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 June 2018



**Final Report For:** The San Juan River Basin Recovery Implementation Program

**Title:** Using Molecular Techniques to Determine Effective Number of Breeders ( $N_b$ ) for Razorback Sucker and Colorado Pikeminnow in the San Juan River

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**Executive Summary**

Recovery plans for many endangered fishes include the production and release of hatchery-reared individuals to augment populations with two major objectives: increase population sizes and promote genetic diversity. In order to meet these recovery criteria, survival, reproduction, and recruitment of wild and augmented individuals must occur at a sustainable scale. Population-level spawning success (i.e., number of reproducing adults) is innately difficult to quantify from field studies, especially for highly fecund species where few individuals can produce a large number of offspring. Fortunately, this question can be addressed by estimating the effective number of breeders ( $N_b$ ) using genetic analyses to determine the annual contribution of adults. Estimates for Colorado Pikeminnow were variable among years. In some years approximately 40% of the adult population contributed to the larvae collected in the San Juan River. These results suggest if environmental factors (e.g., flow, temperature, etc.) can be identified that correlate with increased  $N_b$  estimates, then it would suggest there is a potential to manage the San Juan River to favor Colorado Pikeminnow spawning. Razorback Sucker estimates were consistently low across years with approximately 2% of the adult population contributing annually. This might suggest the bottleneck to recruitment could be occurring at the spawning or reproductive level.

**Introduction**

Recent advances in population-level genetic analyses are increasingly helping managers monitor and adaptively manage the recovery of endangered populations (Hartl and Clark 2007). Recovery plans for many endangered fishes include the production and release of hatchery-reared individuals to augment populations with two major objectives: increase population sizes and promote genetic diversity (Miller and Kapuscinski, 2003). In order to meet these recovery criteria, survival, reproduction, and recruitment of wild and augmented individuals must occur at a sustainable scale; however, understanding factors that limit success in achieving a self-sustaining population can be difficult to identify. Long-term datasets can provide insight into population responses that might hinder recovery, and genetic monitoring can be an additional tool for providing insight into complex ecological, demographic, and genetic factors that can impede the establishment of self-sustaining populations.

Augmentation of endangered fish populations in the San Juan River using captive-reared Razorback Sucker (*Xyrauchen texanus*) and Colorado Pikeminnow (*Ptychocheilus lucius*) began in the mid-1990s and continues as a recovery action (USFWS 2005; USFWS 2015). Annual monitoring of survival, reproduction, and recruitment of these populations has been supported through the San Juan River Basin Recovery Implementation Program (SJRRIP). Mark-recapture data on PIT tagged individuals have enabled estimation of survival of both stocked species (Franssen and Durst *unpublished data*; Clark et al. *in review*), which in turn has prompted additional research investigating ways to increase survival. Larval fish surveys have documented successful reproduction of both species in the river (Farrington et al. 2015), but recruitment to the adult stage is extremely limited. Successful recovery likely requires a significant portion of the reestablished population to reproduce annually to both increase population sizes and ensure maintenance of genetic diversity. Therefore, quantifying the number of individuals that reproduce annually can provide data to make informed management decisions to aid in the reestablishment of self-sustaining populations.

Population-level spawning success (i.e., number of reproducing adults) is innately difficult to quantify from field studies, especially for highly fecund species where few individuals can produce a large number of offspring. Furthermore, there is substantial evidence that shows reproductive output can depend on environmental conditions and age- or size-related factors (Lauer et al. 2005; Lambert 2008). Both temporal and spatial variation in spawning effort has also been observed for Razorback Sucker with adults vacating a spawning area early in the season and later returning to spawn again that year (Marsh et al. 2015) and with individuals visiting multiple sites during the same spawning period (Modde and Irving 1998). Such reproductive strategies further compound the difficulty in determining individual contribution to cohorts over a reproductive season; however, this question can be addressed by estimating the effective number of breeders ( $N_b$ ) using genetic analyses. For long-lived, highly fecund, iteroparous species with overlapping generations, such as Colorado Pikeminnow and Razorback Sucker,  $N_b$  is an extremely useful metric for understanding population-level spawning success due to its defined seasonal reproductive bouts (Waples et al. 2013; Waples et al. 2014). Single cohort  $N_b$  estimates can reliably quantify the number of individuals that contributed to a given cohort (Waples et al. 2014). Obtaining  $N_b$  estimates for the endangered fishes of the San Juan River will provide the first insight into how natural, population-level reproduction may be affecting progress towards recovery.

The objectives of this study were to: 1.) Estimate  $N_b$  for Razorback Sucker annually from 2009-2015 and 2.) Estimate  $N_b$  for Colorado Pikeminnow when samples sizes for any given year were greater than 25 between 2009-2016.

## **Methods**

### *Sample Collection*

Larval fish surveys were conducted annually along a 140 mile reach of the San Juan River between Shiprock, NM, and Clay Hills, UT (Farrington 2015). Since 2009, larval collections were preserved in 95% ethanol making those years suitable for genetic sampling (formalin preservation degrades DNA). Approximately 1,200 samples representing the spatial and temporal distribution of larval sampling efforts were used to

obtain  $N_b$  estimates (Figure 1). In order to ensure  $N_b$  estimates were not artificially lowered due to limited spatial representation of samples, rare collections were targeted while sites with high larval densities were proportionally reflected in samples. Larval Razorback Sucker were collected during much of the sampling season. Early larval stages (e.g., protolarvae to mesolarvae) were targeted throughout larval collections under the assumption that these individuals were from recent spawning events; thus, ensuring sampling was representative of the temporal spawning season. Conversely, Colorado Pikeminnow were only collected later in the sampling season, which made it relatively easy to have those captured individuals reflect the entire seasonal spawning period of adults.

### *Genetic Sampling*

Tissue subsamples from the posterior portion of each specimen were obtained for genetic analysis while the anterior portion was retained for future potential otolith studies. Genomic DNA was extracted using DNeasy® Blood and Tissue Kits (Qiagen, Valencia, CA, USA). A total of nineteen microsatellite loci were used for Razorback Sucker; twelve were developed at Southwestern ARRC for evaluating the genetic diversity of the Razorback Sucker broodstock (Wilson 2012), and seven were developed by Dowling et al. (2011). Twenty-five microsatellite loci were used to amplify Colorado Pikeminnow DNA (Martin et al. 2015). Microsatellite amplification consisted of 10  $\mu$ l reactions containing the following: 1  $\mu$ l DNA, 3  $\mu$ l Qiagen Multiplex Master Mix® (Qiagen, Valencia, CA, USA), 0.2  $\mu$ l of both forward and reverse primers, and 5.6  $\mu$ l of nuclease-free water. Forward primers were labeled with one of four fluorescent dyes (6-FAM, PET, NED, VIC; Applied Biosystems, Inc., Foster City, CA, USA). Amplification for all samples consisted of a “touchdown” protocol, which included an initial denaturing step of 95°C for 15 min, followed by 35 cycles of 94°C for 60 s, 56°C for 45 s, and 72°C for 60 s, with a final extension of 10 min at 70°C; the annealing temperature decreased by 0.2°C for each cycle. Amplified products were processed on an ABI 3500xl Genetic Analyzer. Composite genotypes for individual fish were compiled using GeneMapper™ 4.0 software (Applied Biosystems, Inc., Foster City, CA, USA). A second researcher performed a quality assurance/quality control assessment on 10% of samples to ensure accuracy.

### *Data Analysis*

Departures from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were tested using Genepop version 4.2 (Raymond and Rousset 1995), and alpha (0.05) was adjusted for multiple comparisons using the Benjamini and Yekutieli (2001) method false discovery rate (Narum 2006). PEDANT version 1.0 was used to estimate allelic dropout ( $E_1$ ) and false allele error rates ( $E_2$ ) (Johnson and Haydon 2007). Effective number of breeders ( $N_b$ ) was estimated using the sibship-assignment (SA) method in COLONY version 2.0.4.0 (Jones and Wang 2010). This software uses a maximum likelihood method to estimate relationships among offspring belonging to a single cohort by identifying full and half-sibling families while incorporating genotyping errors (i.e.,  $E_1$  and  $E_2$ ) and allowing for inferences related to the mating strategy of the organism. Analyses were conducted separately for each year to estimate  $N_b$ , the number of adults that contributed at least one offspring, number of sampled offspring produced by each parent, and the number of parental pairs. For both species, male and female polygamy was assumed and parameter settings (i.e., dioecious, diploid, inbreeding, medium run length, full-likelihood with medium likelihood precision, no sibship prior, and updated allele frequencies) were maintained across years. Low error rates were estimated at all loci (dropout

rate  $E_1$  ranged 0.01-0.07 and false allele error rate  $E_2$  0.02-0.05) and were provided independently for each locus (results not reported here).

Adult census estimates (i.e., population sizes) for both Razorback Sucker and Colorado Pikeminnow (Schleicher 2016; SJRRIP 2017) were obtained in order to put  $N_b$  estimates in the context of demographic data. The ratio of  $N_b$  to adult census size provides an estimate of the relative proportion of annual reproductive contribution of adults in the San Juan River. Adult Colorado Pikeminnow (age-5+; > 417 mm TL) and Razorback Sucker (age-4+;  $\geq$  400 mm TL) census sizes were estimated using pass-specific detection probabilities from closed population mark-recapture models (SJRRIP 2017). The relative proportion of spawning adults was estimated by comparing the means obtained for  $N_b$  and adult census estimates.

In an effort to investigate potential sample size bias, we conducted a few rarefaction analyses by resampling annual samples and calculating  $N_b$  estimates at different sample sizes. These simulations were conducted for two years of Razorback Sucker data (see Appendix; Figure A1 and A2). For the 2009 dataset we randomly pulled sample size increments of 10 starting at a sample size of 40. We conducted five runs for each sample size and took the average of those iterations to estimate the mean and 95% confidence intervals. For 2010, we applied the same method but instead used sample size increments of 20.

## Results

### *Colorado Pikeminnow*

Across the 25 microsatellite loci, the number of alleles among cohorts varied from 4 to 35 with an average of 12 alleles across all loci. Overall, mean  $N_b$  estimates averaged 22 across years with a range of 3 to 50 (Figure 2). The lowest mean estimate was observed in 2015 and the highest mean  $N_b$  estimate was found in 2016. Adult census data were not available for 2009, 2010, or 2013 due to limited numbers of recaptures. Mean adult census estimates varied among years with as few as 19 individuals estimated in 2012 to as many as 133 estimated in 2016 (Figure 3). Large variation around these means was observed across years with upper estimates as high as 1,125 adults in 2011 to as few as seven adults estimated in 2012. The relative proportion of spawning adults was variable across years. The lowest annual mean contribution of approximately 3% coincided with the lowest  $N_b$ . Conversely, adult contribution in 2014 and 2016 coincided with the highest mean  $N_b$  estimates with a mean contribution of approximately 40% of adult Colorado Pikeminnow contributing to the larvae collected in the San Juan River (Table 1); however, due to the highly variable adult census estimates we observed extreme variation around these means.

The estimated number of spawning adults and the number of offspring produced by each parent varied among years (Figure 4). Comparison of these measures provides a way to visualize variance in reproductive success by examining the number of sampled individuals each parent contributed. This is important because high variation in offspring contribution can lower  $N_b$  estimates. Years with low  $N_b$  estimates coincided with low estimates of spawning adults. Indeed, in 2015 the lowest number of spawning adults was observed. Conversely, the largest number of spawning adults was observed in 2016, which coincided with the highest  $N_b$  estimate. Individual adult contribution of offspring varied across years with some adults contributing as many as 25 of the sampled offspring, while most contributed very few. This variance in reproductive success within years resulted in a decrease in  $N_b$  estimates. Nonetheless, these estimates were similar most years. For example, adult contribution of offspring was relatively even in 2016 with similar

estimates of  $N_b$  and number of spawning adults. Conversely, in 2014 individual contribution of spring was more variable which contributed to a lower  $N_b$  estimate.

The number of parental pairs varied among years (Figure 5) with estimates of low and high parental pairs coinciding with low or high  $N_b$  estimates and number of spawning adults. Across all years, the number of parental pairs was greater than the estimated number of spawning adults, suggesting a polygamous mating strategy for Colorado Pikeminnow. Full-sibling relationships within parental pairs were also reconstructed. All larval collections and full-sibling relationships were plotted across river mile in order to visualize the extent of larval drift. In some years there was evidence of extensive larval drift with full-siblings collected up to 100 miles apart (e.g. 2009-2015). In terms of distribution of larvae, in years with more larvae, fish were more evenly distributed throughout the system. These results also suggest at least some pairs are spawning relatively high in the system, and in some cases there is evidence for relatively large drift between full siblings.

### *Razorback Sucker*

The number of alleles among cohorts varied from 7-45 across the 19 microsatellite loci with an average of 30 alleles per locus. Mean  $N_b$  estimates did not differ significantly across years with an average of 82 and a range of 65-109 (Figure 6). Adult census estimates were not available for the first two years where we had  $N_b$  estimates. Adult census sizes did not differ among years and means were relatively similar (range = 4,000 - 5,000) (Figure 7). Conversely, the estimated proportion of spawning adults was low with a relatively consistent ratio of approximately 2% (Table 2). Furthermore, we observed very little variation within the  $N_b/N_c$  with the lowest estimate of 0.58% in 2013 and the highest estimate of 4.71% observed in 2015.

The estimated number of spawning adults was relatively similar across years (Figure 8). Similar to Colorado Pikeminnow, the lowest and highest  $N_b$  estimates coincided with the lowest and highest number of spawning adults. Although there is some evidence of variance in reproductive success, overall results indicated relatively even contribution of offspring per parent. This is apparent in the similar measures between the effective number of breeder estimate and number of parents that produced offspring. The number of parental pairs was similar across years, with more parental pairs than estimated number of parent (Figure 9). Similar to Colorado Pikeminnow, there appears to be evidence of extensive larval drift between full siblings with some full-sibling collected over 120 miles apart. In 2009 and 2010 there appears to be downstream stacking of larval fish; however, in 2012- 2015, larvae appear to be relatively evenly distributed throughout the river indicating spatial sampling of larvae was relatively even.

## **Discussion**

Incorporating population-level genetic analyses in the context of population monitoring for endangered species can provide insight into demographic, ecological, and evolutionary process that could be hindering recovery (Hartl and Clark 2007). Estimates of  $N_b$  can provide the opportunity to assess if factors could be limiting the reproductive potential of a population (Bacles et al. 2018). The San Juan River long-term larval monitoring has contributed to an increased understanding of reproduction of the endangered fishes of the San Juan River, and in conjunction with genetic tools can further elucidate if population-level reproductive success is a factor limiting recruitment of Razorback Sucker and Colorado Pikeminnow.

Effective number of breeder estimates for Colorado Pikeminnow were variable among years; however, in some years  $N_b$  estimates were relatively high compared to their adult census

sizes with 40% of the mean estimated number of adults contributing to larval cohorts. Nonetheless, extreme variation was observed around this mean due to large variability in adult census estimates. These highly variable  $N_c$  results are due to few adult Colorado Pikeminnow ( $N=2$  in 2011) contacted during monitoring efforts. Regardless, the large inter-annual variation observed in  $N_b$  estimates suggest that if environmental conditions (e.g., flow, temperature, etc.) can be identified that correlate with increased  $N_b$  estimates, then there is a potential to manage the San Juan River to favor Colorado Pikeminnow spawning. Results for Razorback Sucker, however, were relatively surprising given current population densities and potentially warrant concern. Annual contribution of adults to each larval cohort was low with the highest estimate of 5% observed in 2015 and a consistently low average of approximately 2% across years. These results suggest the bottleneck to recruitment could be occurring at the spawning or reproductive level. Several factors could be causing this including limited spawning habitat availability or high mortality of eggs or early larval stages. At present, current population densities for Razorback Sucker are high, suggesting the ability to find a suitable mate is not a limiting factor.

While these are the first available  $N_b$  estimates for Colorado Pikeminnow,  $N_b$  estimates for Razorback Sucker from this study can be compared to other systems. Annual population monitoring and genetic studies conducted on Razorback Sucker from Lake Mohave between 1997 and 2010 showed a significant increase in mean  $N_b$  estimates (e.g.,  $N_b = 743$  [1998];  $N_b = 49,984$  [2005]) and  $N_b/N_c$  (mean 0.708) over a fourteen-year period (Dowling et al. 2013; Carson et al. 2016). These results suggest management actions (i.e., off shore rearing of larvae) effectively reduced the variance in population-level reproductive success for Razorback Sucker in Lake Mohave (Carson et al. 2016). However, this population is not subjected to the same environmental conditions or management activities as the San Juan River population, and although  $N_b/N_c$  estimates seem relatively high they may not be comparable. Nonetheless, these results showed management positively affected  $N_b$ , suggesting regular  $N_b$  monitoring of the San Juan River population could inform managers when management actions (e.g., increasing fish passage) increase population-level reproductive success.

Certainty in the relative magnitude of  $N_b/N_c$  within natural populations is often unknown. Available data on 62  $N_b/N_c$  estimates were found to vary considerably (0.01-0.95) across taxa (i.e., fishes, amphibians, invertebrates) with a median estimate of 0.225 (Palstra and Fraser 2012). Most taxa have little or no available  $N_b/N_c$  data; however, sufficient data (i.e., both spatial and temporal sampling) have recently become available for several salmonids (Bernos et al. 2017; Yates et al. 2017). For example Bernos et al. (2017) found up to a 20-fold increase in  $N_b/N_c$  estimates for Atlantic Salmon (mean 0.20; range 0.06-0.56) relative to Brook Trout (mean 0.02; range 0.01-0.05). They concluded this difference was due to subtle ecological differences in the ecology of the two species with Brook Trout exhibiting a stronger spawning habitat dependency (i.e., groundwater seepage) whereas Atlantic Salmon tend to be more of generalist in regard to their spawning habitat preferences (Bernos et al. 2017). In addition, their results for Brook Trout, although low, were within the range documented by Whiteley et al. (2015) (mean 0.38; range 0.06-0.77) and Bernos and Fraser (2016) (mean 0.10; range 0.01-0.45). In contrast, results for San Juan River Razorback Sucker were not found within the range documented by Dowling et al. 2013 or Carson et al. 2016 (range 0.07-9.34). This would

suggest factors could be limiting the reproductive potential of Razorback Suck in the San Juan River.

In natural populations,  $N_b$  is typically less than  $N_c$  (Waples et al., 2013), and factors that cause  $N_b$  to be lower than  $N_c$  are often poorly understood. Disparity in  $N_b$  to  $N_c$  is often attributed to biological processes (i.e., demographic characteristics, habitat availability, or life-history) (Palstra and Fraser 2012; Kanno et al. 2015; Bernos et al. 2017). Nonetheless,  $N_b/N_c$  is critically dependent on the accuracy of estimates for both  $N_b$  and  $N_c$ . In many cases,  $N_c$  can be difficult or inaccurately estimated depending on the population being studied, sampling techniques, and/or the ability to determine age at maturation (Luikart et al. 2010). The accuracy of  $N_b$  estimates can be hindered when genetic information is scarce or the sample size is small compared to the real (unknown)  $N_b$  of the population (Wang 2016; Sánchez-Montes et al. 2017). Optimal cohort sample size for estimating  $N_b$  is most accurately estimated from a sample size that is close to or two times greater than the real  $N_b$  (England et al. 2006; Wang 2016; Sánchez-Montes et al. 2017; Bacles et al. 2018). This would suggest the sample sizes used for Colorado Pikeminnow were appropriate for accurate  $N_b$  estimates. Similarly, sample sizes for Razorback Sucker are likely sufficient. This is most apparent in the 2010 collections where additional samples were inadvertently collected. The increase in sample size, yet similar results to other years, would suggest sample sizes were sufficient. Finally, biases in spatial and temporal sampling can result in lower  $N_b$  estimates (Bacles et al. 2018). Temporal sampling for both Razorback Sucker and Colorado Pikeminnow were proportionally represented as best as possible. Spatial sampling was constrained between Shiprock, New Mexico and Clay Hill, Utah; thus, if spawning occurred relatively low in the system and a majority of larvae were lost to Lake Powell, then  $N_b$  estimates might be artificially lowered.

### **Acknowledgements**

This work could not be possible without access to the long-term larval collections. With that we thank Jen Kennedy, Michael Farrington, Howard Brandenburg, Steve Platania and the countless individuals that have contributed to this dataset. We thank the Program Office for providing adult census data and we want to give additional thanks to Eliza Gilbert for assisting with larval fish staging. Assistance with larval collections were provided by Lex Snyder along with other staff at the Museum of Southwestern Biology. We are sincerely thankful to staff at Southwestern ARRC for assistance with data collection and support. This study was approved by the San Juan River Basin Biology Committee through the San Juan River Basin Recovery Implementation Program and funded under a U.S. Bureau of Reclamation, Salt Lake City Project Office.

**Figures**

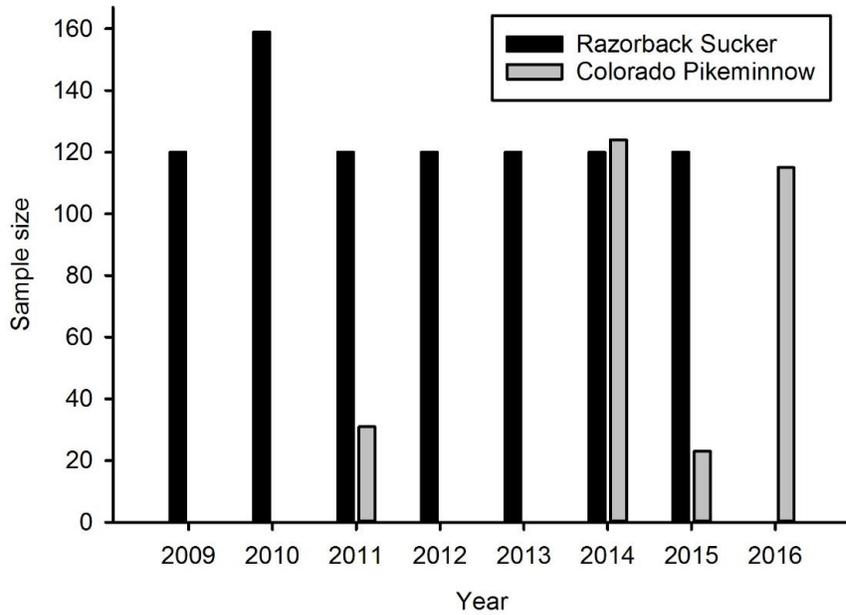


Figure 1. Sample sizes used for effective number of breeder ( $N_b$ ) estimates between 2009-2016. Razorback Sucker are represented in black and Colorado Pikeminnow are shown in gray.

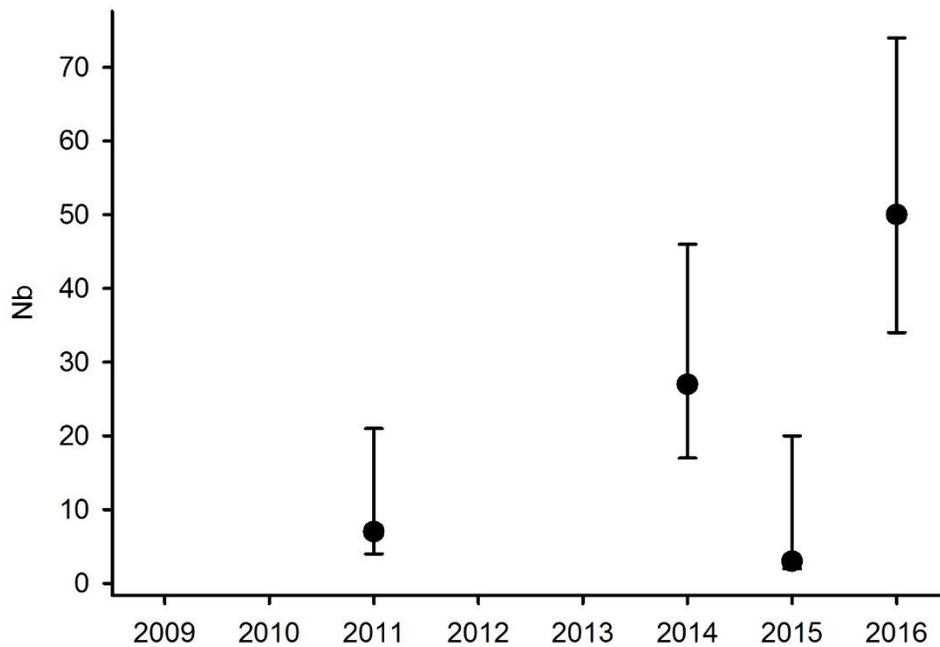


Figure 2. Effective number of breeder ( $N_b$ ) estimates for Colorado Pikeminnow in years samples were available. Circles represent mean estimates with 95% confidence intervals.

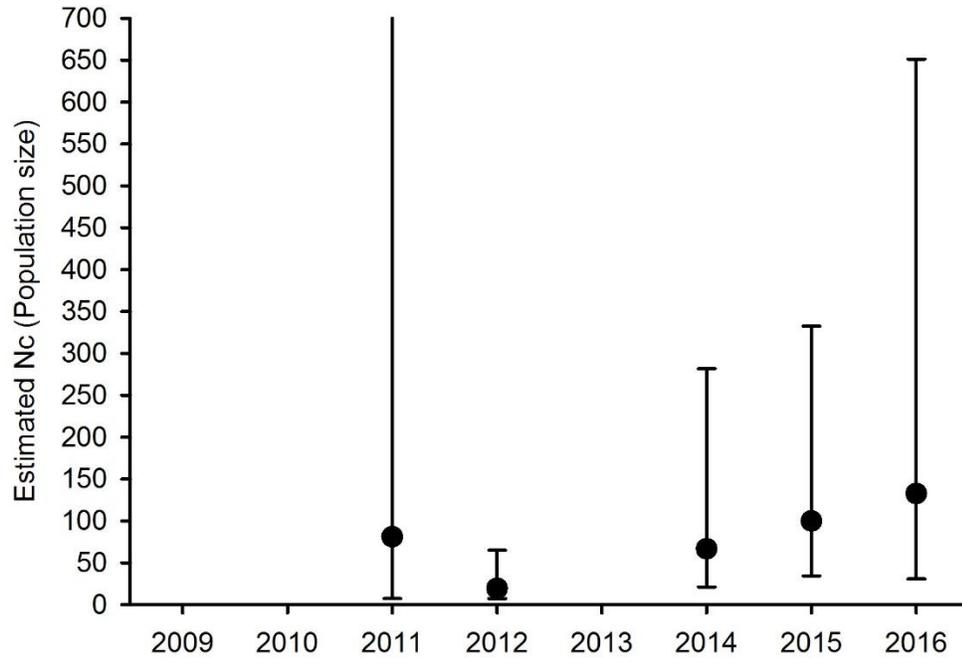


Figure 3. Estimated adult census sizes ( $N_c$ ) for Colorado Pikeminnow. Circles represent mean adult census sizes and bar represent 95% confidence intervals.

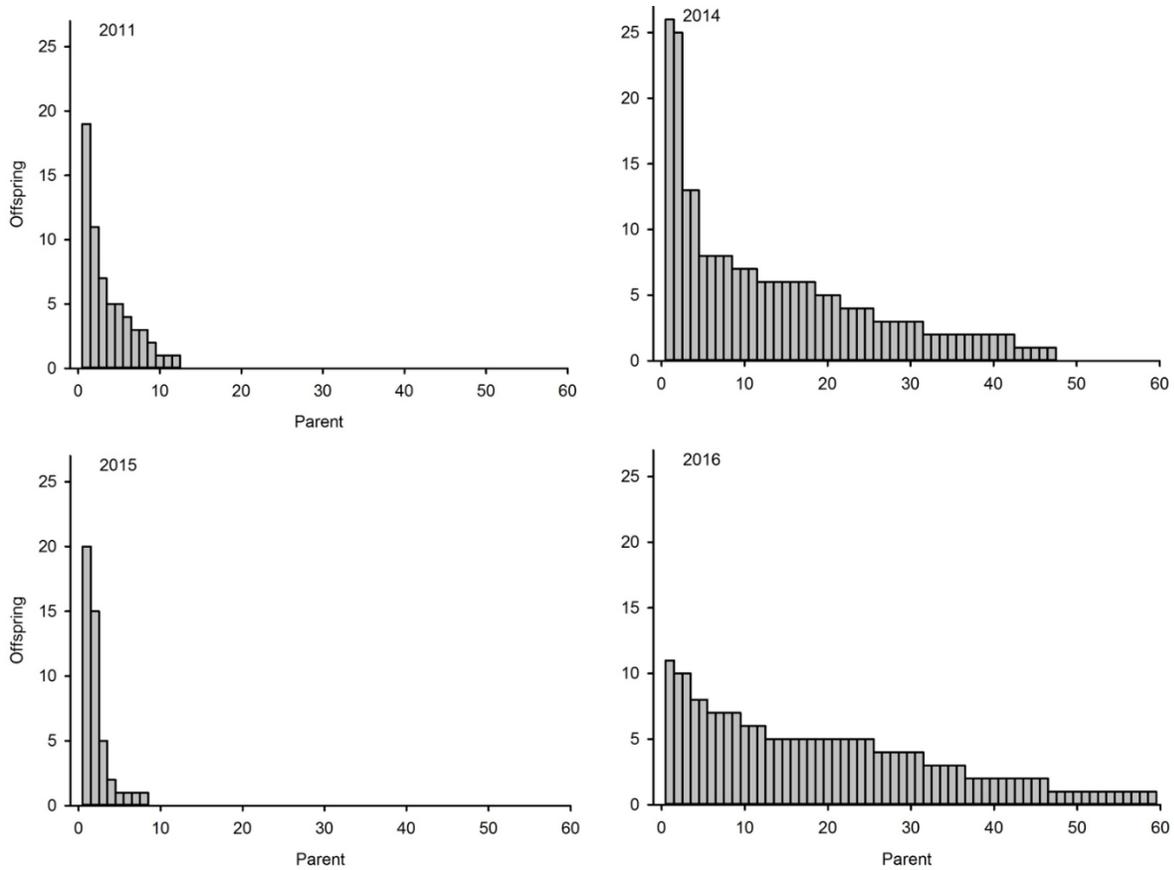


Figure 4. Adult contribution of offspring for Colorado Pikeminnow for 2011, 2014, 2015 and 2016.

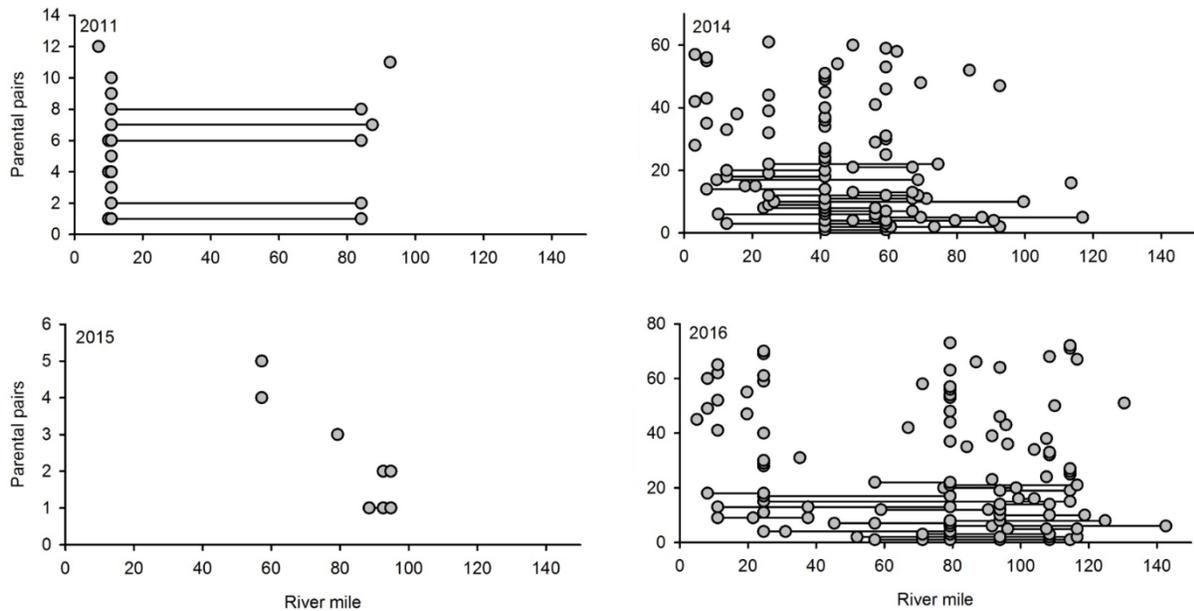


Figure 5. Estimated number of parental pairs for Colorado Pikeminnow. Each circle represents a larval collection within the San Juan River. Lines connecting each circle indicate full sibling relatedness. Circles do not reflect the frequency of larval collections at any given site.

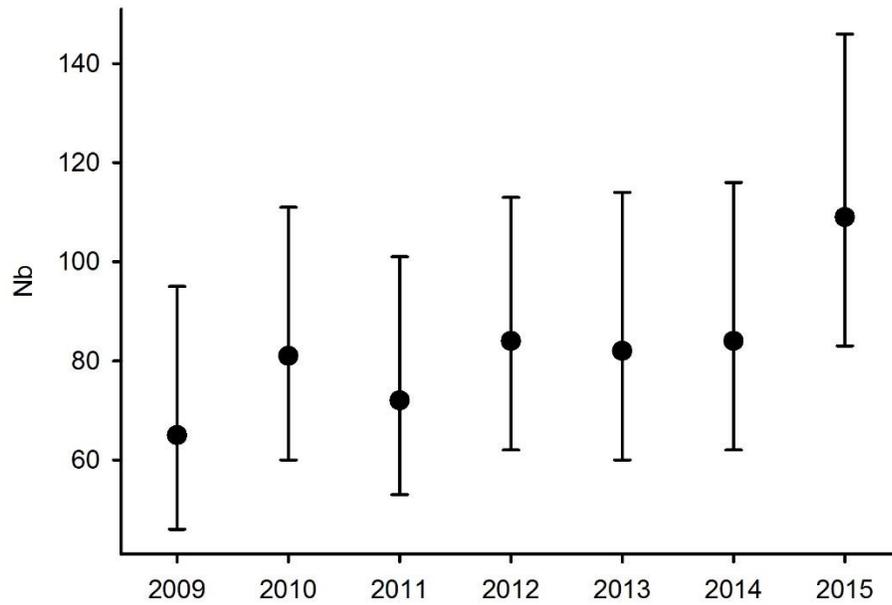


Figure 6. Effective number of breeder ( $N_b$ ) estimates for Razorback Sucker between 2009-2015. Circles represent mean  $N_b$  estimates with 95% confidence intervals.

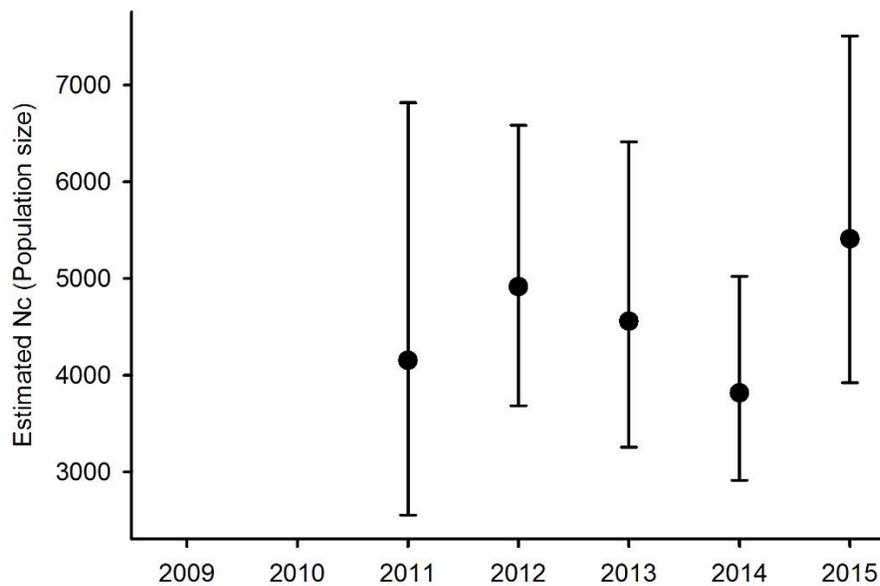


Figure 7. Estimated adult census sizes ( $N_c$ ) for Razorback Sucker. Circles represent mean adult census sizes and bar represent 95% confidence intervals.

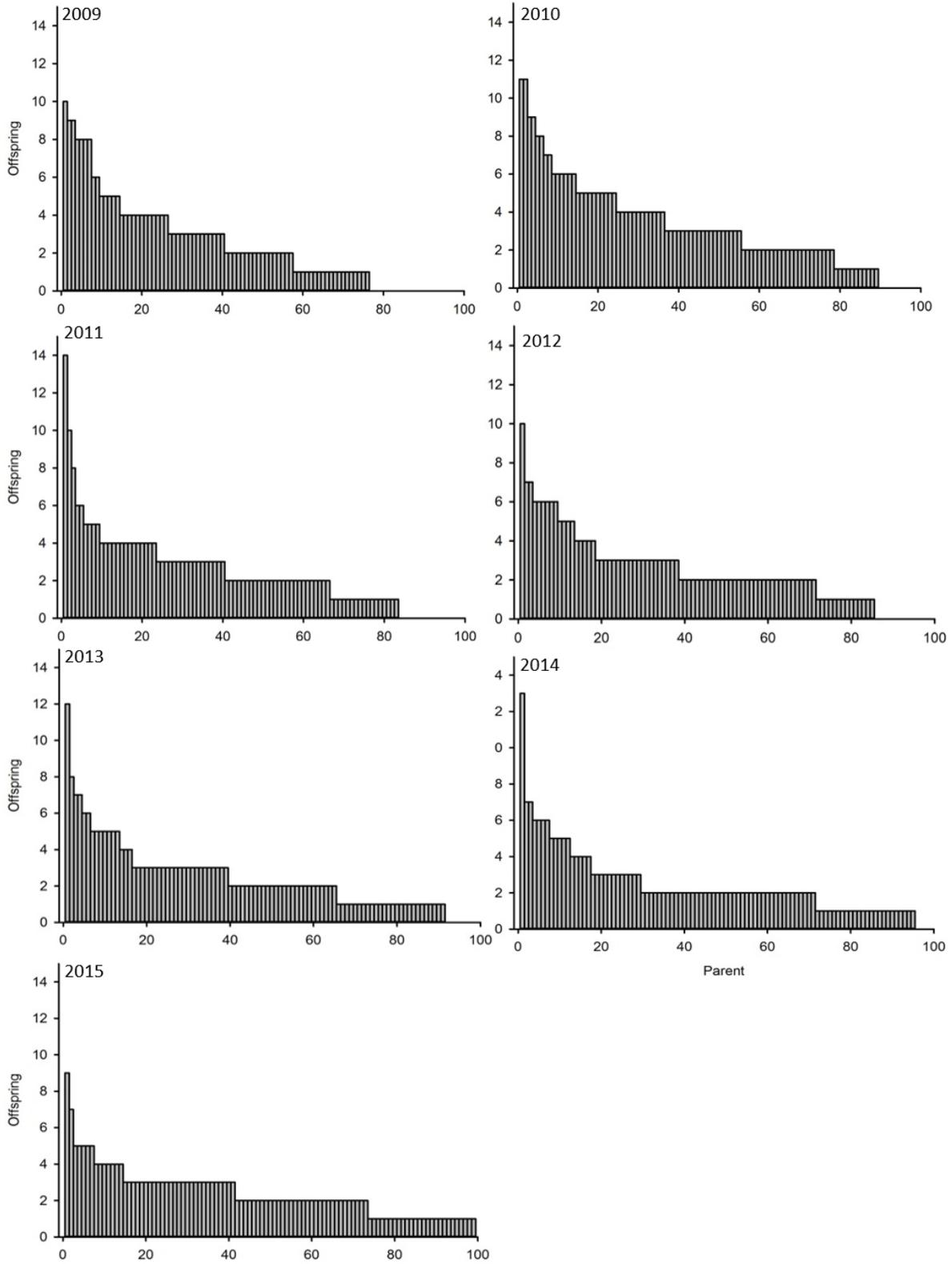


Figure 8. Adult contribution of offspring for Razorback Sucker for 2009-2015.

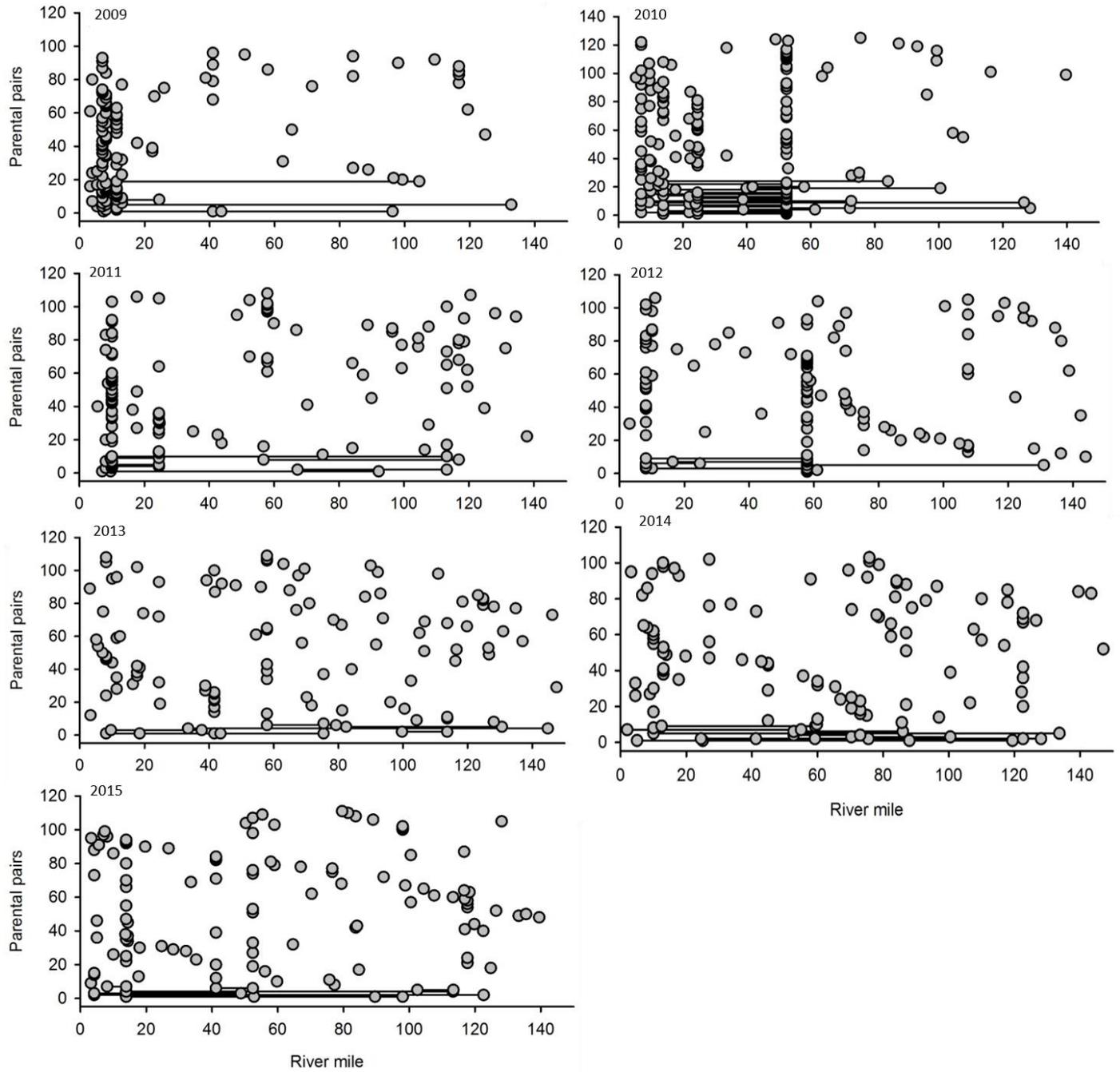


Figure 9. Estimated number of parental pairs for Razorbacks Sucker. Each circle represents a larval collection within the San Juan River. Lines connecting each circle indicate full sibling relatedness. Circles do not reflect the frequency of larval collections at any given site.

## Tables

Table 1. Mean adult census ( $N_c$ ) estimates with 95% confidence intervals in parentheses and mean effective number of breeder ( $N_b$ ) estimates with 95% confidence intervals in parentheses for Colorado Pikeminnow. The estimated proportion of spawning adults ( $N_b/N_c$ ) calculated from mean  $N_b/N_c$  with upper and lower 95% confidence intervals in parentheses. Areas with dashes indicate data were not available for those years.

	2011	2012	2013	2014	2015	2016
$N_c$	81 (8-1,125)	19 (7-65)	-	67 (21-282)	100 (35-333)	133 (31-652)
$N_b$	7 (4-21)	-	-	27 (17-46)	3 (2-20)	50 (34-74)
$N_b / N_c$	<b>8.6%</b> <b>(0.36-262.5%)</b>	-	-	<b>40.3%</b> <b>(6.02-219.0)</b>	<b>3%</b> <b>(0.60-57.1)</b>	<b>37.5%</b> <b>(5.21-238.7)</b>

Table 2. Mean adult census ( $N_c$ ) estimates with 95% confidence intervals in parentheses and mean effective number of breeder ( $N_b$ ) estimates with 95% confidence intervals in parentheses for Razorback Sucker. The estimated proportion of spawning adults ( $N_b/N_c$ ) calculated from mean  $N_b/N_c$  with upper and lower 95% confidence intervals in parentheses. Areas with dashes indicate data were not available for those years.

	2009	2010	2011	2012	2013	2014	2015
$N_c$	-	-	4,154 (2,554-6,818)	4,915 (3,687-6,585)	4,558 (2,915-5,023)	3,818 (3,923-7,508)	5,411 (3,099-4,752)
$N_b$	65 (46-95)	81 (60-111)	72 (53-101)	84 (62-113)	82 (60-114)	84 (62-116)	109 (83-146)
$N_b / N_c$	-	-	<b>1.73%</b> <b>(0.78-3.95)</b>	<b>1.71%</b> <b>(0.94-3.06)</b>	<b>1.80%</b> <b>(0.58-4.01)</b>	<b>2.22%</b> <b>(0.83-2.96)</b>	<b>2.01%</b> <b>(1.75-4.71)</b>

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## Appendix

Table A1. Larval field data for all Razorback Sucker collected between 2009-2015. Results show Year of collection, field identification (field ID), total number of larval Razorback Sucker collected, the number of individuals subsampled for genetic ( $N_b$ ) sampling, total length and developmental stage, date of collection, and river mile. With the exception of the genetic subsampling, these data were pulled from the larval fish reports on the San Juan River Basin Recovery Implementation Program webpage.

Year	Field ID	Field Collections (N)	Genetic Sampling (N)	Total Length	Larval Stage	Collection Date	River Mile
2009	WHB09-041	1	1	10.5	protolarva	19-May-09	132.8
2009	WHB09-044	3	1	13.3 -14.4	mesolarvae	19-May-09	124.8
2009	WHB09-046	2	1	11.1, 13.6	proto - mesolarvae	19-May-09	119.4
2009	WHB09-049	9	4	11.1 -15.9	proto - mesolarvae	20-May-09	116.7
2009	WHB09-051	1	1	10.7	protolarva	20-May-09	109.2
2009	WHB09-053	1	1	11.6	mesolarva	20-May-09	104.5
2009	WHB09-055	2	1	11.9, 13.7	mesolarvae	20-May-09	99.3
2009	WHB09-056	2	1	11.1, 12.0	proto - mesolarvae	21-May-09	96.6
2009	WHB09-060	1	1	14.4	mesolarva	21-May-09	88.8
2009	WHB09-062	8	3	10.1 -15.1	proto - mesolarvae	21-May-09	84.1
2009	WHB09-070	3	1	10.9 -11.9	protolarvae	22-May-09	62.5
2009	WHB09-071	2	1	12.9, 13.0	mesolarvae	22-May-09	57.9
2009	MAF09-029	1	1	11.4	mesolarva	20-May-09	24.5
2009	MAF09-030	4	2	12.2 -14.4	protolarva	20-May-09	22.4
2009	MAF09-031	1	1	13.0	mesolarva	20-May-09	17.7
2009	MAF09-033	13	5	12.4 -17.3	mesolarva	20-May-09	13.0
2009	MAF09-034	57	23	11.1 -18.1	proto - mesolarvae	21-May-09	11.4
2009	MAF09-037	52	21	11.3 -20.7	proto - metalarvae	21-May-09	8.1
2009	MAF09-038	67	26	11.0 -17.4	proto - mesolarvae	21-May-09	7.0
2009	MAF09-039	8	3	11.8 -17.9	mesolarvae	21-May-09	5.4
2009	MAF09-040	4	2	13.7 -19.2	mesolarvae	21-May-09	3.3
2009	WHB09-086	1	1	10.8	mesolarva	18-Jun-09	98.0
2009	WHB09-087	1	1	10.7	protolarva	18-Jun-09	96.3
2009	WHB09-096	1	1	10.7	protolarva	19-Jun-09	71.5
2009	WHB09-098	1	1	12.0	mesolarva	19-Jun-09	65.3
2009	MAF09-042	1	1	11.5	protolarva	16-Jun-09	50.8
2009	MAF09-045	1	1	11.7	protolarva	16-Jun-09	43.6
2009	MAF09-047	12	5	10.9 -11.9	proto - mesolarvae	16-Jun-09	41.0
2009	MAF09-048	1	1	12.6	mesolarva	16-Jun-09	38.8
2009	MAF09-050	2	1	11.0, 11.2	protolarvae	17-Jun-09	26.0

2009	MAF09-052	1	1	11.2	mesolarva	17-Jun-09	23.0
2009	MAF09-060	2	1	14.4, 14.6	mesolarvae	18-Jun-09	8.0
2009	MAF09-061	3	1	11.3 -11.7	mesolarvae	18-Jun-09	7.4
2009	MAF09-062	7	3	10.9 -30.2	meso -juvenile	18-Jun-09	3.9
2010	MAF10-033	17	1	9.4-11.1	protolarvae	19-May-10	107.6
2010	MAF10-034	6	1	11.1-12.4	proto-mesolarvae	19-May-10	107.6
2010	MAF10-037	1	1	10.9	mesolarvae	19-May-10	100.5
2010	MAF10-038	2	1	10.2-10.3	mesolarvae	19-May-10	99.3
2010	MAF10-039	6	1	9.5-11.8	proto-mesolarvae	20-May-10	99.4
2010	MAF10-040	14	1	10.1-12.7	proto-mesolarvae	20-May-10	93.3
2010	MAF10-043	1	1	11.2	protolarvae	20-May-10	87.5
2010	MAF10-044	2	1	10.9, 11.9	proto-mesolarvae	20-May-10	84.1
2010	MAF10-047	1	1	10.4	mesolarvae	20-May-10	75.5
2010	MAF10-048	3	1	10.2-10.9	protolarvae	20-May-10	74.9
2010	MAF10-050	1	1	11.2	protolarvae	20-May-10	72.7
2010	MAF10-051	7	1	10.6-12.2	proto-mesolarvae	20-May-10	72.6
2010	MAF10-052	3	1	10.2-11.3	proto-mesolarvae	20-May-10	69.3
2010	MAF10-054	18	1	9.6-13.2	proto-mesolarvae	20-May-10	61.3
2010	WHB10-041	1	1	11.4	mesolarvae	17-May-10	52.4
2010	WHB10-042	1	1	10.5	protolarvae	17-May-10	49
2010	WHB10-048	1	1	11.2	mesolarvae	18-May-10	39.9
2010	WHB10-049	2	1	11.2,11.6	proto-mesolarvae	18-May-10	38.9
2010	WHB10-050	2	1	11.1, 11.9	mesolarvae	18-May-10	38.8
2010	WHB10-051	22	2	936-12.0	proto-mesolarvae	18-May-10	33.7
2010	WHB10-053	3	1	10.6-11.5	mesolarvae	19-May-10	25
2010	WHB10-054	142	19	9.9-12.1	proto-mesolarvae	19-May-10	24.6
2010	WHB10-055	2	1	9.5,11.4	proto-mesolarvae	19-May-10	24.5
2010	WHB10-056	89	5	9.7-12.3	proto-mesolarvae	19-May-10	22
2010	WHB10-057	152	11	9.5-12.2	proto-mesolarvae	19-May-10	20.9
2010	WHB10-058	14	1	10.5-11.5	proto-mesolarvae	19-May-10	17.8
2010	WHB10-059	4	1	11.4-11.6	mesolarvae	19-May-10	17.7
2010	WHB10-060	126	30	9.8-12.7	proto-mesolarvae	19-May-10	13.9
2010	WHB10-061	45	11	10.1-12.4	proto-mesolarvae	19-May-10	12.4
2010	WHB10-062	30	1	11.5-14.3	mesolarvae	19-May-10	11.4
2010	WHB10-063	1	1	12.8	mesolarvae	19-May-10	10
2010	WHB10-064	36	3	11.1-13.9	mesolarvae	20-May-10	8.1
2010	WHB10-065	3	1	10.6-12.4	mesolarvae	20-May-10	7
2010	WHB10-066	266	17	9.4-14.6	proto-mesolarvae	20-May-10	6
2010	MAF10-059	2	1	10.4,11.5	proto-mesolarvae	14-Jun-10	139.7
2010	MAF10-064	3	1	12.0-18.1	proto-mesolarvae	15-Jun-10	128.5
2010	MAF10-067	3	1	15.7-18.1	mesolarvae	15-Jun-10	126.6
2010	MAF10-071	2	1	9.8,11.1	protolarvae	16-Jun-10	116.2
2010	MAF10-077	1	1	11.8	mesolarvae	16-Jun-10	104.4

2010	MAF10-081	1	1	12.8	mesolarvae	17-Jun-10	96.3
2010	MAF10-093	3	1	12.4-12.6	mesolarvae	18-Jun-10	75.1
2010	MAF10-094	1	1	12.2	mesolarvae	18-Jun-10	72.2
2010	MAF10-098	2	1	10.1, 10.5	mesolarvae	18-Jun-10	65.2
2010	MAF10-099	5	1	11.0-20.7	mesolarvae	18-Jun-10	63.5
2010	MAF10-100	1	1	10.4	meso-metalarvae	18-Jun-10	57.9
2010	MAF10-102	2	1	13.3,14.6	mesolarvae	19-Jun-10	52.9
2010	WHB10-067	11	1	11.0-26.7	meso-juvenile	14-Jun-10	52.4
2010	WHB10-070	2	1	11.3,17.0	mesolarvae	15-Jun-10	41.8
2010	WHB10-076	25	2	11.8-22.4	meso-metalarvae	16-Jun-10	22.4
2010	WHB10-078	5	1	12.6-26.5	meso-metalarvae	16-Jun-10	17.7
2010	WHB10-079	7	1	13.8-22.3	meso-metalarvae	16-Jun-10	16.4
2010	WHB10-080	9	1	10.6-21.2	meso-metalarvae	16-Jun-10	13.9
2010	WHB10-082	61	5	11.0-26.3	meso-juvenile	16-Jun-10	11.4
2010	WHB10-083	1	1	18.2	mesolarvae	16-Jun-10	10
2010	WHB10-084	79	4	11.1-21.3	meso-metalarvae	17-Jun-10	9.6
2010	WHB10-085	1	1	17-Jan-00	metalarvae	17-Jun-10	9.5
2010	WHB10-086	21	2	12.0-20.3	meso-metalarvae	17-Jun-10	8.1
2010	WHB10-087	3	1	11.5-21.1	meso-metalarvae	17-Jun-10	7
2010	WHB10-088	2	1	10.6,11.3	mesolarvae	17-Jun-10	5.4
2010	WHB10-093	1	1	30	juvenile	20-Jul-10	44.8
2010	WHB10-094	1	1	51	juvenile	20-Jul-10	43.8
2011	WHB11-036	6	1	9.9-11.5	proto-mesolarvae	16-May-11	137.9
2011	WHB11-038	4	1	10.9-11.3	proto-mesolarvae	16-May-11	134.5
2011	WHB11-040	1	1	11.6	mesolarvae	16-May-11	131.3
2011	WHB11-042	9	1	10.5-11.1	proto-mesolarvae	16-May-11	128.1
2011	WHB11-046	1	1	10.9	mesolarvae	17-May-11	120.5
2011	WHB11-047	2	1	10.4,10.7	proto-mesolarvae	17-May-11	119.5
2011	WHB11-048	18	2	9.8-12.0	proto-mesolarvae	17-May-11	118.5
2011	WHB11-049	22	2	10.0 -11.7	mesolarvae	17-May-11	116.9
2011	WHB11-050	84	8	9.5 -12.0	proto-mesolarvae	17-May-11	113.2
2011	WHB11-051	22	2	10.0 -11.7	mesolarvae	18-May-11	107.6
2011	WHB11-052	4	1	10.1 -11.2	mesolarvae	18-May-11	106.4
2011	WHB11-054	2	1	9.1, 10.4	mesolarvae	18-May-11	104.4
2011	WHB11-056	3	1	10.1 -11.2	mesolarvae	18-May-11	99.3
2011	WHB11-057	6	1	9.6 -12.0	proto-mesolarvae	18-May-11	92.2
2011	WHB11-058	2	1	10.7, 10.9	mesolarvae	18-May-11	96.4
2011	WHB11-059	3	1	10.4 -13.2	mesolarvae	18-May-11	88.8
2011	WHB11-060	4	1	9.1 -13.0	proto-mesolarvae	18-May-11	87.4
2011	WHB11-062	1	1	10	mesolarvae	19-May-11	84.1
2011	MAF11-035	1	1	12	mesolarvae	16-May-11	74.9
2011	MAF11-037	1	1	10	mesolarvae	16-May-11	70.2
2011	MAF11-038	2	1	10.0,11.0	mesolarvae	17-May-11	67.2

2011	MAF11-040	1	1	10.7	mesolarvae	17-May-11	66.8
2011	MAF11-042	12	1	9.0 -13.5	mesolarvae	17-May-11	59.9
2011	MAF11-043	76	6	9.4 -14.9	mesolarvae	17-May-11	57.9
2011	MAF11-044	18	2	10.2 -11.6	mesolarvae	17-May-11	56.7
2011	MAF11-046	1	1	10.9	mesolarvae	17-May-11	48.5
2011	MAF11-047	6	1	9.2 -11.2	proto-mesolarvae	17-May-11	43.8
2011	MAF11-048	8	1	9.8 -12.8	mesolarvae	18-May-11	42.6
2011	MAF11-050	10	1	11.2 -15.1	mesolarvae	18-May-11	35
2011	MAF11-051	14	1	9.9 -14.2	mesolarvae	18-May-11	24.7
2011	MAF11-052	137	13	8.2 -13.1	proto-mesolarvae	18-May-11	24.5
2011	MAF11-054	22	2	11.5 -17.4	mesolarvae	19-May-11	17.7
2011	MAF11-055	1	1	12.2	mesolarvae	19-May-11	16.4
2011	MAF11-056	347	30	9.4 -16.3	proto-mesolarvae	19-May-11	10
2011	MAF11-057	3	1	9.6 -10.6	mesolarvae	19-May-11	8.8
2011	MAF11-058	47	4	10.0 -17.0	meso-metalarvae	19-May-11	8.1
2011	MAF11-059	1	1	12	mesolarvae	19-May-11	5.6
2011	WHB11-074	9	1	11.0-13.8	mesolarvae	14-Jun-11	124.8
2011	WHB11-077	8	1	11.0-14.5	mesolarvae	14-Jun-11	119.5
2011	WHB11-079	18	2	12.0-15.5	mesolarvae	14-Jun-11	116.9
2011	WHB11-083	1	1	17.4	metalarvae	15-Jun-11	104.4
2011	WHB11-085	2	1	11.9,13.4	mesolarvae	15-Jun-11	99.3
2011	WHB11-086	1	1	10.7	mesolarvae	15-Jun-11	96.4
2011	WHB11-088	1	1	11.5	mesolarvae	15-Jun-11	90
2011	WHB11-091	5	1	11.8-13.7	mesolarvae	15-Jun-11	84.1
2011	MAF11-066	36	3	10.4-18.4	meso-metalarvae	14-Jun-11	57.9
2011	MAF11-068	1	1	12.3	mesolarvae	14-Jun-11	52.4
2011	MAF11-079	1	1	19.7	metalarvae	16-Jun-11	17.7
2011	MAF11-082	38	3	10.8-19.4	meso-metalarvae	16-Jun-11	10
2011	MAF11-083	20	2	12.6-24.1	meso-juvenile	16-Jun-11	9.6
2011	MAF11-084	13	1	14.8-23.8	meso-juvenile	16-Jun-11	8.1
2011	MAF11-085	8	1	11.5-23.8	meso-juvenile	16-Jun-11	7
2011	WHB11-106	1	1	26.2	juvenile	19-Jul-11	52.4
2012	MAF12-052	1	1	10.7	mesolarvae	14-May-12	143.9
2012	MAF12-054	5	1	10.6 -11.3	proto-mesolarvae	14-May-12	142.4
2012	MAF12-056	8	1	10.2 -13.0	proto-mesolarvae	15-May-12	138.8
2012	MAF12-058	3	1	10.4 -11.0	proto-mesolarvae	15-May-12	136.3
2012	MAF12-059	1	1	11.6	mesolarvae	15-May-12	134.5
2012	MAF12-065	1	1	11.3	mesolarvae	15-May-12	127.2
2012	MAF12-066	37	2	11.0 -14.9	mesolarvae	15-May-12	124.8
2012	MAF12-069	2	1	11.2, 12.8	mesolarvae	16-May-12	118.9
2012	MAF12-071	1	1	10.8	mesolarvae	16-May-12	116.9
2012	MAF12-075	140	8	10.1 -17.3	proto-mesolarvae	16-May-12	107.6
2012	MAF12-076	1	1	17	mesolarvae	16-May-12	105

2012	MAF12-079	13	1	9.2 -14.0	mesolarvae	16-May-12	100.5
2012	MAF12-080	8	1	10.5 -17.5	mesolarvae	17-May-12	99
2012	MAF12-082	8	1	10.5 -12.3	proto-mesolarvae	17-May-12	94
2012	MAF12-083	2	1	11.9, 13.0	mesolarvae	17-May-12	92.7
2012	MAF12-086	10	1	9.5 -15.6	proto-mesolarvae	17-May-12	86.8
2012	MAF12-088	3	1	10.7 -12.3	mesolarvae	17-May-12	83.7
2012	MAF12-089	7	1	9.7 -11.5	mesolarvae	17-May-12	81.8
2012	WHB12-061	76	4	9.9 -12.6	proto-mesolarvae	14-May-12	75.4
2012	WHB12-062	2	1	11.8, 12.5	mesolarvae	14-May-12	71.2
2012	WHB12-063	46	3	11.6 -19.8	meso-metalarvae	14-May-12	69.9
2012	WHB12-064	5	1	10.1 -13.5	mesolarvae	15-May-12	69.4
2012	WHB12-067	17	1	13.8 -15.8	mesolarvae	15-May-12	62.2
2012	WHB12-068	3	1	10.6 -13.7	mesolarvae	15-May-12	61.2
2012	WHB12-070	435	20	8.6 -21.2	proto-mesolarvae	15-May-12	57.9
2012	WHB12-070B	244	13	9.5 -20.7	meso-metalarvae	15-May-12	57.9
2012	WHB12-071	7	1	10.7 - 12.4	mesolarvae	15-May-12	52.9
2012	WHB12-074	2	1	10.5, 11.0	mesolarvae	15-May-12	43.8
2012	WHB12-076	11	1	10.5 -13.5	mesolarvae	16-May-12	38.8
2012	WHB12-077	17	1	10.3 -12.9	mesolarvae	16-May-12	33.7
2012	WHB12-079	3	1	10.4 -12.4	mesolarvae	16-May-12	26.4
2012	WHB12-082	3	1	10.5 -11.5	mesolarvae	16-May-12	24.8
2012	WHB12-084	7	1	13.7 -21.0	meso-metalarvae	17-May-12	17.7
2012	WHB12-087	8	1	10.5 -12.5	mesolarvae	17-May-12	16.4
2012	WHB12-089	3	1	10.1 -11.5	mesolarvae	17-May-12	11
2012	WHB12-090	128	6	9.9 -16.3	proto-mesolarvae	17-May-12	10
2012	WHB12-092	468	21	10.0 -30.0	meso-juvenile	17-May-12	8.1
2012	MAF12-096	1	1	14.3	mesolarvae	11-Jun-12	136.4
2012	MAF12-097	1	1	16.8	mesolarvae	12-Jun-12	131
2012	MAF12-101	1	1	11.3	mesolarvae	12-Jun-12	128
2012	MAF12-104	1	1	28.3	juvenile	12-Jun-12	122.2
2012	WHB12-113	1	1	12.7	mesolarvae	11-Jun-12	69.8
2012	WHB12-115	1	1	13.2	mesolarvae	11-Jun-12	67.7
2012	WHB12-116	2	1	18.1,20.4	mesolarvae	12-Jun-12	66.2
2012	WHB12-118	1	1	20.5	metalarvae	12-Jun-12	61
2012	WHB12-119	1	1	28.1	metalarvae	12-Jun-12	58.9
2012	WHB12-120	22	1	14.3-25.4	meso-juvenile	12-Jun-12	57.9
2012	WHB12-123	5	1	10.0-31.8	meso-juvenile	12-Jun-12	49
2012	WHB12-130	1	1	15.5	mesolarvae	13-Jun-12	29.5
2012	WHB12-134	3	1	17.2-26.5	meso-juvenile	13-Jun-12	22.9
2012	WHB12-146	1	1	29.6	juvenile	14-Jun-12	3.1
2013	WHB13-035	5	1	11.1 -12.7	mesolarvae	20-May-13	146.2
2013	WHB13-038	15	1	11.6 -19.8	proto-mesolarvae	20-May-13	19.6
2013	WHB13-039	2	1	11.5, 16.0	proto-mesolarvae	21-May-13	134.9

2013	WHB13-044	2	1	10.9, 13.3	proto-mesolarvae	21-May-13	130.6
2013	WHB13-045	19	2	10.5 -13.7	proto-mesolarvae	21-May-13	128.1
2013	WHB13-046	1	1	12	mesolarvae	21-May-13	126.6
2013	WHB13-047	43	3	10.3 -16.1	meso-metalarvae	21-May-13	124.8
2013	WHB13-048	16	1	10.5 -15.9	proto-mesolarvae	21-May-13	123.2
2013	WHB13-051	2	1	11.7, 14.8	mesolarvae	21-May-13	118.4
2013	WHB13-052	3	1	10.5 -12.3	protolarvae	22-May-13	116.2
2013	WHB13-053	34	3	10.1 -15.4	proto-mesolarvae	22-May-13	113.7
2013	WHB13-054	4	1	10.5 -13.2	proto-mesolarvae	22-May-13	110.9
2013	WHB13-056	3	1	11.8 -17.1	mesolarvae	22-May-13	104.2
2013	WHB13-057	5	1	11.3 -14.7	proto-mesolarvae	22-May-13	102.5
2013	WHB13-060	4	1	13.0 -15.7	mesolarvae	22-May-13	99.7
2013	WHB13-062	11	1	12.0 -18.1	mesolarvae	22-May-13	93
2013	WHB13-063	10	1	9.9 -16.3	proto-mesolarvae	22-May-13	92.2
2013	WHB13-064	2	1	14.9 - N/A	mesolarvae	23-May-13	90
2013	WHB13-065	9	1	13.0 -15.7	mesolarvae	23-May-13	88.3
2013	WHB13-066	4	1	13.0 -15.2	mesolarvae	23-May-13	84.1
2013	WHB13-067	5	1	9.9 -16.3	proto-mesolarvae	23-May-13	81.2
2013	WHB13-068	14	1	10.9 -18.1	mesolarvae	23-May-13	79.3
2013	MAF13-042	58	4	10.9 -18.8	proto-mesolarvae	17-May-13	75.4
2013	MAF13-043	1	1	13.1	protolarvae	17-May-13	71
2013	MAF13-044	2	1	11.5, 13.3	mesolarvae	17-May-13	69.5
2013	MAF13-047	6	1	10.5 -14.4	proto-mesolarvae	18-May-13	63
2013	MAF13-049	60	4	11.0 -21.4	proto-metalarvae	18-May-13	57.9
2013	MAF13-053	1	1	13.1	mesolarvae	18-May-13	43.5
2013	MAF13-054	116	9	10.4 -15.4	proto-mesolarvae	18-May-13	41.6
2013	MAF13-055	32	2	9.6 -18.2	proto-mesolarvae	18-May-13	38.8
2013	MAF13-057	16	1	9.5 -15.0	proto-mesolarvae	19-May-13	24.5
2013	MAF13-060	29	2	11.8 -20.7	meso-metalarvae	19-May-13	18.5
2013	MAF13-061	40	3	10.5 -21.2	proto-metalarvae	19-May-13	17.7
2013	MAF13-063	1	1	21.1	metalarvae	20-May-13	16.4
2013	MAF13-064	35	2	108-18.8	meso-metalarvae	20-May-13	11.4
2013	MAF13-065	3	1	15.6,17.7	mesolarvae	20-May-13	10
2013	MAF13-066	1	1	14.2	mesolarvae	20-May-13	9.6
2013	MAF13-067	104	7	9.7 -19.3	proto-metalarvae	20-May-13	8.1
2013	MAF13-068	16	1	11.9 -18.8	mesolarvae	20-May-13	7
2013	MAF13-069	6	1	9.6 -15.4	mesolarvae	20-May-13	5.6
2013	MAF13-070	7	1	11.0 -17.4	mesolarvae	20-May-13	3.3
2013	MAF13-072	2	1	11.4, 12.1	mesolarvae	9-Jun-13	147.5
2013	MAF13-074	3	1	14.6 -16.1	mesolarvae	9-Jun-13	144.8
2013	MAF13-075	1	1	10.7	mesolarvae	9-Jun-13	137
2013	MAF13-079	2	1	10.0, 10.3	protolarvae	10-Jun-13	131
2013	MAF13-082	3	1	17.7 -27.8	meso-juvenile	10-Jun-13	126.4

2013	MAF13-085	11	1	10.0 -22.6	meso-metalarvae	10-Jun-13	119.8
2013	MAF13-089	1	1	11.1	mesolarvae	11-Jun-13	116.6
2013	MAF13-090	11	1	13.1 -23.6	meso-metalarvae	11-Jun-13	113.7
2013	MAF13-092	17	1	14.3 -25.4	meso-juvenile	11-Jun-13	106.7
2013	MAF13-093	4	1	10.8 -24.6	meso-metalarvae	11-Jun-13	106.6
2013	MAF13-094	1	1	9.8	protolarvae	11-Jun-13	105.1
2013	MAF13-097	11	1	12.8 -25.2	meso-metalarvae	11-Jun-13	100.5
2013	MAF13-100	11	1	10.3 -19.8	meso-metalarvae	12-Jun-13	96.1
2013	MAF13-101	1	1	11.4, 12.1	mesolarvae	12-Jun-13	93.8
2013	MAF13-103	11	1	10.9 -19.2	mesolarvae	12-Jun-13	91.7
2013	MAF13-108	1	1	11.3	mesolarvae	13-Jun-13	82.4
2013	MAF13-109	9	1	10.1 -16.9	protolarvae	13-Jun-13	81
2013	MAF13-111	2	1	12.2 -28.0	meso-juvenile	13-Jun-13	78.5
2013	WHB13-071	2	1	11.5, 11.9	mesolarvae	9-Jun-13	71.7
2013	WHB13-074	12	1	11.8 -20.8	meso-metalarvae	10-Jun-13	70.2
2013	WHB13-075	6	1	11.7 -29.0	meso-metalarvae	10-Jun-13	68.7
2013	WHB13-076	7	1	12.9 -37.2	meso-juvenile	10-Jun-13	67.6
2013	WHB13-077	1	1	11.8	mesolarvae	10-Jun-13	67
2013	WHB13-079	3	1	10.4-11.0	mesolarvae	10-Jun-13	64.9
2013	WHB13-081	95	6	13.8-37.3	proto-juvenile	10-Jun-13	57.9
2013	WHB13-082	14	1	9.5 -23.4	mesolarvae	10-Jun-13	56
2013	WHB13-084	2	1	9.8, 11.2	mesolarvae	10-Jun-13	54.5
2013	WHB13-087	2	1	10.4 -15.0	proto-juvenile	11-Jun-13	48.2
2013	WHB13-089	4	1	10.8 -11.9	proto-mesolarvae	11-Jun-13	43.9
2013	WHB13-090	1	1	12.2	mesolarvae	11-Jun-13	41.8
2013	WHB13-091	4	1	11.4 -12.7	mesolarvae	11-Jun-13	39.2
2013	WHB13-092	4	1	9.8 -10.7	proto-mesolarvae	11-Jun-13	37.7
2013	WHB13-093	2	1	10.2, 11.6	mesolarvae	11-Jun-13	33.5
2013	WHB13-097	5	1	11.3 -14.2	mesolarvae	12-Jun-13	24.8
2013	WHB13-098	3	1	10.4 -14.3	mesolarvae	12-Jun-13	24.5
2013	WHB13-099	1	1	12.4	mesolarvae	12-Jun-13	24.4
2013	WHB13-102	2	1	19.2,37.8	meta-juvenile	12-Jun-13	17.7
2013	WHB13-105	6	1	10.4-15.4	mesolarvae	13-Jun-13	12.4
2013	WHB13-106	34	2	9.8-23.0	proto-metalarvae	13-Jun-13	11.4
2013	WHB13-107	3	1	13.2 -15.6	mesolarvae	13-Jun-13	10
2013	WHB13-109	4	1	10.0 -16.6	mesolarvae	13-Jun-13	7.2
2013	WHB13-110	15	1	10.0 -15.8	mesolarvae	13-Jun-13	5.1
2013	WHB13-112	1	1	16.7	mesolarvae	13-Jun-13	3.1
2013	WHB13-126	1	0	38.5	juvenile	16-Jul-13	126.6
2013	WHB13-127	1	0	68	juvenile	16-Jul-13	124.8
2013	WHB13-132	1	0	70	juvenile	17-Jul-13	116.9
2013	WHB13-140	1	0	33.4	juvenile	18-Jul-13	100.5
2013	WHB13-142	1	0	26.2	juvenile	18-Jul-13	98.6

2013	WHB13-148	1	0	54	juvenile	18-Jul-13	84.7
2013	WHB13-151	3	0	26.8 -55.0	juvenile	19-Jul-13	79.4
2014	MAF14-022	1	1	10.9	protolarvae	23-Apr-14	97
2014	MAF14-031	2	1	13.5, 13.6	mesolarvae	24-Apr-14	78.7
2014	JLK14-008	9	1	10 -14.4	proto - mesolarvae	24-Apr-14	59.8
2014	JLK14-010	4	1	9.5 -12.7	proto - mesolarvae	24-Apr-14	55
2014	JLK14-018	1	1	11.3	mesolarvae	24-Apr-14	2
2014	JLK14-023	9	2	10.8 -11.8	proto - mesolarvae	24-Apr-14	27
2014	JLK14-026	2	1	11.4, 11.4	mesolarvae	24-Apr-14	17.7
2014	JLK14-030	3	1	10.7 -13.7	proto - mesolarvae	24-Apr-14	12.9
2014	MAF14-033	3	1	11 -12.4	mesolarvae	19-May-14	147.1
2014	MAF14-035	2	1	11.3, 11.5	proto - mesolarvae	19-May-14	143.4
2014	MAF14-036	1	1	10.9	protolarva	19-May-14	139.5
2014	MAF14-044	2	1	11.4, 11.5	mesolarvae	20-May-14	126.6
2014	MAF14-046	1	1	11.1	mesolarvae	20-May-14	122.2
2014	MAF14-049	7	1	10.7 -13.5	mesolarvae	20-May-14	117.9
2014	MAF14-053	19	2	10.1 -14.2	proto - mesolarvae	20-May-14	110
2014	MAF14-054	2	1	11.2, 11.3	mesolarvae	20-May-14	107.6
2014	MAF14-055	2	1	11.3, 11.5	mesolarvae	21-May-14	106.5
2014	MAF14-057	11	2	10.7 -13.9	mesolarvae	21-May-14	100.5
2014	MAF14-058	1	1	12	mesolarvae	21-May-14	96.4
2014	MAF14-061	1	1	10.7	protolarvae	21-May-14	88.8
2014	MAF14-062	29	4	10.3 -14.2	proto - mesolarvae	22-May-14	87
2014	MAF14-063	8	1	10 -12.2	proto - mesolarvae	22-May-14	86
2014	MAF14-064	14	2	10.7 -13.6	proto - mesolarvae	22-May-14	84.1
2014	MAF14-065	13	2	10.5 -13.9	proto - mesolarvae	22-May-14	82.4
2014	MAF14-067	1	1	15.9	mesolarvae	22-May-14	78
2014	JLK14-033	24	3	10 -11.7	proto - mesolarvae	19-May-14	75.8
2014	JLK14-034	2	1	11.4, 11.5	mesolarvae	19-May-14	75
2014	JLK14-035	26	4	10 -12.9	proto - mesolarvae	19-May-14	73
2014	JLK14-036	11	2	10.7 -12.9	mesolarvae	20-May-14	70.3
2014	JLK14-037	16	2	11.5, 13.5	proto - mesolarvae	20-May-14	70.3
2014	JLK14-038	2	1	11.5, 13.5	mesolarvae	20-May-14	65.4
2014	JLK14-040	20	3	10.2 -12.5	proto - mesolarvae	20-May-14	60
2014	JLK14-042	1	1	10.7	protolarvae	20-May-14	55.6
2014	JLK14-043	2	1	8.8, 9.7	protolarvae	20-May-14	52.7
2014	JLK14-044	1	1	10.1	protolarvae	20-May-14	52.7
2014	JLK14-046	28	4	10.7 -16.2	proto - mesolarvae	21-May-14	44.9
2014	JLK14-047	2	1	11.5, 12	mesolarvae	21-May-14	42.9
2014	JLK14-048	4	1	10.9 -12	proto - mesolarvae	21-May-14	37
2014	JLK14-049	12	2	9.9 -12.4	mesolarvae	21-May-14	27
2014	JLK14-051	1	1	12.1	mesolarvae	21-May-14	19.8
2014	JLK14-052	3	1	11.1 -17	proto - mesolarvae	22-May-14	17.7

2014	JLK14-053	2	1	15.6, 17	mesolarvae	22-May-14	16.4
2014	JLK14-054	3	1	11 -15.7	mesolarvae	22-May-14	13.9
2014	JLK14-055	14	2	9.9 -15.9	mesolarvae	22-May-14	13
2014	JLK14-056	34	4	10.3 -16.3	proto - mesolarvae	22-May-14	13
2014	JLK14-057	79	9	10.3 -17.9	mesolarvae	22-May-14	10
2014	JLK14-058	1	1	14.1	mesolarvae	22-May-14	8.1
2014	JLK14-059	10	1	10.8 -14.5	mesolarvae	22-May-14	7
2014	JLK14-070	1	1	11.8	mesolarvae	23-Jun-14	133.7
2014	JLK14-073	1	1	12.6	mesolarvae	23-Jun-14	128.1
2014	JLK14-076	68	7	12.1 -23.9	meso - metalarvae	23-Jun-14	122.6
2014	JLK14-078	8	1	11.1 -14.2	mesolarvae	23-Jun-14	119.4
2014	JLK14-080	2	1	13.1, 13.2	mesolarvae	24-Jun-14	117.9
2014	JLK14-081	4	1	12.5 -14.2	mesolarvae	24-Jun-14	116.9
2014	JLK14-094	1	1	20.5	mesolarvae	25-Jun-14	93
2014	JLK14-097	2	1	12.7, 13.6	mesolarvae	25-Jun-14	88
2014	JLK14-098	9	1	11.3 -41	meso - juvenile	26-Jun-14	85.7
2014	JLK14-100	1	1	13.4	mesolarvae	26-Jun-14	83.7
2014	JLK14-102	1	1	10.6	mesolarvae	26-Jun-14	78.7
2014	MAF14-068	1	1	12.3	mesolarvae	22-Jun-14	75.5
2014	MAF14-070	1	1	10.1	mesolarvae	22-Jun-14	70.4
2014	MAF14-071	1	0	28.5	juvenile	22-Jun-14	69.8
2014	MAF14-073	1	1	10.4	mesolarvae	23-Jun-14	67
2014	MAF14-075	7	1	25.2 -34.4	meta - juvenile	23-Jun-14	57.9
2014	MAF14-083	8	1	10 -12.2	mesolarvae	24-Jun-14	41.2
2014	MAF14-085	2	1	11.3, 11.7	mesolarvae	24-Jun-14	33.6
2014	MAF14-087	1	1	13.9	mesolarvae	25-Jun-14	25
2014	MAF14-088	1	1	13.2	mesolarvae	25-Jun-14	24.5
2014	MAF14-097	4	1	11.5 -11.8	mesolarvae	26-Jun-14	8.9
2014	MAF14-099	1	0	31.2	juvenile	26-Jun-14	7.1
2014	MAF14-101	4	1	11.1-35.3	meso - juvenile	26-Jun-14	5
2014	MAF14-102	17	2	10.7-23.8	mesolarvae	26-Jun-14	4.5
2014	MAF14-104	1	1	28.7	juvenile	13-Jul-14	75.2
2014	MAF14-107	1	1	N/A	mesolarvae	14-Jul-14	69.4
2014	MAF14-109	1	0	24.9	juvenile	14-Jul-14	67
2014	MAF14-113	1	1	11	mesolarvae	14-Jul-14	59.3
2014	MAF14-114	3	1	21.5 -25.2	metalarvae	14-Jul-14	59.2
2014	MAF14-115	3	0	25.5 -57.6	juvenile	14-Jul-14	57.9
2014	MAF14-116	1	0	28.4	juvenile	14-Jul-14	56.1
2014	MAF14-123	1	1	17.1	metalarvae	15-Jul-14	41.3
2014	MAF14-137	1	1	12.1	mesolarvae	17-Jul-14	12.5
2014	MAF14-141	1	1	16.5	mesolarvae	17-Jul-14	9.6
2014	MAF14-142	1	1	15.2	mesolarvae	17-Jul-14	8.1
2014	MAF14-143	3	1	12.8 -14.2	mesolarvae	17-Jul-14	6.6

2014	MAF14-145	1	1	15.2	mesolarvae	17-Jul-14	3.2
2015	JLK15-020	1	1	10.4	protolarvae	24-Apr-15	122.6
2015	JLK15-021	5	1	10.1 -12.5	proto-mesolarvae	24-Apr-15	119.8
2015	JLK15-024	8	1	11 -11.5	proto-mesolarvae	24-Apr-15	118.3
2015	JLK15-026	29	2	10.5 -11.9	protolarvae	24-Apr-15	116.7
2015	JLK15-027	1	1	10.8	protolarvae	24-Apr-15	113.4
2015	JLK15-032	2	1	11.3, 11.6	proto-mesolarvae	25-Apr-15	102.5
2015	JLK15-034	2	1	10.3, 11.5	proto-mesolarvae	25-Apr-15	98
2015	JLK15-037	42	3	10.6 -12.7	proto-mesolarvae	25-Apr-15	98
2015	JLK15-038	10	1	10.9 -11.8	proto-mesolarvae	25-Apr-15	89.6
2015	JLK15-039	7	1	11 -11.8	mesolarvae	26-Apr-15	89
2015	JLK15-041	21	2	9.6 -12.1	proto-mesolarvae	26-Apr-15	83.7
2015	JLK15-042	2	1	10, 11.6	proto-mesolarvae	26-Apr-15	81.4
2015	JLK15-043	5	1	10.4 -12.1	proto-mesolarvae	26-Apr-15	79.6
2015	JLK15-044	12	1	10 -11.6	proto-mesolarvae	26-Apr-15	77.3
2015	MAF15-001	1	1	11.9	protolarvae	19-Apr-15	75.7
2015	MAF15-003	2	1	10.9, 11.3	protolarvae	19-Apr-15	70.3
2015	MAF15-007	1	1	N/A	N/A	20-Apr-15	59
2015	MAF15-010	2	1	9.9, 10.3	protolarvae	20-Apr-15	56.1
2015	MAF15-011	3	1	10.2 -11.3	protolarvae	20-Apr-15	52.8
2015	MAF15-013	4	1	10.3 -11.1	proto-mesolarvae	20-Apr-15	50.3
2015	MAF15-014	1	1	10.9	protolarvae	21-Apr-15	48.8
2015	MAF15-018	6	1	10.6 -11.6	proto-mesolarvae	21-Apr-15	35.2
2015	MAF15-019	16	1	9.9 -11.7	proto-mesolarvae	21-Apr-15	32.1
2015	MAF15-020	1	1	11.4	protolarvae	21-Apr-15	28.2
2015	MAF15-021	2	1	11.1, 13	proto-mesolarvae	22-Apr-15	24.7
2015	MAF15-023	7	1	10 -11.6	proto-mesolarvae	22-Apr-15	18
2015	MAF15-027	50	3	9.9 -14.6	proto-mesolarvae	22-Apr-15	14.4
2015	MAF15-028	2	1	10.4, N/A	protolarvae	22-Apr-15	13.7
2015	MAF15-033	6	1	10.4 -11.6	proto-mesolarvae	23-Apr-15	7.4
2015	MAF15-034	22	2	10 -11.8	proto-mesolarvae	23-Apr-15	5
2015	MAF15-036	1	1	11.2	protolarvae	23-Apr-15	3.2
2015	JLK15-048	17	1	10.4 -15	mesolarvae	17-May-15	139.5
2015	JLK15-049	3	1	14.1 -15.9	protolarvae	18-May-15	135.5
2015	JLK15-051	5	1	12.8 -15.6	proto-mesolarvae	18-May-15	133.3
2015	JLK15-055	16	1	10.3 -15.7	mesolarvae	18-May-15	128.1
2015	JLK15-056	4	1	15 -18	mesolarvae	18-May-15	126.4
2015	JLK15-058	4	1	14.7 -17	mesolarvae	19-May-15	122.5
2015	JLK15-062	75	5	9.8 -17.9	mesolarvae	19-May-15	117.7
2015	JLK15-063	33	2	10.5 -15.5	mesolarvae	19-May-15	116.9
2015	JLK15-064	32	2	10.4 -16.4	mesolarvae	19-May-15	113.3
2015	JLK15-065	1	1	12.4	mesolarvae	19-May-15	107.6
2015	JLK15-066	1	1	12	mesolarvae	20-May-15	104.4

2015	JLK15-067	25	2	12.5 -16.8	mesolarvae	20-May-15	100.5
2015	JLK15-068	14	1	11.4 -17.1	mesolarvae	20-May-15	98.8
2015	JLK15-070	5	1	10.8 -15.7	mesolarvae	20-May-15	92.2
2015	JLK15-073	2	1	12.8, 14.7	mesolarvae	21-May-15	84.7
2015	JLK15-074	15	1	12.5 -17.8	mesolarvae	21-May-15	79.4
2015	JLK15-075	27	2	13.3 -17.3	mesolarvae	21-May-15	76.6
2015	MAF15-040	2	1	13 -15.9	mesolarvae	18-May-15	67
2015	MAF15-041	5	1	14 -16.6	mesolarvae	18-May-15	64.6
2015	MAF15-042	17	1	11.2 -20	mesolarvae	18-May-15	59.8
2015	MAF15-043	3	1	14.3 -16	mesolarvae	18-May-15	59
2015	MAF15-044	5	1	13.3 -17.9	mesolarvae	18-May-15	57.9
2015	MAF15-045	2	1	14.7 -16.5	mesolarvae	18-May-15	55.3
2015	MAF15-046	120	9	10.9 -19.6	proto-mesolarvae	18-May-15	52.4
2015	MAF15-050	116	8	11.1 -18.6	mesolarvae	19-May-15	41.2
2015	MAF15-052	2	1	15.6, 26	meso-metalarvae	19-May-15	33.6
2015	MAF15-055	14	1	15.6, 26	proto-mesolarvae	20-May-15	26.8
2015	MAF15-058	2	1	11, 15.8	mesolarvae	20-May-15	19.7
2015	MAF15-061	1	1	15	mesolarvae	20-May-15	17.7
2015	MAF15-062	187	14	10.9 -22.4	meso-metalarvae	20-May-15	13.9
2015	MAF15-064	24	2	11.4 -19.2	mesolarvae	21-May-15	10
2015	MAF15-065	28	2	11 -22.4	proto-mesolarvae	21-May-15	8.1
2015	MAF15-066	1	1	17.1	mesolarvae	21-May-15	7
2015	MAF15-067	2	1	17.4, 16.9	metalarvae	21-May-15	5.6
2015	MAF15-068	82	6	10.2 -20.5	proto-mesolarvae	21-May-15	4.2
2015	MAF15-069	3	1	10.5 -21	proto-mesolarvae	21-May-15	3.3
2015	MAF15-077	2	1	19, 21.8	metalarvae	16-Jun-15	124.8
2015	MAF15-093	1	1	15.3	mesolarvae	18-Jun-15	84.1
2015	JLK15-085	1	1	11.1	mesolarvae	15-Jun-15	52.4

Table A2. The proportion of larval Razorback Sucker sampled for effective number of breeder estimates ( $N_b$ ) from field collections. Results show the year, the number of larvae collected in the field, the number of individuals sampled for  $N_b$  estimates, and the proportion of those sampling efforts.

Year	Field Collections (N)	Genetic Sampling (N)	% sampled
2009	272	120	44.12
2010	1251	160	12.79
2011	1065	120	11.27
2012	1778	120	6.75
2013	979	120	12.26
2014	612	120	19.61
2015	1205	120	9.96
Total	7162	880	12.29

Figure A1. Rarefaction simulation of the 2009 Razorback Sucker dataset. Results show the mean and 95% confidence intervals for the averaged iterative runs ( $N=5$ ). Sample size is displayed on the x-axis and the effective number of breeders ( $N_b$ ) are displayed on the y-axis. The diagonal line represents a 1:1 relationship.

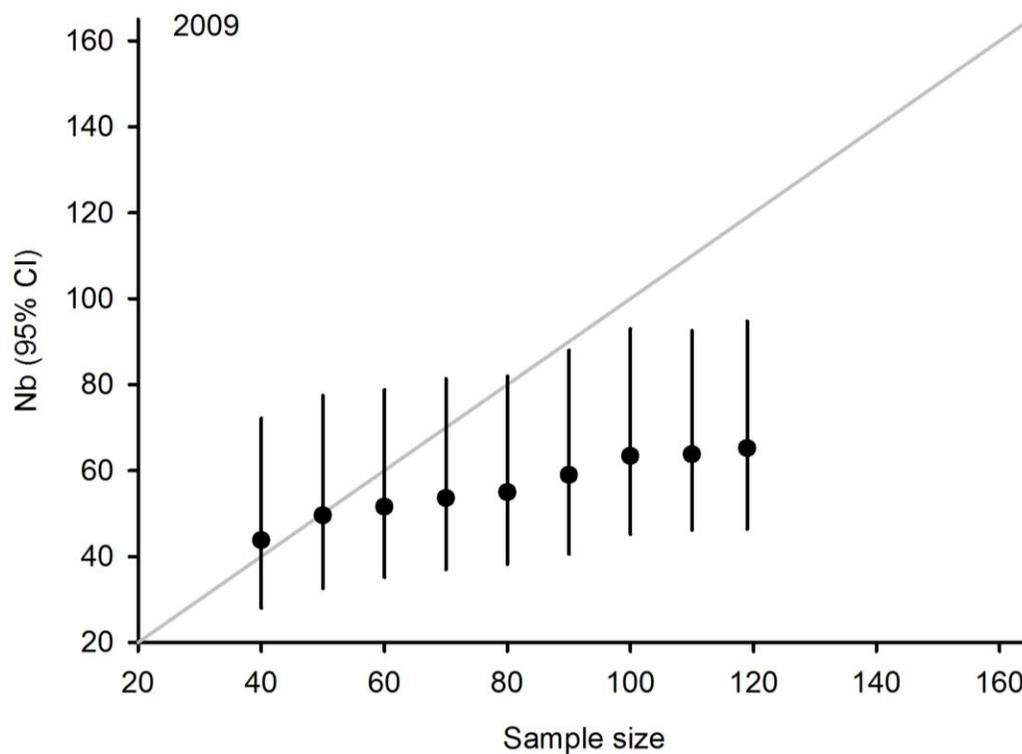
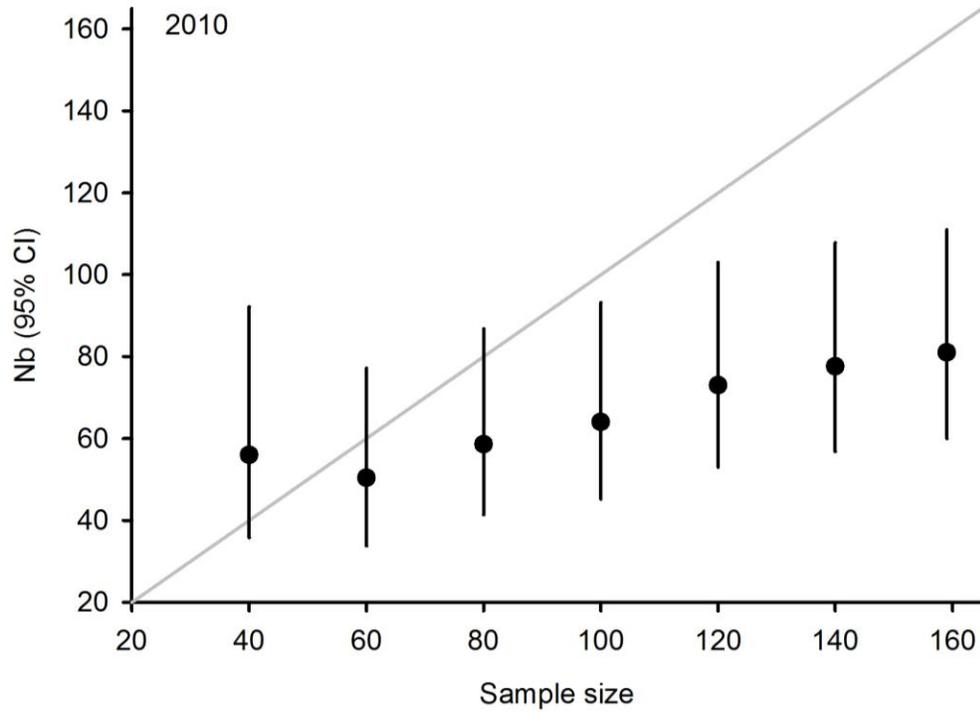


Figure A2. Rarefaction simulation of the 2010 Razorback Sucker dataset. Results show the mean and 95% confidence intervals for the averaged five iterative runs. Sample size is displayed on the x-axis and the effective number of breeders ( $N_b$ ) are displayed on the y-axis. The diagonal line represents a 1:1 relationship.



**Response to comments on:**

**Draft Report – Using Molecular Techniques to Determine the Effective Number of Breeders ( $N_b$ ) for Razorback Sucker and Colorado Pikeminnow in the San Juan River**

**Below we rebut specific comments (in regular font) with our responses (in bold italics).**

**Matt Zeigler’s comments:**

Line 222: “suitable mate?”

***Typo fixed.***

Line 253-254: “Are there some citations for this statement? I think it would be beneficial to have some.”

***The following citations were added here and in the literature cited: Palstra and Fraser 2012; Kanno et al. 2015; Bernos et al. 2017.***

Line 258: “scarce?”

***Typo fixed.***

Line 411: “traits?”

***Typo fixed.***

**Steve Ross’s comments:**

Ross comments on Using Molecular Techniques to Determine Effective Number of Breeders ( $N_b$ ) for Razorback Sucker and Colorado Pikeminnow in the San Juan River.

Principal Investigator: Tracy Diver and Wade Wilson, Southwestern Native Aquatic Resources and Recovery Center, Dexter, NM

Congratulations on a well-written report. The clarity and conciseness makes it a pleasure to read. The report provides useful data to the Program and is important for helping to guide recovery efforts.

One of the data shortcomings that your report emphasizes is that tighter confidence limits are sorely needed for population estimates before the Program can really put faith in the proportion of individuals breeding, although this is certainly not an issue under your control.

***We agree it is difficult to have precise estimates for proportion of breeding adults when our census estimates have low precision. Nonetheless, we also included new calculations for the proportion of adults spawning for both species considering this uncertainty (see table below).***

My main comment is that there is a tendency to over interpret the results when you calculate  $N_b/N_c$  ratios. This, as alluded to above, is due to the extreme variation in estimates of  $N_c$ , especially for Colorado Pikeminnow. Confidence intervals for  $N_b$  tend to be tighter, but remember when you calculate  $N_b/N_c$  ratios, both of those measures have inherent variation around the means and the probabilities are additive. For example, in the table below I show the 95% confidence limits for  $N_b$  and  $N_c$  inferred from Figures 2 & 3 for Colorado Pikeminnow. I interpret this as showing that the true value of  $N_b/N_c$  has a 90% chance of being between 5.3 -157.4. I have revisited this general issue in some of the following specific comments.

Nb	35	74
Nc	47	660
	Nb,low/Nc low	74.5%
	Nb, low/Nc, high	5.3%
	Nb, high/Nc, low	157.4%
	Nb, high/Nc, high	11.2%

***We have updated our tables to address this concern; however, updated results for Razorback Sucker include a consistent 95% confidence interval range of ~1-4%, and thus do not change our overall interpretation of the results. We do, however, agree that the extreme variation in  $N_c$  estimates for Colorado Pikeminnow make interpretations around that variation more difficult.***

Lines 55-56. Clarify by adding “but recruitment to the adult stage is extremely limited.”

***Changed as suggested.***

Line 61. Wouldn't reproductive success be measured by the number of offspring surviving through the larval, juvenile, subadult, and adult stages rather than by the number of reproducing adults?" Perhaps spawning success would be a better term here.

***We changed “reproductive success” to “spawning success.”***

Lines 66-67. Marsh et al. (2015) suggests that batch spawning occurs (defined as multiple spawnings by a single female over the reproductive season), but doesn't really go into any details. Considering the three-endpoint model of life-history evolution, catostomids are considered to have a Periodic life history characterized by larger body size, longer life spans, larger clutch sizes, longer generation times, and lower investment in offspring. Fishes that are Periodic are generally larger-bodied and have incorporated long-distance migrations in their life history. (I summarize this on pages 178-182 in my fish ecology book). In working on this chapter (prior to 2013), I didn't find really well documented examples of batch spawning

in catostomids, whereas it is common in cyprinids. It could be a case of some females spawning early and others later in the season, rather than the same individual spawning twice.

*At present, very little information pertaining to the spawning ecology (e.g., synchronous vs asynchronous) of Razorback Sucker exists. Most studies report spawning site fidelity, habitat preference (e.g., gravel size), and spawning cues (e.g., flow criteria, temperature). Based on the description provided by Marsh et al. (2015), batch spawning was interpreted as a strategy used in Lake Mohave populations. Further evidence provided by Modde and Irving (1998) showed individuals move between spawning sites within the same spawning season. Interpretation of these behaviors as “batch spawning” is premature. Oocyte recruitment and fecundity (i.e., determinant or indeterminate) type in fishes is most accurately determined by histological analysis or oocyte size frequency distributions, and such analyses are potentially warrant for further understanding the spawning strategy of Razorback Sucker. Regardless, we have removed the implication of “batch spawning” from the report; however, we want to retain the observation by Marsh et al 2015 and include Modde and Irving 1998 observations to illustrate the variation and complexity of spawning in Razorback Sucker.*

Line 84, Methods. It would be helpful to show the actual collection sites of the larvae used in the genetic analyses. Also, for subsequent studies, I would really be interested in seeing if larval Razorback Suckers collected from the San Juan arm of Lake Powell had  $N_b$  values different from those in the river above the waterfall. The same is true for Colorado Pikeminnows if sufficient larvae could be obtained.

*Genetic subsampling of larval collections have now been included in an Appendix of the report.*

Lines 168-169. For example, adult contribution of offspring was relatively even in 2016 with estimates of  $N_b$  and number of spawning adults were relatively similar.

*The sentence was revised to “For example, adult contribution of offspring was relatively even in 2016 with similar estimates of  $N_b$  and number of spawning adults.”*

Lines 152-153. The sentence “Mean adult census estimates varied among years with as few as 19 individuals estimated in 2012 to as many as 133 estimated in 2016 (Figure 3)” is misleading given that the confidence intervals of  $N_c$  overlapped for all years analyzed. About all you can really say is that  $N_c$  values did not differ among the years sampled. Using such highly variable numbers of  $N_c$  to determine the proportion of breeders in Table 1 is not very meaningful as I have discussed above.

*We disagree. This sentence gave the mean estimates and does not state anything about significant differences among years. While it is true there may not be significant differences among years, that doesn’t discount the fact that the predicted mean estimates varied by almost an order of magnitude.*

Line 158-159. Figure 4 for 2014 and 2016 is really encouraging!

*We agree.*

Line 178-179. Rewrite as: In terms of distribution of larvae, in years with ~~more increased numbers of~~ larvae, ~~they generally were more~~ evenly distributed throughout the system.

*Changed as suggested.*

Lines 184-185. Rewrite as: ~~Overall, Mean  $N_b$  estimates were low across years with an average of 82 and a range of 65-109. These results were similar between years (Figure 6).~~ **Overall, Mean  $N_b$  estimates did not differ significantly across years, were low**

***Changed as suggested.***

Lines 186-188. Rewrite as: Adult census estimates were not available for the first two years ~~where~~ **where** we had  $N_b$  estimates. ~~Overall, Adult census sizes were relatively high with a general (range of = 4,000 to 5,000) adults estimated in the system (Figure 7).~~ **Overall, Adult census sizes did not differ among years and means were relatively high**

***Changed as suggested.***

Lines 188-189. In the sentence “Conversely, the estimated proportion of spawning adults was low with a relatively consistent ratio of approximately 2% (Table 2)” it would be more meaningful to give the upper and lower ranges based on the confidence intervals for  $N_c$ . As written, the wide range of  $N_c$  distorts the interpretation of the ratio, although not as severely as shown in Colorado Pikeminnows.

***Changed as suggested.***

Lines 211-213 & 215-218. These statements need to consider the variation involved in the two estimates ( $N_c$  &  $N_b$ ). You do treat the issue of variation later in the paragraph in looking at other studies of  $N_b$  and  $N_c$ ; however, I would like to see a little more consideration of the high variation (especially  $N_c$ ) in your discussion.

***Changed as suggested.***

Lines 268-271. This is an important statement and one that I hope you can address in future studies.

***Thank you. If funded for FY19, we hope we will be able to address this statement further.***

**Wayne Hubert’s comments:**

I want to congratulate the PIs on a well-written draft report. It is very nice to read a report that is well organized and without grammatical errors.

However, my skepticism regarding the findings was raised when I read the succinct conclusions within the Executive Summary (Lines 31-33): “Razorback Sucker estimates were consistently low across years with approximately 2% of the adult population contributing annually. This might suggest the bottleneck to recruitment could be occurring at the spawning or reproductive level.” Given the description of the sampling methods and the variances associated estimates of  $N_b$  and  $N_c$ , I am not convinced that such a firm conclusion can be made regarding  $N_b/N_c$  estimates.

***This is a valid point. We recalculated the proportion of adults contributing to the larval cohorts for considering those uncertainties in  $N_c$  and  $N_b$ . However, these results also indicate a consistently low contribution of adults (~1-4%). Thus, we did not change our overall interpretation of the results.***

First, I focus on the Methods and description of the larval fish samples used in the analyses (lines 89-97). It is stated: “Approximately 1,200 samples representing the spatial and temporal distribution of larval sampling efforts were used to obtain  $N_b$  estimates (Figure 1). In order to ensure  $N_b$  estimates were not

artificially lowered due to limited spatial representation of samples, rare collections were targeted while sites with high larval densities were proportionally reflected in samples. Larval Razorback Sucker were collected during much of the sampling season. Early larval stages (e.g., protolarvae to mesolarvae) were targeted throughout larval collections under the assumption that these individuals were from recent spawning events; thus, ensuring sampling was representative of the temporal spawning season. Conversely, Colorado Pikeminnow were only collected later in the sampling season, which made it relatively easy to have those captured individuals reflect the entire seasonal spawning period of adults.” The sample sizes for 2009-2016 for both species are illustrated in Figure 1 (line 285). I am highly skeptical that the annual target sample size of 120 larvae can come close to representing the “spatial and temporal distribution of larvae” of either species over the length of the San Juan River and the duration of the spawning periods. I fear that the samples that were analyzed represent very small proportions of both the spatial and temporal distributions of larvae of each species and, thus, the parental sources of the larvae in the samples are limited and estimates of  $N_b$  are likely biased low. The researchers indicate that they may also have skepticism about their estimates of  $N_b$  in the Discussion (Lines 255-258): “Nonetheless,  $N_b/N_c$  is critically dependent on the accuracy of estimates for both  $N_b$  and  $N_c$ ” and “The accuracy of  $N_b$  estimates can be hindered when genetic information is scarce or the sample size is small compared to the real (unknown)  $N_b$  of the population.” Substantial work is needed to assure that an annual sample size of 120 larvae adequately represents both the spatial and temporal distribution of larvae of a species. However, assuming that is unlikely, effort is needed to identify how truly representative larval samples (i.e., spatial and temporal distributions) of each species can be obtained in the future.”

***We agree our  $N_b$  estimates could be biased if the spatial and temporal scales of reproduction were not sampled; however, we worked under the assumption the larval field samples adequately represented the spatial and temporal scale of endangered larval fish in the river. While we agree potential sampling issues should be considered when interpreting results of these types of analyses, there is little evidence to suggest our estimates are biased low due to poor sample sizes and the published peer review literature also supports our level of sampling.***

***Nonetheless, in an effort to investigate potential sample size bias, we conducted a few rarefaction analyses for Razorback Sucker by resampling annual samples and calculating  $N_b$  estimates at different sample sizes (see Appendix). Overall, the results suggest there is no appreciable increase in  $N_b$  estimates with increased sample sizes (e.g., no significant differences in  $N_b$  when sample sizes are doubled). This should not be too surprising given the large spatial distances between predicted full and half siblings. Overall, the results indicate increasing the number of samples won't increase  $N_b$  estimates because new samples are likely full or half sibs to already sampled individuals.***

***Moreover, we have also included the following peer reviewed literature that supports the adequacy of our sample sizes relative to our  $N_b$  estimates: England et al. 2006; Wang 2016; Sánchez-Montes et al. 2017; Bacles et al. 2018.***

“Second, I am concerned about the failure of the researchers to consider the proliferation of error in their estimates of  $N_b/N_c$ . The figures indicate large 95% confidence intervals for estimates of both  $N_b$  (Figures 2 and 6) and  $N_c$  (Figures 3 and 7). The limited precision of both the  $N_b$  and  $N_c$  estimates is not indicated in Tables 1 and 2 and in the computation of  $N_b/N_c$ . If appropriate computations were made to compute the 95% confidence intervals around the  $N_b/N_c$  estimates, the limited precision of these estimates

would be shown. It is strongly recommended that 95% confidence intervals be computed and that discussion about the precision of the estimates be included in the report.”

*This is a good point (also suggested by Steve Ross). We have updated our tables to include calculations using both means and their 95% confidence intervals.*