Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Razorback Sucker Genetic Diversity Assessment

2016 Annual Report

July 2020

Work conducted under LCR MSCP Work Task C31

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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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Lower Colorado River Multi-Species Conservation Program

Razorback Sucker Genetic Diversity Assessment

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July 2020

Dowling, T.E., P.C. Marsh, and T.F. Turner. 2018. Razorback Sucker Genetic Diversity Assessment, 2016 Annual Report. Submitted to the Lower Colorado River Multi-Species Conservation Program, Bureau of Reclamation, Boulder City, Nevada, by Wayne State University, Detroit, Michigan; Marsh & Associates, LLC, Tempe, Arizona; and University of New Mexico, Albuquerque, New Mexico, under Agreement No. R14AC00004.

ACRONYMS AND ABBREVIATIONS

AMOVA	molecular analysis of variance
A_R	allelic richness
Arlequin	a new series of programs to perform population genetics analyses under Linux and Windows
CI	confidence interval
CRI	Colorado River inflow
cyt*b*	mitochondrial deoxyribonucleic acid cytochrome *b* gene
D_a	estimate of divergence among groups
DNA	deoxyribonucleic acid
dNTPs	dinucleotide triphosphates
F_{CT}	amount of genetic variation among regions
F_{IS}	variation among individuals within populations (standard inbreeding coefficient)
F_{SC}	amount of genetic variation among samples within regions
F_{ST}	amount of genetic variation among samples
FSTAT	a program to estimate and test gene diversities and fixation indices
H_E	expected heterozygosity
H_R	number of haplotypes corrected for sample size
$MgCl_2$	magnesium chloride
min	minute(s)
mM	micromolar
mtDNA	mitochondrial deoxyribonucleic acid
N	sample size
N_h	number of haplotypes
PCR	polymerase chain reaction
PopART	Population Analysis with Reticulate Trees
POPTREE2	Software for constructing population trees from allele frequency data and computing other population statistics with Windows-interface

Symbols

~	approximately
ºC	degrees Celsius
>	greater than
≥	greater than or equal to
µL	microliter
%	percent
±	plus or minus
Θ	level of genetic variation among populations

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INTRODUCTION

Genetic monitoring has been a useful tool for studying population dynamics, providing a valuable complement to traditional ecological and demographic monitoring (reviewed in Dowling et al. 2014). It has been especially useful in studies of razorback suckers (*Xyrauchen texanus*), as it provides information on the levels and patterns of variation in wild adults, larvae, and repatriated individuals (Dowling et al. 2005, 2014; Carson et al. 2016).

Razorback suckers were once abundant and broadly distributed, occupying much of the Colorado River Basin (figure 1). Genetic monitoring of the levels of genetic variation within and among samples of adult and larval razorback suckers from Lake Mead (Reach 1) and Lake Mohave (including the main basin and the reach above Willow Beach – Reach 2), as well as the lower Colorado River in the Lake Havasu region (Reach 3), continues, and the results from 2016 are reported herein.

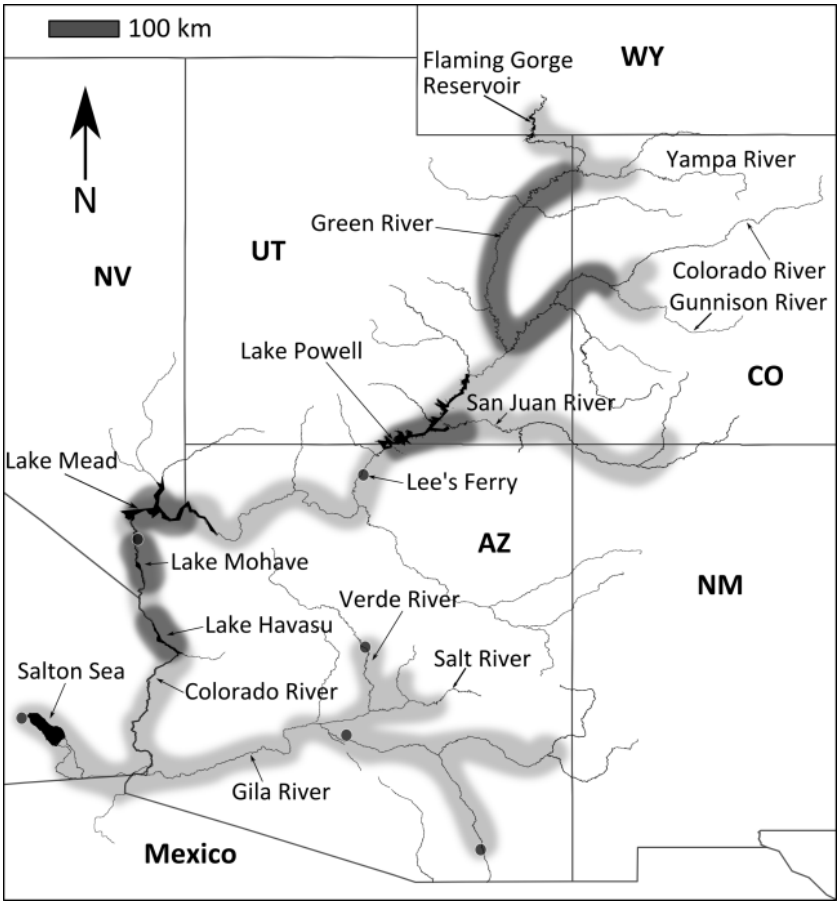

Figure 1.—Historical (light gray) and present (dark gray) distributions of razorback suckers in the Colorado River Basin of Western North America.
Point locations (circles) represent archaeological sites (taken from Marsh et al. 2015).

METHODS

Characterization of Genetic Variation

Deoxyribonucleic acid (DNA) was extracted from larvae and fin clips using a standard phenol-chloroform extraction protocol (Tibbets and Dowling 1996). The quantity and quality of DNA were assessed using a NanoDrop ND-1000 Spectrophotometer (V3.0.1). Genetic variation among samples was assessed using a small (310-base pair) fragment of the mitochondrial deoxyribonucleic acid (mtDNA) gene cyt*b* (Dowling et al. 2005) and 14 dinucleotide microsatellite loci. Variation in cyt*b* was characterized by direct sequencing of polymerase chain reaction (PCR) products from all individuals as described previously (Dowling et al. 2005, 2014). Microsatellite loci were obtained from a library for razorback suckers (Turner et al. 2009; Dowling et al. 2014). Microsatellite fragments were amplified in the following multiplexed PCRs using an Eppendorf Mastercycler thermal cycler: multiplex 1: Xte1, Xte7, Xte20; multiplex 2: Xte2, Xte10, Xte16, multiplex 3: Xte11, Xte12, Xte19, Xte24; and multiplex 4: Xte8, Xte17, Xte18, Xte25. Multiplexed amplifications were completed in 5-microliter (µL) reactions consisting of 2.5 µL of Qiagen Master Mix (Taq DNA Polymerase, PCR buffer, magnesium chloride [$MgCl_2$], and dinucleotide triphosphates [dNTPs]), 0.01 to 0.45 µL of each primer (varies from 0.2 to 9 micromolars), 1 µL of water, and 0.5 µL of genomic DNA (100–1,000 nanograms). Multiplex 1 was amplified using a profile that followed a traditional PCR format. The first step was a denaturation of 15 minutes (min) at 95 degrees Celsius (°C), followed by 25 cycles of 94 °C for 30 seconds for denaturation, an annealing step of 53 °C for 90 seconds, and 72 °C for 1 min, and the amplification was completed with a single step at 72 °C for 10 min. Multiplexes 2, 3, and 4 were amplified using a profile that followed a touchdown format: initial denaturation of 15 min at 95 °C; 25 cycles of 94 °C for 30 seconds, annealing at 65 °C for 1.5 min, and 72 °C for 1 min. The annealing temperature decreased by 1 °C in each of the first 15 cycles and remained at 50 °C for the remaining 10 cycles. Amplification was completed with a single step at 72 °C for 10 min. A microsatellite fragment analysis was completed using a Li-Cor 4300 DNA Analyzer. Five lanes of size standard (50–350 base pairs from Li-Cor) were incorporated on each run, and each gel was scored using the computer software Saga (version 3.3). The software determines the size of each fragment using the size standards and assigns a genotype to each individual. Consistency of allele assignment across gels was obtained by manual comparison of results from multiple gels simultaneously.

Statistical Analysis

MtDNA sequences were visualized and corrected with ChromasLite (version 2.4.4, https://technelysium.com.au/wp/chromas/). Corrected sequences were aligned using BioEdit (Hall 1999, version 7.2.3, http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Aligned sequences were entered into MEGA 7.0 (Kumar et al. 2016) with representatives of known haplotypes (A through II) and unknown haplotypes identified by constructing a neighbor-joining tree. Arlequin (version 3.5.2.2) (Excoffier and Lischer 2010) was used to quantify the number of alleles, calculate gene diversity, and to perform a molecular analysis of variance (AMOVA). Standard statistical analyses (e.g., regressions) were performed using Microsoft Excel.

Relationships among mtDNA haplotypes and their relative abundance in samples were visually examined using minimum spanning networks (Bandelt et al. 1999) generated with Population Analysis with Reticulate Trees (popART) (version 1.7, http://popart.otago.ac.nz). AMOVA was used to partition overall genetic differences among samples (F_{ST}) into the portions attributable to differences among regions (F_{CT}) and among temporal samples within regions (F_{SC}). The number of alleles was corrected by rarefaction (Heck et al. 1975) with the program HPRare (Kalinowski 2005), yielding haplotypic richness (H_R – number of haplotypes corrected for sample size, Kalinowski [2005])). Neighbor-joining trees were also generated from pairwise estimates of F_{ST} (a measure of differentiation among samples) using MEGA.

Microsatellite data were downloaded from Saga into a tab-formatted sheet, uploaded into Excel, and converted to various formats for analysis. Population genetic parameters (allelic richness [A_R], expected heterozygosity [H_E], and the standard inbreeding coefficient [F_{IS}]) were calculated with FSTAT (Goudet 2001). A_R was corrected by rarefaction, in which the group of samples was standardized using the smallest sample (Heck et al. 1975). Corrections for multiple tests followed the B-Y method (Narum 2006). These parameters measure the level of genetic diversity, with higher values of A_R and H_E indicating more genetic diversity and more breeding individuals. F_{IS} examines the relationship of expected and observed heterozygosity, providing a population level measure of relatedness among individuals.

Among-group differences for microsatellite data were also estimated using FSTAT. The significance of among-group variation was examined using the program POPTREE2 (Takezaki et al. 2010), and estimates of divergence among groups (D_a) were clustered using the neighbor-joining method.

RESULTS AND DISCUSSION

Lake Mohave (Reach 2)

Sampling of adults and larvae yielded 128 and 503 individuals, respectively, from four regions (Nine Mile area, Tequila Cove, Yuma Cove, and above Owl Point) in

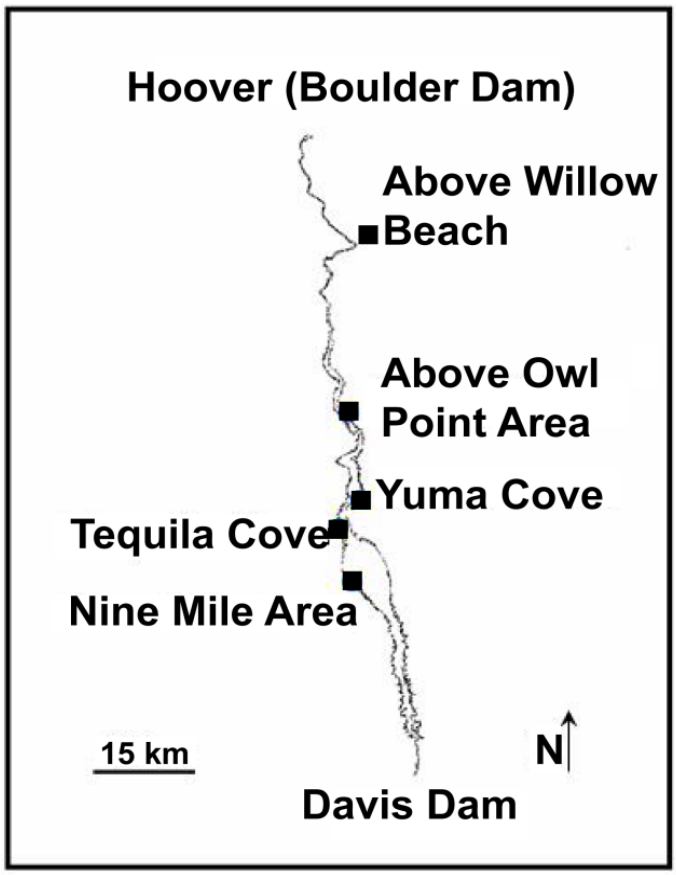

Figure 2.—Major regions of the Lake Mohave basin (modified from Dowling et al. 2005).

the main basin of Lake Mohave in 2016 (figure 2). All larval and adult samples have been characterized for mtDNA, and all adults and 117 randomly selected larvae have been genotyped for 14 microsatellite loci. All haplotypes sampled from all regions since 1997 were similar to each other, differing by at most two mutational steps (figure 3). An analysis of mtDNA in adults and larvae sampled identified 15 and 19 haplotypes, respectively, due to 27 variable positions. Of these 27 changes, 8 resulted in amino acid substitutions; however, 7 of them did not change types of amino acids and were not likely to impact protein function.

Haplotype distribution was similar to previous years (table 1). Measures of genotypic variation (number of haplotypes, gene diversity) for each larval sample met expectations as compared to the original wild population, with only two Lake Mohave samples exhibiting reduced numbers of alleles and genetic diversity (table 2). To examine patterns of variation in the larval samples over time, average H_R and average gene diversity were compared across all larval samples. H_R and gene diversity are standard measures of genetic variation with H_R generally more sensitive to reductions in population size than gene diversity.

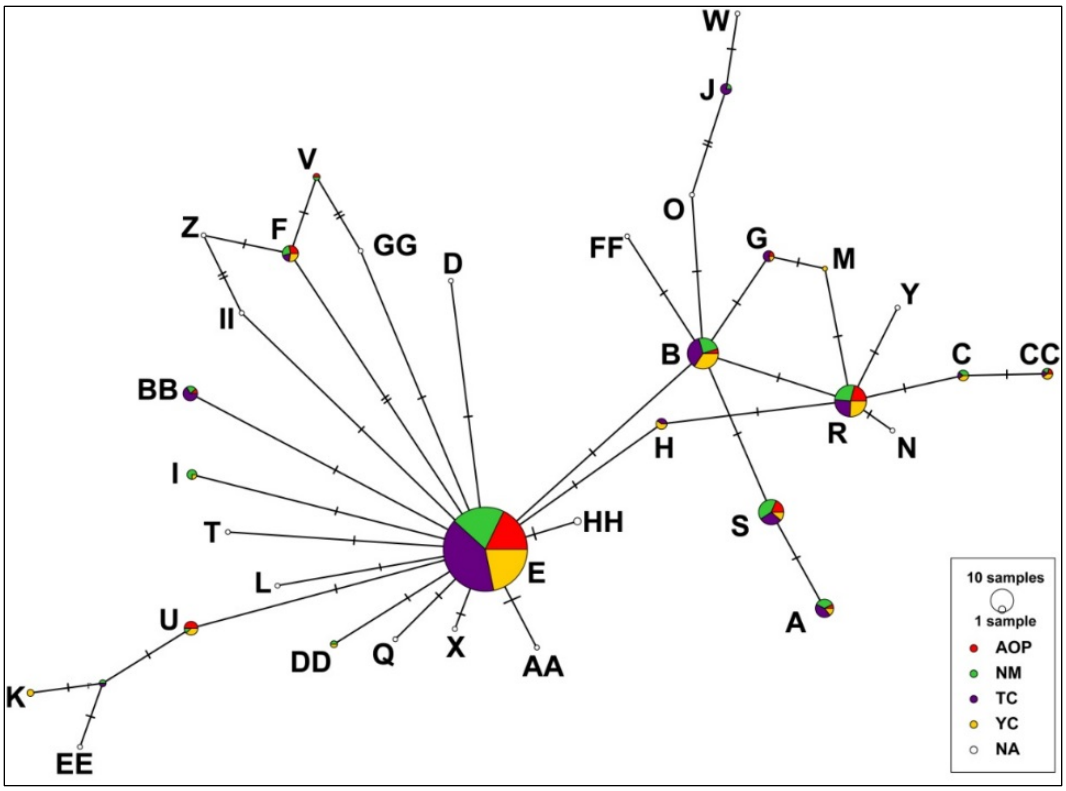

Figure 3.—Minimum spanning network showing relationships of all mtDNA cyt*b* haplotypes found in razorback suckers.
Hash marks on branches identify the number of base differences between haplotypes. The size of the circle identifies numbers of larvae collected from Lake Mohave in 2016, with specific colors in the specific haplotypes representing spawning region of origin (AOP = above Owl Point; NM = Nine Mile reach; TC = Tequila Cove; YC = Yuma Cove, and NA = haplotypes not sampled in 2016).

Average H_R rebounded in 2016 from the decline in 2015 and was similar to 2014 (figure 4A), and there was a positive association between time and allelic richness (slope = 0.063, R^2 = 0.59, P < 0.0001). Average gene diversity was the same as 2015 which was reduced relative to previous years (figure 4B); however, there was evidence of an overall increase in gene diversity over the last 20 years (slope = 0.004, R^2 = 0.36, P = 0.005).

Microsatellite variation was characterized from 117 randomly sampled larval individuals (with respect to space and time) and contrasted with samples from the previous 20 years. One of the loci examined in the 2016 sample exhibited significant deviations from the Hardy-Weinberg equilibrium (after correction for multiple tests), similar to estimates from previous years. Levels of microsatellite allelic richness and gene diversity were comparable to previous years (table 3), with no statistically significant increase in microsatellite variation over time based on linear regression analysis (allelic richness, slope = -0.02, R^2 = 0.05, P = 0.167, figure 5A; gene diversity, slope = 0.0004, R^2 = 0.10, P = 0.273, figure 5B).

Table 1.—Distribution of mtDNA haplotypes for larval and adult samples collected in Lake Mohave, Arizona and Nevada, for the years 2012–2016

	Main basin											Above Willow Beach					
	2012		2013		2014		2015		2016			2014		2015		2016	
Haplotype	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Total	Adults	Larvae	Adults	Larvae	Larvae	Total
A	3	20	9	24	5	10	5	12	6	14	108	2	7	1	0	6	16
B	4	42	5	45	13	34	5	29	13	41	231	9	9	0	10	11	39
C	1	22	0	4	2	4	5	6	1	5	50	0	1	0	1	4	6
D	0	0	0	1	0	1	0	1	0	0	3	0	0	0	0	1	1
E	82	311	80	269	89	222	92	250	64	307	1766	61	67	11	73	63	275
F	5	11	2	16	3	6	2	5	5	11	66	3	2	1	1	1	8
G	1	16	2	1	4	6	2	8	2	5	47	1	0	0	0	5	6
H	3	4	1	6	2	6	3	9	2	5	41	1	2	0	1	1	5
I	0	1	1	0	0	2	1	0	0	4	9	0	0	0	1	0	1
J	0	4	2	2	2	3	3	1	0	5	22	1	0	0	2	4	7
K	1	3	0	3	0	2	0	1	0	2	12	0	0	0	5	0	5
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
M	0	1	0	8	1	5	1	2	0	1	19	1	1	0	1	0	3
N	0	1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0
P	1	7	2	3	5	5	1	6	2	2	34	0	2	0	0	0	2
Q	1	5	0	9	0	7	0	3	1	0	26	0	0	1	0	0	1
R	10	57	9	31	13	35	10	29	13	48	255	6	10	3	4	10	33
S	6	30	15	41	5	12	15	30	8	27	189	14	11	3	10	17	55
U	0	6	0	9	0	2	1	2	0	8	28	1	0	0	2	1	4
V	0	1	1	5	0	1	0	1	3	2	14	0	0	0	2	2	4
Z	0	1	1	1	0	1	0	0	0	0	4	0	0	0	0	0	0
BB	1	6	1	10	0	10	1	1	3	10	43	1	1	0	1	1	4
CC	3	0	5	3	16	0	6	6	4	5	48	1	0	0	2	1	4
DD	0	0	0	0	0	0	0	2	1	1	4	0	0	0	1	0	1
GG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HH	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
II	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1
Total	**122**	**550**	**138**	**491**	**160**	**374**	**153**	**404**	**128**	**503**	**3,023**	**102**	**114**	**20**	**117**	**129**	**482**

Table 2.—Estimates of measures of mtDNA variation for each larval sample collected in 2016 from Lake Mohave and Reach 3

N, N_h, H_R are sample size, number of haplotypes, and haplotype richness (number of haplotypes corrected for sample size, Kalinowski 2005), respectively. Gene diversity is the expected proportion of heterozygotes (± standard error). "*" identifies measures that are significantly lower than variation in the wild adult population (statistical analysis described in Dowling et al. 2014).

Area	Date	N	N_h	H_R	Gene diversity
Lake Mohave					
Nine Mile area	Jan 27	25	6	4.06	0.4267 ± 0.1216
Nine Mile area	Feb 16	19	9	7.71	0.8538 ± 0.0680
Nine Mile area	Mar 2	25	7	5.53	0.7800 ± 0.0587
Nine Mile area	Mar 15	25	9	6.47	0.7567 ± 0.0825
Nine Mile area	Apr 13	22	6	4.91	0.5931 ± 0.1162
Tequila Cove	Jan 27	25	3*	2.49	0.2900 ± 0.1095*
Tequila Cove	Feb 2	30	7	4.92	0.6253 ± 0.0933
Tequila Cove	Feb 16	27	6	4.46	0.6724 ± 0.0742
Tequila Cove	Mar 1	18	4	3.70	0.4771 ± 0.1338
Tequila Cove	Mar 15	26	4	3.32	0.4954 ± 0.1020
Tequila Cove	Mar 29	30	6	3.80	0.4552 ± 0.1062
Tequila Cove	Apr 13	25	8	5.54	0.6367 ± 0.1055
Yuma Cove	Jan 25	25	5	4.30	0.6967 ± 0.0678
Yuma Cove	Feb 2	25	8	5.36	0.6900 ± 0.0837
Yuma Cove	Feb 16	11	7	NA	0.8182 ± 0.1191
Yuma Cove	Feb 29	14	2*	2.00	0.1429 ± 0.1188*
Yuma Cove	Mar 15	25	6	4.42	0.5333 ± 0.1136
Yuma Cove	Apr 11	24	12	8.03	0.8225 ± 0.0741
Above Owl Point	Mar 29	24	6	4.78	0.6014 ± 0.1082
Above Owl Point	Mar 30	26	6	4.76	0.6462 ± 0.0936
Above Owl Point	Apr 12	25	4	2.94	0.2967 ± 0.1150
Above Owl Point	Apr 12	7	4	NA	0.8095 ± 0.1298
Above Willow Beach	Jan 20	15	6	5.80	0.7905 ± 0.0785
Above Willow Beach	Feb 1	32	7	4.39	0.4839 ± 0.1067
Above Willow Beach	Feb 16	25	6	4.68	0.5833 ± 0.1085
Above Willow Beach	Feb 29	25	9	7.03	0.8400 ± 0.0555
Above Willow Beach	Mar 21	25	8	6.47	0.8100 ± 0.0629
Above Willow Beach	Apr 25	7	5	NA	0.8571 ± 0.1371
Reach 3					
Park Moabi	Feb 17, 21	18	7	7	0.6340 ± 0.1269
Needles	Feb 19	25	8	6.72	0.6800 ± 0.0983
Park Moabi	Mar 1	32	7	5.66	0.6331 ± 0.0901
Pulpit	Mar 1	32	9	6.55	0.6976 ± 0.0804
Sand Dunes	Mar 1	25	8	6.72	0.6800 ± 0.0983
Mesquite Bay	Mar 2	29	7	5.42	0.6010 ± 0.0974
Park Lagoon	Mar 16	71	14	7.27	0.7006 ± 0.0580
Park Moabi	Apr 19	26	6	5.5	0.7600 ± 0.0545
Needles	Apr 20	25	3*	2.72	0.5467 ± 0.0544

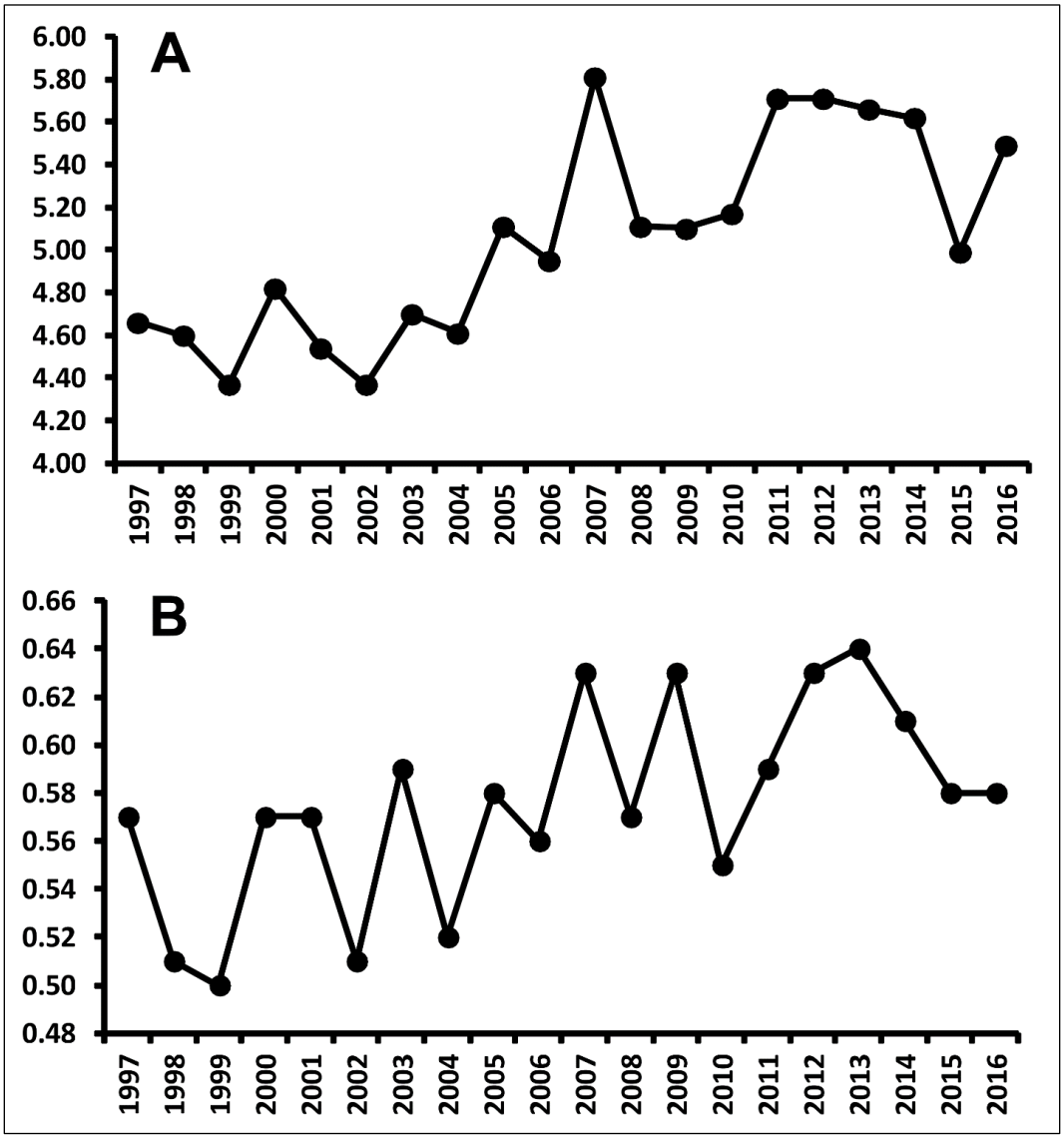

Figure 4.—Mean haplotypic richness (A) and gene diversity (B) estimated with mtDNA from samples of larval razorback suckers (Lake Mohave, Arizona and Nevada).

Table 3.—Average allelic richness and gene diversity (per locus) calculated from microsatellites in larval samples collected in 1997–2016

Year	N	A_R	Gene diversity
1997	120	15.84	0.744
1998	120	15.41	0.730
1999	120	15.50	0.734
2000	120	15.63	0.707
2001	120	15.85	0.731
2002	120	15.47	0.735
2003	120	15.56	0.741
2004	120	15.00	0.714
2005	120	15.48	0.721
2006	120	15.41	0.721
2007	120	15.25	0.730
2008	120	15.93	0.733
2009	120	15.51	0.744
2010	120	14.91	0.739
2011	120	15.91	0.740
2012	120	15.25	0.740
2013	119	15.38	0.743
2014	120	15.26	0.731
2015	119	15.67	0.739
2016	117	15.02	0.728

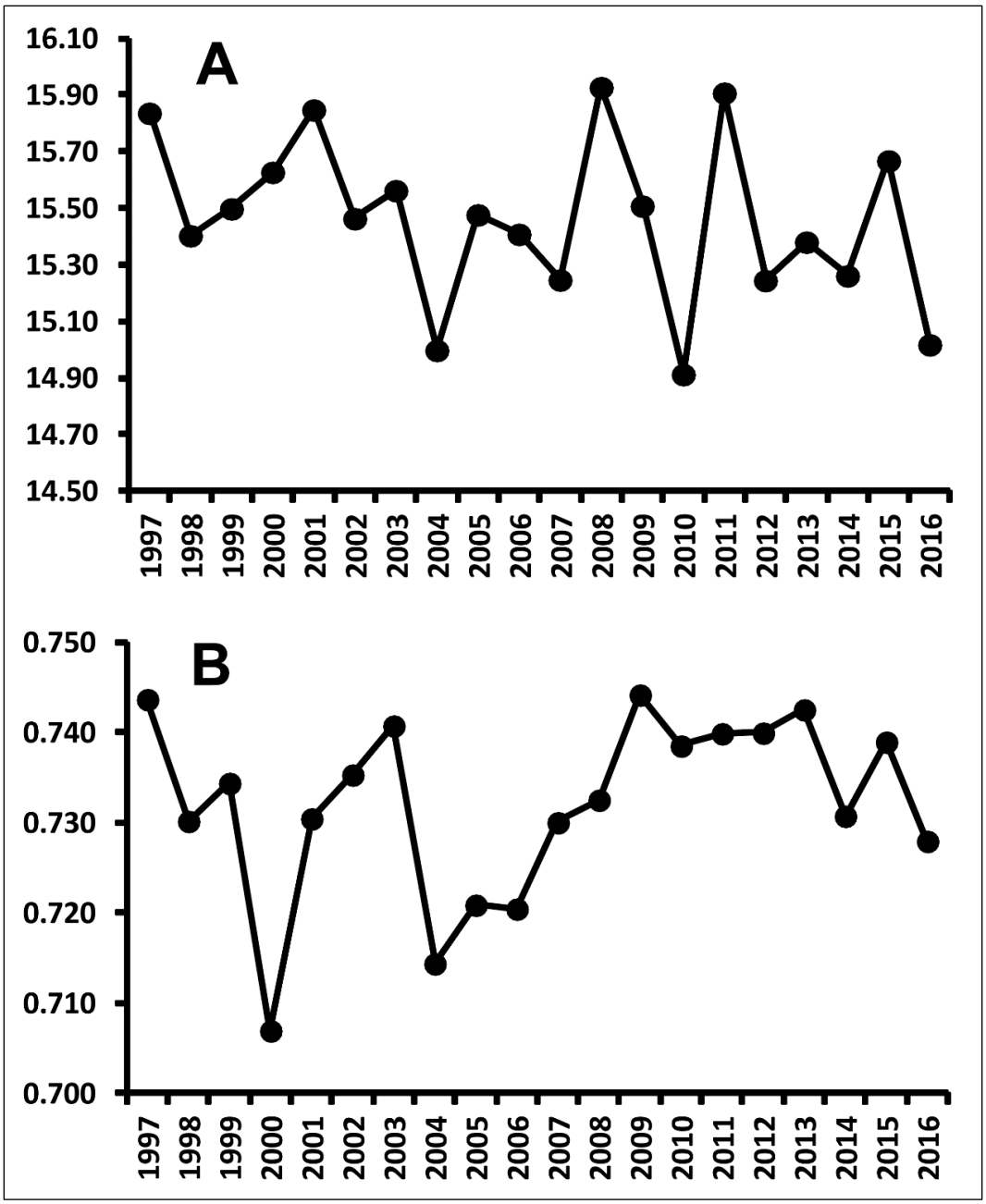

Figure 5.—Mean allelic richness (A) and gene diversity (B) estimated with microsatellites from samples of larval razorback suckers (Lake Mohave, Arizona and Nevada).

The impact of geographic and temporal sampling on the distribution of genetic variation was also examined using AMOVA (Excoffier and Lischer 2010) to calculate F_{ST} and to partition the variation among the four sampling regions (F_{CT}) and among groups within those regions (F_{SC}). Like previous years, there was no significant difference in the distribution of mtDNA variation among the four regions, but samples within regions were significantly different (table 4). This is the typical pattern for razorback suckers, likely reflecting changes in haplotype frequencies among the finite number of spawners at a given time and place (Dowling et al. 2005, 2014).

Table 4.—Results from AMOVA analysis of mtDNA haplotype frequencies for razorback suckers from Lake Mohave, Arizona and Nevada, for the years 1997–2016

Year	Number 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	291	0.039	< 0.0001	-0.012	0.715	0.050	0.001
2000	16	366	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	344	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	559	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	515	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	21	550	0.033	< 0.0001	0.006	0.163	0.027	< 0.0001
2013	20	491	0.045	< 0.0001	-0.001	0.514	0.046	< 0.0001
2014	15	374	0.022	0.007	0.002	0.372	0.020	0.014
2015	16	403	0.031	< 0.0001	0.008	0.101	0.023	< 0.0001
2016	22	503	0.022	< 0.0001	0.003	0.233	0.019	< 0.0001

Selection of random samples of individuals for microsatellite analysis precluded testing for spatial and temporal differences as for mtDNA. However, an F-statistic analysis could be used to quantify distribution of variation within and among annual samples. The total estimate of genetic variance across all 20 years was similar to previous estimates ($F = 0.053$, 95% confidence interval [CI] = 0.038–0.068). Most of the observed variation resulted from the deficiency of heterozygotes described above ($f \sim F_{IS} = 0.045$, 95% CI = 0.033–0.058) and not differences among years ($\Theta \sim F_{ST} = 0.008$, 95% CI = 0.003–0.014), indicating that this sampling strategy is not causing changes in allelic frequencies or levels of variation over time.

The discovery of a sizable population of adult razorback suckers above Willow Beach has led to questions regarding their discreteness relative to the main basin and the role these fish might play in management of this species in the lake; therefore, larvae and adults from this reach were sampled for characterization and comparison with samples from the main basin of Lake Mohave. A total of 129 larvae (representing 6 time periods) and 10 adults were obtained from above Willow Beach in 2016. Larval samples have been characterized for mtDNA but not microsatellites; adults have yet to be genotyped.

Larval samples collected above Willow Beach in 2016 had 16 mtDNA haplotypes (see table 1) and an average gene diversity of 0.727. None of the individual values were significantly different from the original source population. These larval samples from above Willow Beach were compared with the four other regions of the main basin (Nine Mile, Tequila, Yuma, above Owl Point) to see if they were significantly different. Levels of variation among samples collected in 2016 were low but statistically significant ($F_{ST} = 0.039$, $P < 0.0001$), as were the differences among the five regions ($F_{CT} = 0.012$, $P = 0.030$) and among samples within regions ($F_{SC} = 0.027$, $P < 0.0001$). Visualization of the position of these samples showed that four of the six samples were distinct relative to others from the main basin (figures 6 and 7). There are shifts in allele frequencies of major alleles (e.g., E and S, figure 6; see table 1); however, two of the temporal samples are small (January 20, N = 15; April 25, N = 7) as compared to the remaining four samples ($N \geq 25$). A comparison across additional samples will help address the biological significance of this difference.

In summary, mean haplotype richness rebounded in Lake Mohave in 2016, slightly lower than the high levels exhibited in 2011–14 (see figure 4A). While it is difficult to interpret results from the last 2 years as an indicator of stability or decline, recent declines in levels of genetic variations raise concerns and illustrate

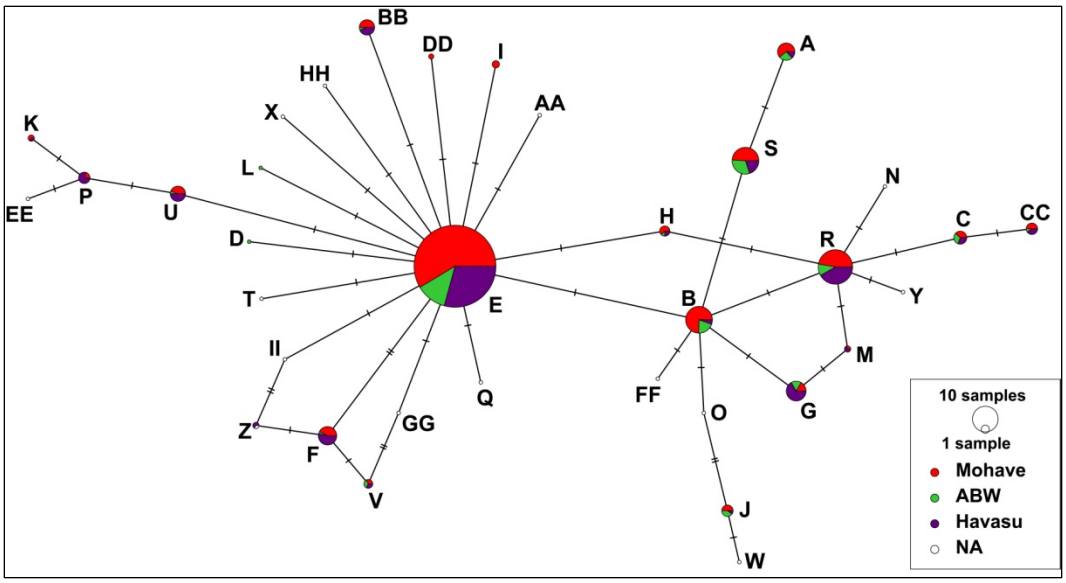

Figure 6.—Minimum spanning network showing relationships of all mtDNA cyt*b* haplotypes found in razorback suckers.
Hash marks on branches identify the number of base differences between haplotypes. The size of the circle identifies numbers of larvae collected in 2016 from the main basin and reach above Willow Beach in Lake Mohave and from Reach 3, with specific colors in the specific haplotypes representing the region of origin (Mohave, ABW = above Willow Beach, Havasu – Reach 3, and NA = haplotypes not sampled in 2016).

the necessity for due diligence in continued genetic monitoring, maximizing survivorship of individuals in Lake Mohave, and development of alternate strategies for conservation.

Analyses of samples from above Willow Beach indicate that some temporal samples are distinct from the main basin in 2016. This is the first year of focused temporal sampling that is comparable to the main basin; therefore, it is not possible to place this result in context. The difference between the two regions is not large ($F_{CT} = 0.012$) and may not be biologically meaningful, and an assessment of discrete temporal collections from additional years is necessary to determine the significance of this result. In the meantime, efforts should continue to incorporate individuals from above Willow Beach into annual larval sampling.

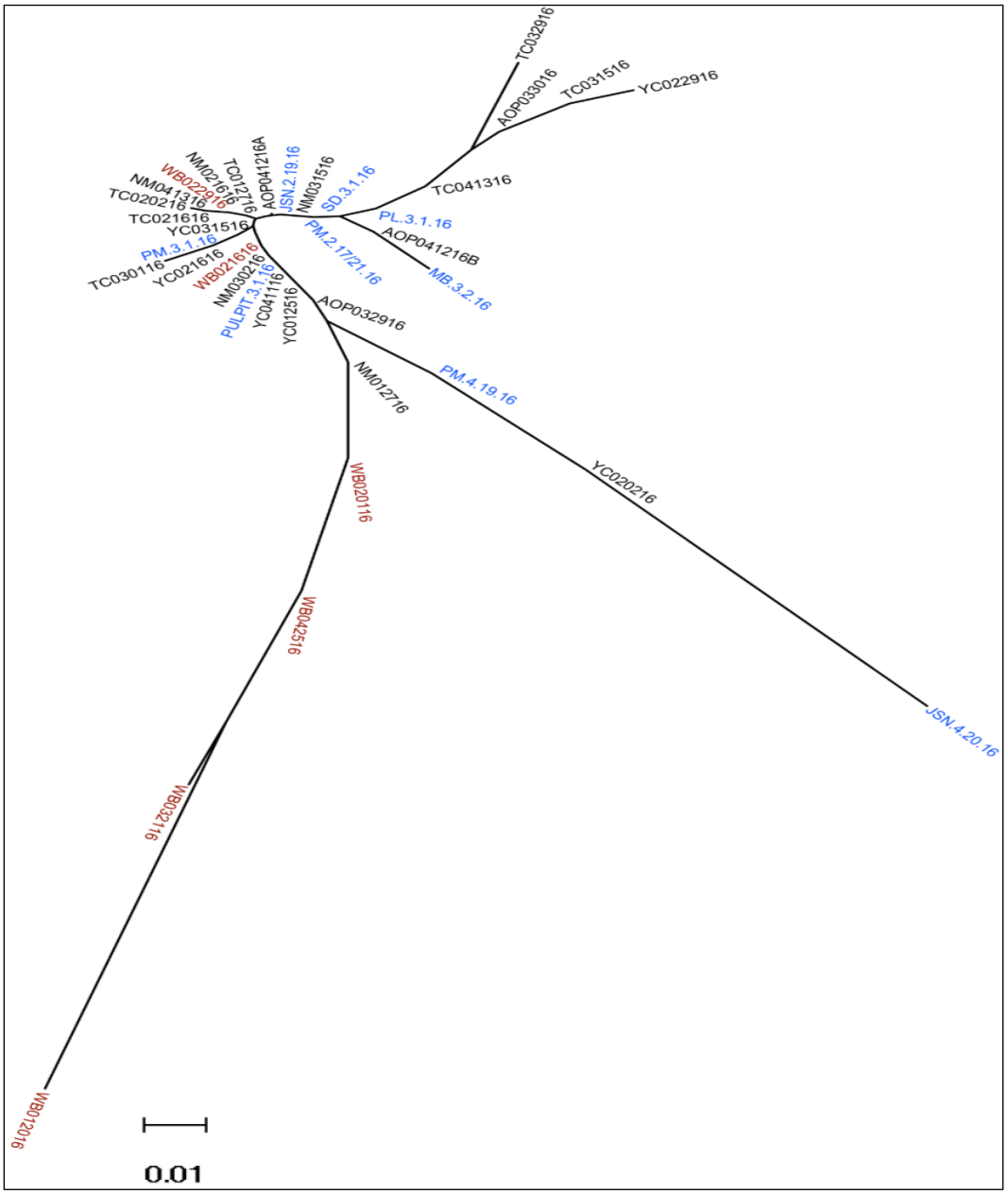

Figure 7.—Neighbor-joining tree constructed from pairwise estimates of F_{ST} among temporal samples from the main basin of Lake Mohave (samples with black labels), above Willow Beach (samples with red labels), and Reach 3 (samples with blue labels).
Scale provided at the lower left.

Lake Havasu Area (Reach 3)

Reach 3 has received razorback suckers from a diversity of sources, including relatively recent stockings of hatchery fish reared from wild caught larvae from Lake Mohave. Samples from lower Colorado River Reach 3 included 283 larvae (9 spatial and/or temporally distinct samples – see table 2; figure 8) and 116 adults. All larvae have been characterized for mtDNA variation but not microsatellites. Adult DNA has been extracted and is in the process of being characterized. Eighteen haplotypes were identified in the nine samples examined (table 5). These samples exhibited similar or higher levels of allelic and gene diversity than Lake Mohave, with only one sample exhibiting a significant reduction in the number of alleles expected as compared to the original Lake Mohave source population (see table 2).

Partitioning of genetic variance among these nine samples also yielded a similar estimate ($F_{ST} = 0.037$, $P < 0.0001$) to those found in Lake Mohave (see table 4). Inclusion of these nine samples with those from the main basin of Lake Mohave and above Willow Beach identified significant differences among locations ($F_{ST} = 0.045$, $P < 0.0001$), with most of this variation reflecting differences among samples within these three regions ($F_{SC} = 0.030$, $P < 0.0001$) rather than differences among regions ($F_{CT} = 0.015$, $P < 0.0001$). Visualization of individual samples shows that most Reach 3 samples clustered among those of the main basin from Lake Mohave, with only a sample from Needles, California (April 20) being distinct (see figure 7). This sample has only three haplotypes, one of which is rare, making it quite distinct from the remainder of the Lake Mohave main basin and Reach 3 samples.

In summary, Reach 3 contains a genetically diverse population that is similar to that found in Lake Mohave. Given the similarities, it is prudent to continue utilizing this reach for maintenance and studies of razorback suckers. Reach 3 has been stocked with fishes from a diversity of natural and hatchery sources. Restricting future stockings to Lake Mohave born fish may avoid potential issues associated with the small number of breeders in traditional hatcheries and adaptation to hatchery conditions.

It is especially interesting to note that flannelmouth suckers (*Catostomus latipinnis*) also are found in Reach 3, yet genetic evidence of introgression with razorback suckers has not been observed. This result is somewhat surprising given considerable introgression between these species observed in Lake Mead (see below). It would be of interest to understand the differences in species interactions between these two locations.

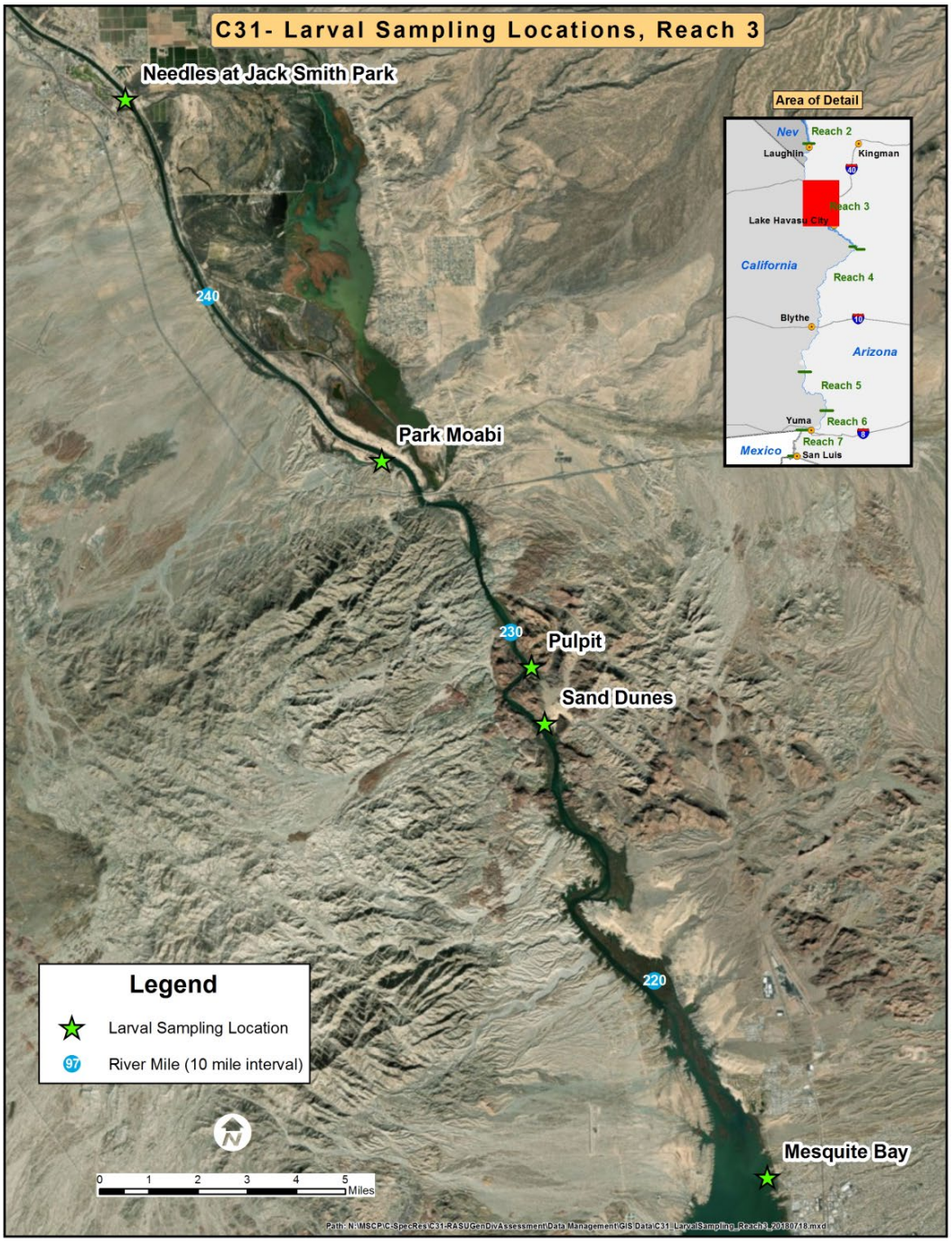

Figure 8.—Map of sampling locations in Reach 3.

Table 5.—Distribution of mtDNA haplotypes for larval samples collected in Reach 3, Arizona, California, and Nevada, from 2016

	Park Moabi				Needles		Pulpit	Sand Dunes	Mesquite Bay	
Haplotype	**2.17 & 21.16**	**03.01.16**	**03.16.16**	**04.19.16**	**02.19.16**	**04.20.16**	**03.01.16**	**03.01.16**	**03.02.16**	**Total**
A			1		1				1	3
B	1							2		3
BB	1		3	3			1			8
C			4							4
CC			2		1			1		4
E	11	19	38	10	14	10	17	14	18	151
F			6		2		1	2	3	14
G	2	4	1		3		3	3	4	20
H			2							2
J		1								1
K			1							1
M		1					1			2
P			4	2		1	1			8
R	1	2	4	8	2	14	5	1	1	38
S	1	3	1	2	1		2	1		11
U		2	3	1	1		1	1		9
V			1						1	2
Z	1								1	2
	18	**32**	**71**	**26**	**25**	**25**	**32**	**25**	**29**	**283**

Lake Mead (Reach 1)

Adult (N = 60) and larval (N = 81) samples collected from four locations from Lake Mead (figure 9) in 2016 have been characterized for mtDNA and microsatellite variation. They were compared to randomly selected adults (collected in 2000, N = 50) and larvae (2011 year-class, N = 120) to provide perspective. MtDNA diversity in 2016 Lake Mead samples of adults and larvae were comparable to earlier years and are significantly different from the original Lake Mohave source population (table 6). Examination of patterns of variation (figure 10) identifies only four haplotypes (E, F, P, and R), with P much more frequent in Lake Mead than Lake Mohave.

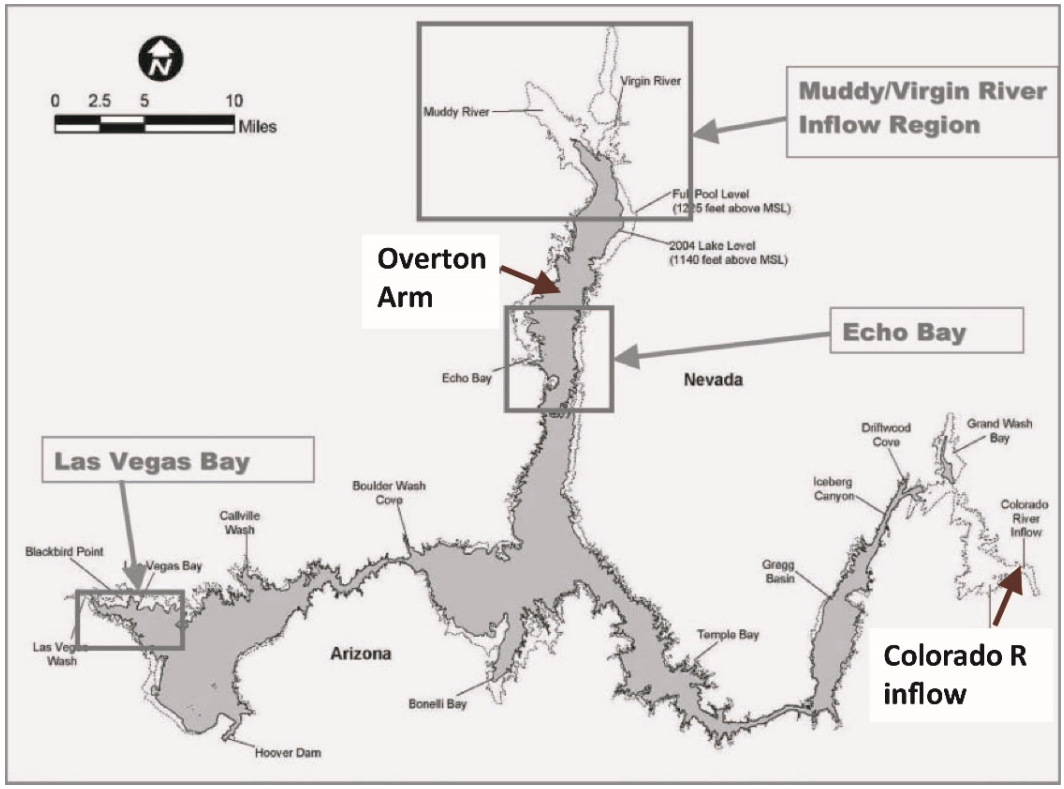

Figure 9.—Sampling localities in Lake Mead (modified from Albrecht et al. 2011).

Quantification of the distribution of genetic variation identified significant differences among samples ($F_{ST} = 0.093$, $P < 0.001$). F_{ST} was further partitioned into within and among-group components (F_{SC} and F_{CT}, respectively) to estimate the influence of various features of their biology (e.g., life history, lake of origin [Mead or Mohave], and time periods [pre- and post-2003]) on patterns of genetic variation. In these three analyses, there were significant differences among samples within groups (life history, lake of origin [Mead or Mohave], and time periods [pre- and post-2003]), with differences between time periods and lakes also explaining a significant amount of variation (table 7). This difference is readily visible when the samples are clustered by F_{ST} values, with samples collected prior to 2003 (except for the adult sample from 2002) most similar to those from Lake Mohave (figure 11A).

Microsatellite diversity in 2016 Lake Mead sample of adults was higher than previous years, while the pooled sample of larvae exhibited lower diversity than earlier years (table 6); however, they both were lower than comparable estimates from Lake Mohave. Distribution of genetic variation within and among samples collected in different years from different locations was characterized using F-statistics. The jackknife average of total genetic variation ($F \sim F_{IT}$) across microsatellite loci was 0.068 (95% bootstrap CI = 0.049–0.086). The

Table 6.—Diversity indices based on mtDNA and microsatellite variation for year from Lake Mead and representative samples from Lake Mohave, Arizona and Nevada

N, N_h, and A_R are sample size, number of haplotypes (not corrected for sample size), and number of alleles (corrected for sample size), respectively. Gene diversity for mtDNA is the probability that two randomly sampled haplotypes are different and, for microsatellites, is also the expected proportion of heterozygotes (± standard error). Measures of variation are averaged across all loci for microsatellites. "*" identifies estimates of mtDNA variation that are significantly different from the Lake Mohave source population.

Location	mtDNA			Microsatellites		
	N	N_h	Diversity	N	A_R	Diversity
Lake Mead						
Adults						
1988	16	2*	0.1250 ± 0.1064*	15	8.28	0.729
2002	29	5	0.6626 ± 0.0685	29	8.17	0.735
2011	15	2*	0.2476 ± 0.1307*	15	7.77	0.745
2012	61	3*	0.5104 +/ 0.0449	61	6.61	0.707
2013	51	4*	0.5537 ± 0.0386	50	7.21	0.721
2014	61	4*	0.4634 ± 0.0570*	61	7.50	0.730
2015	24	5	0.6304 ± 0.0652	26	8.36	0.734
2016	60	5*	0.4983 ± 0.0595	60	8.40	0.742
Larvae						
1997	25	3*	0.6100 ± 0.0588	25	6.53	0.668
2002	57	6*	0.7613 ± 0.0330	57	7.62	0.706
2012	50	2*	0.1502 ± 0.0646*	50	7.31	0.718
2013	78	5*	0.5291 ± 0.0355	77	7.37	0.736
2014	47	3*	0.4394 ± 0.0613	51	7.84	0.722
2015	77	4*	0.4679 ± 0.0637*	71	8.10	0.752
2016	81	4*	0.5037 ± 0.0440*	82	7.08	0.711
Lake Mohave						
Adults	49	11	0.6420 ± 0.0766	50	8.96	0.748
Larvae – 2011	120	14	0.5965 ± 0.0512	120	8.86	0.740

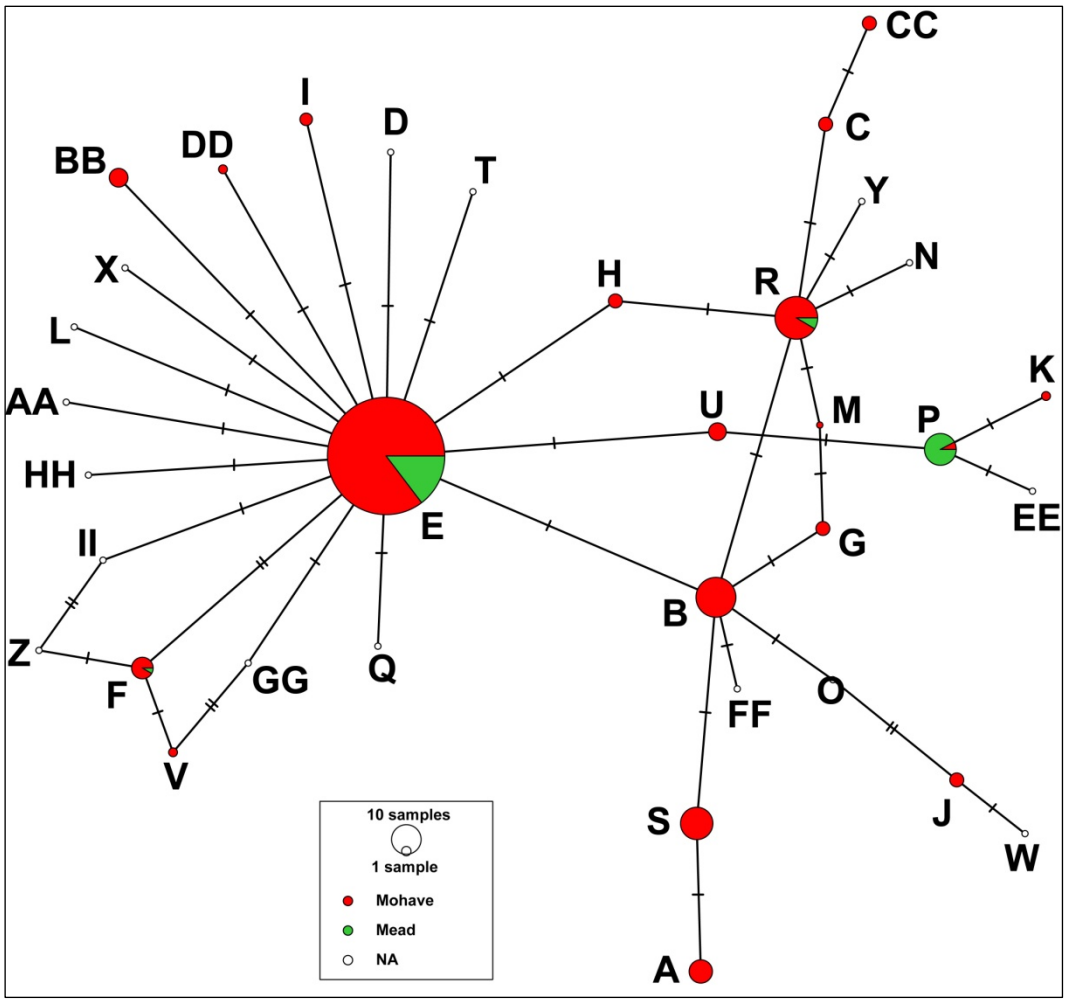

Figure 10.—Minimum spanning network showing relationships of all mtDNA cyt*b* haplotypes found in razorback suckers from Lakes Mead and Mohave.
Hash marks on branches identify the number of base differences between haplotypes. The size of the circle identifies the numbers of larvae collected in 2016 from the main basin of Lake Mohave (red) and Lake Mead (green). Haplotypes not sampled in 2016 (NA) are also shown (white circles).

within-population component ($f \sim F_{IS}$) was significantly different from 0 (average = 0.042, 95% bootstrap CI = 0.022—0.060), indicative of deviations from the Hardy-Weinberg equilibrium reported previously (Dowling et al. 2013). The among-sample component ($\Theta \sim F_{ST}$) was smaller than that within populations, with a significant jackknife average of 0.027 (95% bootstrap CI = 0.020–0.035 indicates the observed value of theta is significantly >0). These values are comparable to previous estimates reported by Dowling et al. (2012a, 2013), indicating that little change in levels of genetic diversity has occurred in this population over the last year.

Table 7.—Results of F-statistic analyses for mtDNA and microsatellites from Lakes Mead and Mohave, Arizona and Nevada
Variation among groups (F_{ST}) can be further partitioned to among samples within groups and among-group components (F_{SC} and F_{CT}, respectively).

Comparison	mtDNA			Microsatellites		
	Percent of variation	Estimate	P	Percent of variation	Estimate	P
Between lakes						
F_{CT}	6.79	0.680	0.049	0.59	0.006	0.093
F_{SC}	6.49	0.070	< 0.001	2.41	0.024	< 0.001
Between adults and larvae						
F_{CT}	0.39	0.004	0.279	0.00	0.000	0.395
F_{SC}	8.92	0.090	< 0.001	2.61	0.026	< 0.001
Between years (pre- and post-2003)						
F_{CT}	11.24	0.112	< 0.001	1.29	0.013	< 0.001
F_{SC}	3.25	0.037	< 0.001	1.98	0.020	< 0.001

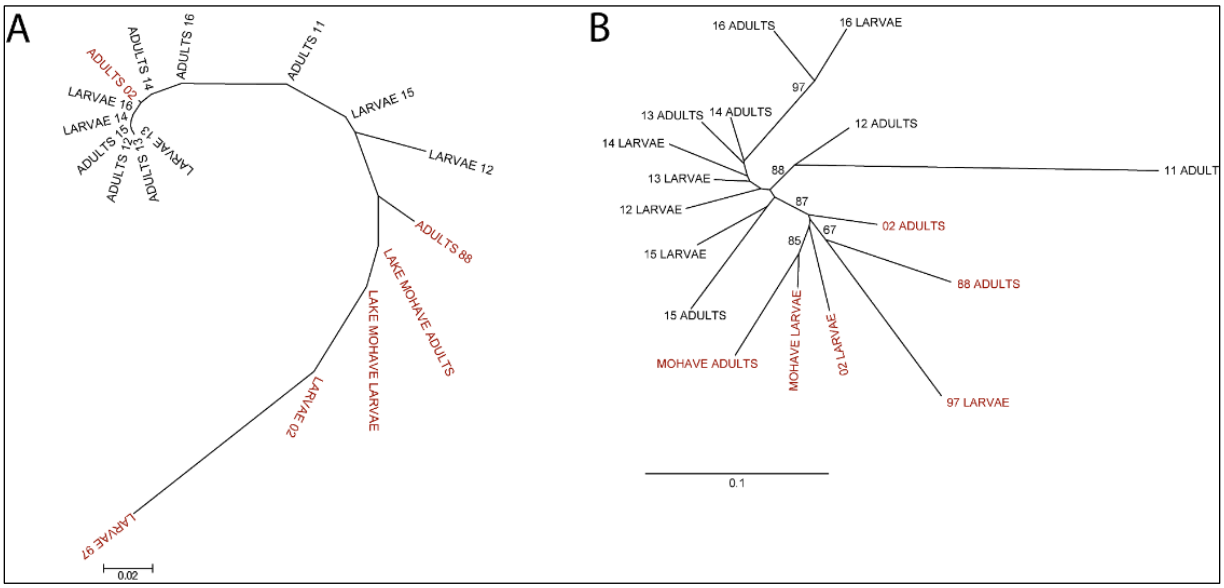

Figure 11.—Neighbor-joining tree for samples from Lakes Mead and Mohave, Arizona and Nevada: (A) pairwise estimates of F_{ST} based on mtDNA and (B) among-population divergence (D_a) from microsatellite data.
Pre- and post-2003 samples are labelled in red and black, respectively. Samples are from Lake Mead unless labelled as from Lake Mohave. The numbers on the nodes of the microsatellite tree (B) are bootstrap values (percentage of 1,000 replicates where the same node is recovered), with only values >65% shown. Branches are drawn to scale (scale provided at the lower left of each figure).

Further partitioning F_{ST} into within and among-group components (F_{SC} and F_{CT}, respectively) yielded results similar to those previously described for mtDNA (see table 7). In each of the three cases, there were significant differences among samples within defined groups; however, the difference among groups was not significant for the larvae versus adult comparison or the Lake Mead versus Lake Mohave comparison, but it was significant for the comparison between the pre- and post-2003 time periods. To assess the significance of among-group variation in these latter two analyses, the level of divergence among groups (D_a) was estimated and clustered. Samples collected before and after 2003 are different, significantly so for microsatellites (see figure 11B), with adult and larval samples from Lake Mohave nested among the pre-2003 samples.

Patterns of variation were further examined using the Bayesian analysis (STRUCTURE), and included flannelmouth suckers, for an additional perspective. This analysis identified the same three groups as reported in Dowling et al. (2013): flannelmouth suckers and groups most common in Lakes Mead and Mohave. The probability of assignment to each of these groups was generated for each individual (summarized in table 8), allowing individuals of mixed ancestry to be identified (defined as >10% assignment probability to two or more groups). Flannelmouth suckers and hybrids with razorback suckers were mostly restricted to later samples and the Colorado River inflow (CRI) (table 8); however, adult hybrids were also rarely observed in Echo Bay and the Overton Arm. In 2016, 12 of 14 adults collected in the CRI were identified as flannelmouth suckers or hybrids (5 and 7, respectively). No larvae were provided from CRI; however, a single larva from Las Vegas Bay appeared to be introgressed with flannelmouth suckers. A single adult and two larvae from Lake Mohave exhibited evidence of limited introgression with flannelmouth suckers, reflecting the past presence of this species further downstream.

Patterns of assignment to the Mead and Mohave groups (A and B, respectively) were similar between adults and larvae, with the exception of the Echo Bay samples from 2012, where the Mead form was more common in the adults than the larvae (table 8). In general, the Mead form was more common in the Overton Arm and the CRI, whereas the Mohave form was prevalent in Echo and Las Vegas Bays. Note, however, that the Mead form (A) remained common in larval samples from the Overton Arm but increased in abundance in both Echo and Las Vegas Bays in 2016. The Mead form was more abundant in adults from 2016 (table 8); unfortunately, most individuals (67 of 81) were not assigned to specific locations this year, so it is difficult to evaluate this result.

These patterns could reflect changes in sampling. The Mead form was rare prior to 2011, potentially explaining the temporal difference discussed above. Prior to 2011, samples came mainly from Echo and Las Vegas Bays, respectively; therefore, the observed patterns may have existed in prior years but were undetected.

Table 8.—Summary of assignments to five categories (Mead [A], Mohave [B], admixed Mead + Mohave [A + B], flannelmouth sucker-razorback sucker hybrid, and flannelmouth suckers) provided by STRUCTURE for samples from Lake Mead, Arizona and Nevada

Location	Year	Mead (A)	Mohave (B)	A + B	Hybrid	Flannelmouth sucker	Total
Adults							
Unknown	1988	0	13	2	0	0	15
	2016	19	11	6	0	0	36
Colorado River inflow	2011	0	1	1	0	2	4
	2012	12	2	1	1	0	16
	2013	0	0	1	2	0	3
	2014	0	1	0	4	2	7
	2015	1	1	2	1	2	7
	2016	2	0	0	7	5	14
Echo Bay	2002	0	8	3	0	0	11
	2011	5	1	1	1	0	8
	2012	29	11	5	0	0	45
	2013	1	4	1	0	0	6
	2014	4	8	2	0	0	14
Las Vegas Bay	2002	0	15	3	0	0	18
	2013	0	3		0	0	3
	2014	1	6	1	0	0	8
	2015	1	9		0	0	10
Overton Arm	2011	2	0	1	0	0	3
	2013	33	2	1	2	0	38
	2014	25	5	2	0	0	32
	2015	4	1	4	0	0	9
	2016	6	3	1	0	0	10
Mohave		0	48	1	1	0	50
	Totals	**145**	**153**	**39**	**19**	**11**	**367**
Larvae							
Colorado River inflow	2014	1	0	2	2	3	8
	2015	0	0	0	4	2	6
Echo Bay	1997	0	24	1	0	0	25
	2002	0	26	4	0	0	30
	2012	5	16	4	0	0	25
	2013	1	3	3	0	0	7
	2014	4	3	3	0	0	10
	2015	6	22	8	0	0	36
	2016	14	6	5	0	0	25
Las Vegas Bay	2002	0	27	0	0	0	27
	2012	2	20	3	0	0	25
	2013	3	26	11	0	0	40
	2014	1	18	4	0	0	23
	2015	0	23	6	0	0	29
	2016	10	2	5	1	0	18
Overton Arm	2013	26	1	3	0	0	30
	2014	7	1	2	0	0	10
	2016	28	2	9	0	0	39
Mohave		0	110	8	2	0	120
	Totals	**108**	**330**	**81**	**9**	**5**	**533**

In summary, these analyses reinforce earlier conclusions that the population in Lake Mead has changed over time, becoming significantly different from what it once was (at least for these genetic markers; see figure 11). It is unclear if this divergence reflects a change in sampling and/or increased infusion of distinctive fish from a divergent source (e.g., Grand Canyon or off-channel sources [B. Senger 2014, personal communication]).

Additional analyses indicate that levels of genetic variation are significantly reduced in Lake Mead relative to Lake Mohave (see table 6), as indicated by numbers of alleles and estimates of gene diversity. The Lake Mohave population is appropriate as the baseline for comparison because previous studies (Dowling et al. 1996b, 2012b) have indicated that Lakes Mohave and Mead were part of the same population prior to the closure of Hoover Dam that currently isolates them. Fortunately there are samples of wild fish born prior to the collapse of the current Lake Mohave population that provide valuable perspective for comparison.

The combination of the results and consistently small census size raises concerns for the welfare of the Lake Mead population. Individuals from the Lake Mead population were only rarely collected in the 1970s and disappeared in the 1980s before being rediscovered in the 1990s at reduced population size (Dowling et al. 2012b). Drastically reduced population size is expected to reduce levels of genetic variation, consistent with the results provided by samples from the late 1980s – early 1990s to date. Such reductions increase the potential harmful effects of inbreeding depression, which has been shown to be important in razorback suckers (Dowling et al. 1996a), raising concerns over the future of the Lake Mead population. Because razorback suckers in Lake Mead and Lake Mohave were historically contiguous until closure of Hoover Dam in 1935 and were part of the same population (Dowling et al 2012b), it would be possible to infuse additional variation into the Lake Mead population through limited augmentation (10s of individuals) with Lake Mohave fish to reintroduce genetic variation and reduce the potential negative impacts of reduced genetic variation. This management action has been discussed by the Lake Mead Work Group but has not been implemented at this point.

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