Received: 8 January 2019 | Revised: 7 October 2019 | Accepted: 21 February 2020

DOI: 10.1002/zoo.21538

RESEARCH ARTICLE

ZOOBIOLOGY WILEY

Inbreeding depression in sperm quality in a critically endangered amphibian

Kristin M. Hinkson | **Sinlan Poo**

Memphis Zoo, Department of Conservation and Research, Memphis Zoological Society, Memphis, Tennessee

Correspondence
Kristin M. Hinkson, Department of Conservation and Research, Memphis Zoological Society, 2000 Prentiss Place, Memphis, TN 38112.
Email: kmhinkson14@gmail.com

Funding information
Institute of Museum and Library Services, Grant/Award Number: MG-30-15-0076

Abstract

Small, isolated populations often experience increased inbreeding and decreased heterozygosity, which increases the potential risk of inbreeding depression. The relationship between inbreeding and sperm health is well-documented in a variety of taxa, but has yet to be explored in amphibians. The dusky gopher frog, *Lithobates sevosus*, is a critically endangered species with years of documented inbreeding and low genetic variability as a consequence of isolation and population size reduction. This study investigated the effects of inbreeding on sperm quality in captive *L. sevosus* using an outbred, sister species (*Lithobates pipiens*) as a standard for comparison. We found *L. sevosus* to have severely reduced sperm quality in terms of total motility, forward progressive motility, concentration, and viability. Additionally, we observed a significant, negative relationship between total sperm motility and mean kinship within captive-bred individuals. These data serve to enhance our understanding of the role inbreeding plays in amphibians, and to provide valuable insight into new risk factors declining amphibian populations may face.

KEYWORDS

assisted reproductive technologies, captive breeding, dusky gopher frog, fertility, *Lithobates sevosus*

1 | INTRODUCTION

Inbreeding provokes an increase in homozygosity and can reduce individual and population fitness—a phenomenon known as inbreeding depression (Charlesworth & Charlesworth, 1999). Inbreeding depression is most commonly explained by two mechanisms: decreased heterozygosity at loci with heterozygote advantage (overdominance) or increased homozygosity for recessive deleterious alleles (dominance; Roff, 2002). Fitness losses associated with inbreeding can include reduced survival, reduced fertility, increased disease susceptibility, and growth deformities (Acevedo-Whitehouse, Gulland, Greig, & Amos, 2003; Aulstad & Kittelsen, 1971; Jiménez, Hughes, Alaks, Graham, & Lacy, 1994; Keller & Waller, 2002). Depending on the trait being acted on, inbreeding depression can pose a great threat to the viability of a population (Hedrick & Kalinowski, 2000).

An inverse relationship between sperm health (i.e., sperm motility, morphology, and fertilization ability) and inbreeding has been documented in many species. This trend has been most frequently investigated in mammals. For example, Florida panthers (*Felis concolor coryi*) have remarkably low levels of genetic diversity, and as such are seen to exhibit lower sperm motilities than less-threatened subspecies (Barone et al., 1994; Roelke, Martenson, & O'Brien, 1993). In wild rabbits (*Oryctolagus cuniculus*), there is a direct association between homozygosity and percent abnormal sperm cells, with more homozygous individuals producing larger proportions of abnormal sperm cells (Gage et al., 2006). This relationship is further bolstered by evidence in Mexican gray wolves (*Canis lupus baileyi*), with Asa et al. (2007) revealing a significant negative correlation between inbreeding and normal sperm morphology and motility. More recent studies have explored this relationship in fishes, with Zajitschek, Lindholm, Evans, and Brooks (2009) and Mehlis, Rahn, and

Bakker (2015) showing that inbreeding impairs sperm competitiveness in guppies (*Poecilia reticulata*) and threespine sticklebacks (*Gasterosteus aculeatus*), respectively. The link between sperm health and inbreeding has even been studied and upheld in invertebrates, with male crickets (*Teleogryllus oceanicus*) displaying a reduction in competition of sperm due to high levels of inbreeding (Simmons, 2011). However, surprisingly, this relationship has yet to be examined in amphibians.

Given the well-documented amphibian extinction crisis (Stuart et al., 2004; Wake & Vredenburg, 2008), investigating the more enigmatic causes of decline (e.g., reduction in gamete quality) places a spotlight on areas that may warrant more attention amidst our collective effort to prevent further biodiversity loss. In fact, years of drastically low reproductive success and juvenile survival have been reported in multiple endangered species, including the dusky gopher frog (*Lithobates sevosus*; Richter, Young, Johnson, & Seigel, 2003) and the Houston toad (*Anaxyrus houstonensis*; Swannack, Grant, & Forstner, 2009), which calls for further research into the reproductive health of at-risk populations. Ideally, targeted research efforts investigating the relationship between inbreeding and sperm health should also be done in reference to a closely-related, outbred species or population to serve as an adequate standard for comparison (Asa et al., 2007; Barone et al., 1994; Johnson, Butts, Smith, Wilson, & Pitcher, 2015; Wildt, Baas, Chakraborty, Wolfle, & Stewart, 1982).

L. sevosus is critically endangered (IUCN, 2019) and extirpated from the majority of its historical range, which once spanned throughout the longleaf pine forests of Louisiana, Mississippi, and western Alabama (Parris & Redmer, 2005). Currently, *L. sevsosus* exists as one population in coastal Mississippi, with reliable breeding events only occurring in two main ponds (Pechmann & Tupy, 2013; Richter, Young, Seigel, & Johnson, 2001). The current census population size is estimated to be between 100 and 200 individuals, with an effective population size estimate of 33–59 individuals (Hinkson & Richter, 2016; Richter & Seigel, 2002). As a consequence of population isolation and small population size, *L. sevosus* is plagued with reduced genetic variation and fluctuating inbreeding levels that have reached estimates expected of full sibling (brother × sister) mating (Hinkson & Richter, 2016; Richter, Crother, & Broughton, 2009). Richter and Nunziata (2014) report evidence of inbreeding depression, revealing positive genetic-fitness associations for survival of egg clutches and for survival to metamorphosis. While these genetic-fitness associations may serve to expose and purge deleterious alleles (Ficetola, Garner, Wang, & De Bernardi, 2011; Richter & Nunziata, 2014), they also compromise the reproductive recruitment of an already reduced population.

To combat further decline, many in situ and ex situ conservation management efforts have been implemented, including habitat restoration, wetland creation, and head-starting and captive breeding programs (U.S. Fish and Wildlife Service, 2014). The captive breeding population was established in 2003 after years of drastically low recruitment to safeguard against extinction and to preserve current, albeit low, levels of genetic variability (Hinkson, Henry, Hensley, & Richter, 2016). Due to a suite of unknown factors, *L. sevosus* does not breed naturally in captivity, and assisted reproductive technologies (ARTs; e.g., in vitro fertilization) are employed for every captive breeding event (Graham, Langhorne, Vance, Willard, & Kouba, 2018). The ultimate success of many breeding efforts through ARTs rests on gamete quality, and if a species or individual can no longer reliably produce healthy, viable gametes, assisted reproduction will likely fail.

Given *L. sevosus*' well-documented history of inbreeding and a captive breeding program that necessitates the use of ARTs, it is an ideal species to investigate the more cryptic consequences of inbreeding depression. More important, the success of conservation efforts, specifically captive breeding, hinges on the ability to produce offspring each year, and declines in sperm quality could severely compromise these efforts. Therefore, our objectives were (a) to compare sperm quality of *L. sevosus* to a sister-species with no evidence of inbreeding (*Lithobates pipiens*; Hoffman, Schueler, & Blouin, 2004) and (b) to investigate the relationship between mean kinship and sperm quality in *L. sevsosus*. Through these objectives, we intend to provide the first account of how inbreeding effects and is related to sperm health in amphibians.

2 | METHODS

2.1 | Taxonomic comparison

We chose the dusky gopher frog (*L. sevosus*) and the northern leopard frog (*L. pipiens*) as focal species for this study. We selected *L. pipiens* as a related, outbred standard of comparison for *L. sevosus* due to their phylogenetic history. The genus *Rana* (Family: Ranidae) first dispersed to North America during the Eocene Epoch (~48–43 Ma) from East Asia through Beringia to western North America. Within the clade, rapid speciation gave rise to the *Rana pipiens* group (also referred to as "*R. pipiens* complex" and "Pantherana") in the Miocene Epoch (~18 Ma; Hillis & Wilcox, 2005; Yuan et al., 2016). Subgroup "Nenirana" is housed within this clade, which encompasses four species (*Lithobates palustris*, *Lithobates capito*, *Lithobates areolatus*, and *L. sevosus*; Hillis & Wilcox, 2005). Within this group, *L. sevosus* exemplifies the effects of small population size and increased inbreeding, and is an ideal study species for uncovering possible links between gamete health and inbreeding. The eastern population of *L. pipiens* is stable and shows no evidence of inbreeding or reduced genetic variability (Hoffman et al., 2004), making it a model species for comparison.

2.2 | Animals

Carolina Biological Supply (Burlington, NC) collected male *L. pipiens* locally and transported the individuals to the Memphis Zoo (Memphis, TN). Upon arrival, individuals were given at least 1 week for acclimation. Male *L. sevosus* were housed indoors at the Memphis

Zoo as part of the dusky gopher frog captive breeding program. The captive population of *L. sevosus* consists of a combination of both captive-bred and wild-caught founder individuals. Zoo staff maintained *L. sevosus and L. pipiens* in groups of one to three in 10-gallon glass aquaria (50.8 × 25.4 × 30.5 cm, length × width × height) and outfitted enclosures with cover, aged water, and a sphagnum moss substrate. Both species were housed in same-sex groupings. Zoo staff fed all individuals a variety of insects (i.e., crickets, mealworms, and superworms) ad libitum. Memphis Zoo Animal Care and Use Committee approved all animal procedures (Approval 16-102 and 18-102). We conducted experiments from January to August 2018.

2.3 | Sperm collection

We administered 10 IU/g body weight of hCG (human chorionic gonadotropin; Sigma-Aldrich, St. Louis, MO) and 0.4 μg/g body weight of GnRH (des-Gly^{10}, D-Ala^{6}; Sigma-Aldrich) intraperitoneally to frogs to induce the release of spermic urine. These hormone dosages follow previously developed protocols for both *L. pipiens* and *L. sevosus* (Graham, Kouba, Langhorne, Marcec, & Willard, 2016; Kouba, Vance, & Willis, 2009). All injections were given using a 0.3 ml syringe and 29 gauge 1/2" needle.

Immediately following injections, we placed frogs individually in 2.4 L plastic boxes filled with 1 cm of aged water to promote urine production. To capture peak sperm production, we collected urine 1 hr postinjection (Graham et al., 2016; Kouba & Vance, 2009). Urine collection was facilitated by inserting medical-grade, plastic catheter tubing (0.86 mm inner diameter × 1.32 mm outer diameter, Scientific Commodities, Inc., Lake Havasu City, AZ) into the cloaca.

2.4 | Sperm assessments

We assessed each urine sample for the presence of sperm cells. For samples containing sperm, we immediately evaluated percent total motility, percent forward progressive motility, and concentration at ×400 using an Olympus CX41 phase-contrast microscope. We determined percent total motility by counting all cells with flagellar movement within 100 cells. We determined percent forward progressive motility by counting all cells exhibiting forward movement within 100 cells. All sperm cell motility estimates followed methods outlined by Della Togna et al. (2017) and were conducted by the same observer to reduce potential observer bias. We determined sperm concentration using a Neubauer-ruled chamber hemocytometer.

We assessed sperm cell viability using an eosin-nigrosin stain. For each sample, we mixed 5 μl of spermic urine with 10 μl eosin solution (0.5% eosin Y stain in aqueous NaCl; Fisher Scientific, Hampton, NH). Thirty seconds later, we added 15 μl of nigrosin saturated solution (Fisher Scientific, Hampton, NH) to the sample. The sperm solution was smeared onto a glass slide, air-dried, and analyzed under ×400 magnification. Cells with intact plasma membranes (i.e., viable cells) displayed whiteheads, while those with non-intact membranes (i.e., nonviable cells) displayed pink heads. Two separate observers analyzed each slide, wherein a total of 100 cells were counted per observer and an average was taken.

We used mean kinship values taken from the Association of Zoos and Aquariums' Species Survival Plan for *L. sevosus*. Values were estimated from an analytical studbook with pedigree assumptions and were calculated as the reciprocal of two times the founder genome equivalents (FGE), where FGE is the number of wild-caught individuals that would produce the same amount of genetic diversity as the study population (Association of Zoos and Aquariums, 2018). Individuals with few relatives in the population have low mean kinship values.

2.5 | Statistical analyses

We performed Mann–Whitney *U* tests to detect differences in sperm total motility, forward progressive movement, concentration, and viability between both species. We also assessed differences in male body weight by species using Mann–Whitney *U* tests. For a subset of *L. sevosus* males with known mean kinship values, we plotted total motility against mean kinship and calculated an R^2 value via ordinary least squares regression. We performed statistical analyses in Program R (v. 3.4.1; R Core Team, 2017), with statistical significance considered at $p < .05$. Values are given as mean ± standard error.

3 | RESULTS

We found no significant difference in body weight between species ($N = 35$ per species, $W = 623$, $p = .91$), with *L. pipiens* and *L. sevsosus* averaging weights of 39.44 ± 1.58 g and 38.69 ± 1.01 g, respectively. In contrast, *L. pipiens* had significantly greater sperm quality and quantity than *L. sevsosus* (Figure 1). Specifically, *L. pipiens* ($N = 36$) exhibited greater sperm motility than *L. sevsosus* ($N = 38$; $W = 241.5$, $p < .001$), with values of 78.83 ± 3.00% and 48.55 ± 4.74%, respectively (Figure 1a). *L. pipiens* ($N = 36$) exhibited greater sperm forward progressive movement than *L. sevosus* ($N = 38$; $W = 31.5$, $p < .001$), with values of 59.83 ± 3.55% and 6.21 ± 1.89%, respectively (Figure 1b). *L. pipiens* ($N = 35$) released spermic urine with higher sperm cell concentrations than *L. sevosus* ($N = 13$; $W = 95.5$, $p = .002$), with values of $17.38 \pm 2.85 \times 10^6$ and $6.87 \pm 3.40 \times 10^6$ cells/ml, respectively (Figure 1c). And finally, *L. pipiens* ($N = 25$) produced a greater percentage of viable sperm cells than *L. sevosus* ($N = 14$; $W = 0$, $p < .001$), with values of 85.76 ± 1.31% and 41.96 ± 4.45%, respectively (Figure 1d). Additionally, a significant, inverse relationship exists between mean kinship and total motility (Figure 2; $p = .006$).

4 | DISCUSSION

Our study is the first to address how amphibian gamete quality is affected by high inbreeding levels and reduced heterozygosity.

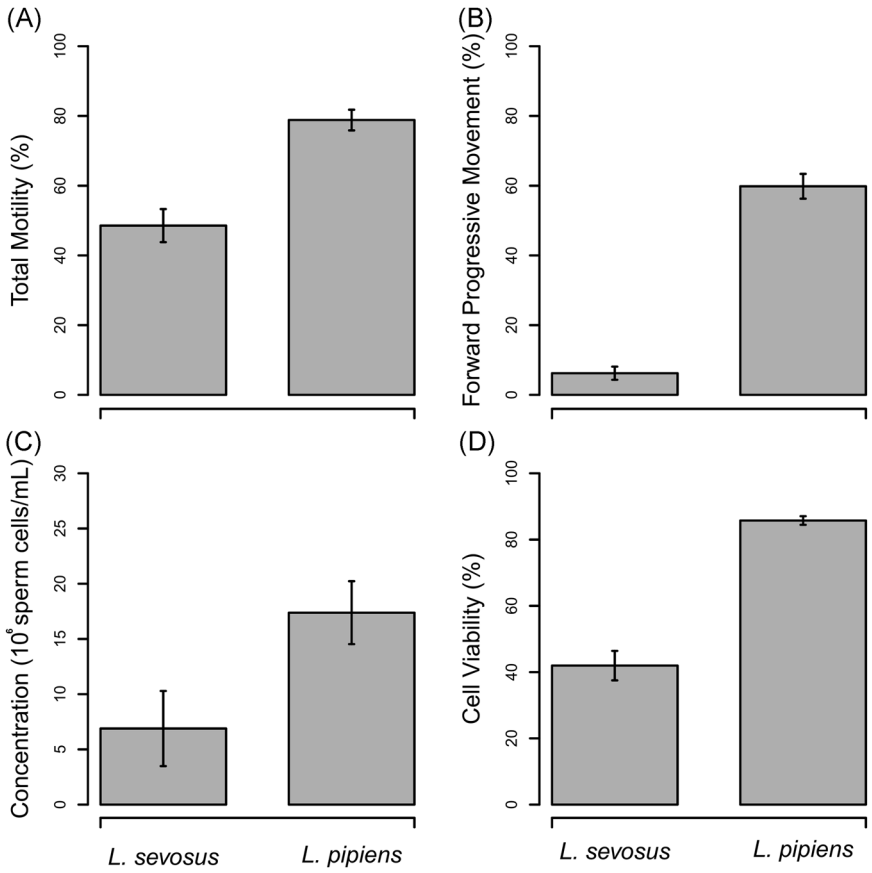

FIGURE 1 Comparisons of sperm total motility (A), forward progressive movement (B), concentration (C), and viability (D) between *Lithobates sevosus* and *Lithobates pipiens*. Data are mean ± standard error

These data serve to enhance our understanding of the role inbreeding plays in amphibians, and to provide valuable insight into new risk factors declining amphibian populations may face. Our findings reveal that *L. sevosus* has significantly reduced sperm quality compared with *L. pipiens* in terms of total motility, forward progressive movement, concentration, and cell viability. Additionally, we show as mean kinship values increase, the amount of sperm cells with flagellar movement significantly decreases. As such, we are able to provide a "first look" into the reproductive consequences of an endangered amphibian with documented inbreeding depression (Richter & Nunziata, 2014) and low microsatellite variation (Hinkson & Richter, 2016; Richter et al., 2009).

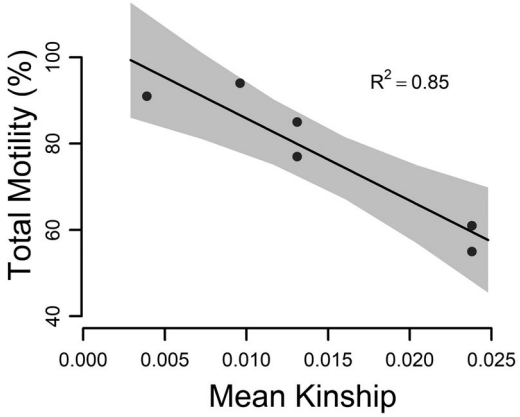

FIGURE 2 Relationship between sperm total motility (%) and mean kinship in *Lithobates sevosus* ($N = 6$). Mean kinship values estimated from an analytical studbook with pedigree assumptions. R-squared value calculated via ordinary least squares regression. The shaded region represents standard error

While the link between inbreeding and sperm health is not unique to any one taxon (Barone et al., 1994; Gage et al., 2006; Michalczyk, Martin, Millard, Emerson, & Gage, 2010; Morato et al., 2001), there does, however, appear to be some threshold that must be met before inbreeding begins to affect sperm quality. For example, Johnson et al. (2015) show that sperm quality was not impaired in captive-bred lake trout (*Salvelinus namaycush*) after one generation of full sibling matings ($F = 0.25$). Gomendio, Cassinello, and Roldan (2000) compare sperm traits across three related species of endangered gazelles and find that only species with elevated inbreeding levels ($F = 0.14$) have reductions in sperm quality. Zajitschek et al. (2009) further tease apart the relationship between inbreeding and reproductive fitness and find that only after four generations of full sibling matings ($F = 0.59$) is inbreeding depression on sperm competitiveness detected in guppies (*Poecilia reticulata*). Taken together, these studies show that sperm traits can be sensitive to genetic stress at variable levels of inbreeding. For *L. sevosus*, positive inbreeding levels have been detected over many years (1997: $F = 0.01$, 2005: $F = 0.02$, 2008: $F = 0.04$, 2013: $F = 0.28$, 2014:

$F = 0.024$; Hinkson & Richter, 2016), showing that even low levels of inbreeding have contributed to inbreeding depression in sperm quality. In fact, results from the current study bolster claims by Hinkson and Richter (2016) that while inbreeding depression has only been investigated in year 1997 (Richter & Nunziata, 2014), it is likely present, perhaps in larger amounts, in other years as well—illustrating the reproductive cost of population isolation and population size reduction in *L. sevosus*.

Low sperm motility, concentration, and viability in *L. sevosus* could help explain low hatching success in both the wild and captive populations of *L. sevosus*. For example, Richter and Nunziata (2014) observe highly variable survival rates within the egg stage (0–100%), with a low average hatching rate per clutch (63%; Richter et al., 2003). The authors attribute these low success rates to unmeasured factors. In captivity, using in vitro fertilization, similar survival rates within the egg stage are seen (range: 0–72%, average: 27%; K. Hinkson and S. Poo unpublished data), showing that even under controlled and stable conditions, hatching success is still low and variable. In light of our findings, we posit that the "unmeasured factors" affecting fertilization rates (and therefore egg stage survival) are likely depressed sperm motility, forward progressive movement, concentration, and viability. This hypothesis is supported by numerous studies which prove that sperm quality (e.g., motility, concentration, and viability) can affect fertility in a diverse group of taxa, including rainbow trout (*Oncorhynchus mykiss*; Ciereszko & Dabrowski, 1994; Lahnsteiner, Berger, Weismann, & Patzner, 1998), sea urchin (*Lytechinus variegatus*; Levitan, 2000), bull (*Bos taurus*; Januskauskas, Johannisson, & Rodriguez-Martinez, 2003), and red deer (*Cervus elaphus*; Malo et al., 2005). In addition to low sperm quality, the low hatching success observed could also be the result of lowered yolk supplies or natural selection within the egg stage against sublethal homozygous genotypes (Larsen et al., 2011), wherein more related individuals would be expected to experience greater reductions in fertilization rates. However, in vitro fertilization rates do not support the latter hypothesis, revealing a 27% hatching success among non-sibling crosses and a 28% hatching success among full sibling crosses (K. Hinkson and S. Poo unpublished data). Consequently, while it is difficult to untangle the interplay of male and female gamete quality, it appears that low sperm quality is the primary driver of reduced reproductive success in *L. sevosus*.

Though our findings illustrate clear connections between population isolation, increased inbreeding, and reduced sperm quality, the negative influence of captivity on reproductive function has been heavily studied (Locatello et al., 2018; Morato et al., 2001; Zupa et al., 2017), and as such, it could be reasoned that our results are the product of an interaction between both life in captivity and inbreeding. This line of logic would then yield *L. pipiens* as an inadequate standard of comparison because of the interactive effect between rearing environment and genetic status. However, founder individuals (i.e., wild-born animals brought into captivity as tadpoles and/or juveniles) are integrated into the captive population of *L. sevosus* regularly due in large part to the success of headstarting efforts (Baxley & Qualls, 2007; Sisson, 2004). In fact, over half of the *L. sevosus* within our study are founder individuals, with less than 3 years spent in captivity. Additionally, previous work reveals equal levels of genetic diversity and relatedness between both populations (Hinkson et al., 2016). Therefore, despite having a 15-year-old captive breeding program, both captive and wild populations of *L. sevosus* are likely genetically similar—showing that reductions in sperm health are most probably due to inbreeding and not captive conditions.

One way to remedy inbreeding depression in sperm quality is to mix subpopulations or distinct lineages. For example, Asa et al. (2007) find that crossing various combinations of three Mexican gray wolf lineages yields individuals with more morphologically normal sperm cells than any one noncrossed individual. Similarly, Johnson et al. (2010) show that survival and fitness metrics in the Florida panther all improve following the translocation of individuals from the Texas subpopulation into Florida. This management tool, however, cannot be directly applied to *L. sevosus*, as it exists as one main breeding population of around 200 individuals. To strengthen the population, since 2004, management efforts, have focused on establishing nearby, interconnected populations through yearly translocations of headstarted individuals to restored wetlands (Lee, 2013; Sisson, 2004)—raising the question of if these measures can promote gene flow and thereby reduce the negative effects of drift and inbreeding (Schwartz & Mills, 2005). Therefore, it is important to follow sperm quality of *L. sevosus* temporally to assess if these management efforts are also able to improve gamete quality in the future.

Alternatively, an argument could be posed that management efforts in conjunction with purging could improve sperm quality. Under the dominance hypothesis, recessive deleterious alleles are unmasked as a result of inbreeding, leading to a decline in vigor (Charlesworth & Charlesworth, 1999; Roff, 2002). Once these deleterious alleles are exposed to natural selection, they can be purged from the genetic architecture. Therefore, purging may restore the normal expression of traits that previously experienced inbreeding depression (Crnokrak & Barrett, 2002; Wang, Hill, Charlesworth, & Charlesworth, 1999). This phenomena is documented in guppies, revealing that within 10 generations of captive inbreeding, inbreeding depression in clutch size and offspring survival decreased after the initial increase (Larsen et al., 2011). In comparison, *L. sevosus* has been isolated for over two decades and has experienced population bottlenecks (Richter et al., 2009), which should serve to reduce lethal alleles exposed through inbreeding (Crnokrak & Barrett, 2002; Ficetola et al., 2011). Additionally, Richter and Nunziata (2014) provide strong evidence for natural selection against sublethal alleles in the year 1997 cohort. Taken together, from these hypotheses, we can speculate that any purging that has occurred or is occurring will not alleviate inbreeding depression in sperm quality, and sperm quality will not improve without alternative measures of intervention (i.e., introduction of gene flow from reintroduced populations). More likely, years of population isolation and reduced genetic variation support the idea that mildly deleterious alleles influencing sperm quality are fixed in *L. sevosus*, as genetic drift can lead to the fixation of mild, nonlethal alleles (Hedrick, 1994; Wang et al., 1999).

Overall, our study provides the first account of the relationship between inbreeding and sperm quality in amphibians, showing that a genetically depauperate species with an extensive history of population isolation has compromised sperm quality when compared to a genetically diverse sister species. Much more, we document new risks facing *L. sevosus*. Since its recognition as a species (Young & Crother, 2001), *L. sevosus* has followed a hapless trajectory, with past research showing reduced genetic diversity, inbreeding depression, low survival to metamorphosis, and low rate of return to the breeding wetland (Richter & Nunziata, 2014; Richter et al., 2003; Richter et al., 2009). These new findings further highlight areas of concern in the long-term survival of *L. sevsosus* and provide an additional tool for monitoring the efficacy of recovery efforts in amphibians. These results signal a need for future research into the connection between inbreeding and sperm quality in other at-risk species to ascertain if this trend is common in amphibians and to determine commonalities between levels of inbreeding and declines in sperm health.

ACKNOWLEDGMENTS

We would like to thank Rachel Snyder and Brittany Nunn for assistance with sample collection and their work analyzing sperm viability. We are grateful to Alex Baecher for making a drab figure beautiful. Special thanks to Memphis Zoo Central Zone staff for taking excellent care of the frogs. And finally, we want to especially thank Dr. Steve Reichling for many years of tireless effort maintaining the captive breeding population of dusky gopher frogs. This study was supported by the Institute of Museum and Library Services (MG-30-15-0076).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Kristin M. Hinkson http://orcid.org/0000-0001-5899-5256
Sinlan Poo http://orcid.org/0000-0002-7767-8173

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D., & Amos, W. (2003). Inbreeding: Disease susceptibility in California sea lions. *Nature*, *422*(6927), 35.

Asa, C., Miller, P., Agnew, M., Rebolledo, J., Lindsey, S., Callahan, M., & Bauman, K. (2007). Relationship of inbreeding with sperm quality and reproductive success in Mexican gray wolves. *Animal Conservation*, *10*(3), 326–331.

Association of Zoos and Aquariums. (2018). *Population analysis and breeding and transfer plan: Dusky gopher frog (*Lithobates *(*Rana*) sevosa*)*.* Chicago, IL: Population Management Center.

Aulstad, D., & Kittelsen, A. (1971). Abnormal body curvatures of rainbow trout (*Salmo gairdneri*) inbred fry. *Journal of the Fisheries Board of Canada*, *28*(12), 1918–1920.

Barone, M. A., Roelke, M. E., Howard, J., Brown, J. L., Anderson, A. E., & Wildt, D. E. (1994). Reproductive characteristics of male Florida panthers: Comparative studies from Florida, Texas, Colorado, Latin America, and North American zoos. *Journal of Mammalogy*, *75*(1), 150–162.

Baxley, D., & Qualls, C. (2007). Monitoring, reproduction, and translocation of the Mississippi gopher frog (*Rana sevosa*). Unpubl. Report to the Mississippi Department of Wildlife Fisheries and Parks.

Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genetics Research*, *74*(3), 329–340.

Ciereszko, A., & Dabrowski, K. (1994). Relationship between biochemical constituents of fish semen and fertility: The effect of short-term storage. *Fish Physiology and Biochemistry*, *12*(5), 357–367.

Crnokrak, P., & Barrett, S. C. (2002). Perspective: Purging the genetic load: A review of the experimental evidence. *Evolution*, *56*(12), 2347–2358.

DellaTogna, G., Trudeau, V. L., Gratwicke, B., Evans, M., Augustine, L., Chia, H., … Comizzoli, P. (2017). Effects of hormonal stimulation on the concentration and quality of excreted spermatozoa in the critically endangered Panamanian golden frog (*Atelopus zeteki*). *Theriogenology*, *91*, 27–35. https://doi.org/10.1016/j.theriogenology.2016.12.033

Ficetola, G. F., Garner, T. W. J., Wang, J. L., & De Bernardi, F. (2011). Rapid selection against inbreeding in a wild population of a rare frog. *Evolutionary Applications*, *4*(1), 30–38. https://doi.org/10.1111/j.1752-4571.2010.00130.x

Gage, M. J., Surridge, A. K., Tomkins, J. L., Green, E., Wiskin, L., Bell, D. J., & Hewitt, G. M. (2006). Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. *Current Biology*, *16*(6), 612–617.

Gomendio, M., Cassinello, J., & Roldan, E. (2000). A comparative study of ejaculate traits in three endangered ungulates with different levels of inbreeding: Fluctuating asymmetry as an indicator of reproductive and genetic stress. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1446), 875–882.

Graham, K. M., Kouba, A. J., Langhorne, C. J., Marcec, R. M., & Willard, S. T. (2016). Biological sex identification in the endangered dusky gopher frog (*Lithobates sevosa*): A comparison of body size measurements, secondary sex characteristics, ultrasound imaging, and urinary hormone analysis methods. *Reproductive Biology and Endocrinology*, *14*(1), 41.

Graham, K. M., Langhorne, C. J., Vance, C. K., Willard, S. T., & Kouba, A. J. (2018). Ultrasound imaging improves hormone therapy strategies for induction of ovulation and in vitro fertilization in the endangered dusky gopher frog (*Lithobates sevosa*). *Conservation Physiology*, *6*(1), Coy020. https://doi.org/10.1093/conphys/coy020

Hedrick, P. W. (1994). Purging inbreeding depression and the probability of extinction: Full-sib mating. *Heredity*, *73*(4), 363–372.

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, *31*(1), 139–162.

Hillis, D. M., & Wilcox, T. P. (2005). Phylogeny of the New World true frogs (*Rana*). *Molecular Phylogenetics and Evolution*, *34*(2), 299–314.

Hinkson, K. M., Henry, N. L., Hensley, N. M., & Richter, S. C. (2016). Initial founders of captive populations are genetically representative of natural populations in critically endangered dusky gopher frogs, *Lithobates sevosus*. *Zoo Biology*, *35*(5), 378–384. https://doi.org/10.1002/zoo.21309

Hinkson, K. M., & Richter, S. C. (2016). Temporal trends in genetic data and effective population size support efficacy of management practices in critically endangered dusky gopher frogs (*Lithobates sevosus*). *Ecology and Evolution*, *6*(9), 2667–2678. https://doi.org/10.1002/ece3.2084

Hoffman, E. A., Schueler, F. W., & Blouin, M. S. (2004). Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. *Evolution*, *58*(11), 2536–2545.

IUCN. (2019). IUCN red list of threatened species. Version 2019-2. Retrieved from www.iucnredlist.org

Januskauskas, A., Johannisson, A., & Rodriguez-Martinez, H. (2003). Subtle membrane changes in cryopreserved bull semen in relation

with sperm viability, chromatin structure, and field fertility. *Theriogenology*, *60*(4), 743–758.

Jiménez, J. A., Hughes, K. A., Alaks, G., Graham, L., & Lacy, R. C. (1994). An experimental study of inbreeding depression in a natural habitat. *Science*, *266*(5183), 271–273.

Johnson, K., Butts, I. A., Smith, J., Wilson, C., & Pitcher, T. E. (2015). The effects of inbreeding on sperm quality traits in captive-bred lake trout, *Salvelinus namaycush* (Walbaum, 1972). *Journal of Applied Ichthyology*, *31*, 62–70.

Johnson, W. E., Onorato, D. P., Roelke, M. E., Land, E. D., Cunningham, M., Belden, R. C., … Shindle, D. (2010). Genetic restoration of the Florida panther. *Science*, *329*(5999), 1641–1645.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241.

Kouba, A. J., & Vance, C. K. (2009). Applied reproductive technologies and genetic resource banking for amphibian conservation. *Reproduction, Fertility, and Development*, *21*(6), 719–737.

Kouba, A. J., Vance, C. K., & Willis, E. L. (2009). Artificial fertilization for amphibian conservation: Current knowledge and future considerations. *Theriogenology*, *71*(1), 214–227. https://doi.org/10.1016/j.theriogenology.2008.09.055

Lahnsteiner, F., Berger, B., Weismann, T., & Patzner, R. (1998). Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. *Aquaculture*, *163*(1-2), 163–181.

Larsen, L. K., Pelabon, C., Bolstad, G., Viken, Å., Fleming, I., & Rosenqvist, G. (2011). Temporal change in inbreeding depression in life-history traits in captive populations of guppy (*Poecilia reticulata*): Evidence for purging? *Journal of Evolutionary Biology*, *24*(4), 823–834.

Lee, J. R. (2013). Mississippi Gopher frog translocation. Unpubished Report to the U.S. Fish and Wildlife Service.

Levitan, D. R. (2000). Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1443), 531–534.

Locatello, L., Bertotto, D., Cerri, R., Parmeggiani, A., Govoni, N., Trocino, A., … Mordenti, O. (2018). Sperm quality in wild caught and farmed males of the European eel (*Anguilla anguilla*). *Animal Reproduction Science*, *198*, 167–176.

Malo, A. F., Garde, J. J., Soler, A. J., García, A. J., Gomendio, M., & Roldan, E. R. (2005). Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. *Biology of Reproduction*, *72*(4), 822–829.

Mehlis, M., Rahn, A. K., & Bakker, T. C. (2015). Sperm quality but not relatedness predicts sperm competition success in threespine sticklebacks (*Gasterosteus aculeatus*). *BMC Evolutionary Biology*, *15*(1), 74.

Michalczyk, Ł., Martin, O. Y., Millard, A. L., Emerson, B. C., & Gage, M. J. (2010). Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1699), 3483–3491.

Morato, R., Conforti, V., Azevedo, F., Jacomo, A., Silveira, L., Sana, D., … Barnabe, R. (2001). Comparative analyses of semen and endocrine characteristics of free-living versus captive jaguars (*Panthera onca*). *Reproduction*, *122*(5), 745–751.

Parris, M. J., & Redmer, M. (2005). Crawfish frog (*Rana areolata*). In M. J. Lannoo (Ed.), *Amphibian declines: The conservation status of United States species* (pp. 526–528). Berkeley, CA: University of California Press.

Pechmann, J. H. K., & Tupy, J. A. (2013). Report of scientific collecting activity 1 October 2012-30 September 2013 (p. 2). Jackson, Mississippi: Unpublished permit report submitted to the Mississippi Museum of Natural Science/Mississippi Department of Wildlife, Fisheries, and Parks.

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Richter, S. C., Crother, B. I., & Broughton, R. E. (2009). Genetic consequences of population reduction and geographic isolation in the critically endangered frog, *Rana sevosa*. *Copeia*, *4*, 799–806. https://doi.org/10.1643/ch-09-070

Richter, S. C., & Nunziata, S. O. (2014). Survival to metamorphosis is positively related to genetic variability in a critically endangered amphibian species. *Animal Conservation*, *17*(3), 265–274. https://doi.org/10.1111/acv.12088

Richter, S. C., & Seigel, R. A. (2002). Annual variation in the population ecology of the endangered Gopher frog, *Rana sevosa* Goin and Netting. *Copeia*, *2002*(4), 962–972.

Richter, S. C., Young, J. E., Johnson, G. N., & Seigel, R. A. (2003). Stochastic variation in reproductive success of a rare frog, *Rana sevosa*: Implications for conservation and for monitoring amphibian populations. *Biological Conservation*, *111*(2), 171–177. https://doi.org/10.1016/s0006-3207(02)00260-4

Richter, S. C., Young, J. E., Seigel, R. A., & Johnson, G. N. (2001). Postbreeding movements of the dark gopher frog, *Rana sevosa* Goin and Netting: Implications for conservation and management. *Journal of Herpetology*, *35*(2), 316–321. https://doi.org/10.2307/1566123

Roelke, M. E., Martenson, J. S., & O'Brien, S. J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, *3*(6), 340–350.

Roff, D. A. (2002). Inbreeding depression: Tests of the overdominance and partial dominance hypotheses. *Evolution*, *56*(4), 768–775.

Schwartz, M. K., & Mills, L. S. (2005). Gene flow after inbreeding leads to higher survival in deer mice. *Biological Conservation*, *123*(4), 413–420.

Simmons, L. (2011). Inbreeding depression in the competitive fertilization success of male crickets. *Journal of Evolutionary Biology*, *24*(2), 415–421.

Sisson, M. A. (2004). Gopher frog reproduction and conservation in Mississippi. Unpublished Report to the U.S. Fish and Wildlife Service.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783–1786. https://doi.org/10.1126/science.1103538

Swannack, T. M., Grant, W. E., & Forstner, M. R. (2009). Projecting population trends of endangered amphibian species in the face of uncertainty: A pattern-oriented approach. *Ecological Modelling*, *220*(2), 148–159.

U.S. Fish and Wildlife Service. (2014). Technical/agency draft recovery plan for the dusky gopher frog (*Rana sevosa*). Atlanta, GA.

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences*, *105*(Suppl 1), 11466–11473. https://doi.org/10.1073/pnas.0801921105

Wang, J., Hill, W. G., Charlesworth, D., & Charlesworth, B. (1999). Dynamics of inbreeding depression due to deleterious mutations in small populations: Mutation parameters and inbreeding rate. *Genetics Research*, *74*(2), 165–178.

Wildt, D., Baas, E., Chakraborty, P., Wolfle, T., & Stewart, A. (1982). Influence of inbreeding on reproductive performance, ejaculate quality and testicular volume in the dog. *Theriogenology*, *17*(4), 445–452.

Young, J. E., & Crother, B. I. (2001). Allozyme evidence for the separation of *Rana areolata* and *Rana capito* and for the resurrection of *Rana sevosa*. *Copeia*, *2*, 382–388. https://doi.org/10.1643/0045-8511(2001)001[0382:aeftso]2.0.co;2

Yuan, Z.-Y., Zhou, W.-W., Chen, X., Poyarkov, J. N. A., Chen, H.-M., Jang-Liaw, N.-H., … Che, J. (2016). Spatiotemporal diversification of the true frogs (Genus *Rana*): A historical framework for a widely studied group of model organisms. *Systematic Biology*, *65*(5), 824–842. https://doi.org/10.1093/sysbio/syw055

Zajitschek, S., Lindholm, A., Evans, J., & Brooks, R. (2009). Experimental evidence that high levels of inbreeding depress sperm competitiveness. *Journal of Evolutionary Biology*, *22*(6), 1338–1345.

Zupa, R., Fauvel, C., Mylonas, C., Pousis, C., Santamaria, N., Papadaki, M, … Passantino, L. (2017). Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, *Seriola dumerili* (Risso, 1810). *Journal of Animal Science*, *95*(9), 4085–4100.

How to cite this article: Hinkson KM, Poo S. Inbreeding depression in sperm quality in a critically endangered amphibian. *Zoo Biology*. 2020;1–8.
https://doi.org/10.1002/zoo.21538