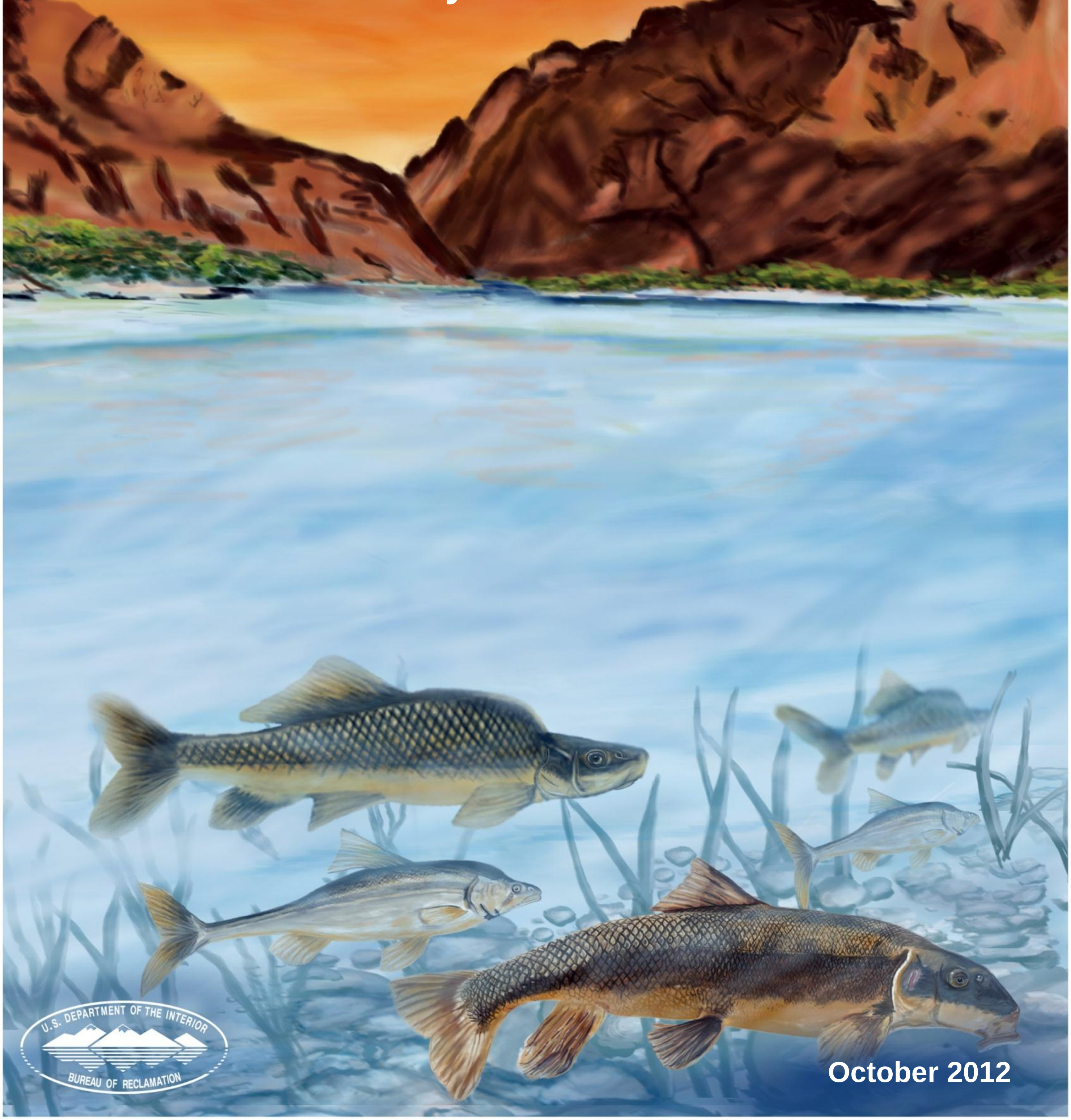
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

RASU Genetic Diversity Assessment



October 2012

Lower Colorado River Multi-Species Conservation Program
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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
Central Arizona Water Conservation District
Cibola Valley Irrigation and Drainage District
City of Bullhead City
City of Lake Havasu City
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City of Somerton
City of Yuma
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Mohave Water Conservation District
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Conservation Participant Group

Ducks Unlimited
Lower Colorado River RC&D Area, Inc.
The Nature Conservancy

Lower Colorado River
Multi-Species Conservation Program

RASU Genetic Diversity Assessment

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Multi-Species Conservation Program
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Lower Colorado Region
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October 2012

Project title: Work task C31 of the LCR MSCP - RASU Genetic Diversity Assessment

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In partial fulfillment of Agreement Number R12AC3001
Between U.S. Bureau of Reclamation and Arizona State University

In 2012, we continued to monitor levels of genetic variation within and among samples of adult and larval razorback sucker from the lower Colorado River and its reservoirs. DNA has been extracted from all samples. Mitochondrial DNA was characterized for all adult and larval samples, while microsatellite variation was analyzed from a subset of individuals in order to characterize variation in the nuclear genome (Dowling et al. 2011). Population genetic parameters were estimated and tested using the programs FSTAT (Goudet 2001) and Arlequin (Excoffier and Lischer 2010).

Lower River.— MtDNA was characterized for 219 and 54 adults and larvae, respectively, collected from the lower Colorado River (Table 1), identifying 12 and 8 haplotypes respectively. These values are comparable to those reported previously for this region (Dowling and Marsh 2011).

Lake Mead.— We characterized 61 adult and 50 larval samples collected from Lake Mead in 2012 for patterns of variation with mtDNA and microsatellites. Number of haplotypes was low, especially when compared to other locations (Table 1). We also compared this sample with previous samples from Lake Mead. Levels of mtDNA and microsatellite variation in adults were similar to those from samples collected in Lake Mead in 2011, with mtDNA variation lower than in samples from 2002 (Table 2). There were no larval samples collected in 2011, however, the two larval samples from 2012, Echo Bay and Las Vegas Bay, exhibited considerably lower levels of mtDNA diversity than those from 2002. Microsatellite diversity in the 2012 sample of larvae from Echo Bay was comparable to earlier samples, and the 2012 sample from Las Vegas Bay was slightly elevated. Levels of mtDNA variation in larvae sampled in 2012 were still lower than those found in the 2012 adult sample, with variation at microsatellite loci comparable.

Distribution of genetic variation within and among samples was estimated with both mtDNA and microsatellite data. Analysis of mtDNA data identified significant structure (F_{ST} = 0.142, P < 0.0001) that reflects variation among samples (F_{SC} = 0.134, P < 0.0001), but not life history stage or lake (F_{CT} = 0.008, P = 0.211). Similar analysis of microsatellite data provided essentially the same result. The jackknife average of total genetic variation ($F \approx F_{IT}$) across microsatellite loci was 0.069 (95% bootstrap confidence interval 0.045 - 0.095). The within population component ($f \approx F_{IS}$) was significantly different from 0 (average = 0.040, 95% bootstrap confidence interval 0.014 to 0.067), indicative of deviations from Hardy-Weinberg equilibrium reported previously (Dowling et al. 2012). The among-sample component ($\Theta \approx F_{ST}$) was smaller than that within populations, with a significant jackknife average of 0.031 (95% bootstrap confidence interval 0.026 - 0.035). These values are comparable to previous estimates that did not include 2012 samples (Dowling et al. 2012).

These analyses indicate that levels of genetic variation remain low in Lake Mead, causing some concern. Because razorback sucker in Lake Mead are not significantly different from those in Lake Mohave, it would be possible to infuse additional variation into the Lake Mead population through augmentation with Lake Mohave fish. This approach, however, could hamper efforts to examine genetic and demographic factors associated with putative recruitment in Lake Mead, information critical for informed management of razorback sucker. Therefore, such actions should be carefully examined before implementation.

Lake Mohave.— We characterized 122 adult and 550 larval samples collected from Lake Mohave in 2012 for patterns of variation with mtDNA, and all adults and 120 larvae for microsatellites. Analysis of mtDNA in adults and larvae identified 14 and 21 haplotypes, respectively, comparable to previous years (Table 3).

To examine patterns of variation over time, we calculated allelic richness (A_R – number of alleles corrected for sample size) for the larval sample (Table 4). This estimate from mtDNA (A_R = 5.00) was smaller than 2011 but higher than many previous years, with a positive slope and strong correlation between time and allelic richness (slope = 0.06, R^2 = 0.52, Figure 1A). Characterization of microsatellite variation also yielded similar patterns of variation to previous years, indicating little change in levels of allelic richness over time (slope = -0.017, R^2 = 0.07, Figure 1B).

Examination of the structure of mtDNA variation in larvae over time and space yielded values consistent with previous years (Dowling et al. 2011), with no geographic structure but considerable differences among temporal samples (Table 5). Analysis of microsatellite variation across years from 1997-2012 yields a jackknife average (across loci) of total variation ($F \approx F_{IT}$) of 0.062 (95% bootstrap confidence interval 0.043 - 0.084). The within population component ($f \approx F_{IS}$) exhibited a jackknife average of 0.057 that was significantly different from 0 (95% bootstrap confidence interval 0.039 to 0.078), a result that is consistent with HWE results discussed in Dowling et al. (2011). The among-year component ($\Theta \approx F_{ST}$) was an order of magnitude smaller than that within years, with a significant jackknife average of 0.006 (95% bootstrap confidence interval 0.002 - 0.011). These results are nearly identical to those calculated in 2011, indicating no change in distribution of allele frequencies over time.

We were also interested in fine scale variation in larval production; therefore, we obtained multiple samples collected in the same week from two locations (Carp Cove – 13-15 March 2012, N = 25, 17, and 27 respectively; Tequila Cove – 14-15 March 2012, N = 25 and 27, respectively). Analysis of microsatellite variation failed to identify significant differences among regions (F_{CT} = 0.004, P = 0.110) or samples (F_{SC} = 0.001, P = 0.274), indicating that samples of larvae collected during the same week are not genetically different. Therefore, collection of one sample per week is adequate and would presumably continue to represent genetic variation of the adult population.

While there was a decline in mtDNA variation in 2012 compared to 2011, overall patterns of variation are generally consistent with those found in previous years, indicating that levels of genetic variation continue to be maintained by the current management program. As long as adult population size remains low, however, there are concerns over the impact of random effects on this population. This can only be alleviated by increasing adult population size, by any means possible.

Literature cited

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Table 1. MtDNA haplotypes for larval and adult samples from the Lower River and Lake Mead, lower Colorado River, Arizona and Nevada, collected in 2012.

	Lower River		Lake Mead	
Haplotype	larvae	adults	larvae	adults
A	1	11		
B	2	6		
C		5		
D	1			
E	37	108	45	38
F	2	26		
P			5	20
J		1		
R	7	47		3
S	3	9		
U		1		
Z		3		
BB	1			
CC		1		
FF		1		
Total	54	219	50	61

Table 2. Diversity indices and their standard deviations based on mtDNA and microsatellite variation for each razorback sucker sample, Lake Mead and Lake Mohave, Arizona and Nevada. N, N_h, A_R, H_O, and H_E are sample size, number of haplotypes, corrected number of haplotypes or alleles (Kalinowski 2005), observed heterozygosity, and expected heterozygosity in each sample, respectively. A_R and heterozygosities for microsatellites are averaged across all loci. Estimates of haplotype diversity and heterozygosities include standard errors. An asterisk identifies values significantly lower than expectations ($P < 0.05$) as determined by resampling as described in Dowling et al. (2012). Samples for 2012 are identified in blue type.

	mtDNA				microsatellites			
Location	N	N_h	A_R	haplotype diversity (h)	N	A_R	H_O	H_E
Lake Mead								
Adults - 1988	16	2*	1.92	0.1250 +/- 0.1064*	15	8.00	0.691 +/- 0.253	0.741 +/- 0.262
Adults - Echo Bay 2002	11	4	4.00	0.7091 +/- 0.0990	11	7.07	0.703 +/- 0.278	0.712 +/- 0.270
Adults - Las Vegas Bay 2002	18	5	4.07	0.6601 +/- 0.1020	18	7.99	0.697 +/- 0.229	0.759 +/- 0.226
Adults - 2011	15	2*	1.94	0.2476 +/- 0.1307*	15	7.60	0.707 +/- 0.221	0.756 +/- 0.225
Adults - 2012	61	3*	2.45	0.5104 +/- 0.0449	61	6.55	0.719 +/- 0.211	0.720 +/- 0.217
Larvae - Echo Bay 1997	25	3*	2.44	0.6100 +/- 0.0588	25	6.32	0.725 +/- 0.251	0.730 +/- 0.224
Larvae - Echo Bay 2002	30	5	3.83	0.7057 +/- 0.0493	30	6.78	0.714 +/- 0.190	0.742 +/- 0.193
Larvae - Las Vegas Bay 2002	27	4*	3.22	0.6410 +/- 0.0561	27	6.41	0.786 +/- 0.225	0.754 +/- 0.211
Larvae - Echo Bay 2012	25	2*	1.92	0.2200 +/- 0.0995*	25	6.39	0.664 +/-0.268	0.688 +/- 0.268
Larvae - Las Vegas Bay 2012	25	2*	1.44	0.0800 +/- 0.0722*	25	7.16	0.696 +/- 0.209	0.751 +/-0.219
Lake Mohave								
Adults	49	11	4.84	0.6420 +/- 0.0766	50	8.75	0.709 +/- 0.253	0.762 +/- 0.269
Larvae - 2011	120	14	4.42	0.5965 +/- 0.0512	120	8.63	0.715 +/- 0.233	0.754 +/- 0.261

Table 3. Distribution of mtDNA haplotypes for larval and adult samples collected in Lake Mohave, Arizona and Nevada, for 2010-2012.

	2010		2011		2012		
Haplotype	adults	larvae	adults	larvae	adults	larvae	Total
a	4	15	5	22	3	20	69
b	9	37	9	34	4	42	135
c	1	18	4	13	1	22	59
e	81	301	105	326	82	311	1206
f	1	8	1	8	5	11	34
g	2	4	2	8	1	16	33
h		9	2	1	3	4	19
i		1	1	6		1	9
j	1	5		6		4	16
k	2		1	4	1	3	11
m		1		5		1	7
n						1	1
p	1	2	1	15	1	7	27
q		5		2	1	5	13
r	13	33	11	32	10	57	156
s	10	27	14	29	6	30	116
u				4		6	10
v	1	2		3		1	7
z						1	1
bb		5	4	3	1	6	19
cc	3	1		2	3		9
gg		2					2
hh		2		1			3
ii				1		1	2
Total:	129	478	160	525	122	550	1964

Table 4. Allelic richness from larval samples collected in Lake Mohave, Arizona and Nevada, for 1997-2012.

Locus	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Xte1	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Xte2	4.00	4.00	3.95	3.00	4.00	4.00	3.98	4.00	4.00	4.00	4.00	4.00	4.00	4.00	5.00	4.00
Xte7	19.82	20.87	19.82	14.87	19.92	17.85	17.92	14.90	17.87	17.87	13.95	15.87	16.92	17.97	19.92	21.90
Xte8	19.95	19.95	18.97	21.90	22.87	18.95	21.95	20.90	18.95	21.92	18.92	21.87	21.90	19.90	21.92	17.97
Xte10	10.00	10.00	10.00	11.97	11.00	10.97	11.98	11.98	12.00	11.95	10.98	12.00	9.98	10.98	10.97	11.00
Xte11	17.87	15.87	16.92	17.88	17.95	19.87	14.87	16.90	16.87	14.97	14.92	17.85	16.83	16.87	13.95	16.90
Xte12	12.90	11.97	13.92	10.97	12.92	10.98	13.97	11.95	10.98	11.97	10.98	12.98	13.93	9.97	13.95	11.95
Xte16	31.77	26.92	25.92	30.00	30.00	26.92	29.95	23.98	27.95	25.87	29.82	26.97	27.87	25.90	30.90	23.00
Xte17	21.87	19.95	19.92	19.97	19.95	21.95	16.97	21.90	20.97	19.95	18.90	19.97	20.90	18.95	20.97	21.95
Xte18	8.98	8.98	8.95	8.00	7.98	7.98	7.00	7.00	8.97	8.00	9.97	10.00	7.97	7.00	8.00	8.00
Xte19	17.90	15.92	15.93	15.97	16.97	14.95	15.97	14.97	15.92	15.95	17.92	17.97	16.90	14.95	15.97	16.00
Xte20	29.92	33.92	35.80	35.97	31.95	34.92	34.87	34.95	34.90	33.85	35.90	35.91	33.95	30.98	32.90	35.92
Xte22	34.92	31.98	28.90	34.87	32.95	32.87	32.97	31.95	30.95	32.90	30.92	32.97	31.95	32.87	31.90	33.90
Xte24	22.00	22.95	23.93	23.98	22.95	23.87	23.90	22.97	23.90	24.97	23.92	24.00	22.95	24.93	23.95	21.00
Xte25	4.95	3.98	3.00	4.00	3.00	3.00	4.00	3.00	3.00	4.00	3.00	3.00	3.00	5.93	3.98	3.00
Average	18.35	17.66	17.57	18.10	18.17	17.79	17.88	17.24	17.66	17.73	17.44	18.24	17.79	17.23	18.16	17.61

Table 5. Results from AMOVA analysis of mtDNA haplotype frequencies for razorback sucker from Lake Mohave, Arizona and Nevada, for each of the years represented.

Year	# of collections	N	F_{ST}	P	F_{CT}	P	F_{SC}	P
1997	13	338	0.088	<0.0001	-0.021	0.845	0.110	<0.0001
1998	19	484	0.043	<0.0001	-0.002	0.512	0.045	<0.0001
1999	13	294	0.039	<0.0001	-0.012	0.715	0.050	0.001
2000	16	367	0.049	<0.0001	-0.009	0.758	0.058	<0.0001
2001	10	230	0.102	<0.0001	-0.001	0.522	0.103	0.001
2002	14	348	0.020	0.015	-0.004	0.651	0.024	0.016
2003	14	370	0.060	<0.0001	0.023	0.069	0.037	0.004
2004	24	560	0.147	<0.0001	0.010	0.240	0.138	<0.0001
2005	17	437	0.059	<0.0001	0.001	0.380	0.058	<0.0001
2006	23	571	0.062	<0.0001	0.000	0.430	0.063	<0.0001
2007	13	308	0.043	<0.0001	-0.012	0.740	0.054	<0.0001
2008	24	576	0.057	<0.0001	0.004	0.275	0.053	<0.0001
2009	21	517	0.097	<0.0001	-0.019	0.994	0.113	<0.0001
2010	19	478	0.042	<0.0001	-0.006	0.761	0.047	<0.0001
2011	19	469	0.011	0.059	0.000	0.51	0.011	0.074
2012	22	550	0.033	<0.0001	0.006	0.113	0.027	<0.0001

Figure 1. Mean allelic richness for mtDNA (A) and microsatellites (B) from samples of razorback sucker, Lake Mohave, Arizona and Nevada.