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First natural breeding of the endangered dusky gopher frog (*Lithobates sevosus*) in captivity

Steven B. Reichling | Jessica Cantrell | Elizabeth A. Roznik | Allison Bogisich | Sinlan Poo

Department of Conservation and Research, Memphis Zoo, Memphis, Tennessee, USA

Correspondence
Steven B. Reichling, Department of Conservation and Research, Memphis Zoo, 2000 Prentiss Place, Memphis, TN 38112, USA.
Email: sreichling@memphiszoo.org

Abstract

To date, all captive breeding of the dusky gopher frog, *Lithobates sevosus*, a federally listed endangered species, has been accomplished using in vitro fertilization (IVF). Here, we describe multievent and highly fecund captive reproduction of dusky gopher frogs driven solely by natural environmental factors. Six pairs of *L. sevosus* were kept in a 3.7 × 4.4 m^2 outdoor enclosure designed to resemble their natural breeding habitat, which included a pool and three artificial burrows. Modifications to the enclosure that simulated temperatures and conditions within their natural range during winter were added in October and removed in late February. Following a warm, rainy period, five egg masses were laid between March 5 and 11, 2020. The number of oocytes per egg mass was 2300 ± 409 (range = 1341–3565), with the total across all five egg masses being 11,501. Of these oocytes, the hatching rate was 68.58 ± 10.05% (range = 37.53%–95.59%), with a total of 7887 successful hatchlings overall. Clutch sizes were similar to those in the wild and greater than those typically produced using IVF; thus, natural breeding can substantially increase the number of frogs available for reintroduction programs. Although assisted reproductive technologies such as IVF will continue to be useful for ensuring the success of strategic genetic pairings of captive *L. sevosus*, the new tool of non-assisted reproduction in specifically designed outdoor enclosures is an important advancement for the conservation and recovery of this endangered species.

KEYWORDS
assisted reproduction technologies, captive breeding, dusky gopher frog, endangered

1 | INTRODUCTION

Lithobates sevosus is a critically endangered amphibian endemic to fire-maintained sandy longleaf pine (*Pinus palustris*) uplands along the central Gulf Coast of the United States. Historical records exist from southwest Alabama through the Mississippi coastal plain west as far as the Florida Parishes (Herpnet, 2013). By 2001, populations had declined sharply, due primarily to habitat degradation, and only two known breeding populations totaling approximately 100 individuals remained in Jackson and Harrison counties in Mississippi (United States Fish and Wildlife Service [USFWS], 2015). Consequently, the species was listed as Endangered (USFWS, 2001) and a federal recovery plan focusing on preserving

existing populations and establishing new ones through translocation and reintroduction was put in place (USFWS, 2015). The Dusky Gopher Frog Species Survival Program (SSP®) began in 2001, with the import of 40 larvae from the population in Harrison County. The purpose of the SSP® is to support recovery by maintaining a genetically robust assurance colony in captivity and to augment conservation through the reintroduction of captive-bred frogs onto suitable unoccupied habitats.

To date, captive breeding of *L. sevosus* has only been accomplished with in vitro fertilization (IVF) techniques. During the first 4 years of the SSP®, efforts at four zoos were made to stimulate natural outdoor breeding using environmental manipulations that mimicked in situ breeding-season conditions. These previous attempts shared several characteristics including small and shallow water reservoirs, which were kept filled for the duration of occupancy by frogs, small shelter refuges, which did not resemble the structure of favored natural refugia, and placement of enclosures in locations where the activities of zoo staff presented a potential stressor. Although the replication of natural environmental triggers is successful for breeding many captive anurans, *L. sevosus* failed to respond, and the captive population remained at less than 100 individuals through 2008 (Reichling & Schad, 2012), maintained solely by consistent wild imports. With population growth static and dominated by older individuals nearing senescence, the persistence of the captive population was in doubt. As natural breeding efforts continued to fail, biologists at the Memphis Zoo developed an IVF protocol that was effective for this taxon (Kouba et al., 2012) and in 2008 the first reproduction of captive *L. sevosus* was achieved. Over the next 5 years, IVF reproduction produced a 91% population growth rate (mean $\lambda = 1.906$), thus saving the SSP® from collapse (Reichling & Schad, 2012). Since the advent of the IVF protocol, thousands of *L. sevosus* have been produced at SSP facilities (Reichling & Groome-Bryan, 2018). However, the procedure is complicated and requires experience to conduct successfully, fecundity and fertilization rates are low, and periodic handling involved during the process is arguably stressful for the frogs. Therefore, our goal was to facilitate successful reproduction in captivity by designing an outdoor enclosure that better mimics the conditions experienced by wild frogs, to stimulate breeding. Although previous attempts at facilitating natural breeding failed, we improved on those methods by modifying the enclosure temperature and water level across seasons, constructing burrow refuges more similar to those in native habitat, and situating the pond in a quieter area. Here we describe robust, multievent and highly fecund captive reproduction of *L. sevosus* through natural amplexus driven solely by natural environmental factors.

2 | OUTDOOR ENCLOSURE AND HUSBANDRY

Frogs were kept in a $3.7 \times 4.4\ m^2$ outdoor enclosure on the grounds of the Memphis Zoo (Memphis, TN), consisting of 13 mm plywood walls set 0.3 m below ground surface and extending 0.9 m above ground. Approximately centered within the enclosure was a depression $3.5 \times 2.0\ m^2$ at the widest span with a maximum depth of ~0.8 m and one end declining gradually to the deepest point (Figure 1). This depression was lined

FIGURE 1 Outdoor enclosure at the Memphis Zoo designed to simulate the microhabitat and environmental conditions occurring at natural breeding sites for the dusky gopher frog

with synthetic rubber polymer and served as a spawning pond. When filled, water quality was maintained with an ornamental pond pump and filter purchased from a hardware store. An 8 cm overlay of sand was spread over the ground to provide a more natural environment for the frogs, and the terrestrial portions were planted with herbaceous vegetation similar in its sparse density to the groundcover typical of longleaf pine uplands. The pond itself was furnished with potted emergent vegetation that either occurred naturally within the depressional wetland habitat of *L. sevosus* (*Eupatorium perfoliatum*, *Aristida stricta*) or were aquatic plants suitable for egg mass deposition (*Equisetum hyemale*). The enclosure received direct sunlight from mid-morning to late afternoon from spring through fall, and briefer midday sun through the winter months. To discourage small predators such as snakes, the top of the enclosure was covered with commercial shade cloth (60%).

Three artificial burrows were placed within the outdoor enclosure. The burrows were constructed of 16-quart coolers, which measured $36 \times 30 \times 20\ cm^3$. A hole ~10 cm in diameter was cut into the side of each cooler, and a corrugated plastic drainpipe was attached to provide a tunnel entrance ~71 cm into the burrow. The coolers were buried 20–30 cm into the ground and covered with a thin layer of sand (~5 cm). Crickets (30–40, twice per week), which had been fed a commercial fortified diet and dusted with vitamin-mineral powder, were cast into the enclosure for ad lib consumption, and the diet was supplemented by native invertebrates that entered the enclosure.

Frogs were introduced into the enclosure in July 2019. In late October, as nighttime temperatures began to drop to near freezing, we winterized the enclosure, so the frogs could remain outdoors. Additional sand was added over the artificial burrows, and a Kane™ heat mat (Kane Manufacturing Company Inc.) was placed above the location of each cooler. The thermostat control for the mats was set

at 37.2°C. Polyethylene plastic sheeting was placed over the entire enclosure (over a canopy tent to allow water runoff) to create a mild greenhouse effect. With these modifications, we were able to increase the internal temperature of the burrows and keep temperatures consistently warmer than the external enclosure temperatures (see Section 3). The plastic sheeting was removed in mid-February in anticipation of rainfall events that might trigger reproduction, and all winter modifications were removed in late February.

The pond within the enclosure was drained down to a few inches and allowed to dry in October. The pond was filled again in December to mimic the natural cycles that act as environmental cues for breeding in the native habitat of dusky gopher frogs. The pond pump was removed from October to March to eliminate possible disruptions of breeding behaviors.

3 | ENVIRONMENTAL CONDITIONS

We monitored air temperatures inside and outside the enclosure and within all three artificial burrows from November 2019 through March 2020. Outside temperatures were recorded every 30 min using Thermochron iButton dataloggers (Maxim Integrated; ±0.5°C accuracy). All other temperatures were recorded every 30 min using HOBO dataloggers (MX2303 and MX2304; Onset Computer Corporation; ±0.2°C accuracy). Temperatures inside the enclosure were recorded directly with a datalogger mounted 13 cm above the ground on the inner enclosure wall. Burrow temperatures were measured using probes attached to dataloggers that were inserted into the deepest part of the burrows. Temperature data were automatically transmitted from the HOBO dataloggers to the cloud-based HOBOlink software by an MX Gateway (MXGTW1; software and device manufactured by Onset Computer Corporation), which allowed us to monitor temperatures in real-time and ensure that burrow temperatures remained appropriate. We also downloaded daily rainfall data recorded by the nearest weather station at the Memphis International Airport (11 km away) from the National Oceanic and Atmospheric Administration.

During the period that the enclosure was monitored, air temperatures outside the enclosure ranged from −8.2 to 37.8°C, and air temperatures within the enclosure ranged from −1.8 to 33.3°C. Burrow temperatures remained more stable than enclosure temperatures, at an average of 11.8°C (range = 6–15.4°C). Frog breeding occurred on four nights within a week-long period: egg masses were found on March 5, 6, 10, and 11, 2020. This breeding period began immediately following 3 days of consistently warm temperatures (daily minimum = 7.8–13.3°C, daily maximum = 20.0–22.2°C) with a total of 13.5 mm of rainfall that fell over these 3 days (Figure 2). This warm and rainy period was preceded by a sharp warming trend over the previous 4 days without rain, starting with a day that ranged in temperature from 0.6 to 7.2°C (Figure 2). During oviposition days daily minimum temperatures averaged 9.4°C (range = 5–13.3°C) and daily maximum temperatures averaged 17.8°C (range = 13.9–20.0°C; Figure 2a). A total of 36 mm of rainfall occurred over 3 days (March 9–11, 2020) during the breeding period (Figure 2b). In the wild, breeding in *L. sevosus* and their congeneric species *L. capito* typically occurs from December through March after rains often associated with passing cold fronts (Jensen et al., 2003; Palis, 1998; Young, 1997). Although frogs in our enclosure did not need to make long breeding migrations to the pond, the environmental conditions immediately preceding and during the breeding period closely resembled those associated with breeding in the wild.

4 | REPRODUCTION AND DEVELOPMENT

4.1 | Adult frogs

The pond enclosure held 12 adult dusky gopher frog individuals, including six females and six males. All individuals were adults raised by the Dallas Zoo from wild-collected eggs in 2016–2017 from Glen's Pond (Harrison County, Mississippi). Adults were transferred to the Memphis Zoo in 2018. Two males and two females were 3 years old at the time of the breeding event, and the remaining eight individuals were 4 years old. Postbreeding mass of females and males were 72 ± 5 and 48 ± 4 g (ranges = 56–87 and 33–64 g), respectively. Snout–vent lengths of females and males were 88 ± 3 and 78 ± 2 mm (range = 84–95 and 71–87 mm), respectively. All values are presented as mean ± standard error.

4.2 | Oviposition and monitoring

Five egg masses were laid between March 5 and 11, 2020 (Figure 3). Eggs masses were removed from the pond within 24–48 h of oviposition and brought into an indoor laboratory environment. Each egg mass was placed in a 38-L glass aquarium (51 cm *L* × 25 cm *W* × 31 cm *H*), with an air-stone and plastic aquatic vegetation, exposed to a 12–12 light–dark cycle with ultraviolet B (UV-B) lights and kept at room temperature. Egg masses were monitored daily for embryonic development and hatching. Unfertilized or dead embryos were manually removed from the aquaria upon discovery to prevent fungal growth from infecting the remaining embryos.

4.3 | Fertilization and hatching success

The average number of oocytes per egg mass was 2300 ± 409 (range = 1341–3565), with the total across all five egg masses being 11,501 (Table 1). Of these oocytes, the hatching rate was 68.58 ± 10.05% (range = 37.53%–95.59%), with a total of 7887 successful hatchlings overall. The embryonic period for egg masses ranged from 4 to 5 days, with embryos continuing to hatch for an additional 4–6 days after the first hatchling was observed.

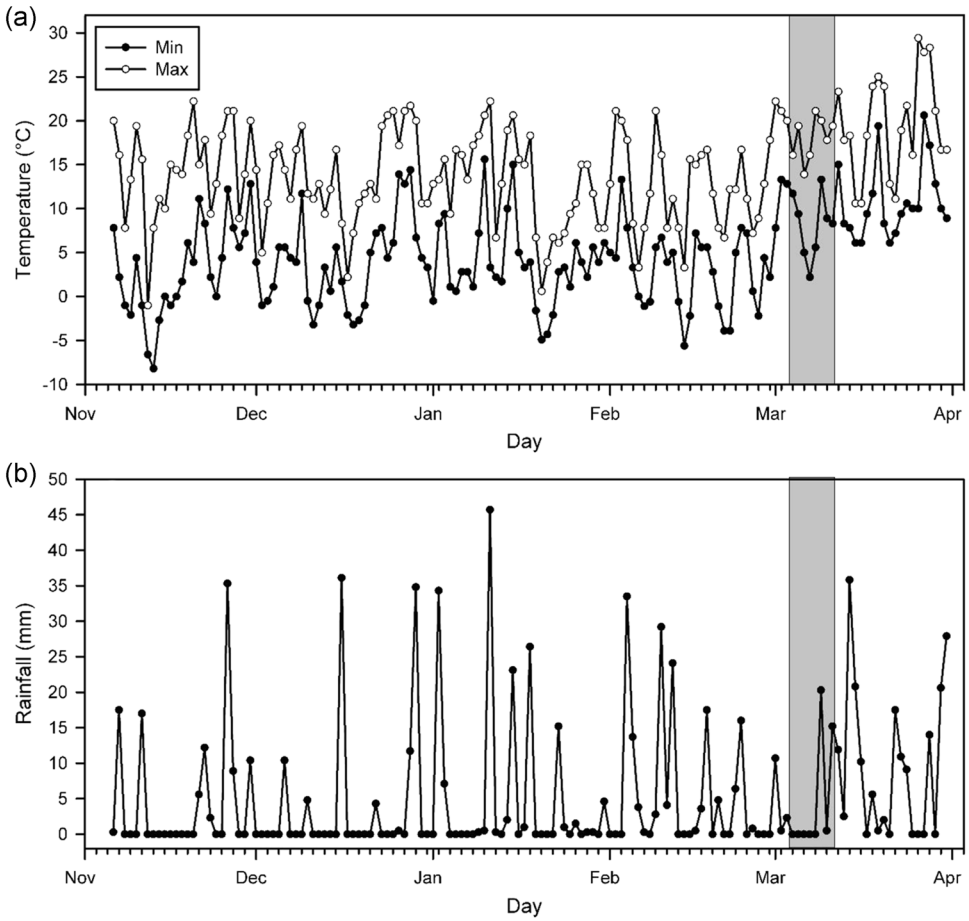

FIGURE 2 Environmental conditions recorded from November 2019 through March 2020 outside the frog enclosure, including (a) daily minimum and maximum temperatures, and (b) daily rainfall. Shaded boxes indicate the breeding period

FIGURE 3 One of five clutches laid on March 6, 2020, the result of breeding of dusky gopher frogs in response to natural environmental conditions

5 | DISCUSSION

The implications for the conservation of *L. sevosus* provided by non-assisted reproduction are significant. An IVF reproduction at the Memphis Zoo in 2020 yielded an average clutch size per female that was approximately 61% of natural in situ clutch size (Richter et al., 2003), and only 27% of the yield from the 2020 captive nonassisted breeding. Four out of the five nonassisted clutches fell well within the range (500–2800) of egg numbers reported for natural in situ reproduction (Richter & Seigel, 2002), with the fifth clutch exceeding the previously reported range. However, the average clutch size was more than two times higher in nonassisted captive breeding compared to in situ natural breeding (2300 and 1134, respectively; Richter et al., 2003). In anurans, female fecundity is often directly related to size and body mass (Pereira & Maneyro, 2012; Prado & Haddad, 2005). Wild postbreeding masses of female ($N = 5$) and male ($N = 7$) *L. sevosus* have been recorded as 49 ± 4 g (ranges = 39–61 g) and 44 ± 2 g (ranges = 35–52 g), respectively (Richter et al., 2001). Comparatively, the postbreeding captive-reared *L. sevosus* females were 23 g (47%) heavier on average compared to their wild counterparts, while captive males were 4 g (9%) heavier than their wild counterparts. Increased feedings and care in captivity in anurans are known to have a

Egg mass number	Oviposition date	Total oocytes	Total hatchlings	Embryonic period (days)	Hatching success (%)
1	3/5	3565	1338	5	37.53
2	3/6	1495	1292	4	86.42
3	3/6	2390	1806	4	75.56
4	3/10	2710	2588	5	95.50
5	3/11	1341	863	5	64.35

TABLE 1 Oviposition and hatching of *Lithobates sevosus* egg masses laid in a man-made pond

positive effect on the body condition and reproductive output of females (Girish & Saidapur, 2000). Consequently, the larger clutch size observed in captive *L. sevosus* may be a result of better body conditions of captive female individuals. Moreover, by head-starting captive offspring and reducing their mortality rate during the more vulnerable stages of development, captive-breeding programs can increase their impact on in situ populations (Germano & Bishop, 2009; Kissel et al., 2014). In *L. sevosus*, egg and larval mortality account for a significant portion of population loss, with only 2.14% survivorship to metamorphosis (Richter et al., 2003). As a result of the nonassisted breeding events reported here, a total of 2384 individuals from Memphis were released onto restored habitat in 2020, compared to 82, 344, and 667 IVF produced individuals during 2017, 2018, and 2019, respectively. Releasing a quantity of frogs sufficient to build a reproductively mature age class, given the expectation of significant mortality among juveniles (Roznik & Reichling, 2021), is an important part of a successful reintroduction effort. As such, the increased captive fecundity is very helpful to the potential recovery of this species.

Our outdoor enclosure successfully facilitated the natural breeding of *L. sevosus* and can be implemented at other breeding facilities in or near their historic range, where environmental conditions are suitable. Maintaining frogs in outdoor enclosures year-round can provide additional benefits that are not available to frogs kept in indoor facilities, including exposure to more space, UV light, and natural climatic conditions than is often available in indoor enclosures. Based on our findings, we believe that larger outdoor enclosures, with more terrestrial space, a larger breeding pond, and more burrows, could be used to sustain a larger number of adult frogs and alleviate the need for assisted breeding in *L. sevosus*. While results from the current study are encouraging, captive colonies face additional pressures, such as low sperm quality and inbreeding depression (Hinkson & Poo, 2020; Poo & Hinkson, 2019). The genetic diversity of the SSP® population is managed through a demographic analysis conducted at 3-year intervals by the Association of Zoos and Aquarium's Population Management Center at the Lincoln Park Zoo in Chicago. These population reviews result in specific pairing recommendations of individual animals. The use of IVF will continue to be the most dependable method for ensuring that important genetic pairings are accomplished precisely and successfully each year, but for producing large numbers of frogs for release into the wild, the new tool of nonassisted reproduction in specifically designed outdoor enclosures is an important advancement for the conservation and recovery of *L. sevosus*.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Elizabeth A. Roznik http://orcid.org/0000-0002-3123-2096
Allison Bogisich http://orcid.org/0000-0003-0555-3280

REFERENCES

Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology: The Journal of the Society for Conservation Biology*, *23*, 7–15.

Girish, S., & Saidapur, S. K. (2000). Interrelationship between food availability, fat body, and ovarian cycles in the frog, *Rana tigrina*, with a discussion on the role of fat body in anuran reproduction. *Journal of Experimental Zoology*, *286*, 487–493.

Herpnet. (2013). *Catalog records for Rana capito*. Retrieved May 3, 2013, from http://www.herpnet2.org

Hinkson, K. M., & Poo, S. (2020). Inbreeding depression in sperm quality in a critically endangered amphibian. *Zoo Biology*, *39*, 197–204.

Jensen, J. B., Bailey, M. A., Blankenship, E. L., & Camp, C. D. (2003). The relationship between breeding by the gopher frog, *Rana capito* (Amphibia: Ranidae) and rainfall. *American Midland Naturalist*, *150*, 185–190.

Kissel, A. M., Palen, W. J., Govindarajulu, P., & Bishop, C. A. (2014). Quantifying ecological life support: The biological efficacy of alternative supplementation strategies for imperiled amphibian populations. *Conservation Letters*, *7*, 441–450.

Kouba, A., Willis, E., Vance, C., Hasenstab, S., Reichling, S., Krebs, J., Linhoff, N., Snozaq, M., Langhorne., & Germano, J. (2012). Development of assisted reproduction technologies for the endangered Mississippi gopher frog (*Rana sevosa*) and sperm transfer for in vitro fertilization. *Reproduction, Fertility, and Development*, *24*, 170–170. https://doi.org/10.1071/RDv24n1Ab116

Palis, J. G. (1998). Breeding biology of the gopher frog, *Rana capito*, in western Florida. *Journal of Herpetology*, *32*, 217–223.

Pereira, G., & Maneyro, R. (2012). Size-fecundity relationships and reproductive investment in females of *Physalaemus riograndensis* Milstead, 1960 (Anura, Leiuperidae) in Uruguay. *The Herpetological Journal*, *22*, 145–150.

Poo, S., & Hinkson, K. M. (2019). Applying cryopreservation to anuran conservation biology. *Conservation Science and Practice*, *1*, e91.

Prado, C. P. A., & Haddad, C. F. B. (2005). Size–fecundity relationships and reproductive investment in female frogs in the Pantanal, South-Western Brazil. *The Herpetological Journal*, *15*, 181–189.

Reichling, S., & Groome-Bryan, C. (2018). Population analysis & breeding and transfer plan. Dusky gopher frog (Lithobates (Rana) sevosa). AZA Species Survival Plan® Yellow. Population Management Center, Lincoln Park Zoo.

Reichling, S., & Schad, K. (2012). *Population analysis & breeding and transfer plan. Dusky gopher frog (Rana sevosa). AZA Species Survival Plan® Yellow*. Population Management Center, Lincoln Park Zoo.

Richter, S. C., Young, J. E., Johnson, 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*, *11*, 171–172.

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*, 316–321.

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

Roznik, E. A., & Reichling, S. (2021). Survival, movements and habitat use of captive-bred and reintroduced dusky gopher frogs. *Animal Conservation*, *24*, 51–63. https://doi.org/10.1111/acv.12599

United States Fish and Wildlife Service (USFWS). (2001). Endangered and threatened wildlife and plants; final rule to list the Mississippi gopher frog distinct population segment of dusky gopher frog as endangered. *Federal Register*, *66*, 62993–63001.

United States Fish and Wildlife Service (USFWS). (2015). *Dusky gopher frog (Rana sevosa) recovery plan*.

Young, J. E. (1997). *Taxonomic status of Rana capito and Rana sevosa: Reproduction and movements of Rana sevosa in Mississippi* (M.S. thesis). Southeastern Louisiana University.

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