Relationship of genetic diversity metrics to density in two Canadian River fishes

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Introduction

Dewatering, fragmentation and associated habitat change of rivers in the Great Plains of North America have substantially altered stream fish communities over the past 50 years (Gido et al. 2010; Hoagstrom et al. 2011). Recently, Perkin et al. (2015a, b) used broad scale analysis to demonstrate that these factors act synergistically to cause declines, particularly in pelagic spawning species. Two members of this guild are the focus of this study, Arkansas River shiner (*Notropis girardi*) and plains minnow (*Hybognathus placitus*). The Arkansas River shiner is native to the Arkansas River drainage in New Mexico (NM), Arkansas, Texas, Oklahoma, and Kansas (KS; Gilbert 1978). Due to changes in timing and duration of flow, channel drying, creation of impoundments, and introduction of non-native species, Arkansas River shiner has now disappeared from about 80% of its former range and is listed as threatened under the Endangered Species Act (US Department of the Interior 1998). Perkin et al. (2014b) also showed that drought accounted for the final demise of populations of pelagic spawning species including Arkansas River shiner, plains minnow, and peppered chub in the Arkansas and Ninnescah Rivers. Specifically, the last reported record of Arkansas River shiner in the Arkansas River (KS) was in 1983 whilst plains minnow persisted there until a moderate drought in 2006 (Perkin et al. 2014). The Canadian River population of Arkansas River shiner is the stronghold for this species and this river also likely hosts a key population of peppered chub. In contrast, plains minnow is widely distributed across the Great Plains (Hubbs et al. 1991) and populations occupying southern drainages are currently considered stable (Warren et al. 2000). Although plains minnow is somewhat more tolerant to drought, it has also experienced local extirpations (Perkin et al. 2015). For this reason, it is imperative to track demographic and genetic status of the Canadian River population of these species in the face of extensive drought conditions that have impacted vast portions of the Great Plains over the past four years. In addition to the native populations in the Canadian River, both plains minnow and Arkansas River shiner have been introduced inadvertently to the Pecos River (NM) where their populations are stable (Hoagstrom et al. 2010).

Genetic monitoring programs track genetic diversity parameters, including genetic effective population size (N_e), over contemporary timescales and can be used to assess the conservation status of imperiled species (Schwartz et al. 2007). Information regarding a population's census size (N_c) or abundance is also important for management as it is acknowledged that small populations will exhibit lower levels of genetic diversity than large populations because of increased genetic drift and/or inbreeding (Hartl & Clark 1997). Typically, a population's effective size is lower than its census size, but the relationship between these metrics differs substantially between species (Frankham 1995). Monitoring changes in N_e over time has been suggested as an alternative to traditional population monitoring (Schwartz et al. 2007; Long et al. 2008). However, many factors affect the relationship between N_e and N_c, so species'

abundance may be correlated with effective size in some cases but not in others. For example, we have shown that in some species (Pecos bluntnose shiner, *Notropis simus pecosensis*) N_e and abundance (catch-per-unit effort) are positively correlated whilst in others (Rio Grande silvery minnow, *Hybognathus amarus*), there is a disconnect between these metrics (Osborne et al. 2010; Osborne et al. 2012). The relationship between N_e and N_c likely depends on species biology, demographics, landscape features, and management protocols. Understanding how these metrics are related in difference species may be informative.

Contemporary N_e was estimated using the single sample linkage disequilibrium method based on six microsatellite loci (Osborne et al. 2013), but a negative estimate was obtained suggesting a large population. In cases where negative estimates are obtained Waples and Do (2010) suggest that the lower confidence interval can provide a plausible estimate of the effective size. In the case of Arkansas River shiner this was 1343. Genetic diversity and genetic effective size have not been obtained to date for Plains Minnow in the Canadian River (NM). However, estimates of N_e have been obtained for this species using the single sample linkage disequilibrium method from the Canadian River in Texas, and from the Platte, Arkansas, and Red Rivers. In all of these other river basins, with the exception of the Platte, N_e is large (Osborne et al. 2014). Precision of estimates can be improved by increasing the number of genetic loci, thereby increasing the total alleles available for N_e estimation (Luikart & Cornuet 1999), and by increasing the number of generations between sampling events (Waples 1989). In this project, we increased the number of microsatellite loci screened and the number of temporal samples for Arkansas River shiner. The accuracy of our estimates of N_e were improved by these measures. This approach also allowed us to estimate variance of N_e using the shift in allele frequencies between generations. This variable has been shown to be a sensitive indicator of population size changes.

The purpose of this project was to generate a genetic monitoring time series for Arkansas River shiner and to establish baseline genetic information for plains minnow. Genetic data on the Arkansas River shiner allowed temporal trends in genetic diversity and N_e to be determined and permitted an assessment of whether recent drought conditions have depressed genetic effective size to be performed. In addition, genetic effective size estimates were paired with density estimates (catch-per-unit-effort [CPUE]) provided by New Mexico Department of Game and Fish (NMDGF) and U.S. Fish and Wildlife Service (USFWS) to assess the relationship between these different metrics of population status for the Arkansas River shiner. For plains minnow, baseline genetic diversity metrics were established for the Canadian River (NM) population and this data was compared to diversity (estimated from microsatellite data) metrics in other populations across the Great Plains (Osborne et al. 2014).

Methods

Sampling

The USFWS and NMDGF personnel collected fish tissue samples from seven sites by seining from the Canadian River, and its tributary Revuelto Creek, in New Mexico (NM) between Ute Reservoir and the NM-TX border. Caudal fin clips were taken from captured fish and stored in 95% ethanol. We deposited these samples at UNM's Museum of Southwestern Biology. Samples for Arkansas river shiner were collected in 2012, 2014, 2015. In addition to the samples collected for this study, we also genotyped archived Arkansas River shiner samples from the Canadian River collected in 2009 (Osborne et al. 2012) for the three microsatellite loci added in this study. Plains minnow samples were collected from the Canadian River in 2014 and 2015 and we included additional samples collected by J. Perkin in 2013 as part of another study.

Molecular methods

Genomic DNA was extracted from air-dried fin clips using standard proteinase-K digestion and standard phenol/chloroform methods (Hillis et al. 1996).

Microsatellites

Plains minnow and Arkansas River shiner were assayed for variation at nine variable microsatellites loci. Microsatellites were amplified as 10 μL reactions, containing 1 μL diluted DNA, 1X Colorless GoTaq® Flexi Buffer, 2 mM $MgCl_2$ solution, 125 μM dinucleotide triphosphates (dNTPs), 0.4 μM of both forward and reverse primers, and 0.375 units of GoTaq® DNA Polymerase. For Arkansas River shiner, Polymerase Chain Reactions (PCRs) were initially denatured at 90°C for 2 min, followed by 30 cycles of denaturing at 90°C for 30 s; annealing at 58°C (*Nme232* [Gold *et al*. 2004], *Ppro126*, *Ppro132* [Bessert and Orti 2003]), 54°C (*Ca12*), 60°C (*Nme208*) [Gold et al. 2004], or 49°C (*Ca6* [Dimsoski et al. 2000], *Lco3*, *Lco6* [Turner et al. 2004]) for 30 s; extension at 72°C for 45 s; and ending with a final extension at 72°C for 30 min. For plains minnow, PCR conditions were the same except for annealing temperatures of 58°C (*Ppro126, Ppro132*, *Nme93* [Gold et al. 2004], *Nme232*), 56°C (*Ca12*), or 49°C (*Lco3, Lco6*, *Lco7* [Turner et al. 2004]). For each sample, one microliter of PCR product was mixed with 10 μl of formamide and 0.4 μl of HD400 size standard and then denatured at 90°C for five minutes. All samples were run on an automated ABI 3130 DNA sequencer and analyzed with Genemapper software (ABI).

Mitochondrial DNA (mtDNA)

Samples were screened for variation at a ~300 base pair fragment of the mitochondrial ND4 gene using single-stranded conformational polymorphism (SSCP) analysis as described in Osborne et al. (2012). Unique haplotypes were sequenced in the forward and reverse direction

to verify haplotypes. Due to the large number of haplotypes identified in Arkansas River shiner using SSCPs in the 2012 and 2014 samples, all 2015 samples were assayed for variation by direct sequencing rather than SSCP analysis. Similarly, for plains minnow, all 2013 and 2015 samples were sequenced.

Data Analyses

Genetic variability- Microsatellites

GENEPOP Version 3.1 (Raymond and Rousset 1995) was used to conduct modified exact tests to determine whether the observed genotype frequencies conformed to Hardy-Weinberg expectations in each temporal sample (analyzed separately). This program was also used to conduct the global test for linkage disequilibrium among loci. Sequential Bonferroni correction (Rice 1989) was applied to account for multiple comparisons. Since all measures of diversity, the number of alleles, gene diversity, and heterozygosity, are dependent on sample size, we used a resampling procedure to calculate the diversity measures. Briefly, for each species 1000 random subsamples were drawn without replacement from each temporal sample. Diversity measures and 95% Confidence Intervals (CIs) were calculated for each locus and temporal sample and a mean was obtained across loci for each statistic (corrected number of alleles [N_{ac}] reflects allelic diversity, gene diversity [H_{ec}], heterozygosity [H_{oc}]). This analysis was conducted in the R statistical package (www.r-project.org; R script available on request). Average inbreeding co-efficients (F_{IS}) were calculated across loci.

Spatial structure

To determine whether significant spatial structure existed between Canadian River and tributary sampling sites, Weir and Cockerham's (1984) F-statistics (based on microsatellite data) were calculated in ARLEQUIN Vers. 3.11 (Excoffier et al. 2005). Significance was assessed by bootstrapping (1000 replicates). This analysis was conducted for both species.

Genetic variability - mtDNA

Haplotype diversity (h) and nucleotide diversity (π) were obtained using ARLEQUIN Version 3.11 (Excoffier et al. 2005). Haplotype richness (H_R; Petit et al. 1998) was obtained using the program Contrib Vers. 1.02 (available at http://www.pierroton.inra.fr/genetics/labo/Software/Contrib/) which uses a rarefaction approach to correct for unequal sample sizes.

Female genetic effective size

Female genetic effective size (N_{ef}; estimated from mtDNA haplotype frequencies) was also calculated using the moments and pseudo maximum likelihood methods (Wang 2001) as implemented in the program MLNE (Wang 2001; 2003).

Contemporary Genetic Effective Size

The single sample linkage disequilibrium method (Hill 1981) was used to estimate the effective number of breeders (N_{eD}) from microsatellite DNA data for Arkansas River shiner and plains minnow using the program NeEstimator Vers. 2.0 (Do et al. 2014). This program implements a correction factor to account for bias that may occur when the sample size is less than the real (unknown) effective size (England et al. 2006). Highly polymorphic loci with many rare alleles, typical of microsatellites, can cause biased estimates of variance genetic effective size (N_{eV}), and N_{eD} (Waples 2006; Hedrick 1999; Turner et al. 2001). NeEstimator calculates estimates after excluding all alleles with frequencies of less than a specified critical value. Here we used P_{crit}= 0.02 as suggested where the the number of individuals sampled is greater than 25 (Waples and Do 2010). This value of P_{crit} generally provides a good balance between precision and bias (Waples and Do 2010). Upper- and lower-bound 95% confidence intervals for N_{eD} and N_{eV} were calculated using the jackknife approach as implemented in NeEstimator. In general, effective size estimators (N_{eV} and N_{eD}) perform the best when effective size is small (and the effects of genetic drift are strong) but when effective size is large, estimators may not be able to distinguish a large from a very large population.

Variance genetic effective size (N_{eV}) and 95% CIs were estimated from temporal changes in microsatellite allele frequencies using the three temporal method estimators (Nei and Tajima 1981; Pollack 1983; Jorde and Ryman 2007) implemented in NeEstimator Vers. 2.0 (Do et al. 2014). Arkansas River shiner and plains minnow were sampled under Plan I (prior to reproduction, with replacement) for all methods; therefore, calculations of N_{eV} via TempoF_s required an estimate of census size (N_c). For both species a generation time of one year was used and N_c was set to 5000. For plains minnow, only a single temporal estimate was obtained between the 2014 and 2015 samples. The 2013 sample was not used for estimation of N_{eV} because the collection locality was different (lower in the Canadian River in Texas) so differences in allele frequencies may be caused by slight differences between localities rather than genetic drift between sampling periods.

Relationship between genetic effective size and CPUE

We used fish collections to estimate abundance of Arkansas River shiner and plains minnow (as measured by catch-per unit-effort [CPUE]). Catch-per-unit-effort is the total number of fish caught divided by amount of effort (measured as the total area covered by a seine net) and is well accepted in the fisheries science as a valid means of estimating abundance (Richards & Schnute 1986). This data was considered along with discharge data (in cubic feet per second) from the USGS gage on Revuelto Creek (Station Number 07227100) (a tributary of the

Canadian). This gage provides a more representative picture of the flows into the Canadian downstream of Ute reservoir as the gaging station on the mainstem of the Canadian is downstream in Texas. As the number of comparisons was small, we visually compared the CPUE and N_{eV} but did not conduct a statistical test of this relationship at this time. For Arkansas River shiner two of the four estimates were infinity for N_{eD} and the other two estimates were very large so aren't included on the figure (Figure 1c).

Results

Genetic variability- Microsatellites

Arkansas River shiner were collected from two to five sites in 2009, 2012, 2014, and 2015 by USFWS and NMDGF personnel. These sites represented the mainstem Canadian River and its tributary, Revuelto Creek. In Arkansas River shiner, departures from Hardy-Weinberg expectations (HWE) were seen in 10 instances from 36 comparisons. These involved four loci including *Ca8* (all temporal samples), *Nme208* (2009, 2014, 2015), *Lco3* (2012) and *Lco6* (2009, 2012). Plains minnow were collected from four sites in 2014 and three sites in 2015. We also included 38 samples collected in 2013 as part of another study (Osborne et al. 2014). In plains minnow, departures from HWE were detected in 12 of 27 total comparisons after Bonferroni correction for multiple comparisons. *Nme232* and *Lco7* departed in all temporal samples, *Ppro126* and *Ca12* departed in two temporal samples, and *Nme93* and *Ppro132* departed in a single temporal sample. Departures from HWE can occur for a variety of reasons which include the presence of null alleles, large allele dropout, and deviations from the assumption of an ideal population. Departures from HWE do not typically affect estimates of diversity or effective size. There was no evidence of linkage disequilibrium among microsatellite loci in either Arkansas River shiner or plains minnow. In Arkansas River shiner, allelic diversity increased over the sampling period whilst observed heterozygosity declined. There was a less clear trend for gene diversity, with higher values in 2012 and 2014 and lower values in 2009 and 2015. In plains minnow, allelic and gene diversity were lower in the 2013 sample when compared to that collected in 2015, however it should be noted that the sampling locality was further downstream in the Canadian River. Observed heterozygosity ranged from 0.56 to 0.58 (Table 1).

Spatial Structure

Pairwise F-statistics (F_{ST}) were small for all comparisons within years and not statistically different from zero in both Arkansas River shiner and plains minnow, indicating no significant genetic divergence between collection localities for either species.

Genetic variability- mtDNA

In Arkansas River shiner, 57 mitochondrial haplotypes were identified from 383 individuals. Haplotype richness varied from 20.61 (2012) to 29.51 (2015) (Table 1). Haplotype diversity was

lowest in 2012 (0.731) and highest in 2015 (0.844). Similarly, high mitochondrial diversity was observed in plains minnow with 53 haplotypes detected from 208 individuals. Haplotype richness varied from 10.98 (2014) to 17 (2013). Haplotype diversity ranged from 0.817 (2014) to 0.940 (2013). In both species, two haplotypes were moderately common and the others were rare. In both species nucleotide diversity (the average number of nucleotide difference per site between two randomly chosen sequences) was low indicating that haplotypes were closely related to each other.

Female genetic effective size

Female genetic effective size estimated using the moments method was infinity for all temporal comparisons for Arkansas River shiner. Maximum likelihood N_e estimates for this species were small (N_{ef}=70.9) for the 2009-2012 comparison but large for the remaining comparisons. For plains minnow, female effective size was 51.2 (2014-2015) when estimated using the moments method. Using the maximum likelihood method, N_{ef} was large (~10,000) for the 2014-2015 estimate (Table 2).

Contemporary Genetic Effective Size

Linkage disequilibrium effective size estimates of number of breeders were large and in some cases negative for both species (Table 1). Negative estimates of N_{eD} indicate that genetic results can be explained entirely by sampling error without invoking genetic drift and in such cases we cannot reject the possibility that the population is very large (Laurie-Ahlberg and Weir 1979; Nei and Tajima 1981). However, the finite lower bounds, according to Waples and Do (2010), can provide plausible limits of the effective size of populations. For Arkansas River shiner, estimates of effective size (N_{eD}) declined from infinity in 2009 and 2012 (lower CI 275 and 726 respectively) to finite estimates of 461,236 (lower 95% CI 409) in 2014 and 3443 (lower 95% CI 430) in 2015. Estimates of N_{eD} obtained for plains minnow was large but finite (N_{eD}= 25,851) for the 2013 collection from the Canadian in Texas and declined from infinite (lower CI 95% 654) to 1899 for the 2015 collection (lower 95% CI 301).

Temporal effective size estimates were consistent across the three different methods used to calculate them (Table 3). For Arkansas River shiner, N_{eV} was less than 500 in all cases and declined between the first and last temporal comparison; consistent with N_{eD} estimates. For the 2014-2015 comparison, N_{eV} was very small (N_{eV}<50). For plains minnow, N_{eV} was indistinguishable from a very large population (2014-2015).

Relationship between genetic effective size and CPUE

For Arkansas River shiner, CPUE estimates were highly variable over the study period from a low of 0.092 fish per m^2 in March 2013 to a high of 2.4 fish per m^2 in November 2014. From

November 2014, CPUE values declined in both 2015 collections (spring and summer; Figure 1b). Catch per unit effort for plains minnow was lowest in fall 2012 (0.07 fish per m^2) and highest in the previous sampling period (1.16 fish per m^2; Figure 1a). Discharge was highly variable between years, with no summer high flow events in 2012 and substantial increases in flow between May and October in 2013 and 2015. In 2014 peak flows occurred earlier (April) and were less protracted (Figure 1c). Genetic effective sizes were consistent with CPUE estimates of abundance in Arkansas River shiner. Specifically, the year with the highest CPUE corresponded to the year of the maximum N_{eV} estimate and the decline seen in abundance between 2014 and 2015 matched the decline in effective size for this temporal comparison. Additional years of data will be required to determine if this trend continues. The N_{eD} effective size estimates show a similar trend of declining N_e over the study period, but effective size estimated using this method was still large (i.e., in the thousands for the lower estimate). Effective size estimates obtained using the temporal (N_{eV}) method and the linkage disequilibrium (N_{eD}) methods track different facets of genetic change and do not estimate effective size in exactly the same generation (Waples 2005). Specifically, single sample N_e methods (such as those provided by linkage disequilibrium; N_{eD}) yield an estimate of the effective number of parents that produced the progeny from which the sample is drawn, and most closely approximates inbreeding effective size (Laurie-Ahlberg and Weir 1979; Waples 2005). Hence there is a lag between estimates obtained using the variance effective size and the linkage disequilibrium effective size. It will be interesting to measure N_{eD} in 2016 to determine if it declines following the trend in N_{eV}.

Discussion

Often, genetic monitoring programs are not initiated until populations are already much depleted, thereby limiting the inferences that can be made from the data. Moreover, many population genetic studies are limited to a point estimate of genetic diversity and genetic effective population size. Such studies do not allow trends in these metrics to be assessed, which can be critical when determining the conservation status of a species. The data collected in this study therefore represents important baseline data for Arkansas River shiner and plains minnow. Our genetic monitoring data for Arkansas River shiner spans a period of extreme (D3) to exceptional (D4) drought conditions (as defined by the the US drought monitor classification scheme; http://droughtmonitor.unl.edu/aboutus/classificationscheme.aspx) that occurred in Northeastern NM between 2011 and 2014. For plains minnow, genetic samples were collected for a period when drought conditions were considered severe (D2). Consequently, abundances of both species varied by an order of magnitude over the monitoring period (2012-2015). Drought conditions impact riverine fishes by reducing available habitat, increasing competition between species, and fragmenting available habitat (i.e., through creation of dry river segments). Additionally, absence of significant rainfall and associated runoff may reduce spawning and recruitment resulting in reduced abundance. Periods of low abundance or reduced numbers of

breeders may be reflected in estimates of genetic effective size and may eventually affect levels of genetic diversity, which may be lost at a rate inversely related to genetic effective size. Specifically, when populations are small, diversity is lost at a faster rate through the random process of genetic drift. However, we have shown previously that, in some cases, species may avoid losses of genetic diversity through a period of dramatic population decline, likely through the use of wetted refugia during dry periods (Osborne et al. 2010).

In Arkansas River shiner, variance effective size estimates were small (100-400) and declining (less than 50) in the most recent time period (2014-2015) mirroring the trend implied by the CPUE data for this time period. Gene diversity and heterozygosity declined between 2014 and 2015 and, interestingly, allelic diversity, haplotype diversity, and haplotype richness increased over this time frame. There may be slight allele frequency differences between sites due to genetic drift that is expected to occur when these populations are disconnected during periods of channel drying that occur during periods of low to zero flow. Hence, one explanation for this observation (increased allelic and haplotype richness) is possible movement of individuals from downstream (Texas portion of the Canadian) where we did not sample, to our sampling localities in New Mexico. Such long range, upstream movements have been recorded for this species (S. Davenport USFWS pers. comm), specifically the collection of a marked Arkansas River shiner in New Mexico portion of the Canadian River. This fish had been tagged and released in Texas. Maintaining connections between localities on the Canadian may help to buffer populations against genetic effects of periods of low density if there are wetted refugial areas that allow for persistence during periods of drying.

We only had two temporal collections (2014 and 2015) for plains minnow, so only a single temporal estimate of genetic effective size was possible. This estimate indicated a large population size. Linkage disequilibrium effective size showed a declining trend from 2014 to 2015 and abundance estimates also declined between the two most recent collections (spring 2015 to summer 2015). It will be important to continue monitoring trends for this species to determine the trend of effective size estimates.

Our genetic monitoring data spans a period of extreme to exceptional drought conditions that occurred in Northeastern NM between 2011 and 2014. Declines in diversity may be a reflection of a smaller breeding populations for Arkansas River shiner in these years. This is consistent with a small variance genetic effective size (N_{eV}) for the 2014-2015 comparison for Arkansas River shiner. Small variance effective size indicates that there was a large shift in allele frequencies from 2014 and 2015 due to genetic drift. The random effects of genetic drift are strongest when the population size is small, which is consistent with the decline seen in CPUE in 2015 compared to 2014. Wetter conditions returned in 2015, so continued genetic monitoring will be important

to determine whether abundances rebound along with effective size. Both Arkansas River shiner and plains minnow are short-lived, so events like drought can impact populations on short time scales. Durham and Wilde (2006) found that the magnitude of stream flow was less important than the presence of water in the stream channel, hence periods of prolonged drought are likely to negatively affect the study species. Inbreeding coefficients were low, thus inbreeding does not currently appear to be occurring at high levels.

Standing mitochondrial diversity was high for both species indicating large historical population sizes for both plains minnow and Arkansas River shiner. Historically, both of these species had broad distributions, and populations of plains minnow persist in four river basins across the Great Plains (Red, Arkansas, Platte, and Canadian). Gene diversity in the Canadian River was higher than that observed in most populations of plains minnow in the Arkansas basin, but lower than populations in the Platte and Red River basins. Heterozygosity was in the range recorded for populations in other basins. Allelic diversity was similar to that recorded in the Arkansas River basin, higher than that seen in the Platte and slightly lower than than in the Red River basin (Osborne et al. 2014). Mitochondrial haplotype diversity in Arkansas River shiner declined between 2009 (Osborne et al. 2013) and the next temporal collection (2012) but then rebounded in 2014 and 2015. In this previous survey of diversity in the Canadian River population in New Mexico and Oklahoma, 38 mitochondrial haplotypes were identified from 190 individuals whilst in the three temporal collections (2012, 2014 and 2015) analyzed here, 57 haplotypes were detected. Haplotype A was the most common in the previous study as well as here, with most haplotypes found at low frequencies.

Conclusions

The results presented here indicate that the Canadian River in New Mexico harbors genetically diverse populations of both plains minnow and Arkansas River shiner. Abundance of Arkansas River shiner has declined in the most recent sampling periods and analysis of the genetic data suggest that the genetic effective size of the population has also declined (which may lead to a loss of genetic diversity over time). The Canadian River is a stronghold for Arkansas River shiner and a recent study suggests that it may also harbor the last remaining population of the peppered chub (K. Gido pers comm.). Hence, continued demographic and genetic monitoring of the species studied here, as well as the peppered chub, are important to track the conservation status of these species. Genetic data is a vital component of conservation plans, particularly if refuge populations need to be established in the future, for example, in the event of catastrophic drought conditions.

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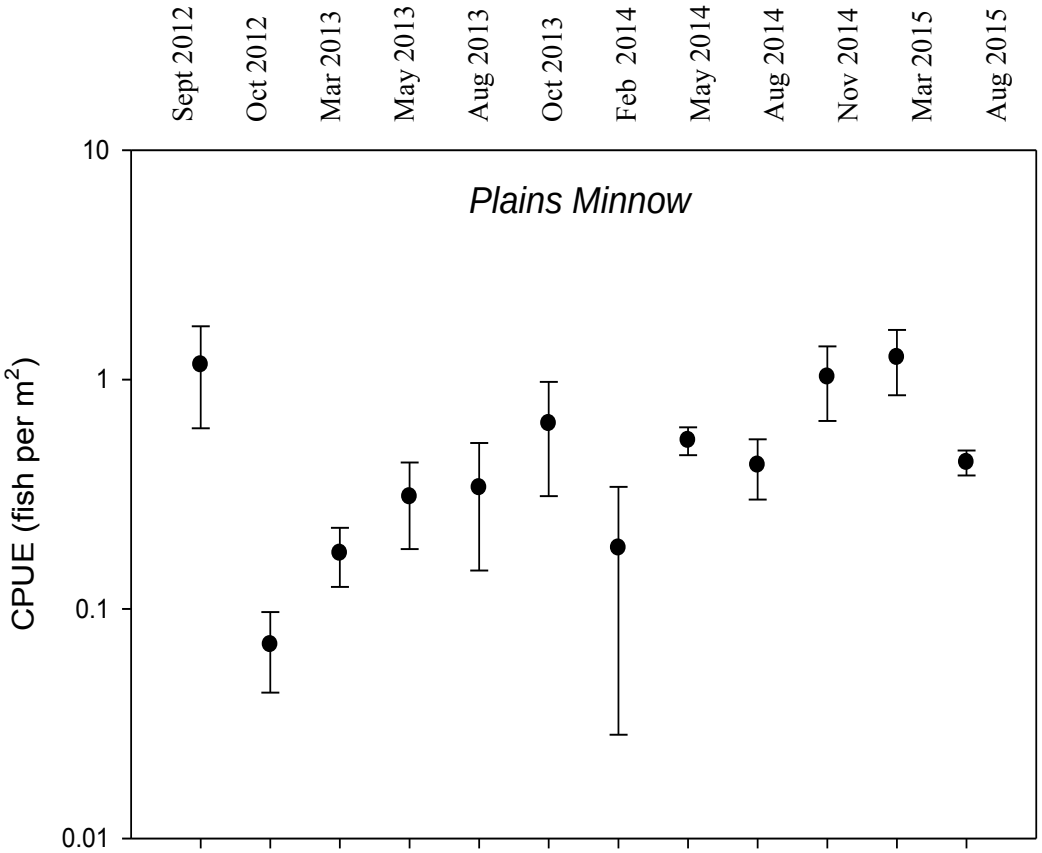
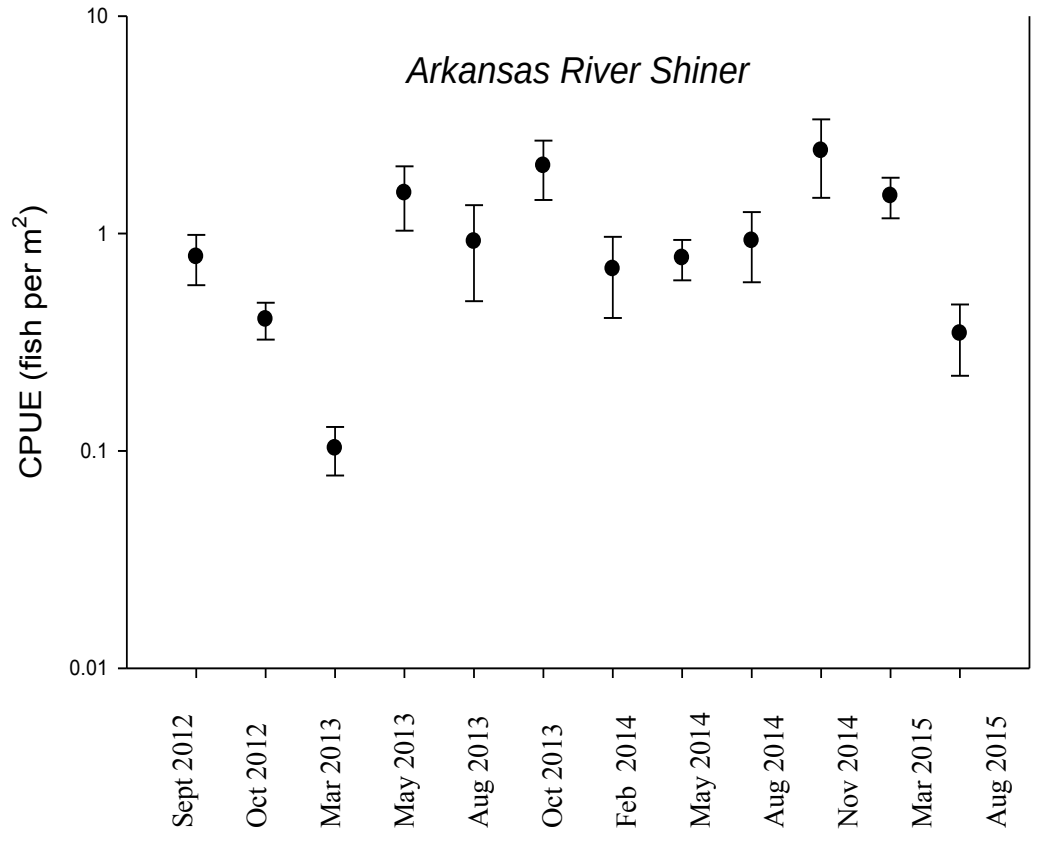
Figure 1. Catch per unit effort (fish per m^2) with 1 standard error bars for (A) plains minnow and (B) Arkansas River shiner by sampling event. (C) Mean monthly discharge at the Revuelto USGS gage for the sampling period, CPUE for plains minnow and Arkansas River shiner, and variance genetic effective population size for Arkansas River shiner for the 2009-2012, 2012- 2014, and 2014-2015 temporal comparisons.

A.

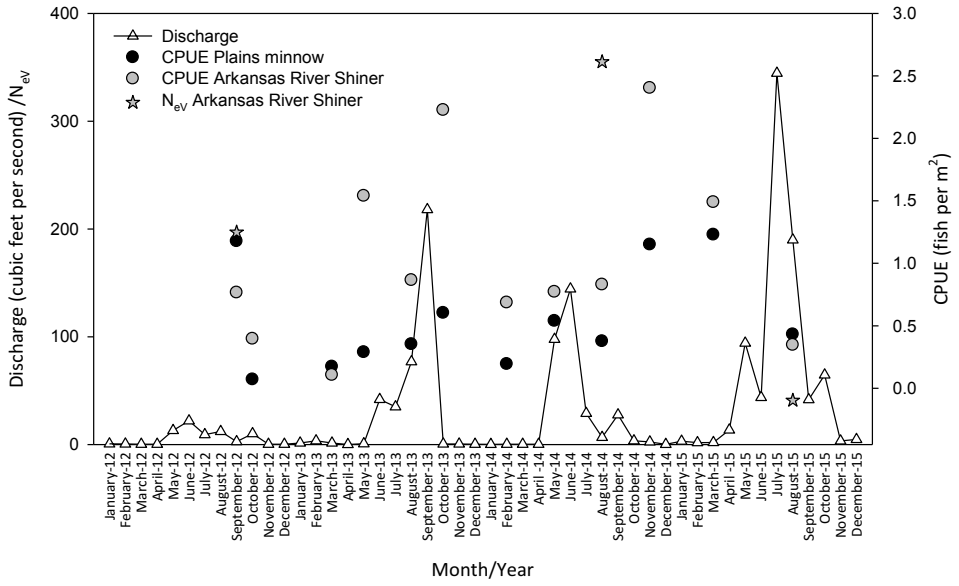


B.



Sampling Period (month/year)

C.


Discharge
CPUE Plains minnow
CPUE Arkansas River Shiner
N_{eV} Arkansas River Shiner
Discharge (cubic feet per second) /N_{eV}
CPUE (fish per m^2)
Month/Year
400
300
200
100
0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
January-12
February-12
March-12
April-12
May-12
June-12
July-12
August-12
September-12
October-12
November-12
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January-13
February-13
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January-15
February-15
March-15
April -15
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June-15
July-15
August-15
September-15
October-15
November-15
December-15

Table 1. Genetic diversity and effective size estimates for Arkansas River shiner and plains minnow from the Canadian River, New Mexico. Variables shown include: 1) Sample sizes (N); 2) genetic diversity metrics (allelic diversity [N_{ac}], gene diversity [H_{ec}], heterozygosity [H_{oc}], and inbreeding coefficient [F_{IS}]) from microsatellite data; 3) genetic variability measures (number of haplotypes [N haps], nucleotide diversity (π), haplotype diversity [h], and haplotype richness [H_R]) from mitochondrial DNA; 4) and genetic effective size of number of breeders estimates (N_{eD}) and upper and lower 95% confidence intervals. Negative estimates of N_{eD} are shown as infinity (∞).

	Microsatellites								mtDNA				
	N	**N_{ac}**	**H_{ec}**	**H_{oc}**	**F_{IS}**	**N_{eD}**	**-95%**	**+95%**	**N**	**N haps**	**π**	**h**	**H_R**
Arkansas River shiner													
2009	91	10	0.579	0.542	0.157	∞	275.3	∞	88	23	0.006	0.807	23.00
2012	146	10.85	0.637	0.622	0.110	∞	726.5	∞	113	27	0.005	0.731	20.61
2014	97	9.838	0.658	0.690	0.078	461,236	409	∞	92	23	0.006	0.805	22.43
2015	99	11.05	0.573	0.496	0.113	3443	430.7	∞	90	31	0.006	0.844	29.51
Plains Minnow													
2013	38	12.111	0.710	0.563	0.209	25,851	206.7	∞	30	18	0.009	0.94	17.00
2014	115	11.605	0.706	0.582	0.270	∞	654.1	∞	86	21	0.007	0.82	10.98
2015	94	11.639	0.698	0.573	0.174	1,899	301.1	∞	92	35	0.009	0.92	15.69

Table 2. Female genetic effective size (N_{ef}) and associated 95% confidence intervals estimated from mitochondrial DNA data for Arkansas River shiner and plains minnow. Moments (Nei and Tajima 1981)) and maximum likelihood (Wang 2001) estimates are given.

	Temporal Comparison	N_{ef}			
		Moments		Maximum Likelihood	
			95% CI		95% CI
Arkansas River Shiner	2009 to 2012	∞	106.9 - ∞	70.5	36.3 - 240
	2012 to 2014	∞	90.4 - ∞	9979	179.1 - ∞
	2014 to 2015	∞	45 - ∞	9992.9	171.4 - ∞
Plains Minnow	2014 to 2015	51.2	17.4 – ∞	9958	135 - 9958

Table 3. Variance genetic effective size estimates using three iterations of the temporal method ($N_{e(PK)}$ -Pollak 1983, $N_{e(NT)}$-Nei and Tajima 1981, $N_{e(JR)}$- Jorde and Ryman 2007) for Arkansas River shiner (*Notropis girardi*) and plains minnow (*Hybognathus placitus*). 95% confidence intervals (obtained using the jack-knife approach) are provided. All methods used P_{CRIT}=0.02.

		Microsatellites N_{eV}								
	Temporal Comparison	**$N_{e(PK)}$**	**- 95% CI**	**+ 95% CI**	**$N_{e(NT)}$**	**- 95% CI**	**+ 95% CI**	**$N_{e(JR)}$**	**- 95% CI**	**+ 95% CI**
Arkansas River Shiner	2009 – 2012	197.3	109.5	421.8	199.6	109.8	423	207.3	151.9	271.5
	2012 – 2014	355.6	140.6	22761.3	318.3	132	3305.4	158.9	115.7	208.8
	2014 – 2015	41.3	25.4	78.4	37.1	22.4	63.9	22.9	16.7	30.1
Plains Minnow	2014 – 2015	∞	139.7	∞	∞	135.5	∞	∞	∞	∞