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Variance in Reproductive Success is Driven by Environmental Factors, not Mating System, in Bonytails

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Abstract

Studying the reproductive ecology of aggregate broadcast spawning fishes is difficult because it generally is not feasible to sample all potential parents and unambiguously assign their offspring. We used molecular-based parentage analysis to gain insights into the reproductive ecology of the endangered Bonytail, and to evaluate whether protected off-channel habitats could be used as an alternative to hatchery production. By genotyping adults and offspring stocked ($n = 4130$) into two experimental backwaters across three years, we determined that most adults (82–97%) contributed to progeny production across years and backwaters, with one exception. Both sexes mated multiply and the number of mates and family size were positively correlated. There was also a positive correlation between adult size and metrics of reproductive success. There were strong interactions between sample years and backwaters suggesting that environmental factors are the primary driver of variance in reproductive success. Knowledge of mating systems and sources of variance in reproductive success is important for management of endangered fish because high variance in reproductive success leads to substantial losses of genetic variation when few individuals reproduce successfully. Although variance in reproductive success was observed, most adults contributed to genetically diverse progeny in experimental backwaters. These results support the use of predator-free, but otherwise natural, backwaters as an effective conservation tool for reintroducing Bonytail to its native habitat.

Introduction

Knowledge of reproductive ecology of bony fishes in general, and endangered species in particular, is vital for implementation of effective management and conservation programs. For example, inter-annual variability in levels of recruitment and contribution of breeding adults to offspring production can significantly impact genetic diversity and abundance (e.g. Hedgecock 1994; Bekkevold et al. 2002; Jay et al. 2014). Mating behaviors such as spatially or temporally synchronous spawning can also make populations vulnerable to exploitation and predation (Rowe and Hutchings 2003) and hence can impact species conservation. Most studies of reproductive ecology, including those that measure reproductive success among

Data Availability

We have deposited the primary data underlying the analysis as follows:

-Individual microsatellite genotypes by year and backwater: Dryad

individuals, have largely focused on mammals and birds because direct observations can often be used to make accurate estimates (Clutton-Brock 1988). DNA fingerprinting however, has revealed that social parents are not always genetic parents in many cases (e.g. Westneat 1990). Molecular parentage analysis also allows studies of reproductive success in species with more cryptic mating systems including broadcast and aggregate spawning fishes (reviewed in Avise et al. 2002; Duong et al. 2011).

In the conventional view of aggregate spawning species, males and females congregate at a neutral location (i.e. not a defended territory) for a brief period of intense mating activity. There typically is little or no monopolization of individuals or space (Wells 1977). Under this scenario, all individuals have a chance to mate but only a few may be successful (Nunney 1993). Consequently, a significant proportion of breeders may leave no offspring (Jones and Hutchings 2002; Seamons et al. 2007; Tatarenkov et al. 2008). In some cases, aggregate spawning species may exhibit more complex breeding behavior (Bekkevold et al. 2002). These sources of variance in reproductive success can depress genetic effective size of the stock produced, thereby accelerating the rate that genetic diversity is lost (Ryman and Laikre 1991). Aggregate breeding behavior does, however, provide a mechanism for large amounts of genetic mixing when fertilization is external. In this situation males and females can mate multiply which makes it more likely that a large number of offspring under optimal spawning, fertilization, and rearing conditions.

Bonytail *Gila elegans* is one of four species of large, long-lived, endemic fishes (including Razorback Sucker *Xyrauchen texanus*, Colorado Pikeminnow *Ptychocheilus lucius*, and Humpback Chub *Gila cypha*) that were once abundant and broadly distributed throughout the Colorado River basin (Minckley et al. 2003). Anthropogenic water use and habitat alterations caused by damming of rivers and establishment of non-native species resulted in widespread declines in both distribution and abundance of these and other native fishes. Bonytail is listed as an endangered species (USFWS 1980) and is functionally extirpated in the wild (Marsh 2004). Efforts to prevent extinction and total loss of genetic diversity of the species began in 1981 with establishment of a captive broodstock. Fewer than 10 individuals were available to found the captive stock due in part to the rarity of the species in the wild (Hamman 1982; Hedrick et al. 2000). Genetic variation in existing captive stocks and all repatriated Bonytail are derived from these original founders. A stocking program for Bonytail also began in the 1980's with releases into numerous sites in the Colorado Basin including the Green River, the upper and lower Colorado River, Lake Mohave and Lake Havasu (summarized in Mueller 2006). These efforts however have failed to improve the status of Bonytail in the wild due largely to predation pressures and resulting recruitment failure (Marsh 2004).

Hatcheries have largely sustained Bonytail since founding of the captive broodstock, but there are significant risks to genetic and phenotypic diversity imposed by hatchery environments (e.g., Araki et al. 2007; Christie et al. 2012). To mitigate hatchery impacts and irreversible changes to the natural river habitat, Minckley et al. (2003) proposed an alternative strategy to hatchery production where native species like Bonytail and Razorback Sucker would breed naturally in isolated and protected off-channel ponds. Offspring would grow in the absence of nonnative predators but still experience natural environmental

conditions. When grown to sufficient size, these fish could be returned to the river and contribute to a genetically well mixed adult population. Successful implementation of this strategy requires thorough understanding of the reproductive ecology of Bonytail and Razorback Sucker.

A few biological details exist of Bonytail reproduction under “natural” conditions. Specifically, Jonez and Sumner (1954) observed: (i) no nest building by either males or females, (ii) females being escorted by three to five males and (iii) breeding aggregations of around 500 individuals, (iv) broadcasting gametes over gravel or other, clean substrate (Minckley 1973; Mueller 2006). Others have observed deposition of adhesive eggs (Mueller 2006; Minckley and Marsh 2009), and spawning in May-July (Vanicek and Kramer 1969). Yet, there are also significant gaps in knowledge. For example, family size variation, variance in reproductive success of males and females, and fidelity of transmission of genetic diversity from parents to offspring are not currently known.

In this study, we use molecular parentage analysis of adults released and allowed to breed in off-channel ponds to provide the first estimates of individual reproductive success for Bonytail. Our primary goal was to identify important sources of variance in reproductive success (e.g., sex, backwater, year) and to better understand the reproductive ecology of Bonytail. A number of studies across a variety of taxa have shown a relationship between genetic diversity of parents and metrics of reproductive success, specifically an association between higher individual heterozygosity and increased reproductive success (e.g., Hoffman et al. 2004; Charpentier et al. 2005). Hence, we also assessed whether genetic diversity of broodstock was correlated with measures of reproductive success (number of mates and family size). Results of this study allowed us to assess whether genetic diversity can be maintained while allowing Bonytail to reproduce in a semi-natural environment with little human intervention. These are the key elements of the Minckley et al. (2003) conservation plan.

Methods

Sample Collection and Molecular Methods

In 2014, 2015 and 2016, an equal number of male and female (Table 1) adult Bonytail were stocked into two isolated backwaters (North Nine Mile subsequently referred to as Nine Mile; and Nevada Egg) that are adjacent to Lake Mohave, a reservoir on the lower Colorado River (Arizona-Nevada). In 2014, stocked adults were obtained from the 2009 year-class produced at Southwestern Native Aquatic Resource and Recovery Center in Dexter, New Mexico. For 2015 and 2016, stocked fish were obtained from the 2012 year-class. All adults were sexed, PIT-tagged and fin clipped prior to release. Males can be distinguished when they are ready to breed as their head, body, anal, pelvic and caudal fins become tuberculated and in females, the egg vent becomes apparent (Mueller 2006; William Knight pers. comm.). For 2015, adult total length (measured prior to release) ranged from 217–300 mm for Nevada Egg and 227 – 290 mm in Nine Mile (Supplementary Figure S1). In 2016, total length ranged from 223 – 300 mm for both backwaters. In 2015, mean female total length was 8.6 mm larger than mean males size and in 2015 this difference was 6.6 mm. In 2014,

fish were not individually measured prior to release but were similarly sized when compared to fish used in 2015 and 2016.

Backwaters are closed systems approximately 1.5–2 meters deep and with surface areas of approximately 2500 m² (Nine Mile) and 4100 m² (Nevada Egg). Once stocked, Bonytail were allowed to mate freely and no manipulation of fish or environment occurred afterward. Experimental backwaters were dried down completely and all remaining fish removed each autumn. New adults were stocked into backwaters each spring. Collections of larvae commenced approximately two weeks after stocking and continued throughout spring and early summer. Young-of-year fish (post-larval juveniles) were collected prior to autumn dewatering. Larvae and young-of-year were collected using dipnets and preserved in 95% ethanol for genetic analysis.

DNA was isolated from fin clips and whole larvae using standard phenol-chloroform extraction methods. Eighteen tetranucleotide microsatellites developed for Bonytail ([Gel] Keeler-Foster et al. 2004), Owens Tui Chub *Siphateles bicolor* [Gbi] (Meredith and May 2002), Cyprinidae [CypG] (Baerwald and May 2004) and Central Stoneroller *Camptostoma anomalum* [Ca] (Dimsoski et al. 2000) were amplified as 10 µL reactions, containing 1 µL diluted DNA, 1X reaction buffer, 2 mM MgCl₂ solution, 125 µM dinucleotide triphosphates (dNTPs), 0.4 µM of both forward and reverse primers, and 0.375 units of Genscript™ DNA Polymerase. Fragments were amplified using the following PCR cycling conditions: initial denaturation at 90°C for 2 min, followed by 30 cycles of denaturing at 90°C for 30 secs (s), annealing at 50°C (Ca8); 51°C (Gbi13/Gel287) or 57°C (Gel225/CypG3; CypG22/Gel300) or 60°C (CypG5) for 30 s, and extension at 72°C for 45 s, and ending with a final extension at 72°C for 30 mins (m). Remaining microsatellite loci (Gel223/Gel226; Gel257/Gel228; Ca12/Gbi87; Ca3/Gel227; Gel234; Gbi34) were amplified using a touchdown protocol with initial denaturation at 90°C for 2 m, followed by 30 cycles of denaturing at 90°C for 30 s, annealing at 56°C (decreasing 0.2°C per cycle) and extension at 72°C for 45 s, and ending with a final extension at 72°C for 30 m. 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 ABI Genemapper software (ABI).

Statistical Analysis—Genetic Diversity

For each year and backwater, collections that were dominated by larval fish, identified based on ontogenetic stages established by Snyder and Muth (2004), were grouped together for subsequent analyses. Remaining fish in the post-larval juvenile stage were grouped separately and referred to as young-of-year samples. GENEPOP'007 (Rousset 2008) was used to conduct a global modified exact test across groups to determine whether observed genotype frequencies conformed to Hardy-Weinberg proportions. Also, global tests for linkage disequilibrium (non-random association of loci) were conducted for all pairs of loci. Sequential Bonferroni correction was used to account for inflated type-I error rates inherent in multiple pairwise comparisons. Progeny sample sizes differed between backwaters and temporal groups so a resampling approach was used to estimate genetic diversity metrics using the R statistical package (www.r-project.org) and custom script (available by request).

To correct for differences in sample sizes across collections, metrics of genetic diversity were based upon estimates obtained from resampling each collection at random without replacement to the minimum sample size observed across all years ($n = 64$ young-of-year in 2016). Microsatellite diversity estimates (sample-size corrected number of alleles [N_{ac}], Nei's gene diversity (Nei 1987) [H_{ec}] and heterozygosity [observed proportion of heterozygotes] [H_{oc}]) were then calculated for randomized samples and the process was repeated for 1000 iterations. Corrected diversity estimates are calculated as mean estimate across all iterations. Diversity statistics were compared between backwaters and years using a two-way ANOVA as implemented in SigmaPlot. Significance was assessed using a Holm-Sidak test to account for multiple tests.

In addition to calculating population level diversity statistics, we calculated a metric of parental relatedness for each individual. Homozygosity per locus (HL) weights the contribution of loci depending on allelic variability (E) (Aparicio et al. 2006). Homozygosity per locus is calculated by $HL = \sum E_h / \sum E_h + \sum E_j$ where E_h and E_j are the expected heterozygosities of the loci that individual carries from homozygosis (h) and heterozygosis (j). Homozygosity per locus was calculated for parents, larvae and young-of-year samples by year and backwater using the GENHET vers. 2.3 package (Coulon 2010). We assessed whether mean values of HL differed between parents and offspring (larvae and young-of-year) in each backwater and year using a univariate test conducted in SPSS (IBM Corp 2013).

Parentage/sibship analysis

The computer program COLONY Vers. 2.0.6.4 was employed to jointly infer parentage and sibship. COLONY considers the likelihood of parentage and sibship over the entire pedigree configuration rather than for pairs of individuals (dyads) (Jones and Wang 2010). For this analysis, mating system was set as polygamous, a 'no inbreeding' model was used, a marker error rate of 0.01 was assumed, and a full likelihood analysis with medium length runs was conducted. We used the 'no inbreeding' model because average inbreeding coefficients (F_{IS}) were small and not significantly different from zero for Bonytail. Under this model, inbreeding is not inferred and the population is assumed to be in Hardy-Weinberg equilibrium. Use of the inbreeding model is not advised when inbreeding levels are low, because computational time is substantially increased and this model does not greatly improve estimation of relationships (Jones and Wang 2010). Medium runs offer a favorable compromise between precision and running time (Jones and Wang 2010). The full likelihood method assigns all samples to inferred relationships including full-siblings, half-siblings or unrelated individuals (Wang 2009). We calculated variance in reproductive success based on the number of mates and offspring by sex using equations in Crow and Denniston (1988). We assessed whether per locus heterozygosity (HL) of adults was correlated with measures of reproductive success using a Spearman correlation.

Genetic Effective Size

Genetic effective size was estimated using the sibship assignment method ($N_{e(SA)}$) (Wang 2009). $N_{e(SA)}$ and 95% CIs were estimated using COLONY version 2.0.6.4 (Jones and

Wang 2010), specifying a polygamous mating strategy. We also calculated the ratio of $N_{e(SA)}$ to the adult census size (N) for each backwater and year.

Relationships between backwaters, years and measures of reproductive success (number of mates and family size) were analyzed using generalized linear models (GLMs). We used the R packages MASS (Ripley 2013) and fitdistrplus (Delignette-Mueller 2015) to identify the most appropriate probability distribution for the response variables in the GLMs. Number of mates was specified as the dependent variable and family size was included as a covariate. We used a negative binomial distribution with a log link function as implemented in the statistical package SPSS.

Results

Genetic Diversity and Effective Population Size

Genetic variation was assessed at 18 microsatellites in 4130 adult and offspring samples collected between 2014 and 2016 from Nine Mile and Nevada Egg backwaters (Table 1). In the 2014 adult samples, four loci (Gel257, Gel227, Gbi87 and Gbi34) departed significantly from HWE in both backwaters. In addition, locus Gel226 and loci Gbi13 and Ca12, departed from HWE in Nine Mile and Nevada Egg, respectively. In 2015 and 2016 all loci departed from HWE proportions in adult samples. Across years, three loci (Gbi87, Gbi34 and Gel257) departed from HWE in the offspring samples while the remaining 15 loci conformed to HWE. Across years and backwaters there was significant gametic disequilibrium between all loci. Mean allelic diversity across loci ranged from 7.5 to 10.5, gene diversity ranged from 0.70 to 0.79 and heterozygosity was between 0.68 and 0.80 (Table 1). Neither gene diversity nor heterozygosity were significantly different between adult, larvae and young-of-year fish in any backwater or year. Allelic diversity differed significantly between backwater and year ($p = 0.014$). Gene diversity also differed significantly between years ($p = 0.001$) with the greatest difference between 2014 and 2015.

In Nine Mile, mean HL differed significantly between adults and offspring in 2014 ($F_{(2, 394)} = 4.39$, $p = 0.01$) and 2015 ($F_{(2, 941)} = 41.96$, $p = 0.0001$) (Figure 1). There was no difference in HL = between adults and offspring in Nine Mile in 2016 ($F_{(1, 262)} = 0.04$, $p = 0.84$). In Nevada Egg in 2015 and 2016, HL increased between the adult and offspring samples ($F_{(2, 995)} = 46.23$, $p = 0.0001$; $F_{(2, 681)} = 24.22$, $p = 0.0001$). In Nevada Egg in 2014, the opposite pattern was observed ($F_{(2, 840)} = 5.95$, $p = 0.004$).

Genetic effective size based on the sibship method ($N_{e(SA)}$) ranged from 70 in 2016 (lower and upper 95% CIs 48–103) in Nine Mile to 156 in Nine Mile in 2015 (lower and upper 95% CIs 125 – 197) (Table 1). The ratio of $N_{e(SA)}$ to the adult census size (N) ranged from 0.35 (2016 Nine Mile) to 0.86 (2014 Nine Mile).

Reproductive Success

Maternity and paternity could be determined for 92% of offspring in 2014 and 91% of offspring in 2016 (Nine Mile). In 2015, maternity and paternity could be established for 84% of offspring from Nine Mile and 73% of offspring from Nevada Egg. For remaining offspring, only one parent could be assigned. We explored whether more missing data in

2015 explained this result; however, levels of missing data ($< 0.5\%$) were comparable to levels in 2014 and 2016. Only samples that could be assigned both maternity and paternity were used in subsequent analyses. Patterns of variation at some microsatellite loci violated assumptions of the COLONY algorithm (e.g., HWE and linkage equilibrium). However, previous analysis of known family groups using this set of microsatellite loci demonstrated parents were accurately identified. For this reason, we retained all loci for subsequent analyses.

Across all years and backwaters, number of mates and number of progeny produced were positively correlated (correlation coefficients ranged from 0.93 to 0.98, with values of $p = 0.0001$ in all cases) for both males and females (Figure 2). Mean number of mates and offspring per individual differed significantly between backwaters and years, with a mean of eight offspring per female (Nevada Egg 2014) and only 0.5 offspring per male and female in Nine Mile in 2016 (Table 2, Supplementary Figure S2). Likewise, variance in metrics of reproductive success differed substantially among years and backwaters. The proportion of males and females producing at least one progeny was high for both backwaters in 2014 (89–92%) and 2015 (87–99%) (Supplementary Figure S3). For 2014 and 2015 in Nevada Egg, 80–90% of individuals mated multiply (Figure 3). Maximum number of mates per individual was 22 (a male in Nevada Egg 2014). In Nine Mile, 70–80% of individuals had more than one mate in 2014 and 2015. In 2016, contribution of adults also was high in Nevada Egg but in Nine Mile 33% of males and 27% of females produced offspring and only 10% of individuals had more than one mate. There was a significant positive relationship between adult total length and number of mates in Nevada Egg in 2015 and 2016, but not for adults in Nine Mile (Figure 4). Larger individuals also tended to contribute a greater number of offspring (Figure 5). GLM analysis of predictors of individual reproductive success revealed that interaction between backwater and year had a highly significant effect ($p = 0.0001$, $df = 2$). There were significant and negative associations between HL and number of mates per male, number of mates per female, and family size, respectively (Figures 6–7).

Discussion

Despite its ubiquity in fishes, few studies of aggregate-spawning mating systems have been conducted because of logistical constraints associated with sampling. Here we present data from a closed, yet natural system where all potential parents were sampled. Using molecular based parentage analysis, we found that across years and backwaters nearly all reproductive adults contributed to the sampled progeny production, with the exception of Nine Mile backwater in 2016. Consequently, genetic diversity (measured at the population level) was maintained between parents and offspring in nearly all cases. Promiscuous mating occurred frequently and there was a strong and positive correlation between the number of mates and family size. Length frequency histograms of offspring suggest that spawning occurred over a brief period in spring, rather than a protracted spawning period that extended into summer.

Surprisingly, there was no difference in measures of reproductive success between sexes. In other words, males and females made approximately equal contributions within backwaters and years. Variance in reproductive success among individuals also was substantial in some

years, such as Nevada Egg in 2014 where an individual had 22 mates and sired at least 40 offspring. This is a conservative estimate because we did not exhaustively sample offspring. We found a positive relationship between measures of reproductive success and adult length in Nevada Egg (2015 and 2016) and a weaker relationship also was observed in Nine Mile in 2015. The main source of variance in reproductive success was the interaction between year and backwater suggesting a strong effect of environmental factors that are localized in space and time.

Plausible explanations for adult Bonytail not leaving offspring include failure to spawn, or mortality prior to spawning. Remote PIT-tag scanning records from Nine Mile in 2016 suggested a fish kill at the end of June as only a single adult was encountered in scanning data after that time, and very few recruits were collected in the fall. A similar event occurred in Nine Mile in 2014 due to a deterioration in water quality in the summer. However, in 2016 adults in Nine Mile were detected prior to the fish kill indicating that they were alive yet there was limited reproduction. During this same period, larvae were collected from Nevada Egg backwater suggesting suitable spawning conditions (e.g., water temperature). It is also likely that failure to detect offspring for a small fraction of adults is attributable to incomplete sampling of progeny which would be more likely for individuals that spawned less often, produced fewer gametes or spawned in habitats less easily sampled.

Parentage analysis in Bonytail is somewhat complicated by the recent demographic history of the species. The founding population of Bonytail at Southwestern Native Aquatic Resource and Recovery Center was exceptionally small (< 10 founders) due partly to its scarcity in the wild (Hamman 1982; Hedrick et al. 2000). This affects our data and results in a number of ways. First, we observed significant linkage disequilibrium (Lewontin and Kojima 1960) among microsatellites used in this study. The extent of disequilibrium throughout the genome is affected by demographic history, recombination rate between loci (Slatkin 1994), breeding system and patterns of geographic subdivision (Slatkin 2008). Extreme reductions in population size can increase linkage disequilibrium and we presume this explains our observations. Additionally, low levels of allelic diversity (and hence reduced statistical power) associated with a small founder population also affected our ability to identify parents of offspring in 2015. Harrison et al. (2013) showed that low allelic diversity negatively affects precision. Other reasons that may have prevented offspring being assigned to parents could include genotyping errors and null alleles however, numerous studies have shown COLONY to be robust to violations of assumptions and presence of genotyping errors, compared to alternative methods.

Small founding size of Bonytail stocks highlights the importance of monitoring individual diversity. To address this, we calculated individual homozygosity (HL). In two thirds of cases this metric increased significantly from adult to offspring samples, suggesting a slight increase in parental relatedness in offspring samples. Studies across a range of species have indicated that HL and other metrics of parental relatedness are negatively associated with components of fitness (survival, disease susceptibility, and reproductive success) (e.g., Amos et al. 2001; Hoffman et al. 2004; Charpentier et al. 2005). However, in only one instance did we observe a relationship of HL (higher in both males and females) with fewer mates and offspring (Nine Mile in 2015). Although values for HL were small in this study, monitoring

this metric will be important as the transition to permanent backwaters occurs during the implementation of the conservation plan for Bonytail. Permanent backwaters are designed to support multiple generations of Bonytail, and there will be increased potential for breeding among related individuals.

Mating system in Bonytail

Genetic data suggest that Bonytail likely congregate as ephemeral breeding assemblages that are distinct in time and space (Emlen and Oring 1977; Domeier and Colin 1997). Emlen and Oring (1977) define breeding assemblages as the convergence of both sexes for a relatively short-lived, synchronized breeding period in which there is minimal sexual selection; consistent with Bonytail results. Our results also support observations of Bonytail reproduction under natural conditions in which aggregations of around 500 individuals were observed; with spawning occurring over gravel bars in Lake Mohave (Jones and Sumner 1954). Use of lentic habitats for spawning by Bonytail may have been an important survival strategy in the face of unpredictable and variable environmental conditions of the Colorado River. Evidence for use of such habitats by Bonytail includes archeological sites containing Bonytail and Razorback remains on ancient Lake Cahuilla, indicating that the lake supported populations of these species for a considerable period of time (Schaefer 1986). Also, propensity of Bonytail to reproduce and recruit in lentic habitats (e.g. Cibola High Levee Ponds; Pacey and Marsh 1998) suggests that flowing water is not necessary for the species to complete its life cycle (Mueller 2006).

Genetic parentage analysis indicates promiscuous breeding for both males and females. Sexual selection theory proposes that in species lacking parental care, males should mate with as many partners as possible (Arnold and Duvall 1994). For males, access to females is more limiting than their ability to produce gametes, hence males of numerous species have adopted various strategies to maximize mating and fertilization success (e.g., mate or resource guarding; Emlen and Oring 1977). Promiscuous mating by males is beneficial because it increases their Darwinian fitness by fertilizing eggs from multiple females (e.g., Bateman 1948; Arnold and Duvall 1994). Other advantages of promiscuity include better offspring quality and performance and bet hedging against infertility. Less obvious are female benefits of multiple mating; particularly in non-resource-based mating systems, in which males only provide gametes. However, in guppy *Poecilia reticulata*, multiply mated females had shorter gestation times, larger broods, better schooling abilities and escape responses compared to singly mated individuals (Evans and Magurran 2000). Other advantages of polyandry include production of broods with potentially adaptive gene complexes and avoidance of inbreeding depression (Fessehaye et al. 2006; Avise et al. 2002; Stockley et al. 1993). When environmental conditions fluctuate dramatically, good genes for the next generation may not persist so a female may improve her chances of acquiring good genes by mating with a variety of males (Petrie and Kempeneers 1998). Promiscuity also is predicted for species such as Bonytail characterized as having a 'periodic' life history strategy (production of large clutches of small eggs and offspring with low survivorship) (Winemiller 1992). This life history strategy is proposed to have evolved in response to large-scale environmental variation (Winemiller 1992).

Variance in Reproductive Success

Although there was variance in family size among Bonytail, the majority of individuals contributed at least one offspring. Males and females made approximately equal contributions to the progeny pool within backwaters and years. There also was not a consistent pattern in the degree of variance in reproductive success between sexes. There were significantly positive associations between adult size and both metrics of reproductive success (number of mates/offspring) in Nevada Egg (2015 and 2016) and for males in Nine Mile in 2015. The strength of the association was weak in some cases, but it is worth noting that although each group of broodstock represented a single age-class, the size distribution of adults was truncated in our study (maximum total length was 300 mm). It is plausible that presence of larger and older adults in the population would strengthen this relationship and differences in reproductive success may be apparent if males and females differ in size. The relationship between reproductive success and body size also has been observed in other fishes (e.g. Bekkevold et al. 2002; Kazakov 1981; Wankowski and Thorpe 1979). For Bonytail, the main source of variance in reproductive success was interaction between year and backwater suggesting a strong effect of local environmental factors such as water temperature and physical chemistry.

One of the main concerns regarding extremely high variance in reproductive success among adults is reduction in genetic effective size and consequently, loss of genetic diversity each generation (Hedrick 2005). Fish that are highly fecund, but have large offspring mortality can be subject to such extreme variances (Hedgcock 1994). Variance in reproductive success was present in adult Bonytail and not sufficiently high, in theory, to result in lowered diversity (Hedrick 2005). Consequently, available genetic variation was transmitted from parents to offspring because the majority of individuals contributed at least one offspring. However, genetic variation will still be lost over time because there will be increased variation in family size between the time of sampling and adulthood. Within generation ratio of $N_{e(SA)}$ to N was 0.72–0.86 (the only exception was in 2016 Nine Mile) which is similar to observations in Lake Sturgeon *Acipenser fulvescens* (Jay et al. 2014) and falls within the range (0.25 – 0.75) reported for a wide variety of other taxa (Nunney 1993, 1996; Waples 2016). The ratio of $N_{e(SA)}$ to N for Bonytail is likely an overestimate because it is derived from combined larval and young-of-year samples and variance will increase by adulthood. The ratio of $N_{e(SA)}$ to N could change when multiple cohorts exist within a population because older/larger Bonytail contribute more offspring (Figure 5).

Conclusions

Results presented here add to our knowledge of Bonytail reproductive ecology, and support prior behavioral observations. Genetic parentage analysis shows that Bonytail is a promiscuous, probably aggregative and synchronous spawner. Results also suggest that allowing reproduction in isolated backwaters can maintain genetic diversity in offspring because of the high contribution of adults. This is an important finding because loss of predator-free habitat, including isolated lentic habitats such as floodplain wetlands (Mueller and Marsh 2002), is the major impediment to their recovery and hence a motivation of the Minckley et al. (2003) conservation plan. Improved understanding of Bonytail breeding behavior may also provide additional opportunities for conservation actions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, and Coulson T. 2001 The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B: Biological Sciences* 268(1480):2021–2027.
- Aparicio JM, Ortego J, and Cordero PJ. 2006 What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology* 15(14):4659–4665. [PubMed: 17107491]
- Araki H, Cooper B, and Blouin MS. 2007 Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 318(5847):100–103. [PubMed: 17916734]
- Arnold SJ, and Duvall D. 1994 Animal mating systems: a synthesis based on selection theory. *The American Naturalist* 143(2):317–348.
- Awise JC, Jones AG, Walker D, DeWoody JA, and collaborators. 2002 Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. *Annual Review of Genetics* 36(1):19–45.
- Bateman AJ 1948 Intra-sexual selection in *Drosophila*. *Heredity* 2(3):349–68. [PubMed: 18103134]
- Baerwald MR, and May B. 2004 Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento–San Joaquin Delta and its tributaries. *Molecular Ecology Resources* 4(3):385–390.
- Bekkevold D, Hansen MM, and Loeschcke V. 2002 Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). *Molecular Ecology* 11(1):91–102. [PubMed: 11903907]
- Charpentier M, Setchell JM, Prugnolle F, Knapp LA, Wickings EJ, Peignot P and Hossaert-McKey M. 2005 Genetic diversity and reproductive success in mandrills (*Mandrillus sphinx*). *Proceedings of the National Academy of Sciences of the United States of America* 102(46):16723–16728. [PubMed: 16275917]
- Christie MR, Marine ML, French RA and Blouin MS. 2012 Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences* 109(1):238–242.
- Clutton-Brock TH (Ed.). 1988 Reproductive success: studies of individual variation in contrasting breeding systems. University of Chicago Press.
- Coulon A 2010 Genhet: an easy-to-use R function to estimate individual heterozygosity. *Molecular Ecology Resources* 10(1):167–169. [PubMed: 21565003]
- Crow JF, and Denniston C. 1988 Inbreeding and variance effective population numbers. *Evolution*, 42(3):482–495. [PubMed: 28564013]
- Delignette-Muller ML and Dutang C. 2015 fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software* 64(4):1–34.
- Dimoski P, Toth G, and Bagley M. 2000 Microsatellite characterization in central stoneroller *Camptostoma anomalum* (Pisces: Cyprinidae). *Molecular Ecology* 9:2187–2189. [PubMed: 11123646]

- Domeier ML, and Colin PL. 1997 Tropical reef fish spawning aggregations: Defined and reviewed. *Bulletin of Marine Science* 60:698–726.
- Duong TY, Scribner KT, Crossman JA, Forsythe PS, and Baker EA. 2011 Environmental and maternal effects on embryonic and larval developmental time until dispersal of lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences* 68(4):643–654.
- Emlen ST, and Oring LW. 1977 Ecology, sexual selection, and the evolution of mating systems. *Science* 197(4300):215–223. [PubMed: 327542]
- Evans JP, and Magurran AE. 2000 Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences* 97(18):10074–10076.
- Fessehaye Y, El-bialy Z, Rezk M, Crooijmans R, Bovenhuis H, and Komen H. 2006 Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in breeding hapas: a microsatellite analysis. *Aquaculture* 256(1–4):148–158.
- Hamman RL 1982 Induced spawning and culture of bonytail chub. *Progressive Fish Culturist* 44:201–203.
- Harrison HB, Saenz-Agudelo P, Planes S, Jones GP, and Berumen ML. 2013 Relative accuracy of three common methods of parentage analysis in natural populations. *Molecular Ecology* 22(4):1158–1170. [PubMed: 23278953]
- Hedgecock D 1994 Does variance in reproductive success limit effective population sizes of marine organisms *Genetics and Evolution of Aquatic Organisms*. Chapman and Hall, London.
- Hedrick PW 2005 Large variance in reproductive success and the Ne/N ratio. *Evolution* 59(7):1596–1599. [PubMed: 16153045]
- Hedrick PW, Dowling TE, Minckley WL, Tibbets CA, DeMarais D, and Marsh PC. 2000 Brief communication. Establishing a captive broodstock for the endangered bonytail chub (*Gila elegans*). *Journal of Heredity* 91(1):35–39. [PubMed: 10739122]
- Hoffman J, Boyd IL, and Amos W. 2004 Exploring the relationship between parental relatedness and male reproductive success in the Antarctic fur seal *Arctocephalus gazella*. *Evolution* 58(9):2087–2099. [PubMed: 15521464]
- IBM Corp. Released 2013 IBM SPSS Statistics for Macintosh Version 22.0. Armonk, NY: IBM Corp.
- Jay K, Crossman JA, and Scribner KT. 2014 Estimates of effective number of breeding adults and reproductive success for white sturgeon. *Transactions of the American Fisheries Society* 143(5):1204–1216.
- Jones MW, and Hutchings JA. 2002 Individual variation in Atlantic salmon fertilization success: implications for effective population size. *Ecological Applications* 12(1):184–193.
- Jones OR, and Wang J. 2010 COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10(3):551–555. [PubMed: 21565056]
- Jonez A, and Sumner RC. 1954 Lakes Mead and Mohave investigations Nevada Fish Games Commission. Carson City.
- Kazakov RV 1981 The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. *Journal of Fish Biology* 19(3):353–60.
- Keeler-Foster C, Spies IB, Bondu-Hawkins V, and Bentzen P. 2004 Development of microsatellite markers in bonytail (*Gila elegans*) with cross-species amplification in humpback chub (*Gila cypha*). *Molecular Ecology Notes* 4:23–25.
- Lewontin RC, and Kojima KI. 1960 The evolutionary dynamics of complex polymorphisms. *Evolution* 14(4):458–472.
- Marsh PC 2004 Threatened fishes of the world: *Gila elegans* Baird and Girard 1853 (Cyprinidae). *Environmental Biology of Fishes* 70(2):144–144.
- Meredith EP, and May B. 2002 Microsatellite loci in the Lahontan Tui Chub, *Gila bicolor obesa*, and their utilization in other chub species. *Molecular Ecology Notes* 2:156–158.
- Minckley WL, Marsh PC, Deacon JE, Dowling TE, Hedrick PW, Matthews WJ, and Mueller G. 2003 A conservation plan for native fishes of the Lower Colorado River. *Bioscience* 53(3):219–234.
- Minckley WL and Marsh PC. 2009 Inland fishes of the greater Southwest: Chronicle of a vanishing biota. University of Arizona Press.
- Minckley WL 1973 Fishes of Arizona. Ariz. Game Fish Department, Phoenix.

- Mueller GA 2006 Ecology of the bonytail and razorback sucker and the role of off-channel habitats in their recovery. Reston, Virginia United States Geological Survey Scientific Investigations Report 2006–5065.
- Mueller GA, and Marsh PC. 2002 Lost, a desert river and its native fishes: A historical perspective of the lower Colorado River (No. USGS/BRD/ITR—2002—0010). Geological Survey Fort Collins Science Center Co.
- Nei M 1987 Molecular evolutionary genetics. Columbia University Press: New York, USA.
- Nunney L 1993 The influence of mating system and overlapping generations on effective population size. *Evolution* 47(5):1329–1341. [PubMed: 28564896]
- Nunney L 1996 The influence of variation in female fecundity on effective population size. *Biological Journal of the Linnean Society* 59:411–425.
- Pacey CA, and Marsh PC. 1998 Resource use by native and non-native fishes of the Lower Colorado River: literature review, summary, and assessment of relative roles of biotic and abiotic factors in management of an imperiled indigenous ichthyofaunal. Final Report submitted to Bureau of Reclamation, Lower Colorado Region, Boulder City, Nevada Arizona State University, Department of Biology, Tempe 59.
- Petrie M and Kempenars B. 1998 Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology and Evolution* 13:52–58. [PubMed: 21238200]
- Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, and Ripley MB. 2013 Package ‘MASS’. CRAN Repos. <http://cran.r-project.org/web/packages/MASS/MASS.pdf>.
- Rousset F 2008 GENEPop’007: A complete reimplementation of the Genepop Software for Windows and Linux. *Molecular Ecology Resources* 8:103–106. [PubMed: 21585727]
- Rowe S, and Hutchings JA. 2003 Mating systems and the conservation of commercially exploited marine fish. *Trends in Ecology and Evolution*, 18(11):567–572.
- Ryman N, and Laikre L. 1991 Effects of supportive breeding on the genetically effective population size. *Conservation Biology* 5(3):325–329.
- Schaefer J 1986 Late Prehistoric Adaptations During the Final Recessions of Lake Cahuilla: Fish Camps and Quarries on West Mesa, Imperial County, California Mooney-Levine and Associates.
- Seamons TR, Bentzen P, and Quinn TP. 2007 DNA parentage analysis reveals inter-annual variation in selection: results from 19 consecutive brood years in steelhead trout. *Evolutionary Ecology Research* 9(3):409–431.
- Slatkin M 1994 Linkage disequilibrium in growing and stable populations. *Genetics* 137(1):331–336. [PubMed: 8056320]
- Slatkin M 2008 Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. *Nature Reviews Genetics* 9(6):477–485.
- Snyder DE, and Muth RT. 2004 Catostomid fish larvae and early juveniles of the Upper Colorado River Basin: morphological descriptions, comparisons, and computer-interactive key. Colorado Division of Wildlife.
- Stockley P, Searle JB, Macdonald DW, and Jones CS. 1993 Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London B: Biological Sciences* 254(1341):173–179.
- Tatarenkov A, Healey CI, Grether GF, and Avise JC. 2008 Pronounced reproductive skew in a natural population of green swordtails, *Xiphophorus helleri*. *Molecular Ecology* 17(20):4522–4534. [PubMed: 18986497]
- Team R Core. 2013 R: A language and environment for statistical computing.
- USFWS. 1980 Bonytail Chub; Determination as an Endangered Species. *Federal Register* 45(80): 27710–27713.
- Vanicek CD, and Kramer RH. 1969 Life history of the Colorado squawfish, *Ptychocheilus lucius*, and the Colorado chub, *Gila robusta*, in the Green River in Dinosaur National Monument, 1964–1966. *Transactions of the American Fisheries Society* 98(2):193–208.
- Wang J 2009 A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology* 18:2148–2164. [PubMed: 19389175]

- Warkowski JWJ, and Thorpe JE. 1979 The role of food particle size in the growth of juvenile Atlantic salmon (*Salmo salar* L.). *Journal of Fish Biology* 14(4):351–370.
- Waples RS 2016 Tiny estimates of the N_e/N ratio in marine fishes: Are they real? *Journal of Fish Biology* 89:2479–2504. [PubMed: 27714807]
- Wells KD 1977 The social behaviour of anuran amphibians. *Animal Behaviour* 25:666–693.
- Westneat DF, 1990 Genetic parentage in the indigo bunting: a study using DNA fingerprinting. *Behavioral Ecology and Sociobiology* 27(1):67–76.
- Winemiller KO. 1992 Life-history strategies and the effectiveness of sexual selection. *Oikos*:318–327.

Figure 1.

Median homozygosity across loci (HL) for Bonytail by year and backwater (Nevada Egg and Nine Mile). Boxes represent the interquartile range, outliers are indicated by points. Significant differences in mean values of HL between adults, larvae and young-of-year (YOY) are indicated by asterisks. ns- not significant, $p < 0.05$ [*], $p < 0.01$ [**], $p < 0.0001$ [****].

Figure 2. Correlation between number of offspring and number of mates for Bonytail by year, backwater (Nevada Egg and Nine Mile) and sex (males– triangles, females– filled circles). Correlation coefficients are not shown but were between 0.93 and 0.99 and highly significant.

Figure 3. Relative number of mates for male (black) and female (light gray) Bonytail by year and backwater (Nevada Egg and Nine Mile).

Figure 4. Relationship between Bonytail adult total length (mm) and the number of mates per individual in 2015 and 2016 by sex (males- circles, females- triangles) and backwater (Nevada Egg and Nine Mile). Pearson correlation coefficients and p-values are shown.

Figure 5. Relationship between adult Bonytail total length (mm) and number of offspring per individual in 2015 and 2016 by sex (males- circles, females- triangles) and backwater (Nevada Egg and Nine Mile). Pearson correlation coefficients and p-values are shown.

Figure 6.

Individual homozygosity across loci (HL) and number of mates for Bonytail by sex (males- circles, females- triangles), year and backwater (Nevada Egg and Nine Mile). Spearman correlation coefficients and p-values are shown (males- top, females- bottom).

Figure 7. Individual homozygosity across loci (HL) of Bonytail and number of offspring by sex (males- circles, females- triangles), year and backwater (Nevada Egg and Nine Mile). Spearman correlation coefficients and p-values are shown (males- top, females-bottom).

Table 1.

Sample size (n) and genetic diversity (gene diversity [H_{ec}], heterozygosity [H_{oc}], average inbreeding co-efficient [F_{IS}], allelic diversity [N_{ac}] statistics and genetic effective size [$N_{e(SA)}$]) for Bonytail by year and backwater (Nevada Egg and Nine Mile), and life-stage (adult, larvae and young-of-year [YOY]). Diversity statistics were calculated using resampling to the minimum sample size of n=64. No larvae were collected from Nine Mile in 2016. The ratio of $N_{e(SA)}$ to N (adult census size) is also shown. Genetic effective size estimates were obtained for the pooled larvae and young-of-year sample.

Year	Backwater	N	Stage	H_{ec}	H_{oc}	F_{IS}	N_{ac}	$N_{e(SA)}$	-95%	+95%	$N_{e(SA)}/N$
2014	Nevada Egg	159	Adult	0.784	0.743	0.052	10.2	--	--	--	--
2014	Nevada Egg	200	Larvae	0.783	0.769	0.02	10.1	--	--	--	--
2014	Nevada Egg	482	YOY	0.776	0.772	0.007	9.8	114	90	150	0.71
2015	Nevada Egg	200	Adult	0.729	0.8	-0.093	7.8	--	--	--	--
2015	Nevada Egg	330	Larvae	0.726	0.698	0.041	7.3	--	--	--	--
2015	Nevada Egg	468	YOY	0.729	0.709	0.031	7.5	152	121	194	0.76
2016	Nevada Egg	200	Adult	0.743	0.797	-0.065	9.5	--	--	--	--
2016	Nevada Egg	252	Larvae	0.753	0.733	0.033	9.3	--	--	--	--
2016	Nevada Egg	231	YOY	0.756	0.747	0.021	9.5	143	115	179	0.72
2014	Nine Mile	158	Adult	0.787	0.773	0.023	10.5	--	--	--	--
2014	Nine Mile	128	Larvae	0.784	0.776	0.014	10.2	--	--	--	--
2014	Nine Mile	111	YOY	0.781	0.738	0.063	10.0	138	109	175	0.86
2015	Nine Mile	200	Adult	0.716	0.794	-0.101	7.7	--	--	--	--
2015	Nine Mile	193	Larvae	0.701	0.683	0.030	7.5	--	--	--	--
2015	Nine Mile	550	YOY	0.721	0.706	0.025	7.6	156	125	197	0.78
2016	Nine Mile	200	Adult	0.738	0.77	-0.037	9.0	--	--	--	--
2016	Nine Mile	--	--	--	--	--	--	--	--	--	--
2016	Nine Mile	64	YOY	0.758	0.777	-0.023	8.4	70	48	103	0.35

Table 2.

Summary statistics for reproductive success for Bonytail by year and backwater (Nevada Egg and Nine Mile) measured by A. Number of mates per male and female and, B. Number of offspring per male and female. Percent of contributing adults, mean number of mates or offspring, variance, standard deviation (Sd) and standardized variance (Sv) of k (number of mates or offspring) are shown.

Sex	%	Variance in reproductive success (mates)			Variance in reproductive success (offspring)				
		Mean	Variance	Sd	Sv $k_{(mates)}$	Mean	Variance	Sd	Sv $k_{(offspring)}$
2014 Nevada Egg									
Male	0.94	5.71	12.41	3.52	2.17	8.00	32.94	5.74	4.12
Female	0.90	5.90	20.81	4.56	3.53	8.30	49.21	7.02	5.93
2014 Nine Mile									
Male	0.91	2.47	2.25	1.50	0.91	2.85	3.23	1.80	1.14
Female	0.91	2.44	3.14	1.77	1.29	2.83	5.26	2.29	1.86
2015 Nevada Egg									
Male	0.89	6.22	13.55	3.68	2.18	7.25	23.58	4.86	3.25
Female	0.94	6.31	13.02	3.61	2.06	7.33	21.19	4.60	2.89
2015 Nine Mile									
Male	0.87	4.48	13.10	3.62	2.92	6.02	24.87	4.99	4.13
Female	0.94	4.54	6.39	2.53	1.41	6.10	15.14	3.89	2.48
2016 Nevada Egg									
Male	0.88	3.44	7.85	2.80	2.28	4.30	16.78	4.10	3.90
Female	0.82	3.28	7.05	2.66	2.15	4.07	11.62	3.41	2.86
2016 Nine Mile									
Male	0.33	0.55	0.82	0.90	1.48	0.59	0.97	0.99	1.65
Female	0.27	0.54	1.36	1.17	2.52	0.58	1.52	1.23	2.62