



# Lower Colorado River Multi-Species Conservation Program

*Balancing Resource Use and Conservation*

## Genetic and Demographic Studies to Guide Conservation Management of Razorback Suckers in Off-Channel Habitats

2010–2015



September 2018

Work conducted under LCR MSCP Work Task C40

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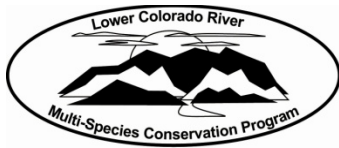
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The Nature Conservancy



# Lower Colorado River Multi-Species Conservation Program

## Genetic and Demographic Studies to Guide Conservation Management of Razorback Suckers in Off-Channel Habitats

**2010–2015**

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# ACRONYMS AND ABBREVIATIONS

AJ	Arizona Juvenile backwater
A <sub>R</sub>	allelic richness
DNA	deoxyribonucleic acid
dNTPs	dinucleotide triphosphates
F <sub>IS</sub>	standard inbreeding coefficient (within populations)
H <sub>E</sub>	expected heterozygosity
m	meter(s)
MgCl <sub>2</sub>	magnesium chloride
min	minute(s)
mm	millimeter(s)
mtDNA	mitochondrial deoxyribonucleic acid
N	sample size
PCR	polymerase chain reaction
PIT	passive integrated transponder
RASU	razorback sucker(s) ( <i>Xyrauchen texanus</i> )
s	second(s)
SNPs	single nucleotide polymorphisms
TL	total length

## Symbols

°C	degrees Celsius
>	greater than
<	less than
<<	much less than
μL	microliter(s)
%	percent

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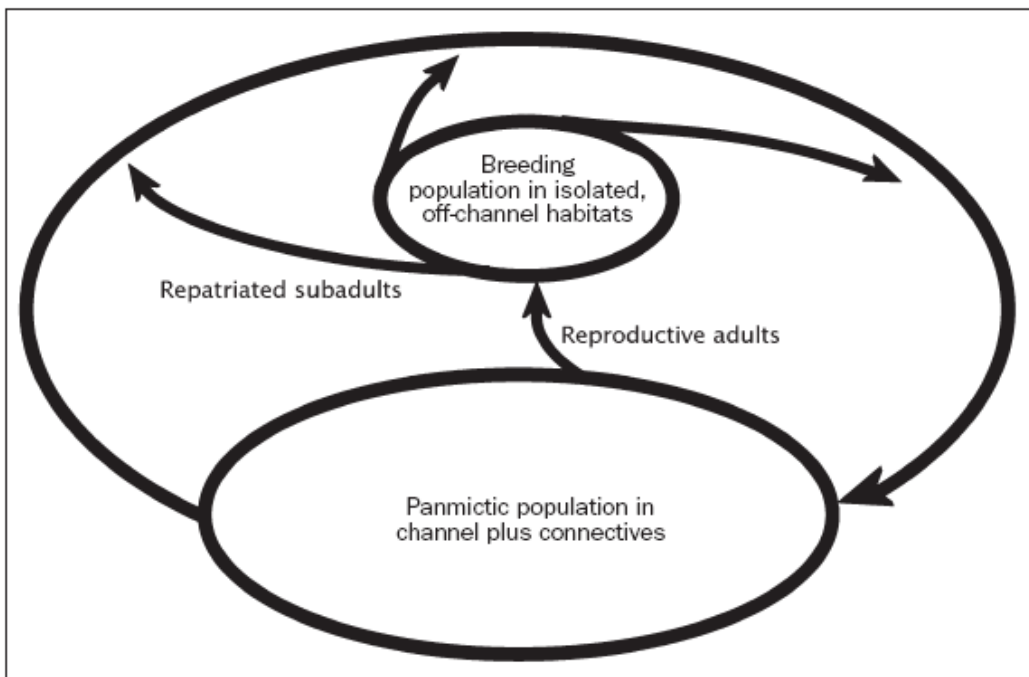
# INTRODUCTION

The razorback sucker (*Xyrauchen texanus*) (hereafter RASU) is one of the four species of large, long-lived, endemic fishes that were once abundant and broadly distributed throughout the Colorado River Basin (Minckley and Marsh 2009). Water development and the introduction and establishment of non-native species resulted in widespread extirpation and declines in the distribution and abundance of these and other native species beginning early in the 20<sup>th</sup> century. The impact of these factors on RASU was profound. This catostomid fish was once abundant throughout much of the river, producing large year-classes in several newly constructed reservoirs. Unfortunately, these and other populations failed to recruit, resulting in dramatic reductions in the number and size of populations as adult fish aged and died, ultimately leading to its listing as endangered (U.S. Fish and Wildlife Service 1991). As one strategy to counter this decline, a repatriation program for restoring RASU in Lake Mohave was begun in the early 1990s (reviewed in Marsh et al. 2015). The Lake Mohave program is unique in that it utilizes wild-produced larvae that are reared in protective custody and repatriated into the lake with the ultimate goal of replacing the pre-existing wild population. In an attempt to ensure accurate genetic representation of wild adults in the lake, larvae are sampled from all major spawning areas throughout the spawning period (January through April). Molecular information indicated that there are high levels of genetic variation in RASU (Dowling et al. 1996b), with the signature indicative of an expanding population (Garrigan et al. 2002). Monitoring levels and patterns of genetic variation within and among temporal and spatial samples of larvae and repatriates indicated that the program thus far has been successful at maintaining genetic variation (Dowling et al. 2005, 2014; Carson et al. 2016). Examination of temporal variation in frequencies of different mitochondrial deoxyribonucleic acid (mtDNA) alleles allowed for estimation of the annual effective number of female breeders and the generational female effective population size (Turner et al. 2007). When this information is considered jointly with annual census data (Marsh et al. 2003, 2005), for quantification of the proportion of the female population that contributes larvae each generation.. This approach indicated that RASU reproductive success is consistent with other long-lived, highly fecund species and that the proportion of the population contributing larvae has increased over time (Dowling et al. 2014).

Despite best efforts, RASU numbers have declined since the species was listed as endangered. The Lake Mohave population of RASU has declined from more than 60,000 in the 1980s to fewer than 3,000 in the recent past (Marsh et al. 2003, 2005), and the current estimate of the population is approximately 3,500, with only 12 estimated wild adults (Wisnall et al. 2017). Given concerns over the success of ongoing programs, it is time to explore additional management strategies for conservation of this species. One possibility suggested by Minckley et al. (2003) was the use of off-channel habitats (figure 1). In this program, native species would breed and their progeny grow in isolated, protected, off-channel



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**Figure 1.—Schematic representation of use of off-channel habitats for management of RASU (from Minckley et al. 2003).**

protected, off-channel ponds in the absence of non-native fishes. Panmictic adult populations would reside in the main channel and connected waters. Levels of genetic diversity would be maintained by bidirectional exchange of adults among main channel populations (e.g., Lake Mohave) and those in isolated habitats. Implementation of the plan has the potential to enhance recovery potential for these fish, as they would spend the critical part of their life history in protective custody while still being maintained in large bodies of water afforded by source populations.

Demographic and genetic factors are important when considering the fate of rare taxa. RASU are iteroparous, long-lived species that are characterized by an enormous reproductive capacity (mature females can produce tens of thousands of eggs per spawn). From a demographic perspective, this life history virtually guarantees a large number of offspring when rearing conditions favor recruitment. The downside from a genetic perspective is that one or a few breeding pairs could produce the majority of offspring that survive each year, and hence pass on only a small subset of the total genetic diversity available in the spawning stock, ultimately leading to increased inbreeding. Because inbreeding leads to reduced viability (Dowling et al. 1996a), it is crucial to monitor genetic diversity in progeny spawned and reared in off-channel habitats and to ascertain the number of parents contributing to offspring that are ultimately repatriated into the river. It is particularly valuable to combine the genetic monitoring approach with demographic studies. A combination of genetic and demographic information

will permit a detailed understanding of how variation in ecological/life history features associate with spawning success and offspring viability of specific individuals. Mortality of RASU progeny is expected to be very high, especially during early life history phases (e.g., larvae and juveniles), and it is possible that certain genotypes are more likely to survive than others, particularly in the quasi-natural habitats in off-channel ponds.

Because of their lack of connectivity with other backwaters and the main river channel, isolated off-channel habitats are atypical for this species, and resulting populations will be considerably smaller than normal. Thus, it is critical to characterize their genetic and demographic factors to assess the potential success of this conservation strategy. RASU were used as a model system to evaluate genetic and demographic factors affecting the use of off-channel habitats as management tools for big-river fishes. The persistence of individuals within backwaters was examined using remote passive integrated transponder (PIT) scanning and assessed the importance of persistence and other factors (e.g., sex, time of spawning, and body size) on reproductive success of individuals within ponds. This is important information for management of ponds, as knowledge of persistence and reproductive success will provide necessary information about the size of ponds necessary and the frequency with which individuals need to be moved among various backwaters.

## **METHODS**

### **Experimental Design**

Initial studies took place in two, predator-free backwaters on Lake Mohave (Arizona Juvenile [AJ] and Dandy) typically used for grow-out of RASU. In 2016, there were not sufficient samples from AJ (due to damage to the pond) or Dandy (due to limited reproduction); therefore, this report covers results from 2010 to 2015. AJ and Dandy have a maximum depth of approximately 1.4 and 2.8 meters (m) at full pool (lake elevation of 202.5 m) respectively, and both are approximately 0.4 surface hectare at full pool. At the start of this experiment, these ponds would dry each year as the lake level is lowered, and they had a history of producing larval RASU each year, making them ideally suited for evaluating variation in reproductive success in this species. In the last 2 years, Dandy has not gone dry, and some adults survived across years. Modifications of AJ in 2016 will likely have the same effect. This was not an issue for this analysis but could be in the future if recruitment occurs.

To examine the role of genetic and demographic factors, each ephemeral backwater was stocked with 200 individuals each year (approximately 100 of each sex). All individuals were PIT tagged and fin clipped prior to release for individual identification in remote scanning and deoxyribonucleic acid (DNA)

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extraction and genotyping, respectively. The sampling period of larvae and juveniles obtained for genetic analyses covered in this report was 2010–15; however, samples from Dandy were only available intermittently (table 1).

The Yuma Cove backwater, an off-channel body of water on Lake Mohave, became available for use in 2013. It is approximately 0.8 surface hectare at full pool (lake elevation of 202.5 m), with a maximum depth of 3.5 m. This is deep enough to have water year around. Like AJ and Dandy, the Yuma Cove backwater was historically used for grow-out, and it has a history of larval production. Because it is a permanent pond, individuals persisted for multiple years, and reproduction was also known to occur. Attempts were made to remove all RASU previously inhabiting the pond; however, it is likely that some individuals (especially juveniles and younger fish) remained.

In 2013, the initial stocking included 200 individuals, 100 of each sex. In each of the next 2 years, 100 additional individuals (50 males and 50 females) were stocked. All stocked individuals were PIT tagged and fin clipped for DNA extraction and genotyping prior to stocking. Samples of larvae and juveniles included in these genetic analyses were obtained in 2013–15 (table 1).

## **Remote Sensing**

Three distinct types of remote PIT scanning units were deployed into AJ and Dandy from 2010 to 2015 to monitor stocked fish. These scanning units were functionally similar to those described in Kesner et al. (2008). From 2010 through 2012, shore-based PIT scanning units utilizing Biomark FS2001 scanners were used exclusively. In 2013, a mix of shore-based units with FS2001 and RM310 scanners were utilized, and in 2014 and 2015, shore-based and submersible RM310 scanner units were used exclusively. Deployment of PIT scanners was conducted at least once each spawning season (February through April), and additional supplemental scanning was conducted in other months as time permitted. A single shore-based RM310 PIT scanning unit was deployed in March 2013 to continuously monitor RASU in the Yuma Cove backwater.

Data from remote PIT scanners were downloaded to a laptop computer at the end of a deployment in AJ and Dandy or on a routine (approximately monthly) basis for the continuous unit in the Yuma Cove backwater. The data were later uploaded to the lower Colorado River Remote Sensing Database through an online Web form (<http://www.nativefishlab.net>) with additional header information, including location, date of deployment, and remote PIT scanning unit type. The data were summarized using Microsoft Access and Excel 2010.

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Table 1.—Samples examined in this study

("\*" indicates samples that were of lesser quality due to issues with the tissue preservative, "NA" identifies samples that were not available, and "+" identifies a small sample from Dandy that was not included in statistical analyses. The Yuma Cove backwater was not initiated until 2013; therefore, there are no samples from previous years.)

Year	AJ				Dandy				Yuma			
	Females	Males	Larvae	Juveniles	Females	Males	Larvae	Juveniles	Females	Males	Larvae	Juveniles
2010	129	71	210	11*	100	100	207	41	–	–	–	–
2011	100	100	306	202	100	100	NA	NA	–	–	–	–
2012	100	100	116	246	100	100	NA	5+	–	–	–	–
2013	102	98	241	44	100	100	65	275	100	100	180	125
2014	102	98	216	59	101	99	NA	NA	50	50	301	122
2015	100	100	606	0	99	101	63	25	50	50	456	NA

## Characterization of Genetic Variation

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 14 dinucleotide microsatellite loci developed from a library for RASU (Turner et al. 2009; Dowling et al. 2014). Microsatellite fragments were amplified in the following multiplexed polymerase chain reactions (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 ( $\mu\text{L}$ ) reactions consisting of 2.5  $\mu\text{L}$  of Qiagen Master Mix (Taq DNA Polymerase, PCR Buffer, magnesium chloride [ $\text{MgCl}_2$ ], and dinucleotide triphosphates [dNTPs]), 0.01 to 0.45  $\mu\text{L}$  of each primer (varies from 0.2 micromolar to 9 micromolars), 1  $\mu\text{L}$  of water, and 0.5  $\mu\text{L}$  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 ( $^{\circ}\text{C}$ ), followed by 25 cycles of 94  $^{\circ}\text{C}$  for 30 seconds (s) for denaturation, an annealing step of 53  $^{\circ}\text{C}$  for 90 s, and 72  $^{\circ}\text{C}$  for 1 min, and the amplification was completed with a single step at 72  $^{\circ}\text{C}$  for 10 min. Multiplex 2, 3, and 4 were amplified using a profile that followed a touchdown format: initial denaturation of 15 min at 95  $^{\circ}\text{C}$ ; 25 cycles of 94  $^{\circ}\text{C}$  for 30 s, annealing at 65  $^{\circ}\text{C}$  for 1.5 min, and 72  $^{\circ}\text{C}$  for 1 min. Annealing temperature decreased by 1  $^{\circ}\text{C}$  in each of the first 15 cycles and remained at 50  $^{\circ}\text{C}$  for the remaining 10 cycles. Amplification was completed with a single step at 72  $^{\circ}\text{C}$  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

Data were downloaded into a tab-formatted sheet, uploaded into Microsoft Excel, and converted to GenePop 3-digit format. A text version of the GenePop file was loaded into the program *Mykiss* (S.T. Kalinowski, <http://www.montana.edu/kalinowski/software/mykiss.html>) to identify parents of each larvae and juvenile based on multilocus genotypes. Parents were

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identified as those individuals with no (or rarely the fewest) mismatches at all loci. When mismatches were detected, individuals were re-examined in Saga (version 3.3) to determine if the result was due to genotyping errors or null alleles. Any genotyping errors were corrected and *Mykiss* rerun on the corrected data to obtain the final assignment.

Population genetics parameters (allelic richness [ $A_R$ ], expected heterozygosity [ $H_E$ ], and the standard inbreeding coefficient [ $F_{IS}$ ]) were calculated with FSTAT (Goudet 2001), including an assessment of significant differences among groups using an included permutation test (1,000 replicates).  $A_R$  was corrected by rarefaction, where 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.

Linear regressions and paired t-tests were calculated using Excel and G-tests using PopTools, an add-in for Microsoft Excel written by Hood (2011). Arlequin (version 3.5.2.2) (Excoffier and Lischer 2010) was used to examine the differences among groups of parents with molecular analysis of variance. Other tests (e.g., Mann-Whitney) were performed using online programs (<http://www.socscistatistics.com/tests/>).

Stocking, harvest, and sampling data from the AJ, Dandy, and Yuma Cove backwaters were recorded by the Bureau of Reclamation, Arizona State University, Wayne State University, or Marsh & Associates, LLC, personnel on paper data sheets and entered into the Lower Colorado River Native Fishes Database. The data were summarized and tabulated using Microsoft Access and Excel 2010. The statistical program R (R Core Team 2015) was used to estimate the probability of being harvested for AJ and Dandy stockings from 2011 through 2015. The probability of a fish being harvested (yes or no) was estimated in a logistic regression model based on the categorical factors year (2011–15) and sex (male/female). Total length (TL) (in millimeters [mm]) at stocking was treated as a continuous predictor. Data for 2010 were not included in this analysis because the size measurements of individual fish were not recorded that year. Each backwater was analyzed separately to avoid the potential for a significant four-way interaction term. The Wald Test (Fox 1997) was used to test the significance of all coefficients involving the categorical factor year (interaction and main effects), and non-significant interaction and main effect coefficients were removed from the final model.

## **RESULTS AND DISCUSSION**

AJ and Dandy have been used as experimental ponds since 2010. These ponds are ephemeral, drying out each autumn/winter due to dam operations; therefore, they provide single point estimates of important demographic parameters. The Yuma Cove backwater became available for use in 2013, and because this backwater is permanent, it is possible to follow adults and recruits in this pond as well as estimate important demographic parameters. Because these ponds are different, ephemeral and permanent ponds are considered separately below for many of the analyses.

### **Patterns of Genetic Variation in All Stocked Adults**

Parents were stocked into backwaters from late January to early February each year, and larvae were captured from early March to early May. Initially, stocked adults were left in the ephemeral ponds until October (through 2014), but in later years, these individuals were harvested in May. Juveniles were typically sampled in October (see table 1). Reproduction occurred consistently in the AJ and Yuma Cove backwaters but was much more sporadic in Dandy as indicated by years where samples were not available (see table 1). The Yuma Cove backwater was never dewatered, but sampling with trammel nets was conducted in November 2013, January 2014, May 2014, and May 2015 to PIT tag and take genetic samples (fin clips) of naturally recruited individuals.

Population genetic parameters were estimated for samples from 2010 to 2015 for AJ, 2013 to 2015 for the Yuma Cove backwater, and only samples where sufficient reproduction occurred in Dandy (2010, 2013, and 2015) to estimate these parameters (table 2). A statistical comparison of  $H_E$ ,  $A_R$ , and  $F_{IS}$  between the two sexes failed to identify significant differences in levels of variation among them ( $P$  values from FSTAT permutation tests, 0.69, 0.88, and 0.96, respectively) as did an AMOVA, which tests for differences in allele frequency among groups ( $P = 0.95$ ). Comparisons of levels and patterns of variation by AMOVA also failed to detect differences among replicates ( $P = 0.487$ ); therefore, each of these replicates are genetically comparable.

Patterns of variation at individual loci were examined for each of the samples to identify deviations from the Hardy-Weinberg equilibrium (excesses or deficiencies of heterozygotes). Because of the large number of tests (24 samples  $\times$  14 loci = 308 tests), the critical value was adjusted to 0.0079 using the B-Y method (Narum 2006). This approach identified 12 significant tests, all reflecting deficiencies of heterozygotes, at 4 loci (Xte 7, Xte 10, Xte 17, and Xte 20). Significant values were scattered randomly across replicates; however, females exhibited significant values more frequently than males (8 and 4, respectively).

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Table 2.—Estimates of population genetic statistics of RASU males and females stocked into AJ and Dandy from 2010 to 2015

Year	Location	Sex	N <sup>1</sup>	H <sub>E</sub> <sup>2</sup>	A <sub>R</sub> <sup>3</sup>	F <sub>IS</sub> <sup>4</sup>
2010	AJ	Females	129	0.737	13.1	0.006
		Males	71	0.743	13.3	0.028
	Dandy	Females	100	0.743	12.9	0.033
		Males	100	0.736	13.3	0.014
2011	AJ	Females	100	0.740	13.0	0.021
		Males	100	0.734	12.7	0.023
2012	AJ	Females	100	0.736	12.8	0.009
		Males	100	0.743	12.6	0.013
2013	AJ	Females	102	0.738	12.9	0.005
		Males	98	0.731	13.0	0.008
	Dandy	Females	100	0.752	12.9	0.01
		Males	100	0.736	13.0	0.008
	Yuma Cove	Females	100	0.739	13.0	0.022
		Males	100	0.735	12.6	0.012
2014	AJ	Females	102	0.735	12.7	0.011
		Males	98	0.732	12.7	0.017
	Yuma Cove	Females	50	0.741	12.8	0.036
		Males	50	0.737	12.7	0.004
2015	AJ	Females	100	0.723	13.0	0.01
		Males	100	0.731	13.2	0.005
	Dandy	Females	99	0.742	13.0	0.021
		Males	101	0.744	13.3	0.008
	Yuma Cove	Females	50	0.721	13.2	0.006
		Males	50	0.734	12.7	-0.007

<sup>1</sup> N = The number of individuals stocked of each sex.

<sup>2</sup> H<sub>E</sub> = A measure of gene diversity.

<sup>3</sup> A<sub>R</sub> = A measure of allelic richness. Estimates of A<sub>R</sub> were generated by rarefaction using a minimum sample size of 46 individuals.

<sup>4</sup> F<sub>IS</sub> = A measure of deviation from the Hardy-Weinberg equilibrium.

Further examination of parent-offspring relationships indicates that these deviations reflect the presence of null alleles at some of these loci (discussed below).



## **Patterns of Variation in Larvae/Juveniles**

Measures of genetic variation were also characterized in the larvae and juveniles were contrasted to determine if they were representative of parents (table 3).  $A_R$  was significantly different among the three life history stages (permutation test,  $P = 0.0001$ ) and always lower in larvae and juveniles than stocked adults (9.5, 8.7, and 10.7, respectively). Estimates of gene diversity were also significant ( $P = 0.001$ ) and yielded a similar pattern, with values in stocked adults ( $H_E = 0.735$ ) always greater than larvae and juveniles ( $H_E = 0.715$  and  $H_E = 0.713$ , respectively).

Levels of variation among larvae and juveniles to directly assess their significance. If sampling of larvae across the spawning season and larval survivorship was random, there should be no difference in the patterns of variation among larvae and juveniles produced by the same adults. This was the case for gene diversity (permutation test,  $P = 0.77$ ); however, the difference in  $A_R$  between larvae and juveniles was nearly significant ( $P = 0.083$ ). This observation will have to be addressed when more samples are available.

Reduced levels of genetic variation in larvae and juveniles relative to adults indicate that progeny represent only a subset of the parents stocked into backwaters. This is not surprising given the mortality observed in stocked adults (see below), potentially reducing or eliminating their ability to reproduce.

## **EPHEMERAL PONDS**

Remote PIT scanning was conducted at least once every year in AJ and Dandy (table 4). PIT scanning that was conducted in February and March typically contacted more than one-half of the fish released, with the exception of Dandy in 2012 and 2013. Contact rates were much lower for PIT scanning conducted in the summer months (May through September). Most of this decline is likely the result of a decline in activity; this is evident because the number of fish harvested each year (table 5) was more than double the number of unique fish scanned after April, except for Dandy in July 2010.

In both AJ and Dandy, proportionally more females were harvested than males for all annually paired data (table 5). The mean of 62.70% of females harvested was 1.5 times higher than the mean for males (39.93%). Mean TL at stocking was also higher for females than males every year from 2011 to 2015 (individual TL was not recorded in 2010, and values reflect overall mean), making it difficult to determine if sex, size at release, or both influence backwater survival.

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Table 3.—Estimates of population genetic statistics of RASU adults (males and females pooled), larvae, and juveniles stocked into AJ and Dandy from 2010 to 2015 and into the Yuma Cove backwater from 2013 to 2015

(Yuma Cove backwater samples from 2014 and 2015 also include all stocked adults from previous year[s], as these individuals could have contributed to production of progeny in those years.)

Location	Year	Rarefaction number	Sample	N <sup>1</sup>	H <sub>E</sub> <sup>2</sup>	A <sub>R</sub> <sup>3</sup>
AJ	2010	200	Adults	200	0.739	16.9
			Larvae + juveniles	211	0.720	14.9
	2011	200	Adults	200	0.737	16.4
			Larvae	306	0.730	14.6
			Juveniles	202	0.718	13.9
	2012	116	Adults	200	0.740	15.0
			Larvae	116	0.719	12.8
			Juveniles	246	0.705	12.3
	2013	43	Adults	200	0.735	12.7
			Larvae	243	0.721	11.5
			Juveniles	44	0.710	10.5
	2014	59	Adults	200	0.734	13.3
			Larvae	216	0.683	8.3
			Juveniles	59	0.664	8.1
	2015	199	Adults	200	0.727	16.9
Larvae			606	0.712	14.1	
Juveniles			59	0.664	8.1	
Dandy	2010	40	Adults	200	0.739	12.7
			Larvae	207	0.707	10.9
			Juveniles	40	0.685	10.1
	2013	64	Adults	200	0.744	13.9
			Larvae	65	0.736	12.7
			Juveniles	275	0.736	12.3
	2015	23	Adults	200	0.743	10.8
			Larvae	63	0.674	7.8
			Juveniles	25	0.658	7.8
Yuma Cove	2013	122	Adults	200	0.737	15.6
			Larvae	180	0.717	13.9
			Juveniles	125	0.717	10.9
	2014	119	Adults	300	0.738	15.6
			Larvae	301	0.730	13.2
			Juveniles	122	0.720	11.9
2015	391	Adults	400	0.736	18.5	
		Larvae	456	0.721	14.9	

<sup>1</sup> = N = The number of individuals stocked of each sex.

<sup>2</sup> = H<sub>E</sub> = Measure of gene diversity.

<sup>3</sup> = A<sub>R</sub> = Measure of allelic richness. Estimates of A<sub>R</sub> were generated by rarefaction for each replicate (as identified by location and year), with the number used for rarefaction provided.

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Table 4.—Remote PIT scanning effort and contact summary information for RASU stocked into AJ and Dandy (Monthly scan-hours were calculated from reported scanning time [recorded minutes of scanning] when provided; otherwise, the values were calculated from recorded date and time of deployment and retrieval. A scanning effort was credited in the month the unit was retrieved if the unit was deployed and retrieved in different months.)

Year-month	AJ		Dandy	
	Scan-hours	Unique PIT	Scan-hours	Unique PIT
<b>2010</b>				
March	30.5	141	87.3	119
April	93.4	134	56.4	20
July	0.0	0	87.6	138
<b>2011</b>				
February	181.3	194	39.9	138
March	70.0	145	53.1	127
April	15.0	41	41.7	68
May	32.5	22	28.7	6
<b>2012</b>				
March	14.1	155	42.0	78
<b>2013</b>				
March	47.2	143	74.8	90
April	41.8	75	27.9	50
September	0.0	0	336.6	34
<b>2014</b>				
March	194.3	176	204.8	192
April	85.6	145	63.9	146
May	0.0	0	84.5	6
September	54.3	1	0.0	0
<b>2015</b>				
April	135.2	91	0.0	0
May	861.0	80	642.2	42
June	400.1	34	0.0	0
July	219.1	3	140.0	2
August	0.0	0	278.6	18
September	312.0	1	448.9	18

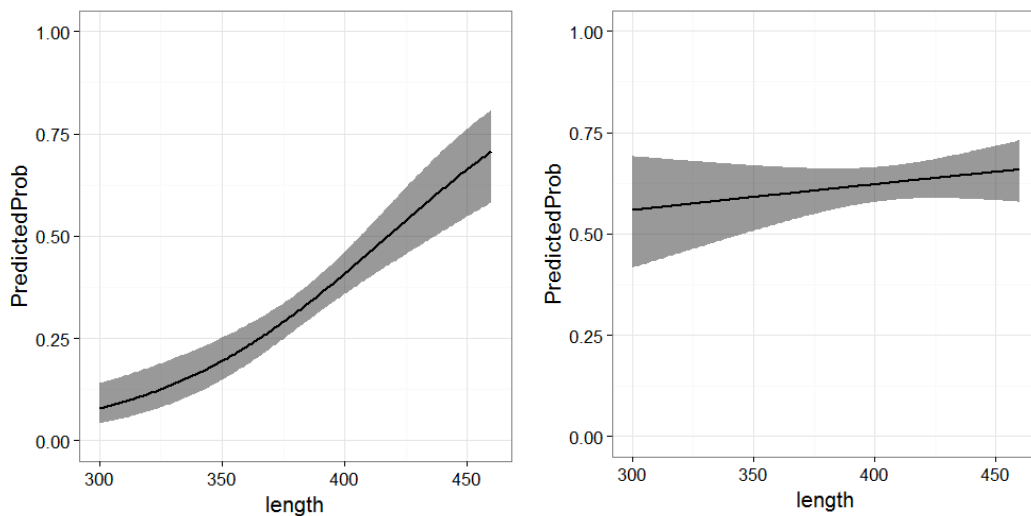
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Table 5.—Stocking and harvest summary data for RASU stocked into AJ and Dandy, Lake Mohave 2010–15

	Number released		Mean release TL (mm)		Number harvested		Mean harvest TL (mm)		Percent harvested	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
<b>AJ</b>										
2010	88	112	378	378	49	72	419	440	55.68	64.29
2011	100	100	392	442	40	70	407	457	40.00	70.00
2012	100	100	403	437	33	61	414	461	33.00	61.00
2013	98	102	397	426	43	55	410	452	43.88	53.92
2014	98	100	378	385	66	75	416	419	67.35	75.00
2015	100	100	355	372	9	32	396	425	9.00	32.00
<i>Backwater subtotals</i>	584	614	384	407	240	365	410	442	41.10	59.45
<b>Dandy</b>										
2010	104	96	378	378	67	81	432	453	64.42	84.38
2011	99	101	393	430	28	63	426	460	28.28	62.38
2012	100	100	403	433	36	41	422	455	36.00	41.00
2013	100	100	392	415	36	85	435	463	36.00	85.00
2014	99	99	370	375	41	64	419	423	41.41	64.65
2015	101	99	352	372	26	59	401	434	25.74	59.60
<i>Backwater subtotals</i>	603	595	381	401	234	393	423	448	38.81	66.05
<b>Total</b>	<b>1,187</b>	<b>1,209</b>	<b>383</b>	<b>404</b>	<b>474</b>	<b>758</b>	<b>416</b>	<b>445</b>	<b>39.93</b>	<b>62.70</b>

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In order to test for differential survivorship of stocked adults, logistic regression was used to estimate the probability of harvest given the known size, sex, and year. For Dandy, all interaction terms involving year of stocking in the logistic regression model were non-significant (Wald test: year\*size\*sex -  $\chi^2 = 2.6$ , df = 4, p = 0.63; year\*length -  $\chi^2 = 8.6$ , df = 4, p = 0.072; sex\*year -  $\chi^2 = 2.4$ , df = 4, P = 0.66), and the main effect of year was also not a significant predictor of harvest probability (Wald test:  $\chi^2 = 6.7$ , df = 4, p = 0.15). Year of stocking was removed from the final logistic regression model for Dandy. The two-way interaction coefficient for sex and release size was statistically significant (z = 4.065, P << 0.001), as was the coefficient for sex (z = -8.194, P << 0.001), indicating that stocking size significantly influenced harvest probability, but that relationship differed among the sexes (figure 2).

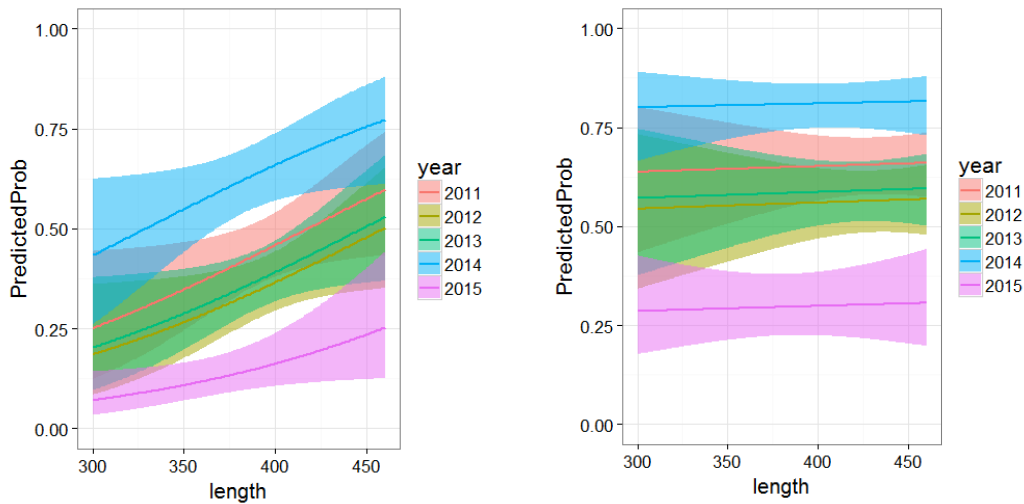


**Figure 2.—Predicted harvest for males (left) and females (right) in Dandy based on logistic regression.**

X-axis represents TL (mm) at stocking. The shaded area represents a 95% confidence interval.

Logistic regression results for AJ were more problematic. All interaction terms involving year were non-significant (Wald test: year\*size\*sex -  $\chi^2 = 7.0$ , df = 4, p = 0.14; year\*length -  $\chi^2 = 9.2$ , df = 4, P = 0.057; sex\*year -  $\chi^2 = 7.5$ , df = 4, P = 0.11), but the main effect of year was statistically significant at an alpha of 0.05 (Wald test:  $\chi^2 = 10.6$ , df = 4, P = 0.032). After the removal of the non-significant interaction terms, sex was a significant predictor of harvest probability (z = -4.263, P = 0.024). The interaction term between stocking size and sex was non-significant (z = 0.008, P = 0.067), but stocking size and the interaction term were kept in the final model for comparison with Dandy (figure 3).

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**Figure 3.—Predicted harvest for males (left) and females (right) in AJ based on logistic regression.**

X-axis represents TL (mm) at stocking. The shaded area represents a 95% confidence interval.

Stocking size had a clear influence on harvest probability in Dandy, but it was less clear in AJ. This was possibly due to the large annual fluctuations in harvest percentages for AJ (see figure 3; between 9 and 67% for males, 32 and 75% for females) compared to Dandy (between 25 and 41% for males, 41 and 85% for females). Annual variation in harvest that cannot be explained by sex or size alone was recorded in both backwaters. Male and female RASU released into both AJ and Dandy in 2014 were smaller on average than the previous 3 years, but harvest percentages in the same year were above average for both backwaters and sexes (see table 5). In order to clarify the sex and size relationship, additional replication would need to be conducted.

AJ and Dandy appear to have environmental differences that consistently affect experimental results. Larvae were collected from AJ every year, and juveniles were collected in all years except 2015. Collections in Dandy were more sporadic. The two backwaters differ in shape, vegetation type, and maximum depth. These factors may impact the susceptibility of RASU to predation by birds, the amount of spawning substrate available, or other biotic and abiotic factors that influence reproduction. Such variables will need to be considered in any future attempts to contrast potentially “replicate” ponds.

Regardless of variation in survival between the two backwaters, sex strongly influenced probability of survival. Based on harvest results, females consistently survived better than males regardless of their size. It is unknown if this occurs in the reservoir as well because most fish are not sexed accurately prior to stocking. There is a strong post-stocking size-survival relationship in the reservoir;

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differential survival between the sexes could explain part of this relationship because females on average, are larger than males. Training hatchery personnel to identify sexes is in the planning stages, and RASU are being stocked at larger sizes (larger fishes are more mature and easier to sex correctly). As these changes are implemented, an assessment of the relationship between sex and survival in the reservoir should be feasible in the next few years.

## Assignment of Parentage to Larvae and Juveniles

Microsatellite data from adults and offspring were input into the program *Mykiss* to assign maternity and paternity for each individual. The method was effective at assigning parentage (table 6), as mothers or fathers were identified in all but 20 instances of 5,848 assignments (0.3% of all assignments). Individual loci were highly consistent with parentage assignment, with mismatched loci occurring at a rate of less than 0.4% per locus per individual. The only exception was Dandy in 2015, where sample sizes were small (table 6). Therefore, microsatellites were useful for identifying parents of larvae and juveniles in ephemeral ponds, with minimal errors.

Table 6.—Error rates (mismatch %) and number of unknown parents for larval and juvenile samples from the AJ, Dandy, and Yuma Cove backwaters

Location	Year	Larvae		Juveniles	
		Mismatch %	Number of unknown parents	Mismatch %	Number of unknown parents
AJ	2010	0.0003	3	NA <sup>1</sup>	NA
	2011	0.0005	0	0.0000	1
	2012	0.0012	0	0.0000	0
	2013	0.0003	0	0.0016	0
	2014	0.0007	0	0.0000	0
	2015	0.0024	6	NA	NA
Dandy	2010	0.0007	0	0.0035	2
	2013	0.0011	4	0.0005	0
	2015	0.0057	3	0.0114	1
Yuma Cove	2013	0.0004	0	0.0051	1
	2014	0.0012	54	0.0069	28
	2015	0.0100	95	NA	NA

<sup>1</sup> NA = Years when samples were not available.

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Known parent-offspring relationships for all 14 loci allowed for detection of null alleles at 4 loci (Xte7, Xte10, Xte17, and Xte18). Null alleles are mutant copies of a locus, where that copy is not expressed due to its deletion from the genome or a mutation in the primer site. Individuals that are heterozygous for null alleles will be scored as homozygous while those that are homozygous for a null allele will be completely missing. Presence of null alleles can possibly lead to reduced estimates of gene diversity in larvae and juveniles, especially if progeny of parents transmitting null variants are overrepresented in the sample. Because the focus at this point is parentage assignment, all loci with null alleles were included, and each instance was manually checked for consistency.

There was considerable variation in the proportional contribution of males and females to reproduction among time periods within each pond and among ponds (table 7). Part of this variation reflects differences in sizes of temporal samples of larvae, as the power to detect the number of males or females contributing to reproduction will be reduced in smaller samples. Because the factors of the most significance are the total contribution of females and males, the best perspective is provided by consideration of all larvae and juveniles collected each year, yielding a minimal estimate of individual contribution. On average, fewer stocked females contributed to progeny than stocked males (39 and 44%, respectively); however, this difference is not statistically significant (paired t-test,  $P = 0.81$ ). The proportion of individuals produced by unique combinations of males and females is also highly variable among spatial and temporal samples, ranging from 11 to 75%, with an average across samples of 44%.

Table 7.—Reproductive contribution of males and females and number of unique pairs (Progeny include larvae that were pooled from all sampling periods and juveniles. For the Yuma Cove backwater in 2014 and 2015 [identified by the “\*”], only progeny where both parents could be identified were included in the calculation of number of unique pairs.)

Location	Number of progeny	Females		Males		Pairs	
		Number of contributors	Percent of stocked	Number of contributors	Percent of stocked	Number of unique pairs	Percent
AJ 2010	210	66	51	39	55	158	75
AJ 2011	508	69	69	74	74	351	69
AJ 2012	362	42	42	48	48	142	39
AJ 2013	285	19	19	50	51	118	41
AJ 2014	275	12	12	7	7	30	11
AJ 2015	606	50	50	57	57	272	45
Dandy 2010	248	39	39	31	31	92	37
Dandy 2013	340	57	56	58	59	171	50
Dandy 2015	88	16	16	17	17	27	31
Yuma Cove 2013	305	56	56	55	55	170	56
Yuma Cove 2014	423*	58	39	25	17	161	47
Yuma Cove 2015	420*	71	47	27	18	225	54



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Reproductive success of individual parents exhibits the expected Poisson-like distribution based on random contributions over time. A large fraction of individuals failed to produce offspring on average; however, the number of individuals failing to contribute any given year at each location is highly variable. For example, the majority of parents contributed progeny in AJ 2011 (68 females, 69 males) while relatively few contributed in AJ 2014 (10 females, 9 males). These numbers may be influenced by the sex of parents, number of progeny sampled, and age of progeny (e.g., larvae, juveniles) considered. Another factor that will influence these numbers is the timing of adult mortality, as early mortality would reduce the probability of adults contributing progeny. Of those individuals that contribute, the majority of them produce few (e.g., one or two) progeny, with a few individuals contributing many progeny. An extreme illustration of this occurred in AJ 2012 (figure 4), where a single female contributed 104 juveniles (> 40% of the 246 juveniles sampled). Therefore, the proportion of adults contributing offspring and individual reproductive success is highly variable among years and locations, with this variability at least partly due to variation in timing of adult mortality.

The association between adult size (as indicated by length) and reproductive success (numbers of larvae, juveniles, and total offspring) with sex was also investigated. Linear regression failed to identify any relationship between length and reproductive success with the exception of two samples from 2015, females from AJ and males from Dandy (table 8). Failure to detect a consistent relationship between these variables indicated that size at stocking had little influence on individual reproductive success in ephemeral ponds. This result was somewhat surprising, as fecundity is generally related to size (Minckley and Marsh 2009); however, perhaps this relationship is not as strong in newly reproductive individuals.

Males contributed to more temporal samples than females (averages of 2.29 and 1.98, respectively); however, only two of the nine annual samples were statistically significant (table 9). The two samples that were significant were likely anomalous, as one (AJ 2010) had many more females stocked, potentially leading to the observed bias in reproductive output for males, while reproduction was severely reduced in the other (AJ 2014), with only 12 females and 7 males reproducing. Therefore, there appears to be no difference between length of time that males and females are reproductively active.

Conditions in the ponds can be highly variable throughout the year; therefore, survivorship of larvae to juveniles produced by specific males and females relative to their date of hatching was also investigated. Because of annual and geographic variation in when samples were collected, adults that contributed larvae collected in March were considered to be “early” producers, while those that contribute in April through early May were considered to be “late” producers.

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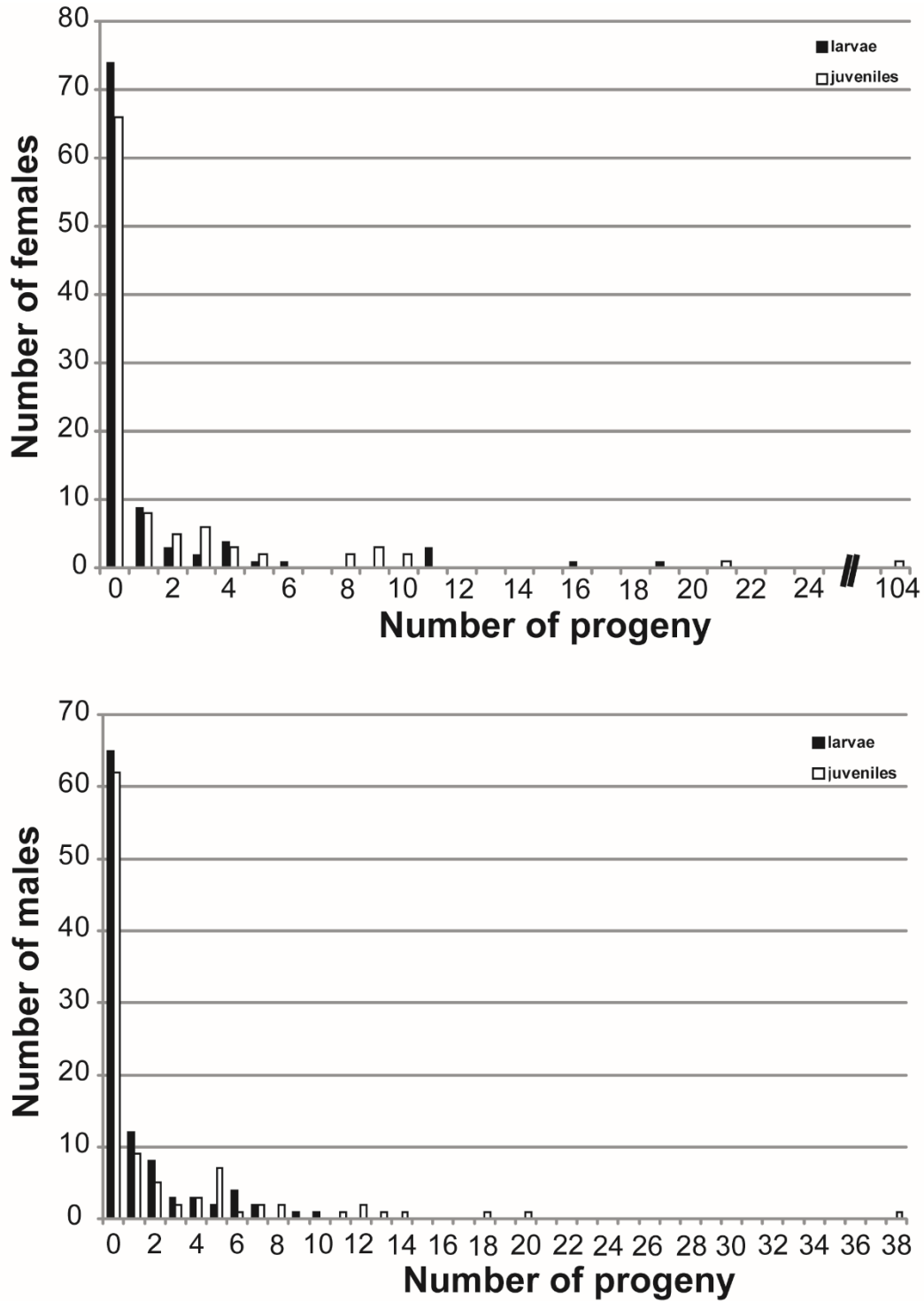


Figure 4.—Relationship between number of females and males (top and bottom panels, respectively) and the number of larvae and juveniles (dark and light bars, respectively) each produced from AJ in 2012.

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Table 8.—Results from linear regression analyses of adult length and reproductive success for each year-class

(Length measurements were unavailable in 2010, and there were no juveniles captured in AJ in 2015.)

Sample	Female						Male				Total	
	Larvae		Juvenile		Total		Larvae		Juvenile			
	F	P	F	P	F	P	F	P	F	P	F	P
AJ 2011	0.02	0.89	0.17	0.68	0.10	0.75	3.17	0.08	0.47	0.49	2.48	0.12
AJ 2012	0.12	0.73	0.00	0.96	0.01	0.90	0.04	0.84	0.78	0.38	0.38	0.54
AJ 2013	0.29	0.59	0.01	0.91	0.26	0.61	0.34	0.56	0.12	0.73	0.38	0.54
AJ 2014	0.03	0.87	0.46	0.50	0.10	0.76	0.31	0.58	0.01	0.94	0.18	0.68
AJ 2015	15.80	0.00	NA		NA		0.52	0.47	NA		NA	
Dandy 2013	0.43	0.52	0.09	0.77	0.14	0.71	1.49	0.22	1.98	0.16	2.06	0.15
Dandy 2015	1.79	0.18	2.04	0.16	1.88	0.17	4.27	0.04	4.77	0.03	4.47	0.04

Table 9.—Contrast of the number of temporal samples in which males and females contributed progeny

Sample	Females		Males		U <sup>2</sup>	Z <sup>2</sup>	P <sup>3</sup>
	N <sup>1</sup>	Average number of times	N	Average number of times			
AJ 2010	66	1.6	39	2.3	702.5	-3.87299	0.0001
AJ 2011	68	1.97	69	2.14	2137	0.89759	0.36812
AJ 2012	25	1.24	36	1.44	357.5	1.34915	0.17702
AJ 2013	18	2.44	47	1.94	296.5	-1.84716	0.06432
AJ 2014	12	2	7	3.57	15	-2.23966	0.0251
AJ 2015	50	3.26	57	3.86	1159	1.65776	0.09692
Dandy 2010	34	2.47	31	2.48	526.5	0	1
Dandy 2013	26	1.12	34	1.21	413.5	0.41769	0.67448
Dandy 2015	13	1.69	15	1.67	97.5	0.02303	0.98404

<sup>1</sup> N = The number of individuals contributing in each sample and the average number of times each individuals contributed.

<sup>2</sup> U and Z = Test values from the Mann-Whitney statistic.

<sup>3</sup> P = The significance value.

Individuals that contributed larvae in both time periods were considered to be a third group. Five of 12 G-tests performed were significant at  $P < 0.05$  (table 10); however, none were significant after correction for multiple tests (B-Y corrected  $P$  value = 0.016). Overall, males that produced larvae early in the reproductive period, or in both time periods, were more likely to be represented in the juveniles ( $G = 26.4$ ,  $P < 0.0001$ ). This is not the same pattern found in females, where early and late producers contributed similarly to juveniles; however, individuals that contribute to both time periods were far more likely to be represented in the juveniles sampled than not ( $G = 9.1$ ,  $P < 0.011$ ).

## **YUMA COVE BACKWATER**

The single shore-based remote PIT scanner deployed in the Yuma Cove backwater was actively scanning in all months since March 2013 except for October, November, and December 2013 (table 11). Minor interruptions in continuous scanning due to power or scanner failure continued throughout the study period. Unique PIT scanning contacts peaked in July 2015 (229 fish) due to survival of the original stocking cohort and the tagging of natural recruits in the pond in May 2015 (table 11). Based on remote PIT scanning data, few RASU stocked in 2014 and 2015 or captured and tagged in November 2013 survived into summer 2015.

Based on remote PIT scanning contacts, female survival was markedly better than male survival to summer 2015, with four times as many females contacted from the original stocking (74 of 100) compared to males (18 of 100) (table 12). Although the number of survivors from the 2014 and 2015 stockings are much lower, the trend continues with those stockings as well. Size at stocking covaried with sex in two of the three stockings. Mean TL of females stocked in 2014 was shorter than that of males, but females were still contacted at a higher rate, although contact was low for both sexes (five females and one male).

Similar to AJ and Dandy, survival in the backwaters is sex dependent. A time series plot of minimum population size (scanned on or after a given date) for the original 2013 stocking into the Yuma Cove backwater illustrates the timing of mortality (figure 5). The largest decline occurred in the first year after release within a few months post-stocking. This time period is coincident with spawning activity. Lower survival for males may be related to differences in behavior between males and females during spawning. Each female is tended to by more than one male (Minckley and Marsh 2009). Male RASU spawning in the shallows of the backwaters would have a higher probability of becoming a victim of avian predation than a female RASU during this period. Mortality in subsequent spawning years is much lower, but this could be due to RASU growing beyond a vulnerable size. The timing of the higher mortality (proximal

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Table 10.—Survivorship for larvae of females and males to juvenile stage relative to timing of reproduction (early in season, late, or in both periods)

(Values are the number of parents producing larvae that do or do not survive to the juvenile stage.)

Sample	Females								Males							
	Juveniles			No juveniles			G <sup>1</sup>	P <sup>1</sup>	Juveniles			No juveniles			G <sup>1</sup>	P <sup>1</sup>
Early	Late	Both	Early	Late	Both	Early			Late	Both	Early	Late	Both	Early		
AJ 2011	7	15	19	11	7	5	7.195	0.027	11	9	24	6	5	7	1.204	0.548
AJ 2012	7	10	1	7	3	1	1.947	0.378	8	8	11	7	1	1	6.085	0.048
AJ 2013	5	2	3	1	6	1	5.187	0.075	8	5	4	6	19	3	6.039	0.049
AJ 2014	0	1	4	2	0	1	NA		1	0	5	0	0	1	NA	
AJ 2015	10	5	17	8	1	4	3.256	0.196	4	4	34	5	1	4	6.966	0.031
Dandy 2010	7	11	7	6	21	4	3.288	0.193	10	15	10	2	16	2	7.174	0.028
Dandy 2013	19	5	1	2	1	0	NA		26	7	5	4	2	0	NA	
Dandy 2015	2	3	2	6	6	2	0.651	0.722	5	4	4	6	5	1	1.916	0.384
<b>Overall</b>	<b>57</b>	<b>52</b>	<b>54</b>	<b>43</b>	<b>45</b>	<b>18</b>	<b>9.065</b>	<b>0.011</b>	<b>73</b>	<b>52</b>	<b>97</b>	<b>36</b>	<b>49</b>	<b>19</b>	<b>26.370</b>	<b>0.000</b>

<sup>1</sup> G and P provide the results of G-statistical analyses.

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Table 11.—Remote PIT scanning effort and contact summary information for RASU tagged and released in the Yuma Cove backwater

(The backwater was originally stocked with 200 RASU in 2013 and stocked with an additional 50 fish in each of 2014 and 2015. In addition, individuals produced within the backwater have been netted, PIT tagged, and returned to the backwater. Monthly scan-hours were calculated from reported scanning time [recorded minutes of scanning] when provided; otherwise, the values were calculated from recorded date and time of deployment and retrieval. A scanning effort was credited in the month the unit was retrieved if the unit was deployed and retrieved in different months.)

Row labels	Yuma	
	Scan-hours	Unique PIT
<b>2013</b>		
March	817.5	190
April	714.7	112
May	862.1	92
August	563.7	69
September	329.2	91
<b>2014</b>		
January	260.5	89
February	34.0	105
March	519.6	81
April	116.3	96
May	771.7	101
July	46.4	92
August	442.1	106
September	515.1	111
November	368.9	103
December	294.4	132
<b>2015</b>		
January	676.9	61
February	742.5	206
March	836.7	104
April	782.5	123
May	867.7	228
June	833.9	221
July	846.9	229
August	651.7	191
September	741.9	214
October	811.5	226
November	335.8	208
December	650.8	35

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Table 12.—The number of RASU with PIT tags implanted prior to stocking into or upon capture within the Yuma Cove backwater

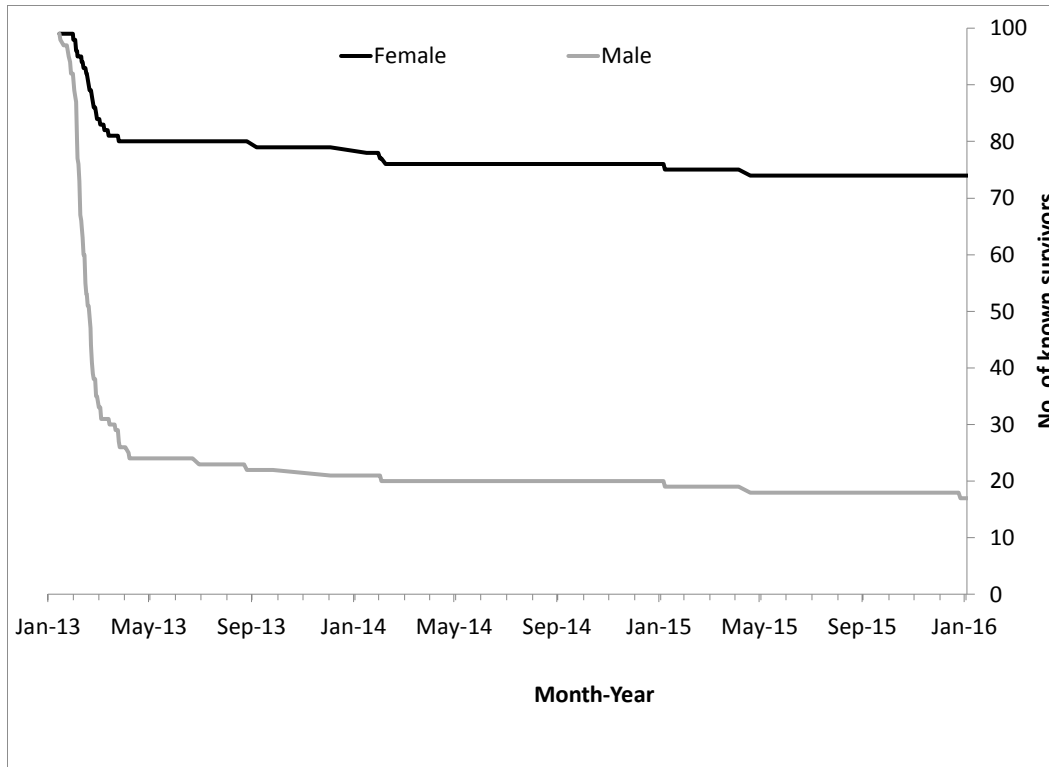
Month-year	Number released			Mean TL (mm)			Contacted after June 2015		
	Female	Male	Unknown	Female	Male	Unknown	Female	Male	Unknown
Stocked									
February 2013	100	100	0	424	386	405	74	18	0
January 2014	50	48	0	369	375	372	5	1	0
January 2015	50	50	0	381	355	368	9	1	0
<b>Totals</b>	<b>200</b>	<b>198</b>	<b>0</b>	<b>399</b>	<b>375</b>	<b>387</b>	<b>88</b>	<b>20</b>	<b>0</b>
Captured									
November 2013	4	3	51	463	446	324	4	2	0
January 2014	1	0	0	480	0	0	1	0	0
May 2015	0	0	124	0	0	422	0	0	120
<b>Totals</b>	<b>5</b>	<b>3</b>	<b>175</b>	<b>466</b>	<b>446</b>	<b>393</b>	<b>5</b>	<b>2</b>	<b>120</b>

to stocking) also implicates the process of stocking itself as a possible cause, but that would not explain the sex bias. Bird predation studies are being conducted on Lower Colorado River Multi-Species Conservation Program Reach 3, although those investigators are not aware of any sex-specific results.

## Assignment of Parentage to Larvae and Juveniles

Microsatellite data from adults and offspring were input into the program *Mykiss* to assign maternity and paternity for each individual. For 2013, parentage was accurately assigned for all 606 instances (e.g., 303 mothers and 303 fathers) except for 1 individual (0.17% of assignments). The effectiveness of assignment declined dramatically in the next 2 years (table 6), as it was not possible to assign parentage in 82 of 1,102 instances (7.4%) and 95 of 926 instances (10.3%) in 2014 and 2015, respectively. The proportion of mismatched loci was consistent with the difficulty of parentage assignment. The proportions of mismatched loci in 2013 and 2014 were comparable to ephemeral backwaters (0.05 and 0.3% per locus per individual, respectively) but increased dramatically in 2015 (1.0% per locus per individual). This difficulty likely reflects the contribution of individuals to progeny that are not included in the sample of adults, possibly individuals that were stocked into the pond prior to the experiment and/or juveniles that became reproductive over the course of sampling. These latter individuals would be especially problematic, as they would be closely related to sampled adults, making parentage assignment especially difficult.

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**Figure 5.—RASU stocked into the Yuma Cove backwater in January 2013 and contacted with remote PIT scanners.**

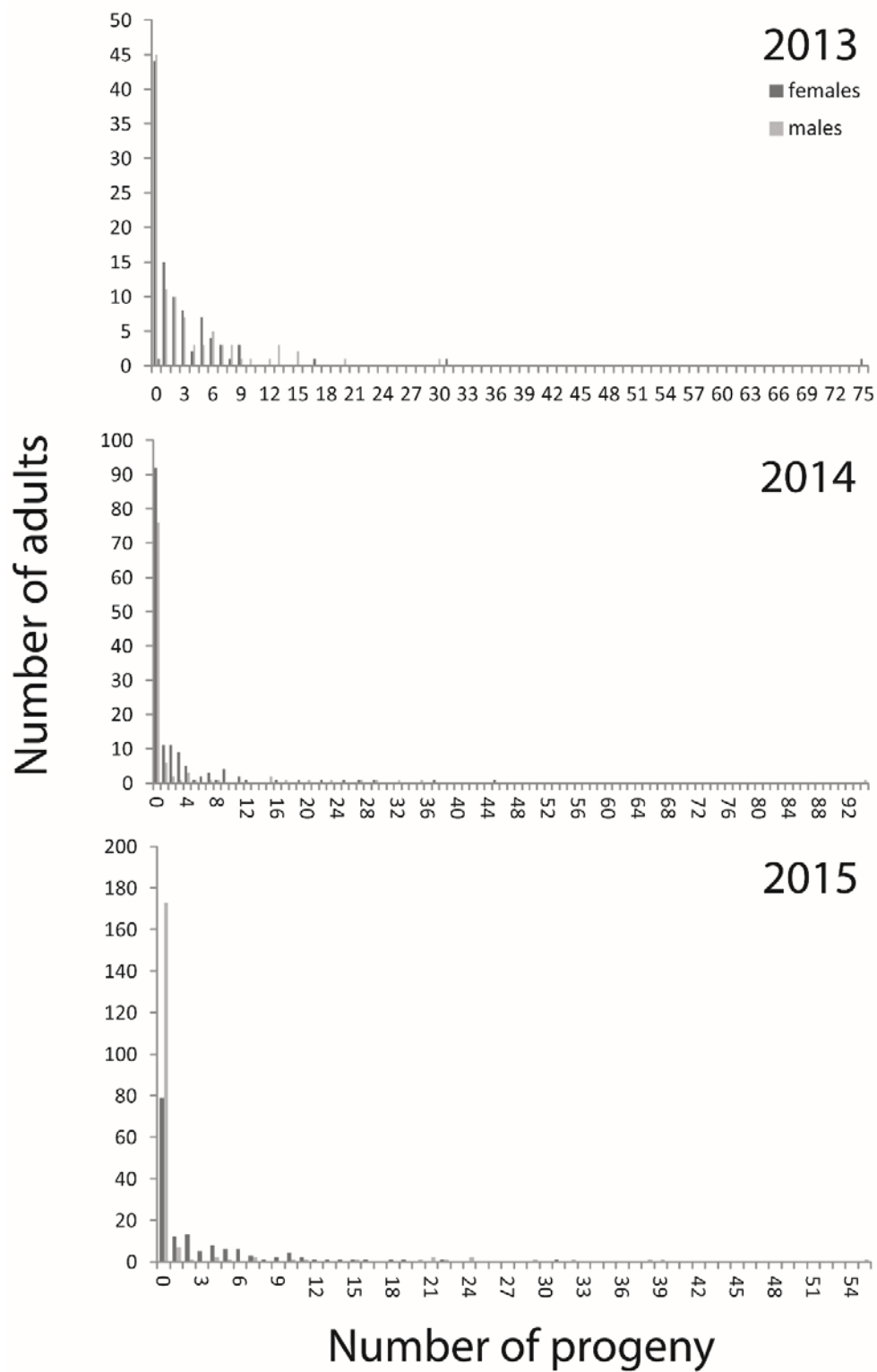
Number of known survivors (y-axis) is the total number of individuals contacted on or after a given date (x-axis).

In 2013, males and females contributed approximately equally to progeny (55 and 56%, respectively), with 56% of the progeny resulting from unique male-female combinations (see table 7). These numbers were similar for the next 2 years, except for contributing males, which declined in 2014 and 2015 (17 and 18%, respectively). In 2014, only one female and four males stocked that year contributed, with all remaining reproducing individuals stocked in 2013. A similar result occurred in 2015, when only three females and three males stocked in 2014 and only six females and two males stocked in 2015 contributed progeny, with the remaining reproducing adults stocked in 2013. Reduced reproduction of fish stocked in 2014 and 2015 is not surprising given their high mortality.

Unusual patterns of adult survivorship and reproductive contribution were also reflected in patterns of reproductive success (figure 6). Male and female



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**Figure 6.—Relationship between number of adults (females [black bars], males [white bars]) and the number of progeny from the Yuma Cove backwater samples collected from 2013 to 2015.**

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contributions in 2013 were similar to those found in ephemeral backwaters; however, they varied in 2014 and 2015 as (1) fewer individuals were detected reproducing and (2) more individuals contributed to larger numbers of progeny than was typical for ephemeral backwaters. This likely reflects reduced reproductive contribution of most individuals stocked, especially those stocked in later years. This is an issue that needs to be understood and examined for the backwater ponds to function as needed for conservation efforts.

## SUMMARY

Genetic and remote sensing methods were used to follow RASU populations in ephemeral and permanent backwater ponds at Lake Mohave. Microsatellites were effective markers for characterizing genetic variation and parentage in ephemeral backwater ponds, indicating that reproductive success for adults stocked into backwaters can be high; however, there is considerable annual variation in the contribution of adults to larvae and juveniles. Unfortunately, these results do not allow for identification of factors that contribute to this variation, making it impossible to develop explicit requirements for individual backwaters at this point; however, this result indicates that local and annual variation will need to be better understood as more backwater habitats are generated in an effort to increase the overall population size of RASU.

Microsatellite markers were less effective in the permanent Yuma Cove backwater because parentage analysis failed to identify one or both parents from many larvae and juveniles in the last 2 years. The inability to identify these parents likely reflects reproduction by adults that had not been sexed and genotyped.

Development of additional DNA markers (i.e., single nucleotide polymorphisms, or SNPs) will provide increased power for parentage analysis, providing an important perspective on interannual variation in permanent backwater ponds.

Remote sensing provided a useful perspective about persistence of adults in these populations. Monthly scans from ephemeral ponds showed considerable variation in detection, with reduced detectability (fewer contacts) after the spawning period. Analysis of harvest data identified differential survivorship of males and females, suggesting that males disappeared at a faster rate than females. Similar patterns of male-female survivorship were also identified in adults stocked into the permanent Yuma Cove backwater in 2013, while supplemental adults stocked into the Yuma Cove backwater in 2014 and 2015 also exhibited extreme mortality as well as biased survivorship. At this point, the reasons for differential survival of males and females and high mortality for supplemental stockings in the

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Yuma Cove backwater are unclear. Identifying and resolving those issues are critical, as it will be necessary to move individuals among backwater ponds for the Lower Colorado River Multi-Species Conservation Program to successfully replicate genetic diversity in the existing population.

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