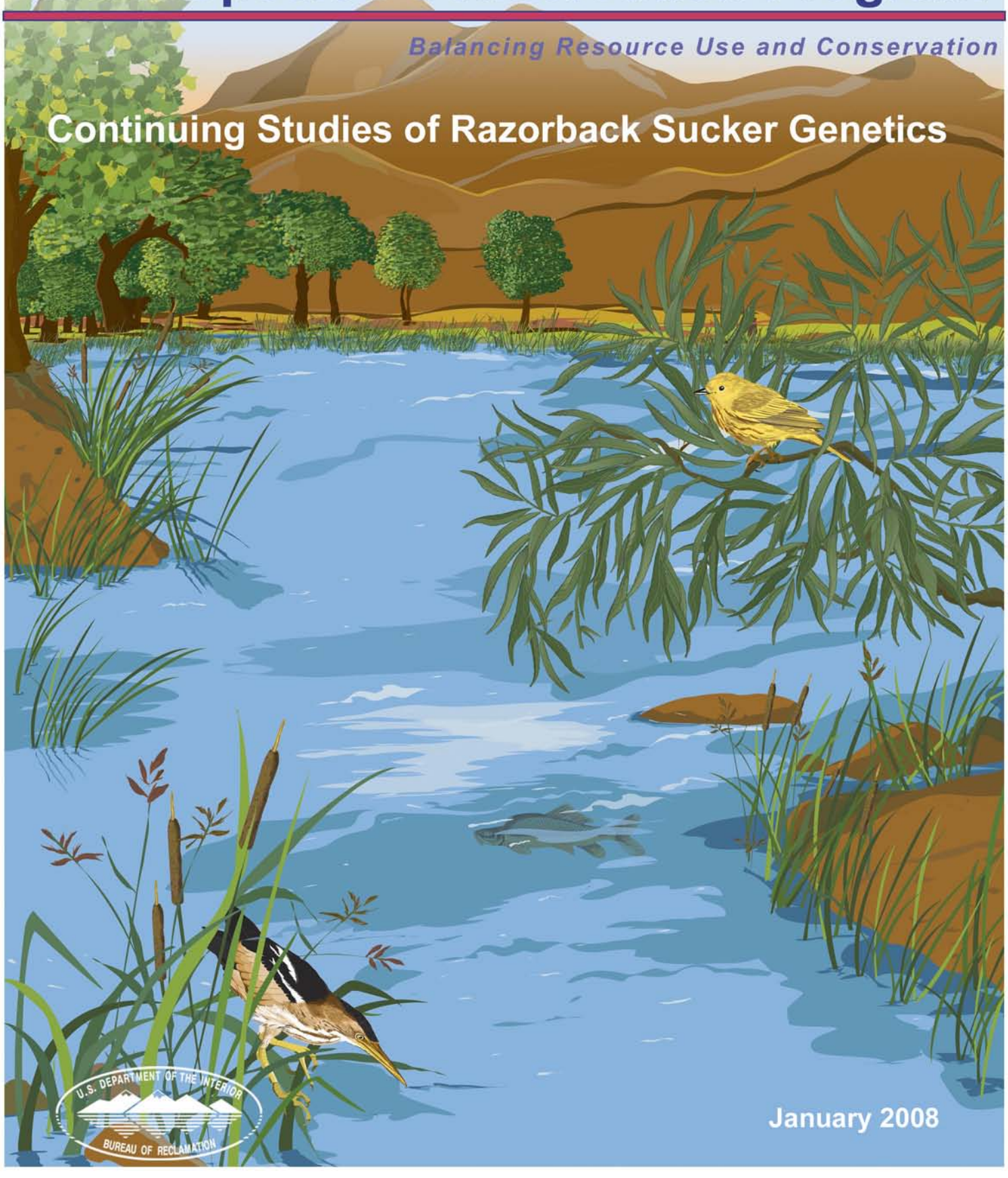
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Continuing Studies of Razorback Sucker Genetics



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January 2008

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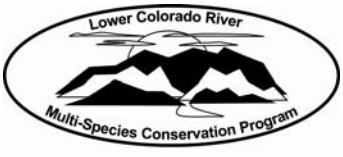

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Lower Colorado River
Multi-Species Conservation Program Office
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
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January 2008

Continuing studies of razorback sucker genetics

Thomas E. Dowling, Paul C. Marsh, and Anne T. Kelsen
School of Life Sciences
Arizona State University
PO Box 874501
Tempe AZ 85287-4501

Final Report Submitted to:

Lower Colorado River Multi-Species Conservation Office
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada

In partial fulfillment of Agreement Number 01-FG-30-0023
Between U.S. Bureau of Reclamation and Arizona State University

4 January 2008

Summary

Larval, repatriate and adult samples of Lake Mohave razorback sucker were characterized for mitochondrial DNA variation to continue assessment of transfer of genetic variation among life history stages. As in the previous study, significant differences were identified at the individual sample level; however, pooling among temporal and spatial samples failed to identify significant differences among larvae, repatriates, and wild adults, supporting the previous conclusion that the Lake Mohave sampling regime is adequately representing genetic variation. Additional larval and adult samples from backwaters and/or the lower Colorado River were also examined. These exhibited considerable variation that was sometimes atypical, highlighting the importance of genetic considerations in management.

Introduction

The major aim of conservation programs is continued persistence of biodiversity. This goal is generally approached through attempts to maintain ecosystems and protect forms threatened with extinction. Even under the best of circumstances, however, many ecosystems have been modified beyond repair, placing their component species in unique and tenuous environments. The ability to survive such conditions is largely determined by levels of genetic variation, which is closely tied to population size (Lande 1988, 1995; Hedrick and Miller 1992; Lynch 1996). Matings between related individuals (inbreeding) are more likely in small populations, resulting in increased frequency of abnormalities in progeny and reduced fitness (inbreeding depression). In small populations, chance effects will result in representation of only a subset of parental genotypes in the next generation, causing continuous loss of variant alleles over time (genetic drift) and ultimately eliminating genetic variation. While the deleterious effects of abnormalities associated with inbreeding depression are immediately obvious, loss of genetic variation also has a profound negative impact. Genetic variation is required for species to adapt to changing environments. Loss of this diversity ultimately reduces their adaptive potential, increasing the probability of extinction in a changing and unpredictable world. The impact of these forces is so profound that conservation biologists have emphasized the significance of genetic factors since inception of the field (reviewed in Soulé and Wilcox 1980), and consideration of genetic parameters has typically played a prominent role in formulation of recovery plans for endangered species.

Razorback sucker is a critically endangered fish endemic to the highly modified Colorado River system. The largest remaining population occupies Lake Mohave, a main stream reservoir in Arizona and Nevada. The species is in immediate danger of extirpation since natural recruitment has been eliminated (Marsh and Minckley 1992, Marsh and Pacey 2005). Razorback sucker remains relatively abundant in Lake Mohave, but estimated numbers have declined precipitously in the past decade from more than 60,000 to fewer than 2,000. Levels of genetic variation in razorback sucker are as high as any other vertebrate examined, indicating that these individuals are remnants of a very large population (Dowling et al. 1996ab).

A cooperative razorback sucker repatriation program was initiated in 1991 under auspices of a multi-agency Native Fish Work Group to prevent extirpation and loss of the Lake Mohave stock (Mueller 1995). The NFWG captures wild razorback sucker larvae from Lake Mohave, rears them in protected sites, then stocks large juveniles back into the lake. Through 2007 more than 500,000 larvae have been harvested, and nearly 128,000 young fish have been stocked (C. Pacey, unpublished data).

We have been using molecular markers to assess and validate the recovery program for razorback sucker from a genetic perspective. Characterization of genetic variation within and among wild and repatriate stocks including wild adults, larvae, and repatriated individuals indicated that the sampling regime has been appropriate for maintenance of genetic diversity (Dowling et al. 2004, 2005, Turner et al. 2007). Here we report results from our continuing studies of Lake Mohave razorback sucker genetics, extending our monitoring efforts of genetic variation within and among samples of larvae and adults (repatriate and wild individuals) across years. The rationale behind this study was two-fold: 1) to continue analysis of temporal variation among larval samples collected at different times from different regions in the lake, and 2) to increase the number of repatriate individuals in the data set. This information is necessary for monitoring levels of genetic variation found in larvae and enhanced estimates of female effective population size. Analysis of additional repatriates will allow for more powerful comparisons of variation in genetic diversity among cohorts. As repatriates become more numerous, we would

predict that the number of females contributing to each larval year class should increase and genetic variation among repatriate cohorts should lessen.

Materials and Methods

Sampling. — To assess patterns of transmission by adults to larvae, we attempted to obtain nominal samples of 25 larvae from each of four different, general areas of Lake Mohave (Nine Mile, Tequila, Yuma, and Wrong) collected four times each year during the January to April spawning period (target of 400 larvae/year). Larval razorback suckers were captured with dip nets at night, after attraction to submersed lights. Individual larvae were transferred immediately to 95% ethyl alcohol in 1.5 mL snap cap vials and transported to the laboratory for processing. Repatriates were represented by samples collected primarily during spawning seasons. These individuals were captured in overnight trammel nets set at the same locations from which larvae were collected as well as other areas of the lake. A small amount (less than 1 g) of tissue was removed with scissors from the right pectoral fin and placed immediately into 95% ethyl alcohol in a 1.5 mL snap cap vial. Fish were identified as repatriates by a unique, 10-digit passive integrated transponder (PIT) tag implanted into each fish at the time of its release. Tissue samples also were obtained as described above from wild adults captured using the same trammel netting technique.

Characterization of mtDNA variation. — Genomic DNA was extracted from whole larvae and fin clips by standard phenol-chloroform protocol (Tibbets & Dowling 1996). All samples were screened for single-stranded conformation polymorphisms (SSCPs — Dowling et al. 1996b; Sunnucks et al. 2000; Gerber et al. 2001; Dowling et al. 2005) in a 311 bp piece of the cytochrome *b* (cyt*b*) gene produced using the primers LE_{RBS} (5′-GCCTACGCCATCCTTCG-3′) and HA (5′-CAACGATCTCCGGTTTACAAGAC-3′). SSCP variation was visualized by two different methods. Prior to 2006, amplification products were visualized as described in Dowling et al. (2005) by labeling with α-^{32}P dATP, electrophoresis through 6% acrylamide gel (37.5:1 acrylamide:bis-acrylamide, 1X TBE), and autoradiography. To distinguish among SSCP patterns for this method, previously loaded samples were arranged by mobility and reanalyzed by grouping them together on a second gel, followed by autoradiography. At least one representative from each presumptive group of haplotypes was sequenced from each set of gels.

In 2006, we started assaying SSCP variation using fluorescent markers on a Licor 4300 DNA analyzer. PCR products were generated using the same primers except that they were labeled with IRD-700 or IRD-800 labels. For amplification, we used the Qiagen multiplex PCR kit (Qiagen) under the following conditions: 94 C, 15 min; 15 cycles of 94 C 30 sec, 50 C 90 sec, 72 C, 60 sec; 72 C, 10 min. Products were diluted 1:25-1:50 with filtered, sterile water. Just prior to loading, 1 uL aliquots of diluted samples were mixed with loading dye, denatured by heating to 95 C, 5 min, then placed on ice. Samples were loaded onto the gel (cast from MDE gel solution [Lonza] in a 41 cm apparatus), and electrophoresis occurred at room temperature for 20 hr at 6 W. The laser tracked the gel at the slowest speed possible. Because image size was generally too large to analyze, images were cropped and imported from the Licor workstation to the accompanying PC computer. Cropped images were analyzed using SAGA Generation 2, allowing for comparison of all lanes across a run. The 10 most frequent haplotypes included as standards on each run. Multiple mobility variants from each run were sequenced to determine haplotype.

***Sequencing.* —** Conditions for amplification were the same as the described above for radioactive SSCP except that a sequencing primer, LERBSSEQ (5′-CGCTATTTTCACCCAACC-3′), was designed to obtain sequence for the entire SSCP fragment. An aliquot of each amplification product (5µLs) was run on a 1% agarose gel prestained with ethidium bromide to check the quality of the reactions. Suitable reactions were purified by centrifugation using Ultrafree MC® filter units (Millipore Corp.) or ExoSAP (US Biochemical) as directed by the supplier. DNA sequences were generated by automated sequencing using an ABI 3730 Sequencer and the Big Dye® vs. 3.1 kit (ABI Perkin-Elmer). Sequences were aligned manually using MacDNASIS v 3.2 (Hitachi Corp.) with the homologous sequence of *Cyprinus carpio* (Chang et al. 1994) as the reference.

***Haplotype assignment.* —** Representative sequences from presumptive groups were entered into a template file containing all known haplotypes and analyzed using PAUP* (version b10, Swofford 1998). Most parsimonious topologies were recovered by heuristic search with simple addition of haplotypes. When a representative sequence matched a known haplotype, all

members of that SSCP group were scored to reflect this designation. New haplotypes were designated alphabetically in order of discovery, not mobility similarity, and added to the template file.

Statistical analysis. — Gene diversity and number of haplotypes were obtained using the program Arlequin (version 3.1, Schneider et al. 2000). Gene diversity is a measure of within population variability, providing the probability that two randomly chosen haplotypes are different in the sample. The number of haplotypes is also related to population size and was corrected for sample size (A_R) by rarefaction using the computer program HP-Rare (Kalinowski 2005). Significant deviation of the number of haplotypes and gene diversity for each larval and repatriate collection relative to the wild adult population was assessed using a bootstrap re-sampling program written in FORTRAN. This program generated null distributions for these two factors by randomly re-sampling haplotypes (with replacement) from a source population (represented by 272 wild adults), followed by counting haplotypes and calculating gene diversity. Confidence intervals were based on distributions of these two parameters for 10,000 replicates, and observed values were considered significant if they fell within the tails of these distributions. Standard statistical analyses were completed using SPSS (version 15.0). Arlequin was also used to examine the distribution of genetic variation within and among populations by molecular analysis of variance (AMOVA — Excoffier et al. 1992). To test for structure associated with location or sampling time, variance was also partitioned into components associated with these two parameters.

Results and Discussion

From 2004-2007, an additional 1912 larvae have been characterized for a total of 4331 collected from Lake Mohave since 1997 (Table 1; see also Dowling et al. 2005 and the final report for the previous funding period). Larvae were also obtained from the Needles area of the Colorado River, Arizona-California (two sites, one on each side of the river, downstream from UTM 11S 720750E 3857275N, which is approximately the location of the Highway 95 [Harbor Avenue] bridge across the river) and A-10 backwater, Arizona, from the lower Colorado River (28 and 51 larvae, respectively). We also characterized 442 fin clips from Lake Mohave, and now have characterized 853 adult fish (342 wild and 511 repatriates — Table 2). Additional fin clip samples

(n = 23) were obtained from the Needles area, captured north of the Needles (Highway 95) bridge. Characterization of all samples identified 33 haplotypes (Figure 1, Tables 1 and 2) that differ by 1-2 mutations. Phylogenetic analysis recovered 54 most parsimonious trees of 34 steps (CI = 0.765, RI = 0.805).

Variation within larval samples. — Larval availability from the four sampled regions of Lake Mohave was highly variable, with Wrong Cove providing the fewest samples and Yuma Cove the most. This was largely because few spawning adults were present and thus few larvae were produced at Wrong Cove relative to other areas. Individual samples with 15 or fewer larvae (n = 4) were excluded from within population analyses but included in analysis of variation among samples.

Estimates of gene diversity were highly variable among collections, ranging from 0.00 to 0.81 (Table 3) with a mean of 0.58. A_R was also highly variable among collections with a mean value of 5.0 and range of 1.0-8.0. These estimates are comparable to those obtained from 1997-2003 collections (means of 0.55 and 4.6, respectively, Dowling et al. 2005), indicating little change in levels of diversity within larval samples. This conclusion was supported statistically as ANOVAs on levels of gene diversity (arcsin sqrt transformed) and A_R among years failed to detect significant differences among years (one-way ANOVAs, F = 0.844, 10 df, P = 0.587 and F = 1.192, 10 df, P = 0.301, respectively).

To further examine potential significance of gene diversity and number of haplotypes, confidence intervals for these measures were generated by bootstrap re-sampling the wild population (as described in Dowling et al. 2005) for each sample size > 15 individuals. Of the 76 larval collections examined, 37 of 152 comparisons exhibited significantly lower diversity and/or fewer haplotypes than expected ($P < 0.05$), more than in the period 1997-2003 where 25 of 186 were significant. As expected, A_R was significant more frequently than diversity (23 and 14 occurrences, respectively, for 2004-2007), reflecting the greater sensitivity of the former measure. To determine if significant deviations were randomly distributed across larval samples, we tested for association between significant deviations and other variables (e.g., location, time of year). Proportion of significant deviations relative to location was nearly significant (Fisher's

exact test, P = 0.054). When split out by years, we found significant differences among locations in 2004 (Fisher's exact test, P = 0.003) while comparisons from 2000 (P = 0.066) and 2003 (P = 0.075) approached significance. Further inspection of the data indicates that 2000 and 2006 results may reflect small samples from Wrong Cove; however, the significant result from 2004 reflects the high proportion of significant test results (8 of 12 tests) from Tequila Cove. High proportions (≥50%, minimum of six tests) of significant deviations were also found in two additional years (1999 and 2005), and Tequila Cove was the only area that produced such deviations. Seasonal variation was also evident as there were differences in the proportion of significant deviations and month the sample was collected (Fisher's exact test, P=0.044). This result reflects more significant deviations early (Jan-Feb, 23% significant) and late (April-May, 30%) in the season relative to the middle (March, 15%). Examination by location indicates a location effect as half of the Jan-Feb significant deviations are from Tequila Cove while the April-May significant deviations were found at Wrong and Yuma Coves. Since initiation of spawning varies from year to year, this analysis is crude; however, it does indicate that there are regional and seasonal differences in the number of spawners contributing to the pool of larvae.

***Variation among larval samples.* —** To examine patterns of variation within and among areas, AMOVA was performed for each year class independently (Table 5). Patterns observed from 2004-2007 were consistent with those observed in the previous seven years (Table 5). Overall fixation indices (F_{ST}) were statistically significant for each of the eleven years, ranging from 0.020 (2002) to 0.147 (2004). Therefore, annual collections exhibit differences in allele frequency and divergence, indicating that different subsets of females from the adult population spawn at different times and/or places. To determine if this pattern results from females homing to specific areas, the fixation index was partitioned to assess variation within (F_{SC}) and among (F_{CT}) geographical areas (Table 5). F_{SC} was also significant for every year (range 0.024-0.138) whereas F_{CT} was never significant (range –0.022-0.023), indicating that haplotype frequencies were significantly different among regions. Previously we expressed concerns about the nearly significant F_{CT} from 2003 (P = 0.069); however, none of the values from 2004-2007 approached significance identifying that result as an anomaly. Therefore, there is no evidence for consistent use of areas by groups of females with similar haplotypes, and variation among temporal

collections within areas explains the observed patterns. These results corroborate demographic studies that showed fish movement among spawning areas (Mueller et al. 2000).

To determine if there were annual differences in the distribution of genetic variation, AMOVA was performed on all data, separating out the temporal collections within years and comparing among years (e.g. ignoring the geographical component). This analysis revealed significant differences among collections (F_{ST} = 0.068, P < 0.001). Partitioning of this variation into within and among year components indicated the majority of variance was attributable to significant differences among temporal collections within years (F_{SC} = 0.069, P < 0.001). Differences among years was not significant (F_{CT} = 0.0005, P = 0.326), indicating that pooled collections were essentially the same among years.

Variation within and among repatriate cohorts. **—** Samples of repatriates have now been obtained from a total of 16 repatriation years (hereafter referred to as "cohorts") from 1992 to 2007. This effort has yielded samples from 511 individuals with 19 haplotypes (Table 2). Collection sizes were small for cohorts 1992-1994 and 2006-2007; therefore, these groups were pooled to form two multi-year cohorts (i.e., 1992-1994, 2006-2007). Levels of genetic variation were variable among cohorts; however, there was no obvious temporal pattern (Table 5). The number of haplotypes ranged from 4 (1992-94 and 2002) to 11 (1996), and when corrected for sampling effects (A_R) they ranged from 3.12 (1997) to 6.19 (2006-07). Gene diversity was also variable, ranging from 0.308 (1997) to 0.759 (2006-07). The potential influence of sampling was examined by randomly re-sampling the parental population (as described in Dowling et al. 2005) for each of the sample sizes for our cohorts. Of the 26 estimates of number of haplotypes and gene diversity, 23 fell within those expected from a random draw of the adult population, with the exceptions being fewer haplotypes than expected in the 1995 cohort and reduced diversity in the 1997 and 2000 cohorts.

AMOVA was used to examine the distribution of variation among cohorts. As with our previous analyses, most of the variation resided within cohorts, with low levels of divergence among them (F_{ST} = 0.003, P = 0.254). In our previous analysis, we were concerned about small sample sizes

for some cohorts (Dowling et al. 2005): however, increased sample sizes failed to change this result, indicating that mtDNA haplotype distributions are similar across stocking year classes.

Variation among adults, larvae, and repatriates. — The ultimate goal of the repatriation program is to ensure that repatriates possess and pass on to their progeny the levels and patterns of genetic variation that are representative of the wild adult population from which they are derived. This requires that sampled larvae represent random reproduction of the adult population and random survivorship of larvae through the custody and repatriation process. The spatial and temporal stratification of larval harvest was specifically designed to ensure transmission of genetic variation in the wild adult population through the larvae and into the repatriate population. Analysis of genetic variation among areas within years supported the use of such a stratified design (see above); however, this does not address the effectiveness of this sampling design in transmitting variation into the repatriate population. To assess the effectiveness of this approach, we performed an AMOVA on adults, larvae, and repatriates, with the larval and repatriate samples partitioned by year of collection (with all samples pooled within each year) and cohort, respectively. This analysis identified significant variation among samples (F_{ST} = 0.003, $P < 0.001$), with the variance explained by differences among larval years or repatriate cohorts (F_{SC} = 0.004, $P < 0.001$) and not among adults, larvae, and repatriates (F_{CT} = −0.001, P = 0.923). Although we were able to detect significant differences among samples within life history groups, the level of variation is so low (e.g. F_{ST} = 0.003) as to not be biologically meaningful. Our ability to detect statistical significance at this level reflects large sample sizes and the power of the method in detecting differences (Hedrick 1999, 2001). Therefore, this significant result should not be construed as important when considering management strategies.

Additional samples. — There are three additional larval and two additional adult samples that require special comment. We were provided a sample of larvae that were volunteer spawn in the Yuma Cove backwater, March 2006. This sample exhibited significantly fewer haplotypes than expected; however, gene diversity was within the range expected for its sample size (Table 3). The frequencies of haplotypes were different from those found in the lake, with the E haplotype occurring in fewer than half the individuals (typical frequency in larvae is 0.62) while the remaining three haplotypes were much more common than expected (Table 1). While this

sample was distinct, it was not atypical as occasional samples will exhibit changes in frequency among the more common alleles (Dowling et al. 2005). When examining results from pairwise F_{ST} comparisons from 2006 larval samples, the mean number of significant comparisons was 10.5, with a standard deviation of 6.3. The Yuma Cove backwater sample exhibited 12 significant pairwise comparisons, well within the range of typical samples. While this is only from a single replicate, this result indicates that the backwater management plan outlined by Minckley et al. (2003) has the potential to maintain genetic variation if implemented properly.

Two additional samples of larvae, one from A-10 backwater and another from the Needles area, were more extreme, but in different ways. The common E haplotype was at a frequency of less than 0.35 while an extremely rare haplotype (U, found in only 15 of 4304 individuals from Lake Mohave, Table 1) was found in 20 of the 51 larvae sampled from A-10 backwater, and like the Yuma Cove backwater sample, exhibited fewer alleles than expected but appropriate levels of diversity. Note, however, all 26 pairwise F_{ST} comparisons were significant, indicating the extreme variation in allele frequency for this sample. The Needles sample exhibited much lower levels of genetic variation than expected as 28 of 30 larvae sampled exhibited the common E haplotype (Tables 1 and 3) and this sample was significantly different from 20 of the 26 larval samples of 2006. The sample of adults from Needles area is typical relative to other adult samples (Table 2), indicating that a limited number of females were involved in production of the larval sample from that reservoir. Finally, we characterized a sample of fin clips from Cibola High Levee pond. These exhibited atypical frequencies with a rare haplotype (BB, found in 2 of 511 repatriates and 0 of 342 wild adults) found in 10 of the 30 individuals characterized and was significantly different from all other adult samples examined.

Implications for management

Results presented here extend those reported previously (Dowling et al. 2005) and indicate that the program continues to be effective at maintaining genetic variation in this managed population. This is especially important as repatriates now are the major component of the adult population in Lake Mohave (repatriates represented 87% of an estimated 1679 adults in 2007), yet levels of genetic variation have not decreased. This indicates that even though the repatriation program has yet to establish large numbers of adults in the lake, the current

population of adults has maintained levels of genetic variation in the adult population and that genetic variation continues to be transmitted into the larvae produced.

Additional samples illustrate why harvest for repatriation should be restricted to larvae directly from the lake instead of additional sources. The sample from Yuma Cove backwater is not distinctive from typical larval samples from the lake; however, because of variance in reproductive success among adults razorback suckers (Turner et al. 2007) and the potential consequences of inbreeding (Dowling et al. 2006a), such samples should be treated with extreme caution. Individuals from the lower river were not collected as larvae from Lake Mohave, but were produced in hatcheries and not intended for use in the Mohave program. Analysis of these samples clearly illustrates issues associated with sampling and why such individuals should not be incorporated into the Lake Mohave repatriation program. Larval samples from A-10 backwater and the Needles area were distinct from all others as was the adult sample from Cibola High Levee Pond. The latter is of special concern as some individuals from this location were stocked into Lake Mohave via Davis Cove. Transfer into the population at large of volunteer spawn from backwaters handled like this one must be discouraged until a protocol is developed to assess the genetic integrity of such repatriates and to ensure that detrimental effects do not accrue.

In addition to examining genetic parameters for the Lake Mohave razorback sucker population, these samples are extremely valuable for assessing population demographics. Turner et al. (2007) used data from 1997-2003 to estimate the effective number of female breeders each year and generation and discussed how changes in this ratio will be useful for monitoring declines in numbers of breeding individuals over time. We (in collaboration with T. Turner) are in the process of estimating these same parameters for the years 2004-2007, extending the estimates and allowing for assessment of stability of these measures. These data, in conjunction with ecological data generated by Marsh's group and cooperators, provide important estimates of demographic and genetic parameters essential for informed management of this species.

Acknowledgements

We are eternally indebted to the late W. L. Minckley for his myriad contributions to this project and his unsurpassed knowledge of razorback sucker biology. We also thank the following Native Fish Work Group members and their staffs, with special appreciation for their leadership roles to Tom Liles (retired) and Andy Clark (Arizona Game and Fish Department), Mike Burrell (Nevada Division of Wildlife), Tom Burke and Ty Wolters (Bureau of Reclamation), C. O. Minckley (U.S. Fish and Wildlife Service, retired), Gordon Mueller (U.S. Geological Survey), and Ross Haley and Kent Turner (U.S. National Park Service, Lake Mead National Recreation Area). Collections were authorized by permits from USFWS, USNPS and the states of Arizona and Nevada.

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Table 1. Haplotypes of razorback sucker larvae from Lake Mohave (11 years), Needles area, A-10 backwater and Yuma Cove (YC) backwater. Number of haplotypes for each sample category is provided in the last line.

	Mohave														
Haplotype	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	total	A-10	Needles	YC backwater
A	9	12	3	20	5	14	20	31	32	20	14	180	0	0	0
B	46	36	27	39	33	19	30	31	36	66	22	385	1	1	4
C	18	8	0	4	0	6	14	13	3	8	10	84	0	0	0
D	0	0	0	0	0	0	1	0	1	1	0	3	0	0	0
E	197	321	200	230	136	239	214	358	267	344	178	2684	16	26	12
F	13	14	14	4	7	9	3	12	8	13	8	105	5	0	0
G	1	1	3	3	0	1	4	3	2	4	1	23	0	0	0
H	2	5	1	3	0	1	0	3	6	5	8	34	0	0	0
I	1	2	0	0	0	0	1	1	2	0	1	8	0	0	0
J	7	9	1	3	1	2	1	2	4	6	2	38	0	0	0
K	3	4	0	0	3	1	4	2	4	3	3	27	0	0	0
L	2	0	0	0	0	0	0	0	0	1	0	3	0	0	0
M	3	2	1	8	1	2	2	0	1	3	2	25	0	0	0
N	1	0	2	0	0	0	0	0	0	0	0	3	0	0	0
O	1	0	0	0	2	0	0	0	0	0	1	4	0	0	0
P	8	5	4	3	1	6	0	2	4	1	2	36	0	0	5
Q	1	2	0	1	0	0	5	1	0	0	1	11	0	0	0
R	18	29	19	17	11	23	29	48	33	45	20	292	0	1	6
S	6	22	12	21	20	24	35	33	25	26	30	254	5	0	0
T	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
U	0	0	3	5	4	0	1	1	0	0	1	15	20	0	0
V	0	5	3	4	5	1	2	14	6	3	0	43	0	0	0
W	0	0	2	1	0	0	1	0	0	0	0	4	0	0	0
X	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0
Y	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Z	0	4	0	0	0	0	0	1	1	2	0	8	0	0	0
AA	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
BB	0	1	0	0	0	0	0	0	1	6	2	10	4	0	0
CC	0	0	0	0	0	0	0	2	1	8	1	12	0	0	0
DD	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
EE	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
FF	0	0	0	0	0	0	0	0	0	3	1	4	0	0	0
GG	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0
total	337	483	295	367	230	348	369	559	437	571	308	4304	51	28	27
# haps	18	19	15	17	14	14	18	19	19	22	20	33	6	3	4

Table 2. Haplotypes from fin clip samples obtained from Lakes Mohave and Needles area. Repatriate year represents year stocked into Lake Mohave. Number of haplotypes in each sample is provided in the last line.

	repatriates																	wild	Needles	Cibola
Haplotype	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total			
A	3	2	0	6	4	0	1	0	1	1	0	0	1	1	1	2	23	12	0	0
B	0	0	0	4	5	0	5	5	2	2	2	3	4	5	2	1	40	24	1	0
C	0	0	0	0	4	1	2	2	0	1	0	0	1	0	0	0	11	8	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	6	0	9	26	42	20	43	39	40	10	11	12	25	35	6	7	331	195	15	16
F	0	0	0	0	1	0	0	2	0	3	1	0	1	0	0	0	8	18	0	0
G	0	0	0	0	0	1	0	0	0	1	0	0	2	2	0	0	6	3	0	0
H	0	0	0	0	1	0	0	0	0	0	0	0	2	1	0	0	4	4	0	0
I	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2	0	0
J	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	3	2	1	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
P	0	0	0	1	2	0	1	0	0	0	0	0	0	1	0	2	7	11	0	0
Q	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2	2	1	0
R	0	1	1	4	7	2	3	5	1	2	0	3	2	3	2	2	38	35	3	0
S	0	0	0	0	3	0	5	4	5	0	0	0	5	3	0	0	25	16	1	2
T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
U	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	2	3	0	2
V	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	4	1	0	0
W	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Y	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Z	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
AA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BB	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	10
CC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
total	9	4	10	41	71	24	63	59	51	20	15	20	43	53	12	16	511	342	23	30
# haps	2	3	2	5	11	4	10	8	7	7	4	5	9	10	5	7	19	20	7	4

Table 3. Summary statistics for larval collections of razorback sucker characterized during years 2004-2007. N is the sample size, A_R is the corrected number of haplotypes (as described in the methods section), and gene diversity is presented with one standard deviation. Significance values (sig) were generated by re-sampling as described in the methods.

Year	Area	Collection date	N	# of haplotypes	A_R	sig	gene diversity	sig
2004	Nine Mile area	3-Feb	10	2	na	na	0.2000+/-0.1541	na
		6-Feb	15	8	na	na	0.7333+/-0.1244	na
		17-Feb	4	1	na	na	0	na
		20-Feb	25	7	6.36	ns	0.6733+/-0.0957	ns
		1-Mar	25	5	4.56	ns	0.4767+/-0.1154	ns
		15-Mar	23	7	6.34	ns	0.5217+/-0.1241	ns
		29-Mar	25	6	5.40	ns	0.7200+/-0.0579	ns
		13-Apr	21	8	7.76	ns	0.7190+/-0.0992	ns
	Tequila Cove	3-Feb	27	3	2.73	-	0.2707+/-0.1046	-
		18-Feb	25	4	3.80	-	0.6767+/-0.0591	ns
		2-Mar	25	3	2.97	-	0.4533+/-0.1022	ns
		17-Mar	29	3	2.38	-	0.1355+/-0.0845	-
		30-Mar	25	6	5.56	ns	0.7467+/-0.0552	ns
		13-Apr	27	4	3.68	-	0.4387+/-0.1078	-
	Wrong Cove	29-Mar	25	7	5.80	ns	0.4300+/-0.1237	ns
		12-Apr	25	3	3.00	-	0.5467+/-0.0909	ns
		4-May	24	5	4.81	ns	0.5942+/-0.1048	ns
	Yuma Cove	29-Jan	29	7	5.38	ns	0.6429+/-0.0932	ns
		17-Feb	25	5	4.53	ns	0.4233+/- 0.1194	ns
		1-Mar	23	6	5.74	ns	0.7352+/-0.0775	ns
		15-Mar	23	6	5.59	ns	0.5178+/-0.1218	ns
		31-Mar	25	6	5.56	ns	0.6267+/-0.1010	ns
		12-Apr	29	4	3.91	-	0.6108+/-0.0811	ns
		4-May	26	1	1.00	-	0	-
2005	Nine Mile area	26-Jan	26	4	3.68	-	0.3477+/-0.1149	-
		9-Feb	27	8	6.78	ns	0.6040+/-0.1073	ns
		25-Feb	25	5	4.53	ns	0.4233+/-0.1194	-
		7-Mar	28	7	5.77	ns	0.5370+/-0.1091	ns
		21-Mar	28	7	6.30	ns	0.6958+/-0.0861	ns
		5-Apr	25	9	7.96	ns	0.8033+/-0.0632	ns
	Tequila Cove	10-Feb	25	4	3.80	-	0.6567+/-0.0546	ns
		23-Feb	24	5	4.83	ns	0.7609+/-0.0495	ns
		7-Mar	28	3	2.70	-	0.2619+/-0.1022	-
		21-Mar	26	7	6.07	ns	0.6492+/-0.0950	ns
		5-Apr	24	3	2.83	-	0.3587+/-0.1096	-
	Yuma Cove	25-Jan	26	7	6.03	ns	0.6708+/-0.0840	ns
		8-Feb	26	4	3.76	-	0.6031+/-0.0785	ns
		23-Feb	25	7	6.16	ns	0.5867+/-0.1102	ns
		8-Mar	25	5	4.76	ns	0.6500+/-0.0872	ns
		22-Mar	25	6	5.56	ns	0.6633+/-0.0920	ns

		5-Apr	24	6	5.33	ns	0.5833+/-0.1015	ns
2006	Nine Mile area	7-Feb	25	9	7.99	ns	0.8100+/-0.0633	ns
		23-Feb	25	8	6.97	ns	0.7267+/-0.0767	ns
		7-Mar	25	5	4.56	ns	0.4767+/-0.1154	ns
2006	Nine Mile area	15-Mar	26	3	2.99	-	0.4462+/-0.1049	ns
		21-Mar	25	6	5.17	ns	0.4267+/-0.1216	-
		6-Apr	25	5	4.59	ns	0.5267+/-0.1101	ns
	Tequila Cove	8-Feb	25	4	3.97	-	0.6400+/-0.0828	ns
		21-Feb	25	4	3.97	-	0.6667+/-0.0727	ns
		7-Mar	24	6	5.64	ns	0.7572+/-0.0600	ns
		15-Mar	23	6	5.60	ns	0.5692+/-0.1144	ns
		22-Mar	25	5	4.53	ns	0.4233+/-0.1194	-
		5-Apr	24	4	3.93	ns	0.4348+/-0.1190	ns
		27-Apr	24	4	3.81	ns	0.5616+/-0.0917	ns
	Wrong Cove	22-Mar	24	6	5.48	ns	0.5942+/-0.1055	ns
		4-Apr	22	7	6.81	ns	0.7965+/-0.0669	ns
		27-Apr	25	3	2.60	-	0.1567+/-0.0957	-
	Yuma Cove	7-Feb	26	6	4.85	ns	0.3538+/-0.1194	-
		23-Feb	25	7	6.33	ns	0.6333+/-0.1039	ns
		8-Mar	25	8	7.13	ns	0.7133+/-0.0887	ns
		14-Mar	26	4	3.72	-	0.4954+/-0.1020	ns
		22-Mar	25	5	4.59	ns	0.5267+/-0.1101	ns
		5-Apr	24	6	5.60	ns	0.5543+/-0.1158	ns
		25-Apr	28	3	2.98	-	0.6111+/-0.0469	ns
	Yuma Cove backwater	14-Mar	27	4	4.00	-	0.7236+/-0.0543	ns
	A-10 backwater	10-Mar	51	6	5.24	-	0.7257+/-0.0376	ns
	Needles	3-Apr	28	3	2.43	-	0.1402+/-0.0871	-
2007	Nine Mile area	6-Feb	25	7	6.36	ns	0.7533+/-0.0669	ns
		23-Feb	5	3	na	na	0.7000+/-0.2184	na
		27-Feb	20	6	6.00	ns	0.8000+/-0.0537	ns
		15-Mar	25	5	4.53	ns	0.4233+/-0.1194	-
2007	Tequila Cove	7-Feb	25	4	3.96	-	0.6033+/-0.0914	ns
		20-Feb	23	7	6.73	ns	0.8063+/-0.0609	ns
		14-Mar	25	5	4.40	ns	0.4700+/-0.1120	ns
2007	Wrong Cove	5-Apr	25	7	5.97	ns	0.4867+/-0.1211	ns
		17-Apr	25	7	6.00	ns	0.6167+/-0.0984	ns
2007	Yuma Cove	5-Feb	25	6	5.37	ns	0.6133+/-0.0966	ns
		20-Feb	25	9	7.93	ns	0.7233+/-0.0918	ns
		12-Mar	35	7	5.91	ns	0.7244+/-0.0651	ns
		14-Mar	25	8	6.60	ns	0.4900+/-0.1230	ns

Table 4. Summary of results of AMOVAs for larval collections of razorback sucker from Lake Mohave. N is total number of individuals collected in that year. P identifies the significance value for the associated F-statistic.

Year	# of samples	N	F_{ST}	P	F_{SC}	P	F_{CT}	P
1997	13	339	0.083	0	0.101	0	-0.021	0.840
1998	19	485	0.043	0	0.046	0	-0.003	0.500
1999	13	294	0.041	0	0.053	0	-0.013	0.715
2000	16	367	0.049	0	0.058	0	-0.009	0.758
2001	10	230	0.100	0	0.101	0	-0.001	0.522
2002	14	348	0.020	0	0.024	0	-0.004	0.651
2003	14	370	0.06	0	0.037	0	0.023	0.069
2004	24	559	0.147	0	0.138	0	0.01	0.240
2005	17	437	0.059	0	0.058	0	0.001	0.380
2006	24	598	0.062	0	0.063	0	-0.0004	0.430
2007	13	308	0.043	0	0.054	0	-0.012	0.740

Table 5. Summary statistics for repatriate cohorts from Lake Mohave fin clip samples. N is the sample size for cohort, A_R is the corrected number of haplotypes (as described in the methods section), and gene diversity is presented with one standard deviation. Significance values (sig) were generated by re-sampling as described in the methods.

Year	N	# of haplotypes	A_R	sig	gene diversity	sig
1992-1994	23	4	3.54	ns	0.5415 +/- 0.1014	ns
1995	41	5	4.02	-	0.5707 +/- 0.0797	ns
1996	71	11	5.52	ns	0.6346 +/- 0.0625	ns
1997	24	4	3.12	ns	0.3080 +/- 0.1180	-
1998	63	10	4.69	ns	0.5253 +/- 0.0741	ns
1999	59	8	4.67	ns	0.5506 +/- 0.0740	ns
2000	51	7	3.52	ns	0.3796 +/- 0.0840	-
2001	20	7	6.14	ns	0.7368 +/- 0.0939	ns
2002	15	4	4.00	ns	0.4667 +/- 0.1478	ns
2003	20	5	4.48	ns	0.6211 +/- 0.1081	ns
2004	43	9	5.52	ns	0.6467 +/- 0.0777	ns
2005	53	10	5.01	ns	0.5559 +/- 0.0793	ns
2006-2007	28	8	6.19	ns	0.7593 +/- 0.0723	ns

Figure 1. One of 54 phylogenetic trees of mtDNA haplotypes from razorback sucker (*Xyrauchen texanus*). Letters identify haplotypes provided in Table 1. Branch lengths are proportional to the number of mutations.