McCloud River redband trout locations sampled. STRUCTURE analyses were run over a range of K-values (1–7) and contained three repetitions of 10^5 iterations following a burn-in period of 5×10^4 iterations.

In order to estimate specific introgression levels in individual redband trout populations, the proportion of ancestry, or q-value, from STRUCTURE at K equal to 2 was calculated per individual and averaged across populations. This analysis should result in average q-values that vary between 0 (wholly redband trout) and 1 (wholly rainbow trout). Output files were compiled using CLUMPP, version 1.1.1 (Jakobsson and Rosenberg 2007), which produces H'-values that asses the similarity of cluster memberships from multiple STRUCTURE runs. These H'-values and mean log-likelihood values from each STRUCTURE run were compared to ensure that each Markov chain Monte Carlo chain converged on near-identical answers. The outfile from CLUMPP was visualized using Distruct, version 1.1 (Rosenberg 2004). Average q-values and the frequency of the mitochondrial SNP were calculated per population.

Results

Fifteen SNPs were developed into TaqMan assays that are easily standardized across laboratories and changes in genotyping technology. No significant deviations from HWE were detected in the 15 amplified SNP loci. If a pair of SNPs showed significant LD, the more diagnostic of the two was retained for subsequent analysis. Thus, *CRB-2677 117*, *LDH 201*, *RAPD 132*, and *B9 90* were excluded from subsequent analyses; ID1C was also excluded due to poor amplification success in many populations. Due to TaqMan assay failure, all 30 Eagle Lake individuals were sequenced for the D-loop locus. This resulted in the confirmation of a mitochondrial SNP within the TaqMan forward primer sequence originally identified by Bagley and Gall (1998). Although Sprowles et al. (2006) found the nuclear SNPs to be fixed between Sheepheaven Creek and two other rainbow trout populations in their discovery panel, our expanded data set revealed only two SNPs that were fixed between Sheepheaven and North Fork American River rainbow trout (*G6PD 103* and *GH2C 501*), and four SNPs between Sheepheaven and Mount Shasta strain rainbow trout (*G6PD 103*, *GH2C 501*, *CRB 106*, and *RAPD 167*; Table 3). In addition, *LDH 156* did not display a high allelic frequency differential between Sheepheaven Creek and North Fork American River rainbow trout (0.16), and *FGG 259* lacked a noticeable

TABLE 3.—Allele frequencies of nine nuclear single-nucleotide polymorphisms (SNPs), a mitochondrial SNP, and mean and SD of *q*-values from STRUCTURE.

Location	*CHIT 80*	*CRB 106*	*CTSD 33*	*E1 147*	*FGG 259*	*G6PD 103*	*LDH 156*	*RAPD 167*	*GH2C 501*
Redband trout									
Upper McCloud River									
Sheepheaven Creek	0.00	0.00	0.03	0.00	0.43	0.00	0.02	0.00	0.00
Edson Creek	0.00	0.00	0.48	0.00	0.00	0.62	1.00	0.00	0.00
McKay Creek	0.00	0.24	0.48	0.38	0.19	0.60	0.14	0.13	0.22
Moosehead Creek	0.00	0.00	0.04	0.82	0.02	0.80	0.85	0.00	0.03
Raccoon Creek	0.10	0.10	0.48	0.74	0.03	0.71	0.35	0.25	0.32
Swamp Creek	0.13	0.02	0.30	0.00	0.00	0.00	0.91	0.00	0.00
Tate Creek	0.17	0.31	0.30	0.34	0.98	0.31	0.13	0.16	0.41
Trout Creek	0.17	0.17	0.41	0.38	0.00	0.38	0.63	0.28	0.20
McCloud River, Station 4	0.93	0.14	0.43	0.58	1.00	0.00	0.90	0.29	0.42
McCloud River, Station 5	0.92	0.42	0.50	0.35	0.92	0.62	0.81	0.09	0.19
McCloud River, Station 8	1.00	0.00	0.00	1.00	0.20	0.00	1.00	0.80	0.80
McCloud River, Station 10	0.93	0.37	0.46	0.42	1.00	0.42	0.63	0.14	0.43
McCloud River, Station 11	0.92	0.33	0.50	0.42	0.70	0.90	0.83	0.08	0.00
McCloud River, Station 12	1.00	0.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00
Lower McCloud River									
Butcherknife Creek	0.01	0.39	0.51	0.06	0.00	0.95	0.74	0.32	1.00
Claiborne Creek	0.52	0.96	0.61	0.03	0.10	0.58	0.56	0.45	0.97
Rainbow trout									
Coleman strain	0.98	0.98	0.60	0.90	0.81	1.00	0.74	0.60	0.98
Eagle Lake	0.52	1.00	0.80	0.97	0.83	0.97	0.55	0.93	1.00
Pit strain	1.00	1.00	0.83	1.00	0.62	1.00	0.92	1.00	1.00
Mount Shasta strain	0.72	1.00	0.77	0.88	0.48	1.00	0.90	1.00	1.00
Mount Whitney strain	0.98	0.97	0.70	0.93	0.16	1.00	0.48	0.13	1.00
North Fork American River	0.63	0.76	0.60	0.68	0.15	1.00	0.18	0.45	1.00
Navarro River	0.33	0.98	0.65	0.85	0.89	0.98	0.47	0.68	0.98

frequency differential for Sheepheaven Creek and both North Fork American River and Mount Shasta strain rainbow trout populations (frequencies were 0.43, 0.15, and 0.48, respectively). The remaining loci had frequency differentials of at least 0.72 for Mount Shasta strain rainbow trout versus Sheepheaven and 0.45 for North Fork American River rainbow trout versus Sheepheaven Creek.

In the STRUCTURE runs, although additional groupings were found at higher values of *K*, the largest change in log likelihood was from *K* equal to 1 to *K* equal to 2, indicating that this is where the significant genetic division lies. *K* equal to 2 is also supported using the Evanno et al. (2005) Delta *K* method. CLUMPP's *H′*-values were very high at *K* equal to 2–4 (0.994–0.998) but dropped drastically at *K*-values of 5 and 6 (0.706 and 0.695, respectively), indicating that at higher levels of *K* STRUCTURE runs were unable to converge on similar answers. The initial *K* equal to 2 STRUCTURE analyses show Sheepheaven Creek and all other redband trout populations cluster away from rainbow trout samples (Figure 2).

Introgression Estimates

Estimates based on the nuclear SNP data indicated widespread introgression of rainbow trout alleles in many of the redband trout samples. Beginning downriver and moving up the watershed, the two samples from tributaries of the lower McCloud River (Claiborne and Butcherknife creeks) showed 0.660 and 0.418 ancestry from rainbow trout, respectively, and lower values were found at the upper McCloud River stations (0.186–0.307; Table 3; Figure 3). The remaining redband trout samples showed levels of introgression ranging from 0.022 to 0.252. Results from the mitochondrial marker showed that the lower McCloud samples were fixed for the rainbow trout allele, and substantial rainbow trout allele frequencies were found in McKay Creek and the upper McCloud River (0.24 and 0–0.5, respectively). In contrast, the mitochondrial SNP found in Sheepheaven individuals was fixed in the other redband trout populations from the various tributaries higher in the watershed (Edson, Moosehead, Raccoon, Swamp, Tate, and Trout creeks), a much higher frequency than expected given the level of introgression in some populations suggested by the nuclear loci (Figure 3). The intermediate frequencies of rainbow trout alleles for the nuclear loci in the Raccoon Creek and Tate Creek populations suggest some remaining degree of identity with redband trout, while both the nuclear and mitochondrial markers point to

TABLE 3.—Extended.

Location	*D-loop 164*	*q*-value Mean	*q*-value SD
Redband trout			
Upper McCloud River	0.00	0.022	0.00
Sheepheaven Creek	0.00	0.033	0.01
Edson Creek	0.24	0.139	0.12
McKay Creek	0.00	0.090	0.09
Moosehead Creek	0.00	0.228	0.13
Raccoon Creek	0.00	0.035	0.02
Swamp Creek	0.00	0.252	0.16
Tate Creek	0.00	0.158	0.10
Trout Creek	0.20	0.291	0.17
McCloud River, Station 4	0.50	0.262	0.19
McCloud River, Station 5	0.31	0.307	0.10
McCloud River, Station 8	0.36	0.293	0.15
McCloud River, Station 10	0.00	0.186	0.13
McCloud River, Station 11	0.11	0.269	0.14
McCloud River, Station 12			
Lower McCloud River	1.00	0.418	0.15
Butcherknife Creek	1.00	0.660	0.17
Claiborne Creek			
Rainbow trout			
Coleman strain	1.00	0.957	0.04
Eagle Lake	1.00	0.960	0.04
Pit strain	1.00	0.978	0.00
Mount Shasta strain	1.00	0.964	0.01
Mount Whitney strain	1.00	0.930	0.06
North Fork American River	1.00	0.851	0.14
Navarro River	1.00	0.934	0.05

Edson Creek as being minimally impacted by introgression (Table 3; Figure 3).

Discussion

Marker Performance

Although the mitochondrial SNP showed high allelic frequency differentials between almost all redband trout populations and the reference panel of rainbow trout, expanding the sample sizes from those of Sprowles et al. (2006) have revealed mixed results with regards to diagnostic power for the nuclear SNPs. In particular, *FGG 259* had low diagnostic power for all populations, but only one individual from Sheepheaven Creek was sequenced in Sprowles et al. (2006). This type of result should be expected with some frequency when the detection panel is small. The non–rainbow trout variant of *LDH 156* was only fixed in Sheepheaven Creek; other populations that showed little to no signs of introgression in STRUCTURE analyses showed elevated frequencies of the "rainbow trout" variant, suggesting that rather than being a true diagnostic marker of redband trout common to the area, it is instead a product of genetic drift, inbreeding, or both in Sheepheaven Creek. Despite the problems of these loci, their inclusion in STRUCTURE analyses did not appear to significantly change introgression estimates (analyses with excluded loci not shown).

Introgression Analysis

The nature of this data set led to some complications in the analysis. Most studies dealing with hybridization can identify reference populations of (1) the native species free from hybridization, and (2) the type of individuals that were used to stock the system. Poor records of early rainbow trout plantings render it impossible to be certain of what kind of rainbow trout is the best representative of fish introduced to the upper McCloud. Mount Shasta strain has been used and propagated in the region and was originally derived from lower McCloud River rainbow trout (Busack and Gall 1980). Given its geographic proximity to upper McCloud River redband trout, it is likely that most planting events used Mount Shasta strain rainbow trout, although Pit strain rainbow trout were also probably planted (M. Dean, CDFG, personal communication). As the representative of redband trout prior to stocking, Sheepheaven Creek individuals appear to be a reasonable choice since the location is an isolated stream very rarely connected to the upper McCloud, with no known history of prior stocking. Additionally, its location on timber harvesting private property makes it less likely to be stocked, legally or illegally. However, the very factors that make hybridization in Sheepheaven Creek unlikely make it difficult to assume it contains all or most of the genetic diversity found in upper McCloud redband trout prior to human interference. The potential for inbreeding, genetic bottlenecks, or both in a creek this small and isolated are high, which would lead to an overestimate of hybridization in other populations as markers that appeared diagnostic for ancestral rainbow trout were in fact artifacts of drift, inbreeding, or both in Sheepheaven Creek. Some of these analysis concerns are addressed by the use of STRUCTURE to quantify the introgressive hybridization present in the system, as described below.

Although our uncertainty in reference populations slightly complicates using STRUCTURE as a tool to assess introgression under these circumstances, we are relatively confident that this method is the most appropriate for our question. STRUCTURE allows us to not declare set reference populations, while their inclusion in the overall analysis is still informative. STRUCTURE has been used in the literature to detect introgression (Pritchard et al. 2007), even in closely related species or hatchery populations (Susnik et al. 2004; Barilani et al. 2005; Jensen et al. 2005; Sanz et al. 2005). Other studies have shown that STRUCTURE reliably detects and quantifies hybridization (Choisy et

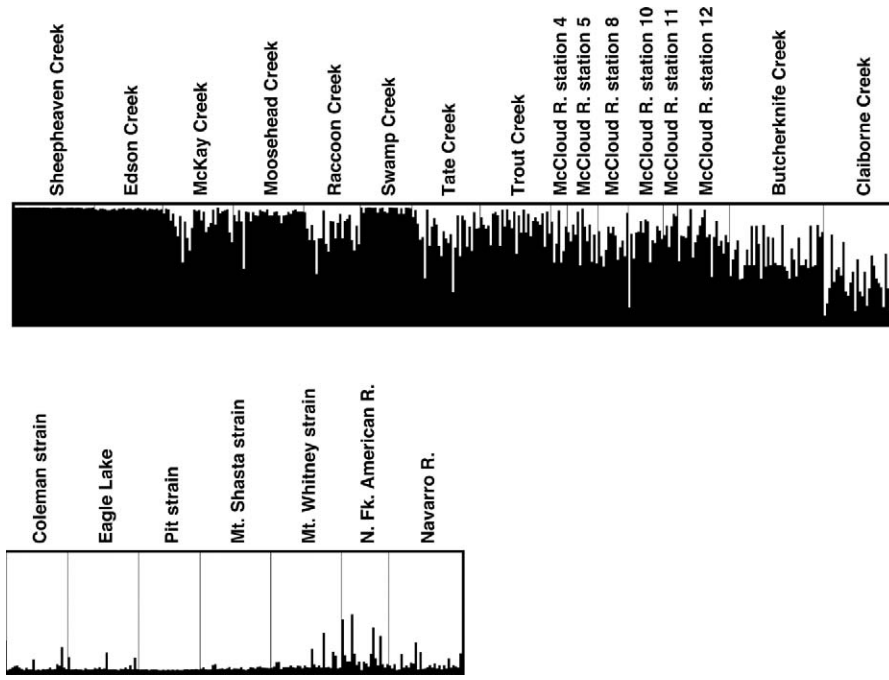

FIGURE 2.—Structure analysis of redband and rainbow trout based on nine nuclear SNP loci. Each vertical bar represents it's the probability of a given individual's being assigned to one of two genetic clusters. Redband trout samples appear in the upper portion of the figure, rainbow trout samples in the lower portion.

al. 2004; Sanz et al. 2009). Using an average *q*-value to characterize a population has been used in previous studies (e.g., Randi and Lucchini 2002; Cegelski et al. 2003; Sacks et al. 2005) and seemed appropriate for this study. Although the SD of individual *q*-values is greater in populations with more intermediate ancestry (Table 3), it is expected in a distribution for values ranging from 0 to 1 that values closest to the extremes would have lower SDs than values nearer to 0.5.

Although introgression estimates between the mitochondrial SNP frequency and STRUCTURE estimates derived from nuclear loci are not directly comparable, as the former is a raw frequency based on one locus and the latter is a derived estimate based on multiple loci, in many cases they provide congruent results. For example, populations such as Edson, Sheepheaven, Swamp, and Moosehead creeks showed low to no introgression with the STRUCTURE analysis and were fixed for the Sheepheaven variant of the mitochondrial SNP. However, where the mitochondrial SNP frequency and STRUCTURE estimates differ is also noteworthy. More than one population fixed for the Sheepheaven mitochondrial SNP variant showed signs of introgression based on nuclear analyses (e.g., Raccoon, Tate, and Trout creeks). As Wirtz (1999) points out, this difference in mitochondrial and nuclear allelic frequencies has many plausible explanations. Though there are a few cases of sex bias in the direction of hybridization in salmonids (e.g., Porath and Nielsen 2003), given the apparent size of these redband trout populations we believe that genetic drift is the most likely explanation. If there were one or few hybridization events, rainbow trout mitochondrial DNA may have failed to enter the gene pool or genetic drift (which is more severe in mitochondrial DNA) could rapidly eliminate a rare allele (Hartl and Clark 1997). One would expect under this scenario that if enough populations were sampled that had undergone similar introgression events, eventually a population fixed for the rainbow trout variant would be discovered. Indeed, Butcherknife and Claiborne creeks show levels of nuclear introgression higher than any of the tributaries to the upper McCloud River but are fixed for the rainbow trout variant of the mitochondrial SNP. However, they also have high-to-fixed frequencies of the rainbow trout variant of *GH2C 501*.

The hydrology of the upper McCloud River watershed appears to play a major role in the introgression levels observed. There is a general pattern of streams north of the McCloud River showing fewer signs of introgression than streams to the south. Most of the streams north of the upper McCloud River have

a greater degree of isolation from the river, major roads, and campgrounds. In the case of Edson and Trout creeks, portions of the channels that would join the stream system are often dry. In the case of McKay Creek, except in flood conditions (no more than once every 2 years) the flow becomes subterranean before ever reaching the river. Similar but even drier conditions apply to Swamp Creek. Both dry channels and subterranean flow contribute to the isolation of Sheepheaven Creek, which only connects to the upper McCloud River via McKay Creek. These streams contrast with Tate and Raccoon creeks, which have no barriers to the upper McCloud and a higher degree of public access (e.g., a nearby campground accessible by paved roads). In fact, Tate Creek is a site of a former trout hatchery constructed in 1934 that was abandoned in the 1940s (StreamWise 2001). Given this lack of migration barriers, it is unsurprising that average q-values of Raccoon and Tate creeks do not differ significantly from those at upper McCloud River sites (one-sample t-test: $P = 0.073$ and $P = 0.406$, respectively). Moosehead Creek appears to share a similar degree of connectivity found in Tate and Raccoon creeks, but only to the remotest headwaters of the McCloud River. Though Trout Creek appears to be an exception to the general north–south pattern, it is noteworthy that both Trout and Swamp creeks were transplanted with individuals from Sheepheaven Creek as a safeguard against extirpation of Sheepheaven Creek redband trout. However, at least in the case of Trout Creek, the previous year's chemical treatment (R. Benthin, CDFG, personal communication) did not appear to eradicate all rainbow trout fish that were presumably in the creek prior to stocking. In all upper McCloud locations, Sheepheaven-specific alleles were present in moderate-to-high frequencies at most loci, indicating potential historical gene flow. This also suggests the genetic distinctiveness of Sheepheaven trout relative to rainbow trout may have been shared with other redband trout populations in the upper McCloud River in the past.

Conservation Implications

Given the importance of introgression status for a species' or subspecies' likelihood of persistence and eligibility for legal protection, an accurate measure of proportional ancestry is an essential tool for management purposes. A conservative measure of introgression may not identify even slightly hybridized populations as "pure," excluding populations with little or nonsignificant introgression. This could needlessly ignore or, worse, destroy populations that could be crucial to a species' recovery when there may be few populations left. Although there are no studies

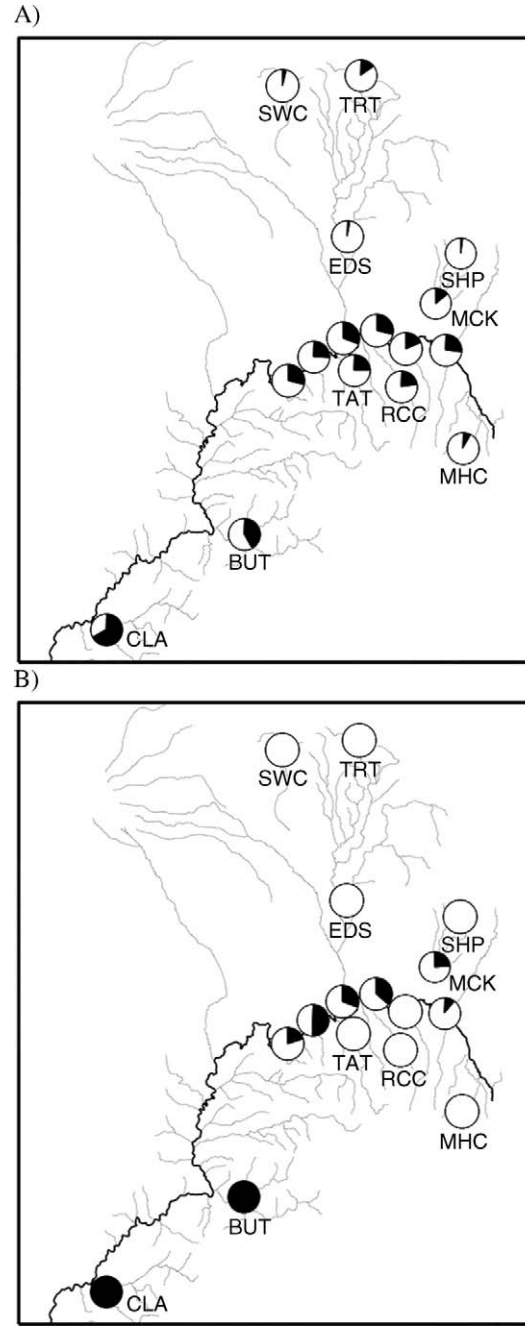

FIGURE 3.—Estimates of introgression among redband trout populations based on **(A)** average q-values from STRUCTURE and **(B)** the frequency of a mitochondrial SNP. The black portion of each circle indicates q-values or allele frequencies characteristic of rainbow trout, the white portion those of redband trout.

to date testing the fitness effect of introgressive hybridization on redband trout, there is some evidence that hatchery and hybrid fish do poorly in this extreme environment (Moyle et al. 1995 and references therein). Some species of salmonids form stable hybrid zones (e.g., Campton and Utter 1985) or hybridization results in little measurable impact on fitness (Rubidge and Taylor 2004; Seiler and Keeley 2007), but other hybrid pairings potentially result in reduced long-term fitness (Young et al. 2001; Ostberg et al. 2004; Cordes et al. 2006; Muhlfeld et al. 2009). Even if there is little effect on fitness of redband trout populations due to hybridization, the uncertain protections offered to introgressed populations make this an important issue to address.

The introgression metrics presented suggest that redband trout in the upper McCloud River watershed may already be imperiled by introgressive hybridization with hatchery rainbow trout strains. The presence of alleles characteristic of Sheepheaven Creek in every upper McCloud redband population sampled suggests that rather than Sheepheaven Creek existing as a sole representative of an ancestral redband lineage, it and its transplant populations (such as Swamp Creek) may instead represent a large portion of nonintrogressed individuals. Using the criteria of Barilani et al. (2005), we believe that Sheepheaven, Edson, Moosehead, and Swamp creeks are not significantly introgressed with rainbow trout. This has important implications for the conservation of upper McCloud redband trout since areas free from the influence of introgressive hybridization are drastically reduced at present. While it is impossible to tell exactly how closely related other redband populations are to Sheepheaven Creek due to the confounding effects of introgression, a phylogenetic analysis excluding individuals likely to be of mixed descent may finally answer questions regarding the taxonomy and systematics of this group.

These introgression results can be used to prioritize future conservation projects. Although certain sampled locations (such as Sheepheaven, Swamp, Moosehead, and Edson creeks) do not appear introgressed with introduced rainbow trout, other locations with fewer barriers to migration or human access (such as McKay, Raccoon, Swamp, and Tate creeks) have undergone introgressive hybridization. Chemical treatment was used in Trout Creek before transplantation of fish from Sheepheaven Creek, with mixed results. For Sheepheaven Creek, a refugial pool was constructed for drier months and years, and the hatchery site on Tate Creek was restored for habitat complexity and better flow conditions, but few barriers to fish migration have been implemented. We suggest that further restoration efforts or barrier construction be prioritized to those streams that have been identified to contain nonintrogressed redband trout. For example, a barrier may be necessary to prevent migration into Moosehead Creek from the McCloud River. Although a few individuals in the upper McCloud show little-to-no signs of introgression with rainbow trout, the connectivity and proximity to so many individuals that appear introgressed provides little justification for prioritizing directed conservation efforts for such a large area at this time.

At the moment, we believe it is premature to make specific conservation decisions regarding whether the introgressed sampled locations should be included in conservation plans, although any further stocking of nonnative trout should be discontinued. Knowledge of the introgression status of the samples described above allows for more objective decisions regarding conservation prioritization. However, as not all habitats that may contain redband trout were included, this work should be extended to determine the extent to which the signal of alleles characteristic of Sheepheaven Creek are found in additional streams and locations containing putative redband trout. An unbiased estimate of the genetic diversity of all of these populations using markers such as microsatellites has yet to be completed. If entire populations displaying some degree of introgression are to be excluded from future conservation plans, it is vital to first assess traits such as population genetic structure, variability, inbreeding, effective population size, and bottlenecks in populations that show little to no introgression. Preliminary microsatellite results suggest that Edson and Sheepheaven creeks have low allelic richness, and Sheepheaven Creek appears to have significant inbreeding (Simmons, unpublished data). These preliminary results emphasize the importance of assessing genetic diversity in other potentially "pure" populations before prioritizing conservation actions for McCloud River redband trout, both in terms of designating critical populations or habitat and discovering populations of potential utility in restoration efforts, thus ensuring that this unique group is likely to persist in the long-term.

Though there are instances of natural hybridization in salmonids (e.g., cutthroat trout *O. clarkii* and steelhead; Campton and Utter 1985; Ostberg et al. 2004), the introgression of nonnative rainbow trout into inland trout populations has become a very common issue in the management of Western native trout. In some cases, such as Deschutes River redband trout *O. m. gairdneri* in Oregon, it appears that little introgression has occurred with hatchery rainbow trout despite the ease with which hybridization could occur (Matala et al. 2008). However, in far more cases regarding salmonid conservation (such as Yellowstone cutthroat

trout *O. c. bouvieri* [Gunnell et al. 2008], Apache trout *O. apache* [Dowling and Childs 1992], and California golden trout [Cordes et al. 2006]), introgression is extensive and has required intervention. Although subspecific hybridization is sometimes used to alleviate low genetic variation (e.g., the Florida panther *Puma concolor coryi*; Hedrick 1995), except in extreme circumstances the risks associated with hybridization are generally too great to consider it to be beneficial to a threatened or endangered species. Further testing will be required to determine if such extreme measures should ever be considered for redband trout in the upper McCloud River watershed. In some taxa such as wolves, hybridization appears to be a rare event due to numerous ecological barriers (Randi et al. 2001). In other taxa, hybridization, especially by the introduction of nonnative species, is an ongoing problem. The latter scenario seems to be a more common occurrence in salmonids (e.g., Campton and Utter 1985; Dowling and Childs 1992; Rubidge and Taylor 2004; Susnik et al. 2004). In some cases, nonnative species may be successful in the short-term, allowing them to replace native species thru hybridization, but they may not be as successful in the long-term as they lack local adaptations (Allendorf 2003).

Just as the effects of hybridization vary greatly, so do current and proposed management actions. In some cases, chemical treatment has been used to eliminate whole populations containing known hybrids (Gall and May 1997), and barriers have been constructed to prevent the spread of introgressed individuals into hybrid-free habitat. Due to the controversy surrounding chemical treatments and their potential environmental impacts (e.g., W. Somer, 2007, presentation at the American Fisheries Society Annual Meeting, San Francisco), some conservation actions have resorted to the physical removal of suspected hybrids despite the disadvantages of variable success rate and cost inefficiency (Elliott and Layton 2004). Other conservation plans dealing with introgressive hybridization from nonnative species rely on restoring refugial habitat and erecting migration barriers to prevent further introgression. While some authorities have suggested excluding all hybridized populations from management plans (Allendorf et al. 2004), others, including the U.S. Fish and Wildlife Service, recommend making decisions on a case-by-case basis and assert that some situations justify retaining populations with low levels of hybridization (O'Brien and Mayr 1991; Dowling and Childs 1992; Campton and Kaeding 2005; Gunnell et al. 2008). The philosophy underlying the latter approach is that it is preferable to retain low levels of hybridization in some populations rather than jeopardize the persistence of the species as a whole. Regardless of which management philosophy is implemented for redband trout in the upper McCloud River watershed, further study is needed to elucidate population genetic structure, bottleneck history, and inbreeding before any populations are excluded from further conservation plans.

Acknowledgments

This work was funded by grants from the California Department of Fish and Game (CDFG; P0480052), the Cantara Foundation (C0310004-O_2), CalTrout (06001544), and by a gift from Hancock Forest Products. We would also like to acknowledge sampling efforts by M. Dean, S. Baumgartner, and B. Jong at CDFG, and J. Phillips and associates at the CDFG Salmonid Tissue Archive. We appreciate the advice and expertise of M. Dean, R. Benthin, and G. Yoshioka (CDFG). M. Stephens, J. Petersen, J. Hull, and M. Finger at University of California–Davis; J. Cordes at the Virginia Institute of Marine Science; and two anonymous reviewers provided invaluable advice and comments during early drafts of this manuscript.

References

Allendorf, F. W. 2003. Introduction: population biology, evolution, and control of invasive species. Conservation Biology 17:24–30.

Allendorf, F. W., R. F. Leary, N. P. Hitt, K. L. Knudsen, L. L. Lundquist, and P. Spruell. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conservation Biology 18:1203–1213.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16(11):613–622.

Aparicio, E., E. García-Berthou, R. M. Araguas, P. Martínez, and J. L. García-Marín. 2005. Body pigmentation pattern to assess introgression by hatchery stocks in native *Salmo trutta* from Mediterranean streams. Journal of Fish Biology 67:931–949.

Bacon, M., P. Brouha, M. Rode, and C. Staley. 1980. Redband trout (*Salmo* sp.): USFS Shasta-Trinity National Forest, California, comprehensive habitat management plan. U.S. Forest Service, Redding, California.

Bagley, M. J., and G. A. E. Gall. 1998. Mitochondrial and nuclear DNA sequence variability among populations of rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology 7(8):945–961.

Barilani, M., S. Deregnaucourte, S. Gallego, L. Galli, N. Mucci, R. Piomboc, M. Puigcerverd, S. Rimondi, J. D. Rodríguez-Teijeiro, S. Spanòc, and E. Randi. 2005. Detecting hybridization in wild (*Coturnix c. coturnix*) and domesticated (*Coturnix c. japonica*) quail populations. Biological Conservation 126:445–455.

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society, Monograph 6, Bethesda, Maryland.

Behnke, R. J. 2002. Trout and salmon of North America. Free Press, New York.

Berg, W. J. 1987. Evolutionary genetics of rainbow trout. Doctoral dissertation. University of California, Davis.

Busack, C. A., and G. A. E. Gall. 1980. Ancestry of artificially propagated California rainbow trout strains. California Fish and Game 66(1):17–24.

CDFG (California Department of Fish and Game). 2008. California Department of Fish and Game catchable trout planting schedule for region 1–northern. Available: www.dfg.ca.gov/. (October 2008).

Campton, D. E., and L. R. Kaeding. 2005. Westslope cutthroat trout, hybridization, and the U.S. Endangered Species Act. Conservation Biology 19:1323–1325.

Campton, D. E., and F. M. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. Canadian Journal of Fisheries and Aquatic Sciences 42:110–119.

Cegelski, C. C., L. P. Waits, and N. J. Anderson. 2003. Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. Molecular Ecology 12(11):2907–2918.

Choisy, M., P. Franck, and J. M. Cornuet. 2004. Estimating admixture proportions with microsatellites: comparison of methods based on simulated data. Molecular Ecology 13(4):955–968.

Cordes, J. F., M. Stephens, M. A. Blumberg, and B. May. 2006. Identifying introgressive hybridization in native populations of California golden trout based on molecular markers. Transactions of the American Fisheries Society 135:110–128.

Dowling, T. E., and M. R. Childs. 1992. Impact of hybridization on a threatened trout of the southwestern United States. Conservation Biology 6:355–364.

Elliott, J., and R. W. Layton. 2004. Lahontan cutthroat trout species management plan for the upper Humboldt River drainage basin. Nevada Department of Wildlife, Reno.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620.

Gall, G. A. E., and B. May. 1997. Trout of the Kern River basin: a genetic analysis of Little Kern River and Golden Trout Creek populations. Report to the California Department of Fish and Game, Threatened Trout Committee, Sacramento.

Gold, J. R. 1977. Systematics of western North American trout (*Salmo*), with notes on the redband trout of Sheepheaven Creek, California. Canadian Journal of Zoology 55(11):1858–1873.

Gunnell, K., M. K. Tada, F. A. Hawthorne, E. R. Keeley, and M. B. Ptacek. 2008. Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and introduced rainbow trout (*O. mykiss*) in the South Fork of the Snake River watershed, Idaho. Conservation Genetics 9:49–64.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics, 3rd edition. Sinauer Associates, Sunderland, Massachusetts.

Hedrick, P. W. 1995. Gene flow and genetic restoration: the Florida panther as a case study. Conservation Biology 9:996–1007.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806.

Jensen, A. B., K. A. Palmer, J. J. Boomsma, and B. V. Pedersen. 2005. Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. Molecular Ecology 14:93–106.

Matala, A. P., S. Marx, and T. G. Wise. 2008. A genetically distinct wild redband trout (>*Oncorhynchus mykiss gairdneri*) population in Crane Prairie Reservoir, Oregon, persists despite extensive stocking of hatchery rainbow trout (*O. m. irideus*). Conservation Genetics 9:1643–1652.

Morin, P. A., G. Luikart, and R. K. Wayne. and the SNP workshop group. 2004. SNPs in ecology, evolution, and conservation. Trends in Ecology and Evolution 19(4):208–216.

Moyle, P. B. 1996. Status of aquatic habitat types, volume I. Assessments and scientific basis for management options. Sierra Nevada Ecosystem Project, Final report to Congress, Center for Water and Wildlife Resources, University of California, Davis.

Moyle, P. B., R. M. Yoshiyama, J. E. Williams, and E. D. Wikramanayake. 1995. Fish species of special concern in California, 2nd edition. Prepared for the California Department of Fish and Game, Rancho Cordova.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters 5(3):328–331.

Nielsen, J. L., K. D. Crow, and M. C. Fountain. 1999. Microsatellite diversity and conservation of a relic trout population: McCloud River redband trout. Molecular Ecology 8(Supplement 1):S129–S142.

O'Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science 251:1187–1190.

Ostberg, C. O., S. L. Slatton, and R. J. Rodriguez. 2004. Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*), and interspecific hybrids. Molecular Ecology 13:2773–2788.

Porath, M. T., and J. L. Nielsen. 2003. Evidence of sexually dimorphic introgression in Pinaleno Mountain Apache trout. North American Journal of Fisheries Management 23:172–180.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(3):945–959.

Pritchard, V. L., K. Jones, and D. E. Cowley. 2007. Estimation of introgression in cutthroat trout populations using microsatellites. Conservation Genetics 8:1311–1329.

Randi, E., and V. Lucchini. 2002. Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conservation Genetics 3:31–45.

Randi, E., V. Lucchini, M. F. Christensen, N. Mucci, S. M.

Funk, G. Dolf, and V. Loeschcke. 2001. Mitochondrial DNA variability in Italian and East European wolves: detecting the consequences of small population size and hybridization. Conservation Biology 14(2):464–473.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86(3):248–249.

Rosenberg, N. A. 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Rosenberg, N. A., M. L. Lei, R. Ward, and J. K. Pritchard. 2003. Informativeness of genetic markers for inference of ancestry. American Journal of Human Genetics 73:1402–1422.

Rubidge, E. M., and E. B. Taylor. 2004. Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). Molecular Ecology 13:3735–3749.

Sacks, B. N., B. R. Mitchell, C. L. Williams, and H. B. Ernest. 2005. Coyote movements and social structure along a cryptic population genetic subdivision. Molecular Ecology 14:1241–1249.

Sanz, N., R. M. Araguas, R. Fernández, M. Vera, and J. García-Marín. 2009. Efficiency of markers and methods for detecting hybrids and introgression in stocked populations. Conservation Genetics 10:225–236.

Sanz, N., M. Cortey, C. Pia, and J. L. García-Marín. 2005. Hatchery introgression blurs ancient hybridization between brown trout (*Salmo trutta*) lineages as indicated by complementary allozymes and mtDNA markers. Biological Conservation 130(2):278–289.

Seiler, S. M., and E. R. Keeley. 2007. Morphological and swimming stamina differences between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), rainbow trout (*Oncorhynchus mykiss*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 64:127–135.

Shriver, M. D., M. W. Smith, L. Jin, A. Marcini, J. M. Akey, R. Deka, and R. E. Ferrell. 1997. Ethnic affiliation estimation by use of population-specific DNA markers. American Journal of Human Genetics 60(4):957–64.

Smith, M. W., and coauthors. 2001. Markers for mapping by admixture linkage disequilibrium in African American and Hispanic populations. American Journal of Human Genetics 69(5):1080–1094.

Sprowles, A. E., M. R. Stephens, N. W. Clipperton, and B. P. May. 2006. Fishing for SNPs: a targeted-locus approach for single nucleotide polymorphism discovery in rainbow trout. Transactions of the American Fisheries Society 135:1698–1721.

Stephens, M. R. 2007. Systematics, genetics, and conservation of golden trout. University of California, Davis.

Stephens, M. R., N. W. Clipperton, and B. May. 2009. Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. Molecular Ecology Resources 9:339–343.

StreamWise. 2001. Tate Creek–McCloud district stream assessment. Report prepared for the U.S. Forest Service, Vallejo, California.

Susnik, S., P. Berrebi, P. Dovc, M. M. Hansen, and A. Snoj. 2004. Genetic introgression between wild and stocked salmonids and the prospects for using molecular markers in population rehabilitation: the case of the Adriatic grayling (*Thymallus thymallus* L. 1785). Heredity 93:273–282.

USFWS (U.S. Fish and Wildlife Service). 1994. Endangered and threatened wildlife and plants: animal candidate review for listing as endangered or threatened species. Federal Register 219:(15 November 1994):58982.

USFWS (U.S. Fish and Wildlife Service). 1996. Proposed policy on the treatment of intercrosses and intercross progeny (the issue of "hybridization"): proposed rule. Federal Register 61:(7 February 1996):4710–4713.

USFWS (U.S. Fish and Wildlife Service). 2000. Endangered and threatened wildlife and plants: notice of reclassification of nine candidate taxa. Federal Register 65(204):(20 March 2000):63044–63045.

Wales, J. H. 1939. General report on investigation of the McCloud River drainage in 1938. California Fish and Game 25:272–309.

Wang, J. 2003. Maximum likelihood estimation of admixture proportions from genetic data. Genetics 164:747–765.

Wirtz, P. 1999. Mother species–father species: unidirectional hybridization in animals with female choice. Animal Behaviour 58:1–12.

Wolf, D. E., N. Takebayashi, and L. H. Rieseberg. 2001. Predicting the risk of extinction through hybridization. Conservation Biology 15(4):1039–1053.

Yang, N., H. Li, L. Criswell, M. Gregersen, R. Alarcon-Riquelme, R. Kittles, R. Shigeta, G. Silva, P. Patel, J. Belmont, and M. Seldin. 2005. Examination of ancestry and ethnic affiliation using highly informative diallelic DNA markers: application to diverse and admixed populations and implications for clinical epidemiology and forensic medicine. Human Genetics 118(3–4): 382–392.

Young, W. P., C. O. Ostberg, P. Keim, and G. H. Thorgaard. 2001. Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). Molecular Ecology 10(4):921–930.