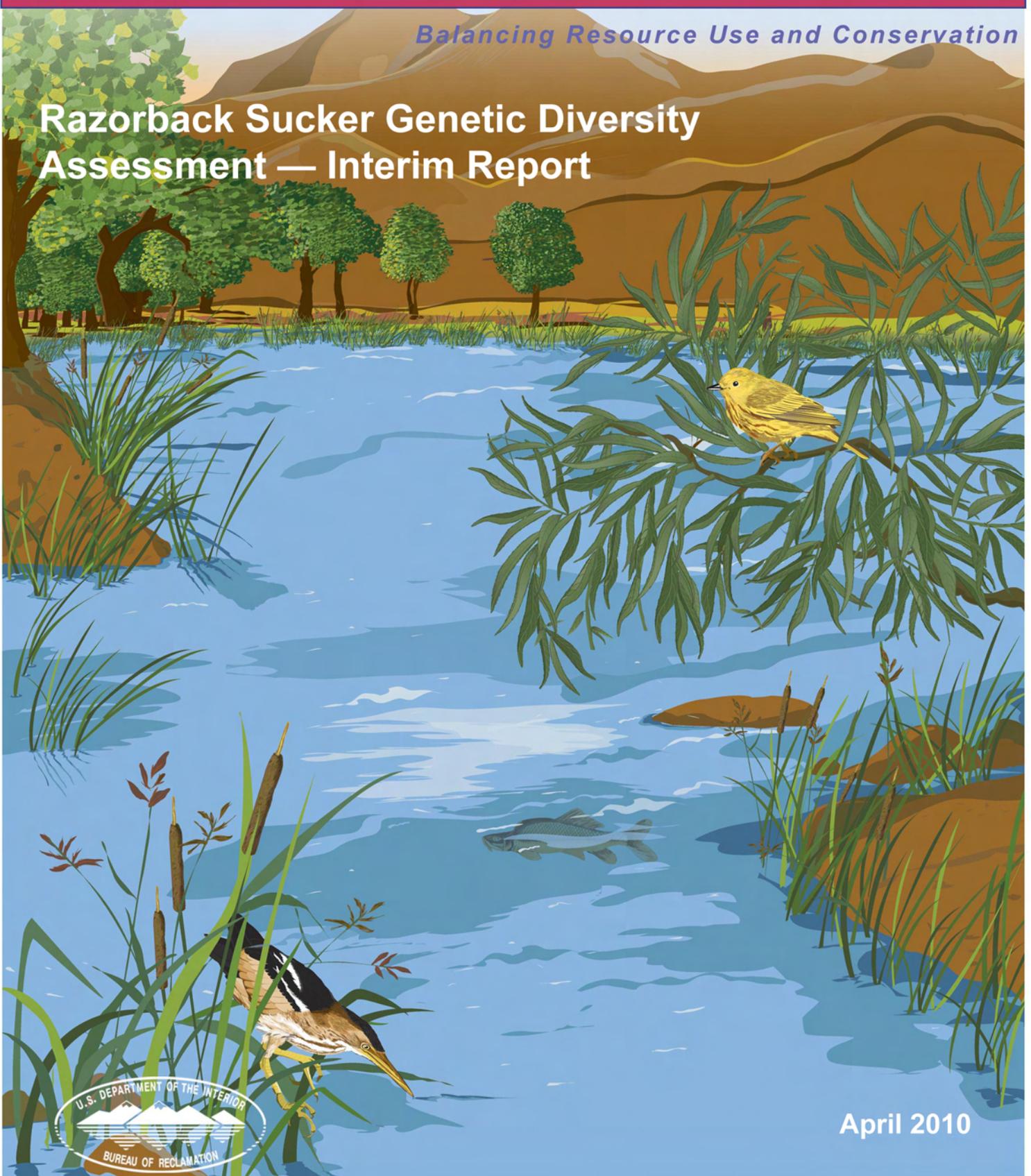




Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Razorback Sucker Genetic Diversity Assessment — Interim Report



April 2010

Lower Colorado River Multi-Species Conservation Program Steering Committee Members

Federal Participant Group

Bureau of Reclamation
U.S. Fish and Wildlife Service
National Park Service
Bureau of Land Management
Bureau of Indian Affairs
Western Area Power Administration

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Arizona Department of Water Resources
Arizona Electric Power Cooperative, Inc.
Arizona Game and Fish Department
Arizona Power Authority
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City of Lake Havasu City
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Yuma Irrigation District
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QuadState County Government Coalition
Desert Wildlife Unlimited

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Los Angeles Department of Water and Power
Palo Verde Irrigation District
San Diego County Water Authority
Southern California Edison Company
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The Metropolitan Water District of Southern California

Nevada Participant Group

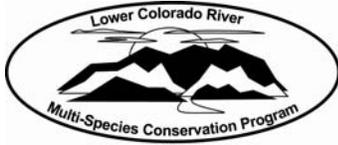
Colorado River Commission of Nevada
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The Nature Conservancy



Lower Colorado River Multi-Species Conservation Program

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In partial fulfillment of Agreement Number 09FG3000001 between Bureau of Reclamation and Arizona State University

Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
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We have continued monitoring levels of genetic variation within and among larval samples of razorback sucker from Lake Mohave and transmission of this variation into adult repatriate individuals. An additional 1327 larvae and 486 adult fin clips were obtained in the years 2008-2009 (Table 1). The larger number of samples includes expanded sampling from Lake Havasu (including the Colorado River downstream from Davis Dam to the head of Lake Havasu), Davis Cove, and the Imperial Ponds as prescribed in the current agreement. DNA has been extracted from most samples (the only exceptions being some of the adults from Lake Havasu). Genotypes have been obtained from all Lake Mohave adults, all 2009 larvae, and 60% of the 2008 larvae. Because characterization is incomplete, we have yet to complete formal statistical analyses; however, resulting patterns of variation appear to be consistent with previous years.

In addition to using our standard methods for characterizing mtDNA variation, a major goal behind this agreement was to expand our perspective on genetic variation. To achieve this goal, we assessed the use of microsatellite variation to characterize variation in the nuclear genome. We developed and optimized 15 sets of microsatellite primers (10 reported in Turner et al. 2009, the remaining five developed after and to be published elsewhere) using technical and statistical methods described in Dowling et al. (in review). To optimize and test these markers, we re-analyzed the basin-wide samples of razorback suckers previously examined by Dowling et al. (1996) for mtDNA restriction site variation. This includes samples from Lakes Mohave, Mead, and Powell, Green-Yampa River, and isolated off-channel ponds in the upper Colorado River basin.

Population genetic parameters were estimated and tested using the program FSTAT (Goudet 2001). As in the analysis of mtDNA, allelic richness was highest in the south and lowest in the upper Colorado River basin (Table 2). All samples were in Hardy-Weinberg

equilibrium for each locus after correction for multiple tests (Table 3). Note, however, many samples exhibited gametic disequilibrium for pairs of loci (Table 4), with nearly half of the significant tests coming from the upper Colorado River sample and the fewest from the Lake Mohave sample. The association between pairwise deviations and the upper Colorado River sample is consistent with production of these individuals by a small number of parents isolated in these off-channel ponds, and consistent with results from analyses of mtDNA (Dowling et al. 1996).

Analysis of population structure identified low but significant levels of variation among populations ($F_{ST} = 0.06$, SE from jackknife analysis across loci = 0.009), less than that identified from analysis of mtDNA ($F_{ST} = 0.23$). This estimate is consistent with the four-fold increase in effective population size in nuclear DNA, decreasing the effects of random factors and rate of differentiation among populations.

To further assess distribution of genetic variation, we used the program STRUCTURE (Pritchard et al. 2000, Falush et al. 2003) to determine the number of distinct groups of populations and the probability of assignment to specific groups as identified by analysis of 15 loci. This is a maximum likelihood approach that maximizes equilibrium within and disequilibrium among groups. The program is run K times (where K is the *a priori* assumed number of groups, in this case for 2 to 5 groups). Several replicates are run for each K (here we used 50 per K) and the fit assessed. Results for each K are best visualized by plotting the probability of assignment of specific individuals (Q) to specific groups. An example is provided in Figure 1 for a subset of *Gila robusta* samples (West Clear, Boulder, and Trout creeks, from Dowling et al. in review) where STRUCTURE was run for three groups ($K = 3$) for 50 replicates. Q values (assignment probabilities) are plotted on the y-axis (summed across replicates) while

individuals are plotted on the x-axis. Probability of assignment to different groups is identified by different colors, in this case blue, yellow, and orange. In this example, individuals of *Gila* from West Clear and Boulder creeks are generally assigned to the blue and brown groups, respectively, across the 50 replicates. The pattern for Trout Creek reflects variation in assignment across the 50 replicates, as all individuals are assigned to the brown group in approximately 1/3 of the replicates and were placed in the orange group in the remaining 2/3 of the replicates. Evidence for variation within replicates is depicted by the West Clear Creek sample, with levels of assignment to blue and orange groups varying among individuals.

To assess population structure among razorback sucker groups, we ran the program for up to five groups ($K = 2-5$), yielding the results in Figure 2. Consistency across replicates (h') is highest for K , and patterns of variation indicate that either 2 or 3 distinct groups exist. For $K = 3$, individuals are generally assigned to three geographically distinct groups representing the upper Colorado River, Green-Yampa-Powell, and Mead-Mohave. The upper Colorado sample is the most distinct, potentially resulting from the small number of individuals isolated in those backwater ponds. While the remaining sets of samples exhibit some distinctiveness, there is evidence for admixture among them as indicated by intermediate probabilities of assignment for some individuals to multiple groups. Results from mtDNA analyses reported by Dowling et al. (1996) were consistent with a single razorback sucker stock in the Colorado River. The microsatellite data reveal finer scale population structure, separating out the isolated upper Colorado River population and providing some evidence for disruption to gene exchange above and below the Grand Canyon.

We are also interested in using microsatellites as markers to identify specific individuals allowing us to examine reproductive contribution of specific individuals. Using 15 loci, we were

able to discriminate more than 80% of the individuals. From Lake Mohave, 96% of the 50 individuals examined possessed distinct multilocus genotypes, indicating it would be possible to genetically identify specific individuals with high probability and to assess their reproductive success.

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Table 1. Summary of larval and adult samples collected in 2008 and 2009.

Year	Location	Larvae	Adults
2008	Mohave	631	55
2009	Davis Cove	0	23
	Havasu	51	228
	Imperial	25	0
	Mohave	620	180
		1327	486

Table 2. Allelic richness (number of alleles corrected for sample size) from 15 microsatellite loci for five razorback sucker samples scattered throughout the Colorado River basin.

Locus	Mohave	Mead	Powell	Green-Yampa	Upper Colorado	mean
Xte01	2.00	2.00	2.00	2.00	2.00	2.00
Xte02	2.40	2.94	2.00	1.60	2.90	2.73
Xte04	6.04	3.87	5.31	5.24	1.00	5.13
Xte07	8.62	6.87	4.79	3.80	2.59	7.16
Xte08	12.18	10.69	8.78	8.08	7.45	11.20
Xte10	8.42	7.99	7.93	7.11	4.00	8.07
Xte11	10.16	8.87	8.74	9.29	3.90	9.96
Xte12	7.96	7.93	6.81	7.26	4.00	7.80
Xte14	6.23	4.81	5.31	5.11	1.00	5.13
Xte16	15.44	13.00	11.28	7.50	5.56	12.54
Xte17	10.22	9.00	5.67	7.93	3.00	8.72
Xte18	6.74	4.94	4.00	4.52	3.68	5.84
Xte19	10.34	8.75	7.62	4.63	2.90	8.80
Xte20	17.95	18.42	11.38	8.68	2.68	15.00
Xte22	17.33	14.55	10.64	10.62	4.97	14.57
mean	9.47	8.31	6.82	6.22	3.44	

Table 3. Tests of Hardy-Weinberg equilibrium for each locus. * identifies significant tests at the $P < 0.05$; however, no tests were significant when corrected for multiple tests ($P < 0.00067$).

Locus	Mohave	Mead	Powell	Green-Yampa	Upper Colorado
Xte01	0.147	-0.304	0.000	-0.047	0.323
Xte02	-0.059	-0.047	-0.063	0.000	-0.122
Xte04	0.004	-0.062	0.101	-0.143	NA
Xte07	0.196*	0.115	0.046	0.197	-0.033
Xte08	-0.027	-0.154	0.064	0.051	0.021
Xte10	0.055	0.091	-0.175	0.223*	-0.102
Xte11	0.007	0.009	0.041	0.009	-0.016
Xte12	-0.051	0.032	0.103	-0.026	-0.157
Xte14	-0.005	-0.079	0.101	-0.138	NA
Xte16	0.018	-0.028	0.147	-0.041	0.009
Xte17	0.075	0.011	-0.102	-0.004	0.106
Xte18	-0.002	-0.193	-0.128	0.010	0.267
Xte19	0.088	-0.048	-0.084	0.019	0.087
Xte20	0.018	0.032	0.100	-0.021	-0.464
Xte22	0.100*	-0.004	-0.090	-0.125	-0.227

Table 4. Number of significant deviations from pairwise gametic disequilibrium for each of the five samples.

	Mohave	Mead	Powell	Green-Yampa	Upper Colorado	All
# sig multilocus tests	4	8	5	10	26	53
# tests	105	105	105	105	78	498
proportion sig tests	0.04	0.08	0.05	0.10	0.33	0.11

Table 5. Number of genetically distinct individuals identified by analysis of 15 microsatellite loci in each of the five populations.

	Mohave	Mead	Powell	Green-Yampa	Upper Colorado
sample size	50	16	18	25	22
# genetically distinct individuals	48	14	15	21	22
percent	0.96	0.88	0.83	0.84	1.00

Figure 1. Example of an assignment probability plot, with probability of assignment (y-axis) to a specific group (identified by different colors) plotted for each individual (x-axis). This plot is for $K = 3$, with 29, 30, and 30 individuals depicted from West Clear, Boulder, and Trout Creek samples of *Gila robusta*.

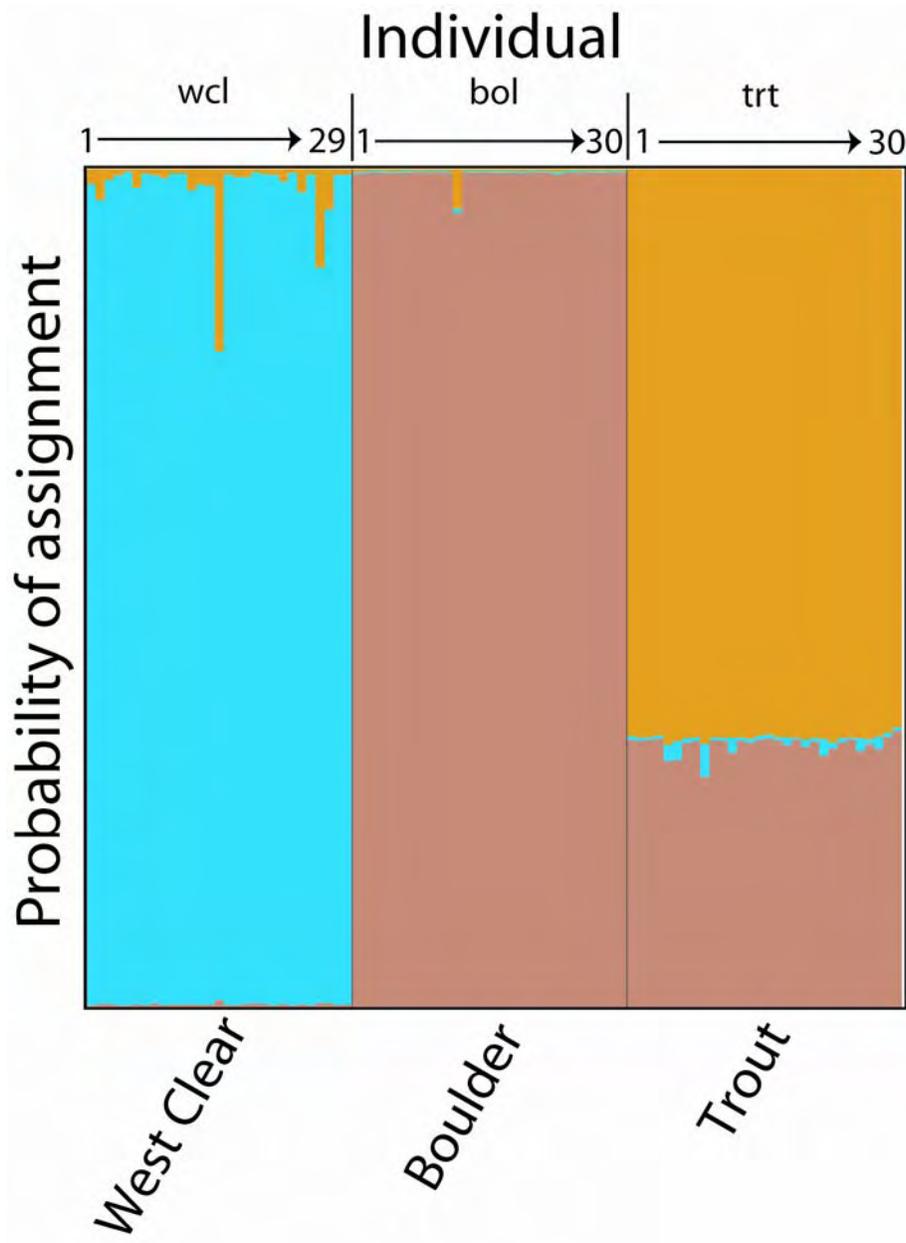


Figure 2. Assignment probability plots for all populations of *X. texanus*. “k#” indicates the assumed group size (2-5) and “h’” the statistic measuring consistency across the 10 replicate runs at that value of *K* (closer to 1 indicates greater similarity among replicates). Colors have no significance across values of *K*.

