Transactions of the American Fisheries Society 136:606–623, 2007

DOI: 10.1577/T06-038.1

[Article]

Genetic Diversity within Fragmented Cutthroat Trout Populations

V. L. PRITCHARD*[1]

Department of Fishery and Wildlife Sciences, New Mexico State University, Las Cruces, New Mexico 88003, USA

K. JONES

Genetic Identification Services, 9552 Topanga Canyon Boulevard, Chatsworth, California 91311, USA

D. E. COWLEY

Department of Fishery and Wildlife Sciences, New Mexico State University, MSC 4901, Post Office Box 30003, Las Cruces, New Mexico 88003, USA

Abstract.—Interior cutthroat trout *Oncorhynchus clarkii* have undergone severe declines over the past 150 years. Many subspecies now persist in a highly fragmented state, primarily within headwater streams. We used 12 microsatellites to investigate the population genetic characteristics of 22 remnant populations of Rio Grande cutthroat trout *O. c. virginalis* isolated in montane streams in New Mexico. Populations varied markedly in the amount of genetic diversity they contained. There was no significant relationship between estimated adult population size or habitat size and heterozygosity; however, populations occurring above natural barriers were significantly less diverse. Seven population samples exhibited significant deviations from Hardy–Weinberg equilibrium. Interlocus variance in the population inbreeding coefficient F_{IS} was correlated with habitat size, and several population samples exhibited a significantly higher variance in interindividual relatedness, or a significantly higher median individual inbreeding coefficient, than would be expected by chance. These results suggest that cutthroat trout populations in headwater streams consist of multiple partially discrete subpopulations in which only a small number of adults successfully reproduce. The potential for such population substructure should be considered when planning management activities for stream-dwelling cutthroat trout.

Like many freshwater fishes, the cutthroat trout *Oncorhynchus clarkii* of interior North America has suffered major declines as a result of human activity over the past 150 years. Of 11 recognized subspecies, 2 are extinct, 3 are listed as threatened under the U.S. Endangered Species Act, and the remainder are considered to be of conservation concern (Behnke 2002). Population declines have been caused by a number of factors. Habitat degradation and overfishing, particularly in the late 1800s and early 1900s, are believed to have severely affected cutthroat trout in many areas. The major threat today, however, comes from nonnative trout, which have been stocked in vast numbers throughout North America over the past century and now occur in self-sustaining or artificially sustained populations throughout most of the cutthroat trout's native range. All interior subspecies of cutthroat trout will hybridize freely with introduced rainbow trout *O. mykiss* and other nonnative cutthroat trout subspecies to produce fertile offspring (Hitt et al. 2003; Weigel et al. 2003). On-going gene flow from a large nonnative population can cause cutthroat trout to be replaced by a hybrid swarm and eventually with fish indistinguishable from the nonnative taxon. Additionally, native cutthroat trout populations are frequently observed to decline to extinction when their habitat is invaded by introduced brook trout *Salvelinus fontinalis* or brown trout *Salmo trutta* (Harig et al. 2000; Dunham et al. 2002), apparently due to competitive exclusion or predation acting at the early life stages (Peterson et al. 2004). As a result of the combined effects of nonnative trout, habitat destruction and overfishing, the majority of interior cutthroat trout subspecies have now become restricted to areas where anthropogenic impacts have been minimal. Frequently they exhibit highly fragmented spatial distributions occurring as geographically isolated populations in relatively small areas of habitat, most commonly in headwater streams. Such small fragmented populations have an elevated extinction risk as a result of demographic, environmental, and genetic stochasticity (Wilcox and Murphy 1985; Morita and Yamamoto 2001). When local extinction events occur natural re-colonization of the

* Corresponding author: vpritcha@nmsu.edu
[1] Present address: Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, California 90089-0371, USA.

Received February 15, 2006; accepted October 23, 2006
Published online April 19, 2007

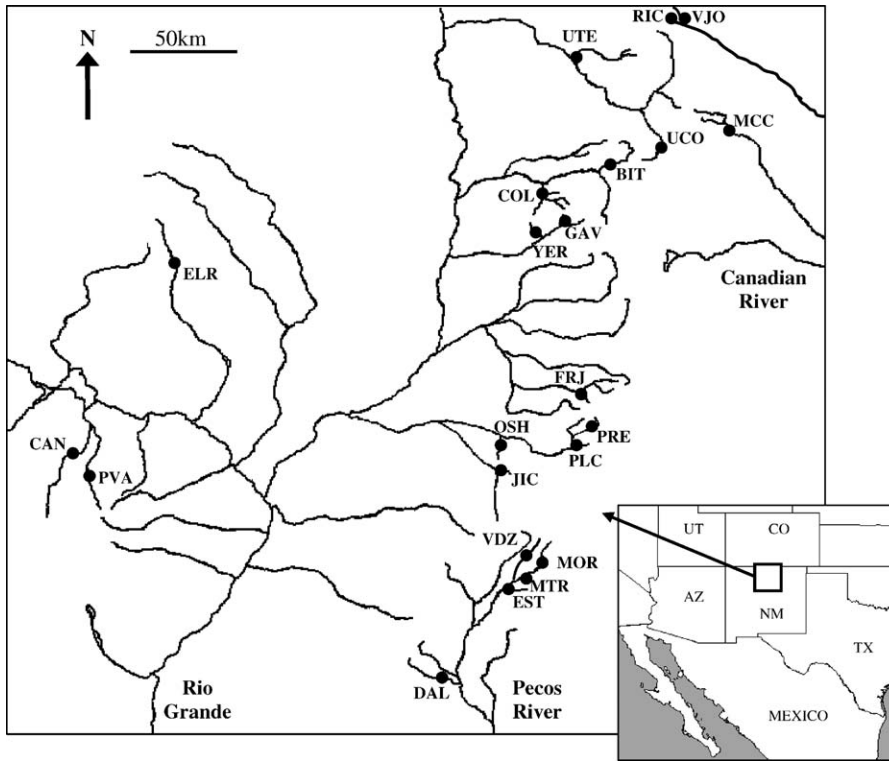

FIGURE 1.—Sampling locations for Rio Grande cutthroat trout in New Mexico. Dots indicate the positions of barriers delineating the downstream extent of the known pure population. Location codes are given in Table 1.

habitat by cutthroat trout is often precluded by the presence of migration barriers or the absence of a nearby source of migrants.

In response to concerns about the status of cutthroat trout, management agencies have developed aggressive conservation programs. Management activities include the identification and monitoring of extant populations, quantification of levels of nonnative genetic material within these populations, construction of artificial fish movement barriers to prevent invasion by nonnative trout, habitat restoration, reintroduction of native cutthroat trout into suitable locations, and development of pure-strain hatchery stocks for reintroduction purposes. Agencies have multiple theoretical and practical tools at their disposal to aid the achievement of conservation goals. For example, several models are available that predict the area of habitat required to support a cutthroat trout population with a sufficiently small probability of going extinct within a specified time frame or a sufficient long-term effective population size (N_e) to maintain the current level of genetic diversity in the population (Hilderbrand and Kershner 2000; Hilderbrand 2003; Pritchard and Cowley 2006). Despite this attention, relatively little is known about the ecology, demography and population genetics of many subspecies of interior cutthroat trout. As a result, many management decisions are based upon assumptions or extrapolation from data collected from other taxa. Clearly, further studies can improve the efficacy of management activities.

We focused on the Rio Grande cutthroat trout *O. c. virginalis* of Colorado and New Mexico. This subspecies has the southernmost distribution of all cutthroat trout and is believed to be native to at least three different river drainages: the Rio Grande, the Pecos River and the Canadian River (Behnke 2002; Figure 1). It is estimated that Rio Grande cutthroat trout currently occupies less than 12% of its former historic range (Pritchard and Cowley 2006). Most extant populations are located in remote, first or second order headwater streams, which often suffer extreme and fluctuating environmental conditions and may represent marginal trout habitat. Mean occupied stream length throughout the current range is 7.9 km (range = 1–28 km; Pritchard and Cowley 2006). Many populations have persisted because they are protected from the incursions of nonnative trout by natural barriers, and artificial barriers have been constructed to protect other populations.

TABLE 1.—Sampling locations for Rio Grande cutthroat trout populations included in the study; sample size (n), stream length, adult population size estimated from one or more multipass electrofishing surveys, and presence or absence of a natural migration barrier are shown. Blank cells indicate that data are unavailable.

Population	Code	N	Drainage	Latitude (°N)	Longitude (°W)	Stream length (km)	Estimated adult population	Mean gradient (%)	Natural barrier
Bitter Creek	BIT	30	Rio Grande	36.74	105.34	6.4	690	4	No
Cañones Creek	CAN	30	Rio Grande	36.13	106.47	17.6	2,950	5	Waterfall
Columbine Creek	COL	30	Rio Grande	36.66	105.52	20.0	1,800	15	Waterfall
Dalton Creek	DAL	30	Pecos	35.68	105.76	8.8		6	Waterfall
El Rito Creek	ELR	40	Rio Grande	36.54	106.27	12.8	5,440	3	No
Frijoles Creek	FRJ	27	Rio Grande	36.27	105.41	4.8		7	No
Gavilan Canyon	GAV	20	Rio Grande	36.58	105.48	2.9	280	20	No
Jicarito Creek	JIC	21	Rio Grande	36.08	105.61	4.0		15	No
Little Vermejo Creek	VJO	30	Canadian	36.97	105.13	12.0	280	8	No
McCrystal Creek	MCC	30	Canadian	36.79	105.13	11.8	280	7	No
Osha Creek	OSH	24	Rio Grande	36.16	105.61	8.7		6	Waterfall
Policarpio Creek	PLC	30	Rio Grande	36.14	105.46	5.3	400	5	No
Polvadera Creek	PVA	30	Rio Grande	36.06	106.44	7.7	646	9	Waterfall
Ricardo Creek	RIC	20	Canadian	36.97	105.13	14.4	260	7	No
Rio Mora	MOR	30	Pecos	35.92	105.51	4.0		7	Waterfall
Rio Mora tributary	MTR	30	Pecos	35.90	105.53	2.9		6	Waterfall
Rio Valdez	VDZ	30	Pecos	35.96	105.52	4.8	50	8	Waterfall
Rito de la Presa	PRE	20	Rio Grande	36.19	105.40	1.6		6	No
Rito los Esteros	EST	31	Pecos	35.59	105.59	4.0		12	Waterfall
Upper Comanche Creek	UCO	30	Rio Grande	36.78	105.59	18.3	1,200	3	No
Ute Creek	UTE	60	Rio Grande	36.94	105.46	15.0		8	No
Yerba Creek	YER	20	Rio Grande	36.57	105.52	4.8	120	17	No

Migration of Rio Grande cutthroat trout between most populations is precluded by the presence of these barriers and by intervening populations of nonnative trout. Although there are successful on-going programs to reintroduce the subspecies to suitable habitat remnant populations continue to be lost, primarily as a result of drought and the invasion of nonnative trout.

We used 12 microsatellites to investigate the population genetic characteristics of 22 extant populations of Rio Grande cutthroat trout. First, we investigated the relationship of genetic diversity and population inbreeding coefficient (F_{IS}) to habitat characteristics. We hypothesized that cutthroat trout populations isolated above a natural barrier such as a waterfall would exhibit lower genetic diversity than those that were historically able to receive a larger number of founders or more frequent migrants. Genetic variation is expected to be lost from a population more rapidly where effective population size is small, hence we also hypothesized that cutthroat trout populations isolated in small stream remnants, and thus containing fewer individuals, would be genetically less diverse. Such populations are also expected to exhibit a higher multilocus F_{IS} due to an increased likelihood of consanguineous matings. For the same reason, higher multilocus F_{IS} might also be observed in streams with a high gradient, since movement of fish within these streams may be limited by multiple cascades separating areas of suitable habitat. Castric et al. (2002) showed that interlocus variation in F_{IS} is negatively related to the number of progenitors able to spawn successfully in each generation. As the number of successful spawners is expected to be limited by the availability of spawning sites, which itself should be limited by habitat size, our final hypothesis was that interlocus variance in F_{IS} would decrease as stream length increased.

Subsequently, after observing significant deviations from Hardy–Weinberg equilibrium in some populations, we investigated intrapopulation structuring in more detail. We tested the hypothesis that observed heterozygote deficiencies were due to consanguineous matings by examining the distribution of individual inbreeding coefficients within each population sample. We also examined the level of relatedness between individuals and used a maximum-likelihood approach to partition individuals into family groups. Finally, many remnant Rio Grande cutthroat trout populations are expected to have suffered rapid reductions in effective population size as a result of recent population fragmentation. Therefore, we examined our data set for genetic evidence of population bottlenecks using three different approaches.

Methods

Tissue collection.—Tissue samples ($n = 643$) were obtained from 22 populations of Rio Grande cutthroat trout comprising 14 populations from the Rio Grande drainage, 5 populations from the Pecos River drainage and 3 populations from the Canadian River drainage (Table 1; Figure 1; sample abbreviations mentioned in

the text refer to streams listed in Table 1). All populations had previously been assessed as having little or no introgression from rainbow trout or the typical or finespotted forms of Yellowstone cutthroat trout *O. clarkii bouvierii*, based on allozyme and microsatellite data (Keeler-Foster 2003; Pritchard et al. 2007a; New Mexico Department of Game and Fish [NMDGF] unpublished data). Tissue samples were in the form of fin clips, collected between 1999 and 2003 from fish captured by means of electrofishing, and preserved by freezing before analysis. Sample size ranged from 20 to 60 individuals per population (Table 1). Samples from each stream were collected in a single sampling period. Exact sampling pattern varied according to fish density and stream characteristics. However, at all sites, fish of multiple age-classes were systematically sampled over a stream reach of several hundred meters or more to minimize the effects of habitat structuring or family grouping (Hansen et al. 1997). For two populations, ELR and UTE, samples were collected from four different stream sections.

Microsatellite analysis.—We used 12 tetranucleotide microsatellite loci in this study. Six of these (*J3, J14, J103, J132, K216, K222*) were isolated from Rio Grande cutthroat trout, and six (*H12, H18, H114, H118, H126, H220*) were isolated from rainbow trout and had previously been checked for cross-amplification in Rio Grande cutthroat trout (Pritchard et al. 2007b). The PureGene DNA Extraction Kit (Gentra Systems, Minneapolis, Minnesota) was used to extract DNA following the manufacturer's instructions. Microsatellites were amplified in 20-μL reactions and products labeled using an M13 procedure (Pritchard et al. 2007b). We used the following reaction mix: 1 μL template DNA, 2 ng/mL; 0.2 mM each reverse and M13-modified forward primers; 0.1 mM M13-labeled oligo; 0.2 mM each premixed deoxynucleotide triphosphates; 1.5 mM $MgCl_2$; 0.25 units Biotaq DNA polymerase (Bioline USA Inc., Canton, Massachusetts); and 1× Biotaq buffer. Polymerase chain reaction was conducted using a MJ Research PTC-100 96V thermocycler with the following conditions: initial denaturation 95°C (5 min), followed by 10 cycles of 94°C (30 s), 57°C (60 s), and 72°C (30 s), followed by 22 cycles of 94°C (30 s), 55°C (60 s), and 72°C (30 s), and terminating with a final extension at 72°C for 10 min. Amplification products were mixed 1:1 with 98% formamide loading dye, denatured for 3 min at 95°C, and then cooled on ice before running on 5% denaturing acrylamide gels at 35W for 70 min. Products were detected using an ABI-377 DNA sequencer and sized using Genotyper 2.5 software and Rox 500 size markers (Applied Biosystems, Inc., Foster City, California). Preliminary results suggested the presence of multiple alleles separated by differences of 1 or 2 base pairs (bps). To examine the contribution of allele sizing error to these results we compared allele sizes between two repeat genotyping runs for 10 individuals. These results, together with observations of variation in allele sizes between samples from the same population genotyped at different times (NMDGF, unpublished data), suggested that the majority of observed 1-bp and 2-bp differences were due to errors in allele sizing. We, therefore, chose to bin all alleles into 4-bp size categories. Allele binning did not create any new homozygotes or heterozygotes within the data set analyzed for this paper.

Other information.—Data on adult population size, stream length occupied, and the presence of natural barriers isolating populations were obtained from NMDGF (2002 and unpublished; Table 1). Population sizes, calculated by estimating number of individuals in stream sections using multipass electrofishing and then extrapolating to the entire habitat area, were available for only a subset of streams included in this study. Where estimates were available for multiple years we used the mean. Since such population estimates may suffer substantial sampling error, and the true number of cutthroat trout in a stream may exhibit substantial interannual variation (House 1995; Schlosser 1995; Pritchard and Cowley 2006), habitat size may provide a better approximation of long-term N_e than point estimates of adult population size. We used stream length as an approximation for habitat size since total available habitat area is difficult to estimate in these high altitude streams and varies according to annual precipitation conditions. Both Kruse et al. (2001) and Young et al. (2005) found abundance of cutthroat trout in montane streams to be a function of the square of occupied stream length. Stream gradients were estimated using the Geographic Information System program ArcView 3.2 (ESRI), using portions of the National Elevation Data set obtained from the U.S. Geological Survey Seamless Data Distribution System (http://seamless.usgs.gov) and Terrestrial Initiative in Global Environmental Research (TIGER) hydrology maps obtained from the New Mexico Resource Geographic Information System Program (http://rgis.unm.edu).

Statistical analysis: Genetic diversity and Hardy–Weinberg equilibrium within populations.—For each population sample, the number of alleles, observed heterozygosity (H_o), expected heterozygosity corrected for sampling bias (H_e; Nei 1987), and single locus and multilocus values of F_{IS} were obtained using the program Genetix 4.04 (Belkhir et al. 2001). Allelic richness of each locus in each population was calculated using Fstat 2.9.3.2 (Goudet 1995, 2001),

which accounts for varying sample sizes using the rarefaction method. Tests for conformation to Hardy–Weinberg equilibrium and for heterozygote deficiency and excess were performed for each locus in each population using an exact test implemented in Genepop 3.4 (Raymond and Rousset 1995). Overall significance of results over all loci in each population, and over all populations for each locus, was assessed by calculating the likelihood of obtaining the observed number of significant single locus tests by chance alone using the binomial likelihood function (Chapman et al. 1999)

$$L = \sum_{i=r}^{n} C(1-\alpha)^{n-r}(\alpha)^{r},$$

where n is the total number of tests, r is the number of significant tests at a given level of statistical significance α, and C is the factorial constant $n!/(r!(n-r)!)$. The null hypothesis, for example, no deviation from Hardy–Weinberg equilibrium, is rejected where $L < 0.05$.

We inspected the distribution of allele sizes within the data set and compared the size of outlying alleles to the allele size range observed in reference samples of the nonnative taxa known to hybridize with Rio Grande cutthroat trout in New Mexico, namely, rainbow trout and the two forms of Yellowstone cutthroat trout (Pritchard et al. 2007b). Following observations of significant deviations from Hardy–Weinberg equilibrium, we also examined our data set for evidence of genotyping artifacts that might contribute to this pattern. We used the program Micro-Checker (van Oosterhout et al. 2004) to identify loci that might contain null alleles and calculate the expected frequency of these alleles following Brookfield (1996). We also compared the results of replicate genotyping runs for 10 individuals.

Statistical analysis: Relationship of genetic diversity to habitat characteristics.—We used a Mann–Whitney *U*-test to investigate whether populations isolated above a natural barrier exhibited significantly lower H_e than those not historically isolated by such a barrier. Because all populations sampled in the Pecos River, but relatively few populations sampled in the Rio Grande, are protected by a natural barrier, the results might be confounded by lower genetic diversity in the Pecos River drainage as a whole. Therefore, we also performed this test using the populations in the Rio Grande drainage only. We examined whether H_e or multilocus F_{IS} was associated with stream length or gradient using Spearman rank correlation tests. We also used a Spearman rank correlation test to investigate the relationship between interlocus variance in F_{IS} and stream length. Since interlocus variance in F_{IS} may be biased where some loci are monomorphic, we performed this analysis twice, once using all samples and once excluding all samples fixed at one or more loci. Statistical tests were performed using SPSS 12.0.1.

Statistical analysis: Genetic structure within populations.—We used several approaches to identify the presence of genetic substructuring within populations. First, we investigated the possibility that observed deviations from Hardy–Weinberg equilibrium were the result of consanguineous matings within a population by calculating Ritland's (1996) individual inbreeding coefficient for each individual in each population. The multilocus estimator of this coefficient is

$$\hat{\rho} = \frac{\sum_{i,l} \frac{S_{il} - P_{il}^2}{P_{il}}}{\sum_{l}(n_l - 1)},$$

where $\hat{\rho}$ represents the individual inbreeding coefficient, P_{il} is the estimate of the frequency of the ith allele at the lth locus (here estimated from the sample), $S = 1$ if the individual is homozygous for the ith allele at the lth locus and 0 otherwise, and n represents the number of alleles at the locus. Individuals with missing data at one or more loci were removed from the analysis. Median observed individual inbreeding coefficient within each population was then compared with the distribution of median individual inbreeding coefficients calculated from 2000 simulated data sets. These simulated data sets were produced by randomly permuting alleles between individuals for each locus in each population to simulate the range of heterozygosities that would be expected if mating was random with respect to relatedness. Individual inbreeding coefficients and permuted data sets were generated using Microsoft Excel.

Second, we looked at the distribution of pairwise relatedness coefficients between individual fish. A higher mean relatedness coefficient than expected by chance would suggest that all fish were derived from a very small number of parents, while a higher variance in relatedness coefficients than expected would suggest the presence of several partially isolated interbreeding groups (Castric et al. 2002). We used the program Identix (Belkhir et al. 2002) to calculate pairwise relatedness between all individuals in each population using the identity coefficient of Mathieu et al. (1990). This estimator shows reduced variance at low allele numbers compared with other relatedness estimators (Belkhir et al. 2002). Identix tests for the significance of means and variances of relatedness coefficients in populations deviating from Hardy–Weinberg equilib-

rium by comparing them with results from simulated data sets created by randomly permuting genotypes between individuals (1,000 permutations).

Third, following Hansen and Jensen (2005) we used the software Colony 1.2 (Wang 2004) to partition the individuals in each sample into full-sib and half-sib groups. As the power of sibship reconstruction is dependent upon the genetic diversity within each sample, we simulated a population of unrelated individuals by randomly drawing alleles, with replacement, from the population sample and compared the number of full-sib and half-sib pairs identified in the real and simulated samples. For all runs we specified a rate of 2% for both allele drop-out and other genotyping errors and allowed one sex to be mated multiply. We performed three runs for each sample using different random number seeds and selected the solution with the highest log-likelihood. Since fish of varying ages were sampled from each stream we noted that in some cases individuals identified as full-sibs may in fact be parent–offspring pairs.

Two population samples, ELR and UTE, included fish sampled at four discrete locations within the stream reach. For these populations we investigated a relationship of relatedness to spatial position. We arbitrarily coded pairs of individuals collected at the same location as 0, and those collected at different locations as 1. We then investigated whether the resulting spatial association matrix was correlated with the matrix of pairwise relatedness using a Mantel test implemented in Genetix (10,000 permutations).

Statistical analysis: Evidence for recent population bottlenecks.—We examined our data set for evidence of recent reductions in effective population size using three different approaches. Since allelic diversity is reduced more quickly than heterozygosity following a reduction in effective population size (Nei et al. 1975), populations that have recently experienced a bottleneck should exhibit a higher level of heterozygosity than that expected given the observed number of alleles. Therefore, we compared the observed number of loci exhibiting such a heterozygosity excess in each sample with null expectations using a Wilcoxon matched-pairs test implemented in the program Bottleneck (Cornuet and Luikart 1996). As recommended by Cornuet and Luikart (1996) we assumed a two-phase model of microsatellite mutation (TPM) with 90% single-step mutations. We also used Bottleneck to qualitatively compare the distribution of allele frequencies with that expected in a nonbottlenecked population: alleles at low frequencies are expected to become relatively less abundant than alleles in intermediate frequency classes following a bottleneck (Luikart et al. 1998). Third, since rare alleles are more likely than common alleles to be lost from a population during a bottleneck, but the frequency of a microsatellite allele is not expected to be correlated with its size, the ratio of microsatellite allele number to microsatellite allele size range (*M*; Garza and Williamson 2001) is expected to be reduced following a population bottleneck. We, therefore, used the program M_P_Val (Garza and Williamson 2001) to investigate whether *M* in each population was reduced in relation to equilibrium expectations. As recommended by the Garza and Williamson (2001) we assumed a two-phase mutation model with 90% single-step mutations and set the average size of multiple-step mutations at 3.5 repeat units. We assumed a per-generation microsatellite mutation rate of 5×10^{-4} and excluded monomorphic loci from the analysis. Because the true N_e before population reduction was unknown we ran the analysis three times for each population using a range of initial N_e values we believed to be realistic for stream-dwelling trout populations: N_e = 500 ($\theta = 1$), 1,000 ($\theta = 2$), and 2,000 ($\theta = 4$). As the value of *M* can be greatly influenced by the presence of nonnative genetic material in a sample, we repeated all analyses for populations, DAL, ELR, UCO, UTE and VDZ, removing all individuals that appeared to contain alleles in a nonnative size range.

Results

Genetic Diversity and Hardy–Weinberg Equilibrium within Populations

The total number of alleles observed per locus ranged from 8 (*K222* and *H18*) to 19 (*K216*), with a mean of 13.1 (Appendix A). Populations exhibited considerable variation in genetic diversity. Nine population samples exhibited fixed alleles at one or more loci, with the samples from Polvadera Creek, Cañones Creek, Osha Creek, and Rio Mora being fixed at 8, 4, 3, and 3 loci, respectively. Expected heterozygosity ranged from a minimum of 0.09 in the PVA sample to a maximum of 0.59 in the RIC sample, with a mean of 0.42. Mean allelic richness within each population sample, based on the minimum per-locus sample size of 12 successfully genotyped individuals, was 3.1, considerably lower than the mean allelic richness of 6.3 calculated for all populations combined.

Five samples contained outlying alleles at *J14*, *K222*, *H114*, *H118* or *H220*, which were within a size range characteristic of nonnative trout (DAL: 6 out of 718 alleles; ELR: 3 out of 942 alleles; VDZ: 7 out of 720 alleles; UCO: 2 out of 718 alleles; and UTE: 9 out of 1,440 alleles). We chose to retain the 26 individuals containing these 27 alleles in the majority of our analyses for several reasons. First, we are confident that these fish represent advanced generation backcrosses and, therefore, are not expected to alter the contempo-

rary demography of the population. Second, we are unable to recognize and exclude other introgressed individuals that are carrying nonnative alleles in an overlapping allele size range between Rio Grande cutthroat trout and other taxa, or are carrying nonnative genetic material at loci other that those genotyped. Third, given the relatively high degree of genetic differentiation known to exist between Rio Grande cutthroat trout populations (global $F_{ST} > 0.4$; Pritchard and Cowley 2006), it is possible that some alleles may mistakenly be classified as "nonnative" due to the limited size of our known pure Rio Grande cutthroat trout reference sample. Exclusion of these 26 individuals from the data set did not substantially change any of the results presented in this paper, except where noted for the M_P_Val analysis.

Of the 22 populations examined, 7 exhibited significant deviations from Hardy–Weinberg equilibrium. Two of these samples (EST and YER) deviated from Hardy–Weinberg equilibrium as a result of heterozygote deficiency at multiple loci, while the remaining five samples (BIT, CAN, ELR, FRJ, UCO) deviated from Hardy–Weinberg equilibrium as a result of heterozygote deficiency at some loci combined with heterozygote excess at other loci. One additional sample, UTE, exhibited overall significant heterozygote deficiency when the one-tailed test was applied. Loci contributing to heterozygote deficiency or excess varied among population samples. When considering loci over all samples combined, three of the 12 loci used (*J3*, *K216*, *H126*) deviated significantly from Hardy–Weinberg equilibrium (Table A.1 in the appendix).

When comparing replicate genotyping runs for 10 individuals, we observed a heterozygote changing to a homozygote, or vice versa, in three cases, each at a different locus (*J14*, *K216*, or *H118*). However, only one of these changes was at a locus that exhibited heterozygote deficiency in any population (*K216*). If all populations within our data set were assumed to be in Hardy–Weinberg equilibrium, Micro-checker found seventeen locus-sample combinations to exhibit evidence for null alleles (*J3*: BIT, CAN ELR; *J14*: none; *J103*: none; *J132*: EST, FRJ; *K216*: CAN, MOR, MTR; *K222*: EST; *H18*: EST, MOR, UTE; *H114*: DAL; *H118*: none; *H126*: COL, FRJ, GAV, UCO). Three of these locus-sample combinations exhibited a sufficient frequency of null alleles that null homozygotes would be expected in the data set, however, none were observed. When nonamplifying individuals did occur (1–21 per locus), they were most commonly seen in populations that did not deviate significantly from Hardy–Weinberg equilibrium at the relevant locus. However, multiple nonamplifying individuals were observed in samples COL and FRJ at locus *H126*. We have also observed evidence for null alleles at *H126* within some population samples of two closely related subspecies, Colorado River cutthroat trout *O. c. pleuriticus* and greenback cutthroat trout *O. c. stomias* (Pritchard et al., in press). The presence of null alleles at this locus seems to be limited to specific samples and may be due to conditions during particular genotyping runs. We, therefore, excluded locus *H126* from samples COL, FRJ, GAV and UCO for all subsequent analyses. Following removal of *H126*, UCO no longer deviated significantly from Hardy–Weinberg equilibrium. While we cannot completely rule out the presence of null alleles at other loci, or the existence of related phenomena such as short allele dominance (Morand et al. 2002), we nevertheless consider it unlikely that all observed heterozygote deficiencies, distributed over multiple loci, are due to genotyping artifacts alone.

Relationship of Genetic Diversity to Habitat Characteristics

Expected heterozygosity was not significantly correlated with estimated adult population size or stream length (H_e and adult population: Spearman rank correlation coefficient $[C_R] = -0.23$, $P = 0.45$; H_e and stream length: $C_R = 0.25$, $P = 0.26$; Figure 2). In contrast, H_e was significantly lower in samples from populations occurring above a natural migration barrier than those from populations not isolated by such a barrier (Mann–Whitney *U*-test, all populations: $z = 2.47$, two-tailed $P = 0.013$; Rio Grande populations only: $z = 2.12$, two-tailed $P = 0.034$). Multilocus F_{IS} was not significantly associated with adult population size, stream length or stream gradient, although we did observe a weak trend of increasing F_{IS} with increasing gradient (F_{IS} and adult population: $C_R = 0.26$, $P = 0.39$; F_{IS} and stream length: $C_R = 0.15$, $P = 0.50$; F_{IS} and stream gradient: $C_R = 0.31$, $P = 0.16$; Figure 2). We observed a negative correlation between interlocus variance in F_{IS} and stream length (all populations: $C_R = -0.41$, $P = 0.056$; populations polymorphic at all loci: $C_R = -0.62$, $P = 0.024$). The increased strength of the relationship when samples monomorphic at one or more loci were excluded was primarily due to the removal of CAN, the second most genetically depauperate sample within our data set (Figure 2).

Genetic Structure within Populations

The distribution of individual inbreeding coefficients exhibited a right skew within most populations, indicating the presence of a small number of highly inbred individuals in each sample. For four population samples median individual inbreeding coefficient was significantly higher than would be expected from

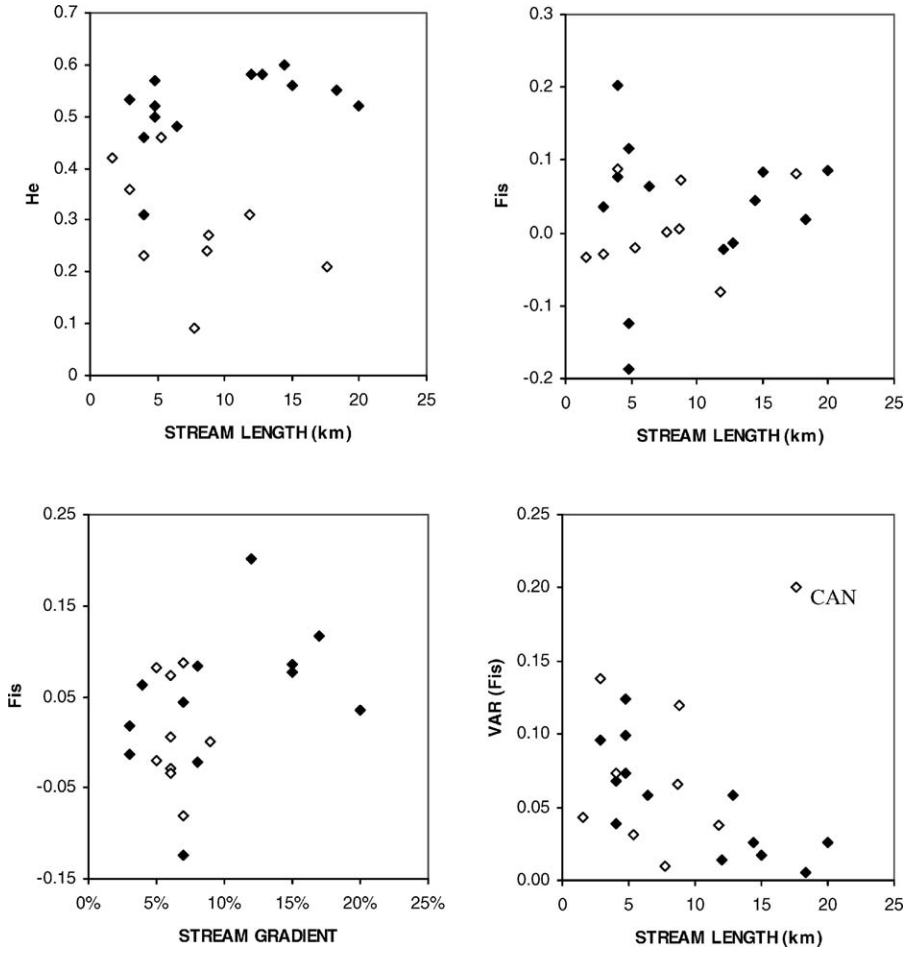

FIGURE 2.—Relationships of genetic diversity indices to habitat characteristics. Populations that are monomorphic at one or more loci are indicated by open symbols. The outlying sample CAN (Cañones Creek) is indicated on the plot of VAR(Fis) against stream length.

random assortment of alleles (RIC, $P = 0.020$; MOR, $P = 0.021$; EST, $P < 0.001$; UTE, $P = 0.015$). In contrast, both FRJ ($P = 0.025$) and VDZ ($P < 0.001$) exhibited a median individual inbreeding coefficient significantly lower than would be expected from random mating.

No population sample exhibited a mean pairwise relatedness coefficient significantly different from that expected under random mating. However, variance in pairwise relatedness coefficients was significantly higher than expected in 4 of 7 samples deviating from Hardy–Weinberg equilibrium (BIT: $P = 0.004$; EST: $P < 0.001$; UTE: $P = 0.004$; and YER: $P = 0.002$). A significantly higher variance in pairwise relatedness coefficients than expected was additionally observed in five samples that did not significantly deviate from Hardy–Weinberg equilibrium (GAV: $P = 0.032$; JIC: $P = 0.007$; VJO: $P = 0.012$; OSH: $P = 0.003$; and VDZ: $P = 0.006$). These results suggest that several partially discrete interbreeding groups were sampled in these streams. For population samples collected from El Rito Creek and Ute Creek, we observed a significant correlation between the matrix of pairwise relatedness and the matrix that indicated whether or not individuals were collected at different stream locations (Mantel test, ELR: $P = 0.016$; UTE, $P < 0.001$). Hence, individuals collected at the same spatial location in a stream tended to be more closely related than those collected from different locations.

Table 2 shows the number of full-sib and half-sib families and maximum full-sib family size as estimated by the Colony software program from the real and simulated data. In an extreme situation, where all individuals were unrelated, the number of half-sib and full-sib families in a sample would both equal the

TABLE 2.—Number of full-sib (FS) and half-sib (HS) families identified in real and simulated (Sim) population samples using Colony, and maximum full-sib family size estimated for each sample. Population codes are given in Table 1.

	FS families		HS families		Maximum FS family size	
Population	Real	Sim	Real	Sim	Real	Sim
BIT	14	17	7	8	6	3
CAN	13	13	5	5	8	4
COL	18	19	9	7	4	3
DAL	15	15	6	5	4	4
ELR	27	28	11	11	4	4
EST	12	18	7	7	5	4
FRJ	16	19	7	7	4	4
GAV	12	15	6	9	5	3
JIC	11	14	4	5	5	3
MCC	15	17	5	8	3	3
MOR	13	13	4	6	5	5
MTR	14	15	6	5	5	4
OSH	11	12	4	5	5	3
PLC	15	18	5	5	5	4
PRE	15	15	7	6	3	2
PVA	13	11	5	4	5	6
RIC	17	16	9	8	2	3
UCO	21	23	11	9	3	3
UTE	38	42	12	13	4	3
VDZ	15	22	5	11	4	2
VJO	22	24	11	10	2	2
YER	9	12	6	6	6	2

number of individuals. Observation of the number of full-sib families and half-sib families estimated from both real and simulated samples using Colony shows that, as expected, the number of families identified depended upon the genetic diversity present in the sample. In general, we observed little difference between the number of families estimated from the simulated and real samples. This suggests that in many cases Colony may not have identified true family structure within our data set, perhaps because allelic diversity is low at several loci in many populations, or because the presence of parent–offspring pairs or multiple mating by both sexes is confounding the analysis. We did observe strong evidence that the sample from VDZ contained several groups of closely related individuals: Colony identified 22 full-sib families nested in 11 half-sib families from the simulated data, but only 15 full-sib families nested in five half-sib families from the real data. Colony also identified fewer and larger families in the real samples from BIT, GAV, JIC, MCC, OSH, PLC, UTE and YER than in the simulated samples, suggesting that the program is identifying at least some true family structure within these populations.

Evidence for Recent Population Bottlenecks

Three population samples, DAL, PLC, and MTR, exhibited a shifted mode in allele size distribution. Two of these samples, PLC and MTR, also exhibited evidence for a recent population bottlenecks using the heterozygosity excess method (one-tailed Wilcoxon test for heterozygosity excess; PLC: $P = 0.0005$; MTR: $P = 0.0048$). In contrast we found no evidence for a recent bottleneck in Policarpio Creek or Rio Mora tributary using the approach of Garza and Williamson (2001), as implemented in M_P_Val. Results of the M_P_Val analysis were strongly influenced by the effective population size assumed before the population bottleneck (Table 3). If we assumed an N_e of 2,000 before population size reduction, only six population samples exhibited a lower value of M than expected under equilibrium conditions. Three of these samples contained outlying alleles that may have originated from a nonnative taxon, and the value of M was no longer significantly lower than equilibrium expectations when individuals containing these alleles were removed. Assuming a lower N_e before the occurrence of a population bottleneck caused an increase in the values of M calculated from the simulated samples (Garza and Williamson 2001), and hence more population samples exhibited a significantly lower M than expected under equilibrium conditions.

Discussion

Remnant Rio Grande cutthroat trout populations clearly vary in the level of genetic diversity that they contain. As has been demonstrated for other inland stream-dwelling salmonids (e.g., Carlsson and Nilsson 2001; Costello et al. 2003; Taylor et al. 2003), populations of Rio Grande cutthroat trout existing

TABLE 3.—Observed ratio of the number of microsatellite alleles to the alleles size range (M) in each sample, and proportion of M values in 10,000 samples from simulated equilibrium populations (M_s) that are lower than the observed M. Proportions less than 0.05 are italicized. Simulated populations were generated assuming three different values of effective population size (N_e). Individuals containing alleles that appear to be within a nonnative size range were removed from samples DAL2, ELR2, UCO2, UTE2, and VDZ2. Population codes are given in Table 1.

		Proportion of $M_s < M$		
Population	M	$N_e = 500$	$N_e = 1000$	$N_e = 2000$
BIT	0.736	***0.010***	***0.028***	0.085
CAN	0.615	***0.000***	***0.001***	*0.003*
COL	0.753	***0.020***	0.060	0.148
DAL	0.692	***0.002***	***0.009***	***0.024***
DAL2	0.747	***0.026***	0.066	0.165
ELR	0.806	0.083	0.206	0.393
ELR2	0.844	0.220	0.435	0.680
EST	0.764	***0.022***	0.078	0.183
FRJ	0.694	***0.003***	***0.008***	***0.024***
GAV	0.723	***0.007***	***0.028***	0.096
JIC	0.788	0.057	0.165	0.389
MCC	0.723	***0.009***	***0.026***	0.072
MOR	0.817	0.150	0.306	0.509
MTR	0.78	0.053	0.136	0.286
OSH	0.822	0.170	0.334	0.575
PLC	0.869	0.378	0.614	0.824
PRE	0.878	0.440	0.690	0.893
PVA	0.875	0.400	0.552	0.717
RIC	0.689	***0.002***	***0.008***	***0.026***
UCO	0.763	***0.026***	0.085	0.197
UCO2	0.767	***0.032***	0.092	0.218
UTE	0.648	***0.000***	***0.000***	***0.001***
UTE2	0.720	***0.004***	0.009	0.028
VDZ	0.615	***0.000***	***0.000***	***0.000***
VDZ2	0.717	***0.005***	***0.023***	0.072
VJO	0.787	***0.048***	0.139	0.314
YER	0.698	***0.002***	***0.009***	0.042

above a natural migration barrier tend to be genetically depauperate compared with those not historically isolated by such a barrier. Such populations, however, are also the most secure from invasion by nonnative trout. Despite an ongoing management effort to protect extant cutthroat trout populations by the construction of artificial barriers, such barriers often fail (Harig et al. 2000). For example, the majority of barriers constructed for the protection of Rio Grande cutthroat trout populations in Colorado since 1975 have failed within 5 years, and the subsequent brook trout invasion has been associated with population declines (Pritchard and Cowley 2006). Additionally, a number of extant pure Rio Grande cutthroat trout populations remain unprotected by any fish movement barrier. Continuing loss of populations not protected by a natural migration barrier is expected to lead to a disproportionate loss of the genetic diversity remaining within the subspecies.

Contrary to expectations, no significant association was observed between the size of a remnant population or its habitat and expected heterozygosity. Indeed, one of the largest populations included this study, in Cañones Creek, is also one of the least diverse. The low levels of genetic variation observed in this population may be due, at least partly, to a recent documented population bottleneck. Surveys in 1975 revealed this stream to contain very few Rio Grande cutthroat trout, apparently as a result of habitat degradation (NMDGF unpublished data). Following habitat restoration, however, cutthroat trout numbers rebounded and Cañones Creek is now considered to be one of the most secure Rio Grande cutthroat trout populations remaining (USFWS 2002). New Mexico Department of Game and Fish currently targets creeks with larger populations as a source of gametes for the establishment of hatchery stocks. Although these populations are likely to be demographically more robust to the impact of gamete collection, there is clearly no guarantee that targeting such populations will also result in a more genetically diverse hatchery stock.

We found conflicting results when using different methods to examine our samples for the genetic signature of population bottlenecks. Both the heterozygosity excess and allele frequency distribution methods suggested that bottlenecks had recently occurred within Policarpio Creek and Rio Mora tributary. In contrast, results of the M_P_Val analysis did not support a hypothesis of recent bottlenecks within these populations, but instead suggested recent severe reductions in N_e in several other streams. The results of all three methodologies should be treated with caution. The performance of the heterozygosity excess and allele frequency methods in populations that deviate from Hardy–Weinberg equilibrium is not known, and genetic signals of recent bottlenecks in other populations may have been confounded by the presence of heterozygote deficiency or excess. The expected value of M in a population that has not undergone a bottleneck varies both with N_e and the microsatellite mutation model assumed. The observed value of M can also be heavily biased by incomplete sampling of the alleles present in the population, and by the presence of introduced genetic material in a sample. Within Rio Grande cutthroat trout in New Mexico, this could not only include genetic material from other *Oncorhynchus* taxa but also that from a Rio Grande cutthroat trout hatchery line that was previously stocked throughout the state (NMDGF unpublished data).

Nevertheless, if we assume that the value of M has not been greatly biased by stocking, comparison of M between different Rio Grande cutthroat trout populations enables us to make some inferences about those populations' recent histories. The low value of M

observed in the samples from Bitter Creek, Cañones Creek, Frijoles Creek, Gavilan Canyon Creek, McCrystal Creek, Ricardo Creek and Yerba Creek strongly suggests the occurrence of recent reductions in N_e within these streams. Populations of Rio Grande cutthroat trout in both Ricardo Creek and Little Vermejo Creek are known to have undergone recent declines, associated with brook trout invasion (Pritchard and Cowley 2006; NMDGF unpublished data). In contrast, the relatively high values of M observed in the samples from Rio Mora, Osha Creek and Polvadera Creek suggest that the low genetic diversity of these populations, which are isolated above waterfalls, is the result of founder effects and long-term low N_e rather than recent bottlenecks. We also observe a high value of M in the sample from El Rito Creek. This stream contains an abundant Rio Grande cutthroat trout population persisting in a relatively large area of high quality habitat, which may have been less affected by fragmentation than populations isolated in smaller stream reaches.

The observation of decreasing interlocus variance in F_{IS} with increasing habitat size, which mirrors that of Castric et al. (2002) for brook trout in small lakes, supports the hypothesis of a low contemporary N_e in many of the remnant Rio Grande cutthroat trout populations included in this study. Such a pattern is expected if relatively few individuals contribute to the next generation, for example, because the availability of suitable spawning sites is limited or survival to reproductive age is low. In several populations we also observed a significantly higher median individual inbreeding coefficient, or a significantly higher variance in relatedness, than expected by chance. Taken as a whole, these results suggest that many remnant cutthroat trout populations may include multiple, partially discrete subpopulations, with only a small number of adults successfully reproducing in each and some highly inbred individuals arising as a result of matings between relatives. The individual inbreeding coefficients observed in Rio Valdez and Frijoles Creek were significantly lower than would be expected by chance alone and may have arisen as a result of interbreeding between individuals from two or more previously isolated subpopulations, perhaps following changes in habitat conditions.

Genetic differentiation over a very small spatial scale has been observed in several other studies of inland salmonids (e.g., Estoup et al. 1998; Ruzzante et al. 2001; Wofford et al. 2005) and is generally attributed to limited movement of individuals within a habitat. No study has yet investigated the mobility of Rio Grande cutthroat trout within montane streams. Young (1996) performed a radiotelemetric study of adult Colorado River cutthroat trout, a closely related subspecies occurring in similar habitat. He found substantial movement, in the range of several hundred to several thousand meters, much of which appeared to be associated with migration to and from spawning habitat. Conversely, in streams isolated by a migration barrier strong selection for low vagility is expected as individuals moving downstream over the barrier are permanently lost from the population (Northcote 1992). Even in the absence of restrictions to movement partially isolated interbreeding groups may arise if fish tend to return to their natal gravel beds to spawn, or if kin or familiar individuals preferentially associate with each other. Juvenile salmonids of several species exhibit a preference for the odor of kin (Krause et al. 2000), and kin associations may persist to adulthood (Fraser et al. 2005).

The results of this study have several implications for the management of cutthroat trout populations in small headwater streams. First, if only a few adults are able to reproduce in each generation then effective population size may be much lower than the census adult population size (N). No study has yet attempted to estimate the relationship of N_e to N in stream-dwelling cutthroat trout. Palm et al. (2003) and Jensen et al. (2005) estimated N_e/N ratios of between 0.2 and 0.5 for stream-resident brown trout. When calculating minimum habitat sizes required to support cutthroat trout populations with $N_e > 500$, Hilderbrand and Kershner (2000) assumed an N_e/N ratio of 0.2. The U.S. Fish and Wildlife Service assumed a ratio of N_e to adult census population size of approximately 0.5 when setting recovery criteria for the threatened greenback cutthroat trout (Young and Harig 2001), and the same criteria are used by the Colorado Division of Wildlife when assessing conservation status of Rio Grande cutthroat trout populations (CDW 2004). Clearly, a better understanding of the relationship of N_e to census population size is required if we are to calculate, for example, how much habitat is required to support a cutthroat trout population with a sufficient N_e to avoid inbreeding depression, or to retain its current level of genetic variation and hence its adaptive potential. Second, as noted by Hansen and Jensen (2005), if the progenitors for a hatchery line are taken from a population consisting of only few families, then that line is at increased risk for inbreeding depression. Hence, if a hatchery stock of native trout is to be developed, it may, in some cases, be preferable to generate genetically diverse stocks by crossing individuals from multiple populations within the same drainage rather than retaining single lines from individual streams. We note, however, that such hatchery lines should be used with caution: although

a genetically diverse mixed stock may be preferred if native trout are to be reintroduced into an isolated area of unoccupied habitat, use of such a stock to supplement existing populations should be avoided due to the risk of outbreeding depression. Alternatively, genetic diversity within restoration populations may be achieved by introducing wild fish or their hatchery-reared progeny from multiple streams, an approach that should minimize the chance of selection in the hatchery environment. Third, the presence of some degree of population subdivision emphasizes the need for a careful sampling protocol when attempting to assess the level of nonnative introgression within a cutthroat trout population. Currently, agencies prioritize populations of cutthroat trout for different management interventions according to their level of genetic purity, which is ideally measured using taxon-diagnostic genetic markers. If samples for genetic testing are collected from only a small section of stream population-level introgression may be over- or underestimated due to the sampling of related groups of individuals.

Acknowledgments

This research was funded by NMDGF through the Federal Aid in Sport Fish Restoration Act and by the New Mexico Agricultural Experiment Station. We are grateful to personnel of NMDGF and the U.S. Forest Service, who collected tissue samples, and staff of Genetic Identification Services who provided technical support. Special thanks go to Yvette Paroz and Kirk Patten of NMDGF for providing data and advice. Comments from two anonymous reviewers substantially improved the manuscript.

References

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

Belkhir, K., P. Borsa, L. Chiki, N. Raufaste, and F. Bonhomme. 2001. Genetix 4.02: logiciel sous Windows pour la génétique des populations. [Genetix 4.02: Windows software for population genetics.] Université de Montpellier II, Montpellier, France.

Belkhir, K., V. Castric, and F. Bonhomme. 2002. Identix: a software to test for relatedness in a population using permutation methods. Molecular Ecology Notes 2:611–614.

Brookfield, J. F. Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5:453–455.

Carlsson, J., and J. Nilsson. 2001. Effects of geomorphological structures on genetic differentiation among brown trout populations in a northern boreal river drainage. Transactions of the American Fisheries Society 130:36–45.

Castric, V., L. Bernatchez, K. Belkhir, and F. Bonhomme. 2002. Heterozygote deficiencies in small lacustrine populations of brook trout *Salvelinus fontinalis* Mitchell (Pisces, Salmonidae): a test of alternative hypotheses. Heredity 89:27–35.

Chapman, R. W., G. R. Sedberry, C. C. Koenig, and B. M. Eleby. 1999. Stock identification of gag, *Mycteroperca microlepis*, along the southeast coast of the United States. Marine Biotechnology 1:137–146.

Colorado Division of Wildlife. 2004. Conservation plan for Rio Grande cutthroat trout *Oncorhynchus clarki virginalis* in Colorado. Available: http://wildlife.state.co.us/Research/Aquatic/CutthroatTrout/. (February 2006).

Cornuet, J.-M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, and E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57:328–344.

Dunham, J. B., S. B. Adams, R. E. Schroeter, and D. C. Novinger. 2002. Alien invasions in aquatic ecosystems: toward an understanding of brook trout invasions and potential impacts on inland cutthroat trout in western North America. Reviews in Fish Biology and Fisheries 12:373–391.

Estoup, A., F. Rousset, Y. Michalakis, J.-M. Cornuet, M. Adriamange, and R. Guyomard. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout *Salmo trutta*. Molecular Ecology 7:339–353.

Fraser, D. J., F. Duchense, and L. Bernatchez. 2005. Migratory trout schools exhibit population and kin associations beyond juvenile stages. Molecular Ecology 14:3133–3146.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

Goudet, J. 1995. Fstat, version 1.2: a computer program to calculate *F*-statistics. Journal of Heredity 86:485–486.

Goudet, J. 2001. Fstat: a program to estimate and test gene diversities and fixation indices, version 2.9.3. Available: www2.unil.ch/popgen/softwares/fstat.htm. (March 2007).

Hansen, M. M., and L. F. Jensen. 2005. Sibship within samples of brown trout *Salmo trutta* and implications for supportive breeding. Conservation Genetics 6:297–305.

Hansen, M. M., E. E. Nielsen, and K.-L. D. Mensberg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Molecular Ecology 6:469–474.

Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. North American Journal of Fisheries Management 20:513–520.

Hilderbrand, R. H. 2003. The roles of carrying capacity, immigration, and population synchrony on persistence of stream-resident cutthroat trout. Biological Conservation 110:257–266.

Hilderbrand, R. H., and J. L. Kershner. 2000. Conserving

inland cutthroat trout in small streams: how much stream is enough? North American Journal of Fisheries Management 20:513–520.

Hitt, N. P., C. A. Frissell, C. C. Muhlfeld, and F. W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences 60:1440–1451.

House, R. 1995. Temporal variation in abundance of an isolated population of cutthroat trout in western Oregon, 1981–1991. North American Journal of Fisheries Management 15:33–41.

Jensen, L. F., M. M. Hansen, J. Carlsson, V. Loeschcke, and K. L. D. Mensberg. 2005. Spatial and temporal genetic differentiation and effective population size of brown trout (*Salmo trutta* L.) in small Danish rivers. Conservation Genetics 6:615–621.

Keeler-Foster, C. L. 2003. Rio Grande cutthroat trout *Oncorhynchus clarki virginalis*: population genetics and hybridization with introduced rainbow trout *Oncorhynchus mykiss*. Doctoral dissertation. New Mexico State University, Las Cruces.

Krause, J., R. K. Butlin, N. Peukuri, and V. L. Pritchard. 2000. The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. Biological Reviews 75:477–501.

Kruse, C. G., W. A. Hubert, and F. J. Rahel. 2001. An assessment of headwater isolation as a conservation strategy for cutthroat trout in the Absaroka Mountains of Wyoming. Northwest Science 75:1–11.

Luikart, G., F. W. Allendorf, J.-M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238–247.

Mathieu, E., M. Autem, M. Roux, and F. Bonhomme. 1990. Epreuve de validation dans l'analyse de structures génétiques multivarées: comment tester l'équilibre panmictique? [Validation tests in the analysis of multivariate genetic structures: how to test a panmictic equilibrium?] Revue de Statistiques Appliqués 38:47–66.

Morand, M.-E., S. Brachet, P. Rossignol, J. Dufour, and N. Frascaria-Lacoste. 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. Molecular Ecology 11:377–385.

Morita, K., and S. Yamamoto. 2001. Effects of habitat fragmentation by damming on the persistence of stream-dwelling trout populations. Conservation Biology 16:1318–1323.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

NMDGF (New Mexico Department of Game and Fish). 2002. Long-range plan for the management of Rio Grande cutthroat trout in New Mexico. NMDGF, Santa Fe.

Northcote, T. G. 1992. Migration and residency in stream salmonids: some ecological considerations and evolutionary consequences. Nordic Journal of Freshwater Research 67:5–17.

Palm, S., L. Laikre, P. E. Jorde, and N. Ryman. 2003. Effective population size and temporal genetic change in stream-resident brown trout (*Salmo trutta* L.). Conservation Genetics 4:249–264.

Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: effects of brook trout on native cutthroat trout. Ecological Applications 14:754–772.

Pritchard, V. L., and D. E. Cowley. 2006. Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*): a technical conservation assessment. U.S. Forest Service. Available: www.fs.fed.us/r2/projects/scp/assessments/riograndecutthroattrout.pdf. (August 2006).

Pritchard, V. L., K. Jones, and D. E. Cowley. 2007a. Estimation of introgression in cutthroat trout populations using microsatellites. Conservation Genetics, DOI: 10.1007/s10592-006-9280-0.

Pritchard, V. L., K. Jones, J. L. Metcalf, A. P. Martin, P. Wilkinson, and D. E. Cowley. 2007b. Characterization of tetranucleotide microsatellites for Rio Grande cutthroat trout and rainbow trout and their cross-amplification in other cutthroat trout subspecies. Molecular Ecology Notes, DOI: 10.1111/j.1471-8286.2007.01695.x.

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

Ritland, K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research 67:175–185.

Ruzzante, D. E., M. M. Hansen, and D. Meldrup. 2001. Distribution of individual inbreeding coefficients, relatedness, and influence of stocking on native anadromous brown trout *Salmo trutta* population structure. Molecular Ecology 10:2107–2128.

Schlosser, I. J. 1995. Critical landscape attributes that influence fish population dynamics in headwater streams. Hydrobiologia 303:71–81.

Taylor, E. B., M. D. Stamford, and J. S. Baxter. 2003. Population subdivision in westslope cutthroat trout *Oncorhynchus clarki lewisi* at the northern periphery of its range: evolutionary inferences and conservation implications. Molecular Ecology 12:2609–2622.

USFWS (U.S. Fish and Wildlife Service). 2002. Endangered and threatened wildlife and plants: candidate status review for Rio Grande cutthroat trout. Federal Register 67:112(11 June 2002):39936–39947.

van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Wang, J. L. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 66:1963–1979.

Weigel, D. E., J. T. Peterson, and P. Spruell. 2003. Introgressive hybridization between native cutthroat trout and introduced rainbow trout. Ecological Applications 13: 38–50.

Wilcox, B. A., and D. Murphy. 1985. Conservation strategy: the effect of fragmentation upon extinction. American Naturalist 125:879–887.

Wofford, J. B., R. E. Gresswell, and M. A. Banks. 2005. Influence of barriers to movement on within-watershed

genetic variation of coastal cutthroat trout. Ecological Applications 15:628–637.

Young, M. K. 1996. Summer movements and habitat use by Colorado River cutthroat trout in small, montane streams. Canadian Journal of Fisheries and Aquatic Sciences 53:1403–1408.

Young, M. K., P. M. Guenther-Gloss, and A. D. Ficke. 2005. Predicting cutthroat trout (*Oncorhynchus clarkii*) abundance in high-elevation streams: revisiting a model of translocation success. Canadian Journal of Fisheries and Aquatic Sciences 62:2399–2408.

Young, M. K., and A. L. Harig. 2001. A critique of the recovery of greenback cutthroat trout. Conservation Biology 15:1575–1584.

Appendix follows

Appendix: Genetic Variation in Rio Grande Cutthroat Trout

TABLE A.1.—Number of alleles (A), allelic richness (R), expected heterozygosity adjusted for sample size (H_e), observed heterozygosity (H_o), and inbreeding coefficients (F_{IS}) for each locus in each population. Single- and multiple-population estimates of R are based on a minimum sample size of 12 successfully genotyped individuals. Single-locus deviations from Hardy–Weinberg equilibrium are indicated by asterisks ($P \leq 0.05$*, $P \leq 0.001$**). The term L(HW) indicates the likelihood that the multilocus or multipopulation sample is in Hardy–Weinberg equilibrium based on the binomial likelihood test. Blank cells indicate that a value cannot be calculated for F_{IS} because the locus is insufficiently polymorphic. Population codes are given in Table 1 in the text.

Locus	Statistic	BIT	CAN	COL	DAL	ELR	FRJ	GAV	JIC	VJO	MCC	OSH
J3	A	5	2	4	1	3	3	3	2	3	1	1
	R	4.13	1.64	3.29	1.00	2.51	2.96	2.60	1.60	2.80	1.00	1.00
	H_e	0.40	0.07	0.54	0.00	0.33	0.57	0.52	0.05	0.33	0.00	0.00
	H_o	0.13	0.00	0.50	0.00	0.20	0.70	0.65	0.05	0.38	0.00	0.00
	F_{IS}	0.67**	1.00*	0.08*		0.40*	−0.24	−0.25		−0.16		
	L(HW)											
J14	A	4	2	3	2	16	3	3	2	5	4	2
	R	3.24	2.00	2.79	2.00	9.78	1.89	2.58	2.00	4.55	3.62	1.95
	H_e	0.32	0.30	0.34	0.51	0.84	0.07	0.23	0.37	0.65	0.61	0.16
	H_o	0.33	0.23	0.30	0.40	0.76	0.07	0.25	0.48	0.59	0.80	0.08
	F_{IS}	−0.03	0.24	0.12	0.21	0.09*	−0.01	−0.09	−0.29	0.10	−0.32*	0.47
	L(HW)											
J103	A	5	4	10	2	9	4	8	4	8	2	4
	R	4.45	3.60	7.70	2.00	6.00	3.12	7.29	3.90	6.85	2.00	3.88
	H_e	0.49	0.45	0.73	0.33	0.78	0.30	0.78	0.57	0.81	0.35	0.63
	H_o	0.47	0.45	0.67	0.27	0.68	0.19	0.75	0.52	0.90	0.37	0.32
	F_{IS}	0.05	0.02	0.08	0.18	0.13	0.38*	0.04	0.08	−0.11	−0.06	0.15
	L(HW)											
J132	A	3	3	3	2	6	4	5	2	5	3	2
	R	2.99	2.93	3.00	2.00	4.55	3.22	4.58	2.00	4.24	2.79	2.00
	H_e	0.56	0.54	0.60	0.33	0.55	0.53	0.69	0.28	0.66	0.38	0.34
	H_o	0.37	0.77	0.48	0.40	0.44	0.48	0.65	0.14	0.60	0.30	0.33
	F_{IS}	0.35*	−0.43*	0.21	−0.23	0.20	0.10	0.05	0.50	0.10	0.22	0.01
	L(HW)											
K216	A	4	3	8	4	7	4	7	2	9	2	2
	R	3.64	2.04	6.72	3.79	4.94	3.14	6.48	1.57	7.57	1.64	2.00
	H_e	0.62	0.10	0.76	0.61	0.61	0.55	0.81	0.05	0.83	0.07	0.51
	H_o	0.67	0.03	0.63	0.62	0.79	0.89	0.70	0.05	0.87	0.07	0.75
	F_{IS}	0.08*	0.66*	0.17	−0.01	−0.32*	−0.64**	0.14		−0.05	−0.02	−0.49*
	L(HW)											
K222	A	2	1	2	2	3	5	2	2	4	3	2
	R	2.00	1.00	2.00	1.99	2.30	4.44	2.00	1.82	3.97	2.64	1.98
	H_e	0.30	0.00	0.29	0.26	0.52	0.75	0.41	0.09	0.70	0.47	0.19
	H_o	0.37	0.00	0.34	0.30	0.70	0.89	0.55	0.10	0.83	0.60	0.21
	F_{IS}	−0.21		−0.19	−0.16	−0.36*	−0.19	−0.36	−0.03	−0.19	−0.28	−0.09
	L(HW)											
H12	A	7	4	9	2	6	6	6	3	4	3	2
	R	5.95	3.29	5.62	1.99	5.14	4.95	5.91	3.00	3.58	2.30	1.50
	H_e	0.76	0.39	0.67	0.24	0.73	0.68	0.78	0.67	0.59	0.16	0.04
	H_o	0.79	0.33	0.73	0.20	0.63	0.70	0.75	0.60	0.57	0.17	0.04
	F_{IS}	−0.04*	0.15	−0.10	0.15	0.15	−0.03	0.04	0.11	0.05	−0.06	
	L(HW)											
H18	A	2	1	2	2	2	3	2	2	5	2	2
	R	2.00	1.00	2.00	1.99	2.00	2.84	2.00	1.57	3.38	2.00	2.00
	H_e	0.36	0.00	0.47	0.26	0.49	0.55	0.48	0.05	0.25	0.50	0.47
	H_o	0.40	0.00	0.31	0.30	0.55	0.59	0.45	0.05	0.27	0.38	0.46
	F_{IS}	−0.10		0.34	−0.16	−0.13	−0.07	0.07		−0.08	0.24	0.02
	L(HW)											
H114	A	3	1	3	2	2	3	2	2	7	3	1
	R	3.00	1.00	2.64	1.96	1.99	2.44	2.00	1.57	5.09	2.96	1.00
	H_e	0.60	0.00	0.46	0.18	0.28	0.53	0.33	0.05	0.70	0.47	0.00
	H_o	0.60	0.00	0.50	0.00	0.28	0.33	0.20	0.05	0.63	0.60	0.00
	F_{IS}	0.01		−0.09	1.00**	0.00	0.37*	0.40		0.09	−0.29	
	L(HW)											
H118	A	2	2	2	2	3	3	2	2	3	1	2
	R	1.88	2.00	2.00	1.96	2.95	2.99	2.00	2.00	2.41	1.00	1.50
	H_e	0.13	0.46	0.46	0.18	0.51	0.62	0.51	0.47	0.30	0.00	0.04
	H_o	0.13	0.28	0.37	0.13	0.70	0.89	0.35	0.33	0.34	0.00	0.04
	F_{IS}	−0.06	0.40*	0.21	0.27	−0.38*	−0.46**	0.32	0.30	−0.17		
	L(HW)											

TABLE A.1.—Extended.

PLC	PVA	RIC	MOR	MTR	VDZ	PRE	EST	UCO	UTE	YER	All
1	1	5	2	1	2	1	2	9	6	3	13
1.00	1.00	4.61	1.41	1.00	2.00	1.00	2.00	5.51	3.79	3.00	4.30
0.00	0.00	0.60	0.03	0.00	0.33	0.00	0.51	0.48	0.60	0.66	
0.00	0.00	0.68	0.03	0.00	0.40	0.00	0.48	0.40	0.53	0.90	
		−0.14			−0.23		0.06	0.16*	0.11*	−0.39	
											<0.001
2	1	8	1	2	7	2	2	7	7	3	18
2.00	1.00	5.94	1.00	1.88	6.01	1.94	2.00	5.73	5.50	2.63	7.05
0.44	0.00	0.53	0.00	0.13	0.80	0.14	0.37	0.76	0.71	0.28	
0.37	0.00	0.55	0.00	0.07	0.79	0.15	0.23	0.73	0.75	0.32	
0.17		−0.03		0.48	0.01	−0.06	0.40*	0.03	−0.06	−0.07	
											NS
3	4	7	4	2	5	7	5	8	7	5	18
2.99	3.64	5.72	2.76	2.00	4.66	6.31	4.35	6.09	6.07	4.89	11.22
0.61	0.61	0.62	0.24	0.50	0.76	0.69	0.63	0.71	0.80	0.71	
0.50	0.63	0.65	0.20	0.55	0.83	0.70	0.32	0.72	0.77	0.60	
0.18	−0.05	−0.06	0.18	−0.10	−0.10	−0.01	0.49**	−0.02	0.04*	0.16	
											NS
3	2	5	2	3	3	3	4	5	4	2	9
3.00	1.40	4.18	2.00	3.00	2.97	2.60	3.50	4.60	3.59	2.00	6.12
0.65	0.03	0.62	0.30	0.66	0.60	0.47	0.58	0.71	0.61	0.46	
0.60	0.03	0.50	0.23	0.73	0.64	0.45	0.55	0.80	0.53	0.17	
0.08		0.20	0.24	−0.11	−0.07	0.03	0.06	−0.13	0.12	0.70*	
											NS
4	2	9	3	2	6	3	5	10	12	5	19
3.62	1.96	7.57	2.36	1.64	5.52	2.85	4.02	8.31	7.46	4.74	8.95
0.62	0.18	0.80	0.21	0.07	0.79	0.51	0.69	0.86	0.83	0.74	
0.60	0.20	0.85	0.10	0.00	0.80	0.75	0.55	0.87	0.75	0.63	
0.04	−0.09	−0.07	0.54*	1.00*	−0.01	−0.48	0.20	−0.01	0.10	0.10*	
											<0.001
2	1	4	1	2	2	4	2	5	3	3	8
2.00	1.00	3.98	1.00	2.00	1.88	3.20	2.00	3.20	2.97	2.85	4.30
0.44	0.00	0.69	0.00	0.44	0.13	0.54	0.40	0.42	0.46	0.43	
0.57	0.00	0.75	0.00	0.43	0.13	0.45	0.23	0.43	0.42	0.25	
−0.29		−0.09		0.02	−0.06	0.17	0.45*	−0.03	0.09	0.68*	
											NS
3	1	6	3	2	3	5	2	11	8	6	18
3.00	1.00	5.03	3.00	2.00	3.00	4.29	1.78	7.85	6.05	5.42	6.40
0.66	0.00	0.66	0.59	0.44	0.66	0.59	0.09	0.82	0.79	0.72	
0.59	0.00	0.60	0.67	0.43	0.67	0.60	0.10	0.77	0.78	0.70	
0.12		0.10*	−0.12	0.02	−0.01	−0.02	−0.03	0.06**	0.01	−0.01	
											NS
2	1	5	3	2	7	2	5	4	4	2	8
2.00	1.00	4.03	2.40	2.00	5.78	2.00	4.91	3.33	2.80	2.00	4.89
0.36	0.00	0.36	0.45	0.48	0.74	0.45	0.75	0.43	0.22	0.50	
0.40	0.00	0.40	0.23	0.50	0.83	0.55	0.35	0.40	0.12	0.42	
−0.10		−0.13	0.49*	−0.04	−0.13	−0.21	0.53**	0.08	0.46**	0.17	
											NS
1	1	6	2	2	3	1	2	3	4	2	10
1.00	1.00	5.59	1.88	2.00	2.40	1.00	1.78	2.64	2.40	1.94	4.07
0.00	0.00	0.82	0.13	0.51	0.52	0.00	0.09	0.36	0.46	0.14	
0.00	0.00	0.65	0.13	0.43	0.93	0.00	0.10	0.33	0.37	0.15	
		0.22	−0.06	0.15	−0.8**		−0.03	0.07	0.07	−0.06	
											NS
2	1	4	3	4	3	3	2	2	2	2	9
2.00	1.00	3.56	2.64	3.72	2.40	2.94	2.00	1.40	1.36	2.00	4.24
0.49	0.00	0.39	0.53	0.63	0.44	0.56	0.28	0.03	0.03	0.23	
0.57	0.00	0.25	0.63	0.70	0.47	0.65	0.32	0.03	0.03	0.26	
−0.49		0.36	−0.21	−0.12	−0.07	−0.18	−0.18		−0.01	−0.13	
											NS

TABLE A.1.—Continued.

Locus	Statistic	BIT	CAN	COL	DAL	ELR	FRJ	GAV	JIC	VJO	MCC	OSH
H126	A	2	1	5	2	7	6	4	3	7	2	2
	R	2.00	1.00	4.53	1.98	4.67	4.74	3.60	2.57	5.57	1.40	2.00
	H_e	0.50	0.00	0.58	0.21	0.61	0.46	0.68	0.32	0.54	0.03	0.51
	H_o	0.41	0.00	0.38	0.23	0.60	0.20	0.35	0.29	0.59	0.03	0.42
	F_{IS}	0.18		0.34*	−0.11	0.02*	0.57**	0.49*	0.12	−0.10		0.19
	L(HW)											
H220	A	5	2	3	2	7	4	4	4	5	4	1
	R	4.82	1.96	2.96	1.93	5.79	3.70	3.45	3.85	3.79	3.40	1.00
	H_e	0.70	0.18	0.50	0.16	0.72	0.70	0.47	0.70	0.59	0.68	0.00
	H_o	0.73	0.20	0.50	0.17	0.73	0.81	0.50	0.75	0.50	0.70	0.00
	F_{IS}	−0.04	−0.09	−0.01	−0.07	−0.01	−0.17	−0.07	−0.08	0.16	−0.03	
	L(HW)											
All loci	Mean A	3.67	2.17	4.50	2.08	5.92	4.00	4.00	2.50	5.42	2.50	1.92
	Mean R	3.34	1.95	3.77	2.05	4.39	3.37	3.71	2.29	4.48	2.23	1.82
	H_e	0.48	0.21	0.53	0.27	0.58	0.53	0.56	0.31	0.58	0.31	0.24
	H_o	0.45	0.19	0.48	0.25	0.59	0.56	0.51	0.28	0.59	0.33	0.25
	F_{IS}	0.06	0.08	0.11	0.07	0.04	−0.07	0.08	0.08	−0.02	−0.08	0.01
	L(HW)	0.006	<0.001	NS	NS	<0.001	<0.001	NS	NS	NS	NS	NS

TABLE A.1.—Extended. Continued.

PLC	PVA	RIC	MOR	MTR	VDZ	PRE	EST	UCO	UTE	YER	All
3	2	6	2	2	2	3	3	9	6	4	11
2.79	2.00	5.16	1.98	2.00	2.00	2.85	2.99	7.09	4.53	3.60	5.62
0.52	0.35	0.65	0.21	0.51	0.51	0.43	0.61	0.80	0.60	0.64	
0.67	0.30	0.60	0.23	0.63	0.97	0.40	0.74	0.63	0.57	0.50	
−0.28	0.13	0.08	−0.12	−0.25	−0.93**	0.08	−0.21	0.21*	0.06	0.20*	
											<0.001
5	1	4	1	1	5	4	2	6	5	6	16
4.74	1.00	3.59	1.00	1.00	3.80	3.60	2.00	4.71	3.92	4.77	8.40
0.66	0.00	0.51	0.00	0.00	0.59	0.65	0.51	0.59	0.64	0.49	
0.73	0.00	0.40	0.00	0.00	0.67	0.50	0.45	0.57	0.57	0.45	
−0.11		0.22			−0.12	0.24	0.11	0.03	0.12	−0.02	
											NS
2.58	1.50	5.75	2.25	2.08	4.00	3.17	3.00	6.58	5.67	3.58	
2.51	1.42	4.91	1.95	2.02	3.53	2.88	2.78	5.04	4.20	3.32	6.30
0.46	0.09	0.60	0.23	0.36	0.57	0.42	0.46	0.58	0.56	0.50	
0.47	0.19	0.57	0.21	0.37	0.68	0.43	0.37	0.56	0.52	0.45	
−0.02	0.00	0.05	0.09	−0.03	−0.19	−0.03	0.20	0.04	0.08	0.12	
NS	NS	NS	NS	NS	NS	NS	0.006	0.045	NS	0.006	