






## CONTRIBUTED PAPER

# Post-release comparisons of amphibian growth reveal challenges with sperm cryopreservation as a conservation tool

Sinlan Poo<sup>1,2</sup>  | Allison Bogisich<sup>1</sup>  | Mariah Mack<sup>3</sup>  | Bryan K. Lynn<sup>4</sup>  | Anne Devan-Song<sup>4</sup> 

<sup>1</sup>Department of Conservation and Research, Memphis Zoological Society, Memphis, Tennessee, USA

<sup>2</sup>Department of Biological Sciences, Arkansas State University, Jonesboro, Arkansas, USA

<sup>3</sup>Department of Biology, Southern Illinois University Edwardsville, Edwardsville, Illinois, USA

<sup>4</sup>Department of Integrative Biology, Oregon State University, Corvallis, Oregon, USA

## Correspondence

Sinlan Poo, Department of Conservation and Research, Memphis Zoological Society, 2000 Prentiss Pl, Memphis, TN 38112, USA.

Email: sheilapoo@gmail.com

## Funding information

Memphis Zoo Conservation Action Network, Grant/Award Number: 5813-6002

## Abstract

Conservation translocation using captive-bred individuals has become increasingly important for species restoration. Despite advancements in technologies for captive-breeding, such as gamete cryopreservation, it remains largely unknown if these artificially-produced offspring can be used to establish a viable wild population. Using an amphibian species with a stable conservation status (*Anaxyrus fowleri*), we measured the post-release growth and survivorship of offspring produced from frozen/thawed sperm and projected fecundity and population differences. Cryo-derived tadpoles and post-metamorphic toadlets were smaller than their natural counterparts. Model projections show that early-life differences in growth can scale up to substantial differences in final life fecundity and population trends. Our findings call for greater attention towards the differences between captive-bred and cryo-derived individuals compared to their natural counterparts, and the need for more investment into developing cryopreservation technologies that are viable at the population level for conservation translocation.

## KEYWORDS

*Anaxyrus fowleri*, assisted-reproductive technology, captive-breeding, captive-release, conservation translocation, reintroduction, reproductive physiology

## 1 | INTRODUCTION

In response to elevated rates of species extinction, interdisciplinary methods are urgently needed to prevent or mitigate further species loss (Blair et al., 2017; Clark et al., 2001). Thinking beyond the confines of traditional disciplines, these interdisciplinary, cross-agency collaborations aim to provide a more comprehensive plan for species conservation by leveraging the

resources and expertise of different fields (Marquardt et al., 2018).

An increasingly important branch of applied conservation biology that directly links ex situ and in situ efforts is that of conservation translocations (IUCN/SSC, 2013). Through the intentional moving of species, individuals can be introduced to reinforce declining populations or to establish new populations of extirpated species. These efforts have been amplified in response to extinction

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Conservation Science and Practice* published by Wiley Periodicals LLC on behalf of Society for Conservation Biology.

threats, with hundreds of conservation translocation programs spanning vertebrate and invertebrate animals (Bubac et al., 2019; Fischer & Lindenmayer, 2000). One of the pillars of conservation translocations is the establishment of a source population (IUCN/SSC, 2013). The number of individuals that are translocated or released can be a significant factor in determining the success of a program (Germano & Bishop, 2009). For species facing extinction threats, insurance populations are often set up in captivity, as a stock for future conservation efforts (Harding et al., 2016). In recent years, advancements in gamete cryobiology and biobanking have been particularly novel fields of development within captive programs (e.g., Comizzoli & Holt, 2019; Silla & Byrne, 2019), because of their potential to contribute to conservation translocations through the propagation of genetically valuable, at-risk species.

For a successful conservation translocation to occur, biological, logistical, ecological, political, public support, and financial factors need to be considered (Berger-Tal et al., 2020; Bubac et al., 2019). A critical obstacle that many programs face is not knowing if translocated individuals are able to establish themselves in their new environment (Msuya & Mohamed, 2019). Oftentimes, translocation programs lack the resources to monitor individuals post-release (Berger-Tal et al., 2020) or are only able to do so for a short period of time (<4 years, Bubac et al., 2019). While statistical models can be used to extrapolate the success of translocation efforts (Kissel et al., 2014), such models require life history data that are often difficult to obtain for rare species and existing models are largely based on information from natural populations. However, captive individuals can differ significantly from their natural counterparts in terms of growth, development, morphology, behavior, and other factors important to their reproduction and survivorship (Milot et al., 2013; Poo & Hinkson, 2020; Suboski & Templeton, 1989).

In the cases of gamete cryopreservation, in particular, a large gap remains between the theoretical impact this tool may have and its practical application in terms of conservation translocation. Though laboratory-based methods using cryopreserved gametes have been successful, we have yet to demonstrate successful establishment of artificially produced offspring in the wild. While undoubtedly a promising frontier for wildlife conservation, the viability of offspring that are captive-bred and cryo-derived individuals is virtually unknown (Poo & Hinkson, 2020). Consequently, the feasibility of cryopreservation for (re-)establishing a self-sustaining population or preserving ecological function in the wild is speculative at best. To assess the efficacy of cryopreservation as a conservation tool, we need to first (1) quantify the underlying differences between individuals produced via

cryopreserved gametes and those produced through natural mating, and (2) understand how these differences can scale up to affect growth rate, survival, final life fecundity, and population-level parameters.

In this study, we examined the growth and development of amphibians produced through cryopreserved sperm after they are released into their natural environment. Using a model species with a stable conservation status (Fowler's toad, *Anaxyrus fowleri*), we compared post-release growth and survivorship between cryo-derived and natural-bred tadpoles in a natural pond. We followed individuals through metamorphosis to determine carry-over effects from the larval to juvenile stage, and projected growth rate, average life fecundity, and population-level effects of cryopreservation. We used our empirical results of experimental and control body sizes and growth rates at metamorphosis to project the population size differences between cryo- and natural-bred populations over a 30-year span, employing a discrete post-breeding age-structured population model. Based on previous findings in controlled laboratory settings (Poo & Hinkson, 2020), we predict tadpoles derived from cryopreserved sperm would be at a disadvantage compared to their natural counterparts, and that this effect would carry over to the subsequent life-stages.

## 2 | METHODS

### 2.1 | Study species

We conducted this study from April to August 2020 in Shelby County, Tennessee. We collected 60 Fowler's toads by hand near permanent or ephemeral lentic habitats adjacent to forested areas during the breeding season and maintained all individuals in 10-gallon glass aquaria (51 cm L × 25 cm W × 31 cm H), with up to four toads per aquarium (Appendix 1).

### 2.2 | Reproduction via in vitro fertilization and natural breeding

Toads were randomly assigned to either the experimental (cryo-derived) group or control (natural-bred) group. For the experimental group, we conducted in vitro fertilization using cryopreserved sperm and fresh eggs, following methods from previous studies (Poo & Hinkson, 2020, Appendix 2). For the control group, we placed male-female pairs in individual plastic boxes (37 cm L × 20 cm W × 12 cm H) filled with 2 cm of aged tap water and allowed them to lay eggs naturally through male-female amplexus.

## 2.3 | Rearing and assessments

From each male–female pairing, we randomly selected 25 tadpoles upon hatching and placed them in a 5 L bucket filled with aged tap water. Tadpoles were reared in captivity until 10 days past oviposition and then released into a natural permanent pond (Meeman Biological Station, Tennessee). Each set of 25 tadpoles was released into a rectangular fine mesh enclosure (61 cm L × 61 cm W × 91 cm H) with 100 g of dry leaf litter, which allowed for nutrient exchange but protected tadpoles from predators, and floating cork barks for metamorphosing toadlets (Figure 1). Tadpole morphology was assessed at 10 days post-oviposition (prior to pond release) and at 20 days post-oviposition (10 days post-release). Total length, tail length, and body width of tadpoles were measured from dorsal photographs using the ImageJ (Rasband, 1997–2018). Upon reaching metamorphosis (Stage 46, Gosner, 1960), snout–vent length (SVL), mass, and age (days since oviposition) of toadlets were recorded.

## 2.4 | Statistical analyses

Assumptions of data normality were tested using the Shapiro–Wilk test. Effects of cryopreservation on offspring growth and development were tested using

generalized linear mixed models (GLMMs), with clutch identity included as a random variable to account for potential clutch effects. GLMM families were chosen based on data distribution. We tested the effects of cryopreservation on total length and tail length of tadpoles at 10 days post-oviposition using GLMMs with underlying beta distribution and body width using GLMM with underlying lognormal distributions. For tadpoles at 20 days post-oviposition, we tested total length, tail length, and body width using GLMMs with underlying lognormal distributions. We tested the effects of cryopreservation on SVL and mass at metamorphosis and larval stage duration using GLMMs with underlying Gaussian, beta, and lognormal distribution, respectively. We tested differences between survivorship to metamorphosis in cryo-derived and natural-bred clutches using Welch's *t* test. We conducted all statistical analyses in the R programming environment (v. 3.6.0) using a significance level of  $\alpha = .05$ . Means are presented with standard errors.

## 2.5 | Growth models

We used a von Bertalanffy equation (von Bertalanffy, 1938, Appendix 3) to model differences in cryo-derived and wild *A. fowleri* growth rates,

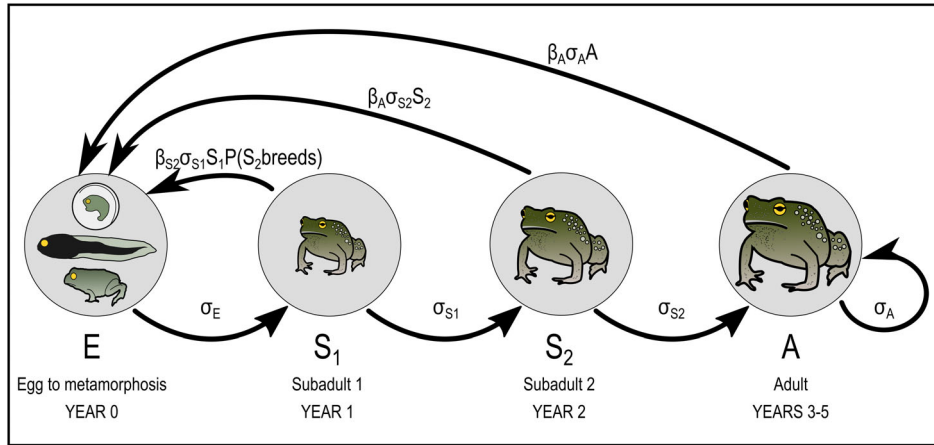
$$SVL_t = SVL_{\max} - (SVL_{\max} - SVL_0)e^{-k(t-t_0)}$$



FIGURE 1 Fine mesh enclosures were used to rear natural and cryo-derived *Anaxyrus fowleri* tadpoles in a natural pond (a). Enclosures were inoculated with leaf litter and included habitat both above and below the water in the vertical column (b)

where  $SVL_t$  is average body size at age  $t$ ,  $SVL_{max}$  is asymptotic maximum SVL,  $SVL_0$  is SVL at metamorphosis,  $t$  is age,  $t_0$  is age at metamorphosis and  $k$  is the growth coefficient. We obtained cryo-derived and natural  $SVL_0$  measurements from our empirical data. We obtained estimates of natural-bred growth efficient  $k$ ,  $SVL_{max}$ , and size at sexual

maturity from previous studies (Appendix 3). We estimated  $k$  for cryo-derived individuals based on the difference in growth rate between cryo-derived and natural tadpoles (Figure 3). Finally, we compared the proportional difference in growth across the maximum lifespan of a toad (5 years, Green & Middleton, 2013).



$$\Delta E = \beta_{S_2} \sigma_{S_1} S_1 P(S_2 \text{ breeds}) + \beta_A \sigma_{S_2} S_2 + \beta_A \sigma_A A$$

$$\Delta S_1 = E \sigma_E$$

$$\Delta S_2 = S_1 \sigma_{S_1}$$

$$\Delta A = S_2 \sigma_{S_2} + A \sigma_A$$

FIGURE 2 Discrete post-breeding age-structured population model of *Anaxyrus fowleri* using a 5-year lifespan, with sexual maturity reached at Year 2. This model assumed that not all Year 2 subadults breed, while all adults from ages 3 to 5 breed annually

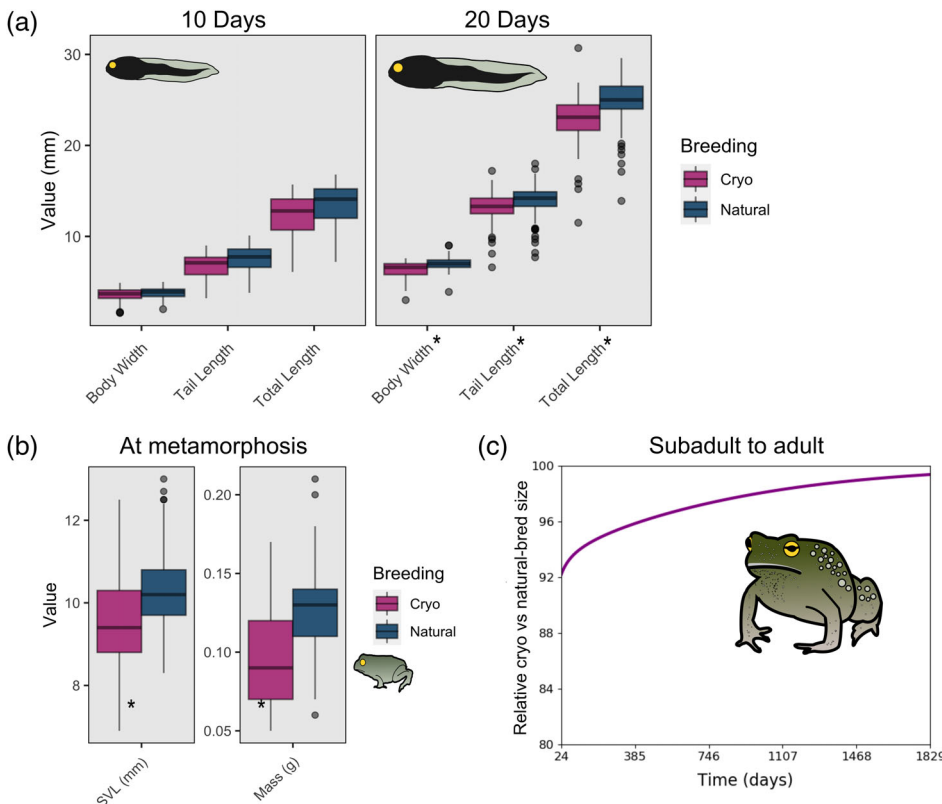
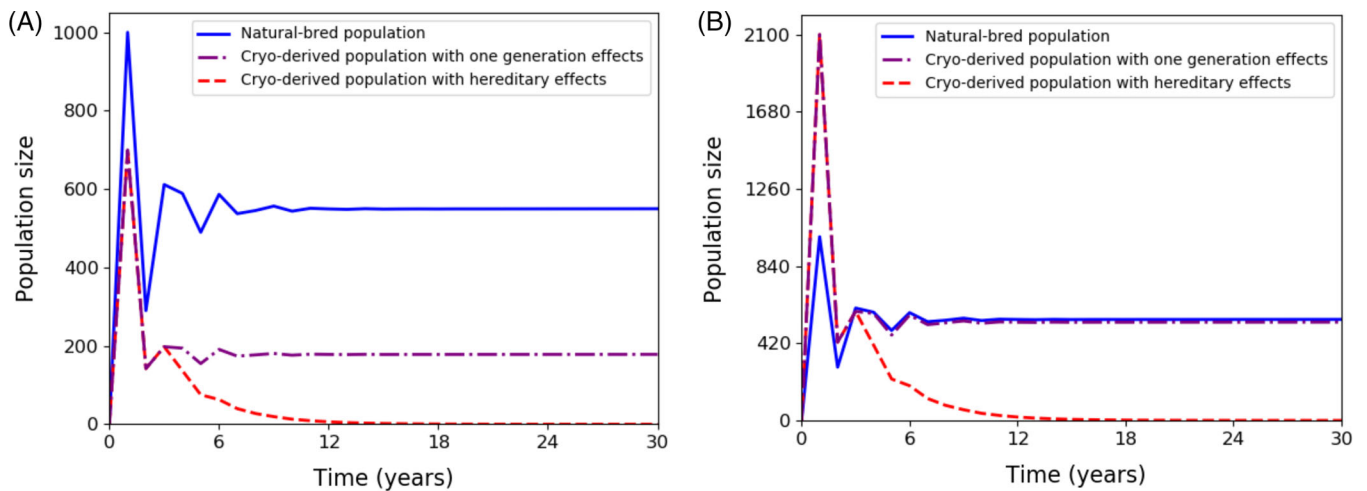


FIGURE 3 Relative sizes of cryo- vs. natural-bred *Anaxyrus fowleri* larvae 10- and 20-days post-oviposition (a) and at metamorphosis (b) from experimental results, and projected percentage size difference across a five-year lifespan between cryo- and natural-bred individuals based on the von Bertalanffy growth model (c). Significant differences are marked with an (\*) next to the variable name



**FIGURE 4** Estimated population size differences over 30 years for initial starting conditions at 1 million female *Anaxyrus fowleri* for all three scenarios (a), and an additional 2 million for only the cryo-derived scenarios (b)

## 2.6 | Population models

Age-structured population models are commonly used to predict population outcomes in a variety of species from plants (Kalisz & McPeck, 1992) to ungulates (White et al., 2018), and have been implemented in the study of several amphibians (Minin and Griffiths, 2011; Kissel et al., 2014; Vimercati et al., 2017). We used a discrete, deterministic, all-female post-breeding age-structured (Kendall et al., 2019; Otto & Day, 2007) population model (Figure 2), using survival and fecundity parameters from wild populations reported in the literature (Appendix 4). We chose a discrete model, rather than the more common continuous model, to reflect that this species does not breed continuously throughout the year, but instead has a distinct breeding season. Further, we take a deterministic approach, removing randomness from the simulations. While natural populations do face a lot of randomness with weather related events, predators, competition and landscape factors, we were particularly interested in seeing the effects of cryopreservation on the outcomes of the populations. Lastly, we chose to develop a post-breeding model (as opposed to a pre-breeding model) to incorporate a class which includes exclusively eggs to metamorphic toadlets, since the empirical aspects of this study focus on this stage of growth.

We estimated differences in cryo-derived versus natural population parameters based on effects of size at metamorphosis from our experimental data, using an initial starting condition of 1 million eggs for each population. We modeled population size across 30 years in three different scenarios: natural

populations, cryo-derived populations where effects of cryopreservation only last one generation, and cryo-derived populations where effects of cryopreservation are inherited across generations. We ran the same model with initial cryo-derived eggs starting at 3 million eggs, while keeping the natural-bred population at 1 million eggs. Finally, we compared the estimated average five-year individual fecundity of cryo-derived and natural-bred cohorts starting at one million eggs for each group.

## 3 | RESULTS

### 3.1 | Effects of cryopreservation

A total of five cryo-derived and eight natural-bred clutches were included in the study, with 25 randomly selected tadpoles per clutch ( $N = 13$  clutches, 325 tadpoles total). At the pre-release assessment (10 days post-oviposition), total length, tail length, and body width were not significantly different between cryo-derived and control tadpoles ( $N = 325$ , Figure 3a). However, at the post-release assessment (20 days post-oviposition), cryo-derived tadpoles were significantly smaller than control tadpoles in all three morphology measurements ( $N = 273$ , Figure 3a). Similarly, at metamorphosis, cryo-derived toadlets were smaller in SVL and in mass compared to their natural-bred counterparts ( $N = 242$ , Figure 3b). Duration of the larval stage was not significantly different from natural-bred individuals, nor was percent survival at metamorphosis (Appendix 5).

### 3.2 | Predicted carry-over effects of cryopreservation on growth rate, population, and average female final life fecundity

Our estimated differences in cryo-derived versus natural-bred *A. fowleri* growth indicated a slower growth rate and older age at sexual maturity for cryo-derived individuals (Figure 3c). Cryo-derived individuals were estimated to catch up in size with natural-bred individuals only towards the end of their lifespan (Figure 3c). These differences scaled up to population-level differences in three different scenarios modeled (natural, cryo-derived effects for one generation, and cryo-derived effects preserved across generations). Natural-bred populations were predicted to remain stable over 30 years, while populations with cryo-derived effects for only one generation remained stable but at lower population numbers than natural-bred populations (Figure 4). For populations where cryo-derived effects were preserved across generations, the post-metamorphic population was estimated to go extinct after approximately 17 years (Figure 4). Estimated average five-year fecundity was 1.99 eggs per individual for an initial cohort of 1 million natural-bred eggs, and 0.78 eggs per individual for an initial cohort of 1 million cryo-derived eggs.

## 4 | DISCUSSION

Through in situ monitoring, we provide the first insights into the post-release adaptiveness of amphibians propagated in captivity using cryopreserved sperm, and modeled how cryopreservation effects may scale up to population-level trends. We show that cryo-derived individuals had similar rates of survivorships at metamorphosis, but were at a disadvantage in terms of size and mass, compared to natural-bred individuals. This sub-lethal effect of cryopreservation is observed during the larval stage and is carried over beyond metamorphosis into the juvenile stage, resulting in smaller size at metamorphosis and projected stunted growth patterns into maturity. Our population modeling estimated smaller population sizes for cryo-bred frogs, and population extinction if cryo-derived effects are heritable. With few, if any, examples of in situ post-release monitoring of cryo-derived animals existing in the scientific literature, this study takes the first step in bridging the gap between the theoretical use of cryopreservation and the actual feasibility of these methods in situ. While cryopreservation can be invaluable in preserving biological and genetic diversity, our findings highlight the challenges of cryopreservation as a conservation tool for at-risk amphibians.

In amphibians, a disadvantage in size during the larval and juvenile stages is known to have a negative effect on a suite of life history characteristics. These effects can have more long-lasting effects on the survivorship and reproductive output. Larger tadpole size is linked to higher efficiency of prey consumption (Crossland, 1998) and predator avoidance (Formanowicz, 1986). Similarly, size at metamorphosis has a direct positive relationship with increased fecundity (Fontenot Jr., 1999; Scott, 1994), earlier sexual maturity (Berven, 1990; Scott, 1994), better foraging abilities (Cabrera-Guzmán et al., 2013; Flowers & Graves, 1995), greater locomotive abilities (Beck & Congdon, 2000; Goater et al., 1993), and higher mating success (Howard & Young, 1998). All of these characteristics affect the most significant parameters of population viability: survival and fecundity. Moreover, these effects can carry-over from one life stage to another, and can be content dependent based on environmental conditions and resource availability (Touchon et al., 2013). Comparing our findings to that of a previous laboratory-based study (Poo & Hinkson, 2020), we found that disadvantages of size and mass in cryo-derived individuals persist even after they are released into natural habitats, which can have a number of implications if they are used in captive-release and translocation programs.

From our population simulations, small differences in size at metamorphosis can scale up to substantial differences at the individual and population level. Our results project a decrease in average final life fecundity in females, and lower population numbers of cryo- versus natural-bred toads. These findings further emphasize the significance of size at metamorphosis as a life history trait that reverberates through many metrics of overall fitness for amphibians (e.g., Cabrera-Guzmán et al., 2013; Howard & Young, 1998; Scott, 1994). Our results highlight the need for model projections to take into account effects of laboratory captive-breeding techniques, instead of solely using empirical values from natural populations to make predictions for captive-release models. As with many other limitations of deterministic simulations, our population and growth models do not take into account demographic and environmental stochasticity, and also overlook potential community-level effects. Despite these caveats, we can see that smaller sizes observed in cryo-derived tadpoles and post-metamorphic toadlets could pose a challenge to the use of cryopreservation as a method for species conservation via captive-breeding and conservation translocation.

Given the increasing importance of captive-breeding programs and cryopreservation technologies in wildlife conservation, it may seem surprising that few, if any, studies have examined the post-release growth and survivorship of cryo-derived individuals. However, cryopreservation has

largely been developed for species of higher conservation priority, such as mammals and birds (e.g., Brown et al., 2018; Gee et al., 1985; Hermes et al., 2009; Huang et al., 2012), or for species of higher economic value, such as livestock (e.g., Morrell & Mayer, 2017; Sieme & Oldenhof, 2015) and aquaculture (e.g., Magnotti et al., 2018; Martinez-Paramo et al., 2017). The findings from this study breaks new grounds by increasing our knowledge of post-release growth and survivorship of amphibians produced using cryopreserved sperm. These explorations and discussions are fundamental to long-term conservation efforts involving assisted artificial reproductive technologies. While cryopreserved sperm can produce individuals capable of surviving in their natural environment, empirical differences in growth and development, as well as model projections for fecundity and population growth suggest disadvantages in the cryo-derived group. These disadvantages are nuanced and complex, both in terms of the cause and mechanism during the process of cryopreservation and in vitro fertilization that led to the discrepancies observed and with respect to the implications it may have on wild populations that are supplemented with or established by using cryo-derived individuals. All of these topics require more attention and dedicated research efforts by future studies. Areas that merit further examination include investigations into the cause of the discrepancies in size between cryo-derived and natural-bred individuals, the heritability of these differences, and the other potential sub-lethal effects of cryopreservation. Though the process of cryopreservation can cause significant damage to sperm cells through DNA fragmentation (Pérez-Cerezales et al., 2009; Zilli et al., 2003), altered rates of DNA methylation (Aurich et al., 2016), and a myriad of processes affecting sperm membrane structure and function (Peris-Frau et al., 2020), little is known about the effects or the heritability of these effects across generations (but see Lavara et al., 2014). In particular, if the effects of cryopreservation are heritable, it may pose significant problems for cryo-derived population viability. In addition, validation of our growth and population models through multi-year studies that follow cryo-bred clutches to sexual maturity would provide a more comprehensive picture of potential long-lasting effects of using cryopreservation in conservation translocation programs. It should be noted, however, that multigenerational experiments in amphibians are logistically difficult to conduct in situ, given their biphasic life history and high rates of larval mortality.

Given the paucity of studies on cryo-derived offspring across life stages, our findings provide salient information for amphibian conservation. Ultimately, the goal is to increase our knowledge of the ecological adaptiveness of captive-bred amphibians in situ, so that we can bolster the post-release survival of at-risk species that may ultimately rely on such breeding methods. By combining empirical

data from post-release monitoring and model projections, our study takes one step forward in understanding the complexities of using sperm cryopreservation in reintroduction efforts and points to the potential barriers we face in using it as an effective wildlife conservation tool.

## ACKNOWLEDGMENTS

We thank the Memphis Zoo Director of Conservation and Research, Dr. Steve Reichling, and University of Memphis Meeman Biological Station Director, Dr. E. Keith Bowers for their support throughout the project. We are grateful for the wonderful assistance from Kameron Hall, Maryella Cohn, and Karen Candia. This work was funded by the Memphis Zoo Conservation Action Network grant. All procedures were approved by Animal Care and Use Committees (Memphis Zoo Approval 2020-1, University of Memphis Approval 0854) and the Tennessee Wildlife Resource Agency (Permit 2430).

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Sinlan Poo conceived of the study. Sinlan Poo, Allison Bogisich, and Mariah Mack carried out the experiment and data collection. Sinlan Poo, Bryan K. Lynn, and Anne Devan-Song analyzed the data. All authors contributed to the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support these findings of this study are available at [https://osf.io/xmt5w/?view\\_only=a90fc91dd31f4d62a4ed9537d5620338](https://osf.io/xmt5w/?view_only=a90fc91dd31f4d62a4ed9537d5620338).

## ORCID

Sinlan Poo  <https://orcid.org/0000-0002-7767-8173>

Allison Bogisich  <https://orcid.org/0000-0003-0555-3280>

Mariah Mack  <https://orcid.org/0000-0002-6834-5537>

Bryan K. Lynn  <https://orcid.org/0000-0003-0690-103X>

Anne Devan-Song  <https://orcid.org/0000-0002-9509-2678>

## REFERENCES

- Aurich, C., Schreiner, B., Ille, N., Alvarenga, M., & Scarlet, D. (2016). Cytosine methylation of sperm DNA in horse semen after cryopreservation. *Theriogenology*, *86*(5), 1347–1352. <https://doi.org/10.1016/j.theriogenology.2016.04.077>
- Beck, C. W., & Congdon, J. D. (2000). Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad, *Bufo terrestris*, metamorphs. *Functional Ecology*, *14*(1), 32–38. <https://doi.org/10.1046/j.1365-2435.2000.00386.x>
- Berger-Tal, O., Blumstein, D. T., & Swaisgood, R. R. (2020). Conservation translocations: A review of common difficulties and promising directions. *Animal Conservation*, *23*(2), 121–131. <https://doi.org/10.1111/acv.12534>

- Berven, K. A. (1990). Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology*, 71(4), 1599–1608. <https://doi.org/10.2307/1938295>
- Blair, M. E., Le, M. D., Sethi, G., Thach, H. M., Nguyen, V. T. H., Amato, G., ... Sterling, E. J. (2017). The importance of an interdisciplinary research approach to inform wildlife trade management in Southeast Asia. *Bioscience*, 67(11), 994–1002. <https://doi.org/10.1093/biosci/bix113>
- Brown, M. E., Singh, R. P., Pukazhenth, B., Keefer, C. L., & Songsasen, N. (2018). Cryopreservation effects on sperm function and fertility in two threatened crane species. *Cryobiology*, 82, 148–154.
- Bubac, C. M., Johnson, A. C., Fox, J. A., & Cullingham, C. I. (2019). Conservation translocations and post-release monitoring: Identifying trends in failures, biases, and challenges from around the world. *Biological Conservation*, 238, 8. <https://doi.org/10.1016/j.biocon.2019.108239>
- Cabrera-Guzmán, E., Crossland, M. R., Brown, G. P., & Shine, R. (2013). Larger body size at metamorphosis enhances survival, growth and performance of young cane toads (*Rhinella marina*). *PLoS ONE*, 8(7), e70121. <https://doi.org/10.1371/journal.pone.0070121>
- Clark, T. W., Stevenson, M. J., & Ziegelmayer, K. (2001). *Species and ecosystem conservation: An interdisciplinary approach* (Vol. 96). Yale School of the Environment Bulletin Series.
- Comizzoli, P., & Holt, W. V. (2019). Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. *Biology of Reproduction*, 101(3), 514–525. <https://doi.org/10.1093/biolre/iox031>
- Crossland, M. R. (1998). Predation by tadpoles on toxic toad eggs: The effect of tadpole size on predation success and tadpole survival. *Journal of Herpetology*, 32(3), 443–446. <https://doi.org/10.2307/1565463>
- Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological Conservation*, 96(1), 1–11. [https://doi.org/10.1016/S0006-3207\(00\)00048-3](https://doi.org/10.1016/S0006-3207(00)00048-3)
- Flowers, M. A., & Graves, B. M. (1995). Prey selectivity and size-specific diet changes in *Bufo cognatus* and *B. woodhousii* during early postmetamorphic ontogeny. *Journal of Herpetology*, 29(4), 608–612. <https://doi.org/10.2307/1564745>
- Fontenot, C. L., Jr. (1999). Reproductive biology of the aquatic salamander *Amphiuma tridactylum* in Louisiana. *Journal of Herpetology*, 33, 100–105.
- Formanowicz, D. R. (1986). Anuran tadpole/aquatic insect predator-prey interactions: Tadpole size and predator capture success. *Herpetologica*, 42(3), 367–373.
- Gee, G. F., Bakst, M. R., & Sexton, T. J. (1985). Cryogenic preservation of semen from the greater Sandhill crane. *The Journal of Wildlife Management*, 49(2), 480–484. <https://doi.org/10.2307/3801557>
- Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology*, 23(1), 7–15. <https://doi.org/10.1111/j.1523-1739.2008.01123.x>
- Goater, C. P., Semlitsch, R. D., & Bernasconi, M. V. (1993). Effects of body size and parasite infection on the locomotor performance of juvenile toads, *Bufo bufo*. *Oikos*, 66, 129–136.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16(3), 183–190.
- Green, D. M., & Middleton, J. (2013). Body size varies with abundance, not climate, in an amphibian population. *Ecography*, 36(8), 947–955. <http://doi.org/10.1111/j.1600-0587.2013.00063.x>
- Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, 30(2), 340–349. <https://doi.org/10.1111/cobi.12612>
- Hermes, R., Göritz, F., Saragusty, J., Sós, E., Molnar, V., Reid, C. E., Schwarzenberger, F., & Hildebrandt, T. B. (2009). First successful artificial insemination with frozen-thawed semen in rhinoceros. *Theriogenology*, 71(3), 393–399. <https://doi.org/10.1016/j.theriogenology.2008.10.008>
- Howard, R. D., & Young, J. R. (1998). Individual variation in male vocal traits and female mating preferences in *Bufo americanus*. *Animal Behaviour*, 55(5), 1165–1179. <https://doi.org/10.1006/anbe.1997.0683>
- Huang, Y., Li, D., Zhou, Y., Zhou, Q., Li, R., Wang, C., Huang, Z., Hull, V., & Zhang, H. (2012). Factors affecting the outcome of artificial insemination using cryopreserved spermatozoa in the giant panda (*Ailuropoda melanoleuca*). *Zoo Biology*, 31(5), 561–573. <https://doi.org/10.1002/zoo.20421>
- IUCN/SSC. (2013). *Guidelines for reintroductions and other conservation translocations. Version 1.0*. IUCN Species Survival Commission.
- Kalish, S., & McPeck, M. A. (1992). Demography of an age-structured annual: Resampled projection matrices, elasticity analyses, and seed bank effects. *Ecology*, 73(3), 1082–1093. <https://doi.org/10.2307/1940182>
- Kendall, B. E., Fujiwara, M., Diaz-Lopez, J., Schneider, S., Voigt, J., & Wiesner, S. (2019). Persistent problems in the construction of matrix population models. *Ecological Modelling*, 406, 33–43. <https://doi.org/10.1016/j.ecolmodel.2019.03.011>
- 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(5), 441–450. <https://doi.org/10.1111/conl.12093>
- Lavara, R., Baselga, M., Marco-Jiménez, F., & Vicente, J. S. (2014). Long-term and transgenerational effects of cryopreservation on rabbit embryos. *Theriogenology*, 81(7), 988–992. <https://doi.org/10.1016/j.theriogenology.2014.01.030>
- Magnotti, C., Cerqueira, V., Lee-Estevez, M., Farias, J. G., Valdebenito, I., & Figueroa, E. (2018). Cryopreservation and vitrification of fish semen: A review with special emphasis on marine species. *Reviews in Aquaculture*, 10(1), 15–25. <https://doi.org/10.1111/raq.12145>
- Marquardt, S. R., Annis, M., Drum, R. G., Hummel, S. L., Mosby, D. E., & Smith, T. (2018). On the cutting edge of research to conserve at-risk species: Maximizing impact through partnerships. *Integrative and Comparative Biology*, 58(1), 140–149. <https://doi.org/10.1093/icb/icy009>
- Martinez-Paramo, S., Horvath, A., Labbe, C., Zhang, T. T., Robles, V., Herraes, P., ... Cabrita, E. (2017). Cryobanking of aquatic species. *Aquaculture*, 472, 156–177. <https://doi.org/10.1016/j.aquaculture.2016.05.042>
- Milot, E., Perrier, C., Papillon, L., Dodson, J. J., & Bernatchez, L. (2013). Reduced fitness of Atlantic salmon released in the wild after one generation of captive breeding. *Evolutionary Applications*, 6(3), 472–485. <https://doi.org/10.1111/eva.12028>



- Minin, E. D., & Griffiths, R. (2011). Viability analysis of a threatened amphibian population: modelling the past, present and future. *Ecography*, *34*, 162–169.
- Morrell, J. M., & Mayer, I. (2017). Reproduction biotechnologies in germplasm banking of livestock species: A review. *Zygote*, *25*(5), 545–557. <https://doi.org/10.1017/S0967199417000442>
- Msuya, C., & Mohamed, N. (2019). Reintroduction of the Kihansi spray toad *Nectophrynoides asperginis* back to its natural habitat by using acclimatizing cages. *Tanzania Journal of Science*, *45*(4), 570–583.
- Otto, S. P., & Day, T. (2007). *A biologist's guide to mathematical modeling in ecology and evolution*. Princeton University Press.
- Pérez-Cerezales, S., Martínez-Páramo, S., Cabrita, E., Martínez-Pastor, F., de Paz, P., & Herráez, M. P. (2009). Evaluation of oxidative DNA damage promoted by storage in sperm from sex-reversed rainbow trout. *Theriogenology*, *71*(4), 605–613. <https://doi.org/10.1016/j.theriogenology.2008.09.057>
- Peris-Frau, P., Soler, A. J., Iniesta-Cuerda, M., Martín-Maestro, A., Sánchez-Ajofrín, I., Medina-Chávez, D. A., Fernández-Santos, M. R., García-Álvarez, O., Maroto-Morales, A., Montoro, V., & Garde, J. J. (2020). Sperm cryodamage in ruminants: Understanding the molecular changes induced by the cryopreservation process to optimize sperm quality. *International Journal of Molecular Sciences*, *21*(8), 2781.
- Poo, S., & Hinkson, K. M. (2020). Amphibian conservation using assisted reproductive technologies: Cryopreserved sperm affects offspring morphology, but not behavior, in a toad. *Global Ecology and Conservation*, *21*, e00809. <https://doi.org/10.1016/j.gecco.2019.e00809>
- Rasband, W. S. (1997–2018). *Image J*. U. S. National Institutes of Health. <https://imagej.nih.gov/ij/>
- Scott, D. E. (1994). The effects of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*, *75*(5), 1383–1396. <https://doi.org/10.2307/1937462>
- Sieme, H., & Oldenhof, H. (2015). Cryopreservation of semen from domestic livestock. In W. F. Wolkers & H. Oldenhof (Eds.), *Cryopreservation and freeze-drying protocols* (pp. 277–287). Springer New York.
- Silla, A. J., & Byrne, P. G. (2019). The role of reproductive technologies in Amphibian Conservation Breeding Programs. *Annual Review of Animal Biosciences*, *7*(1), 499–519. <https://doi.org/10.1146/annurev-animal-020518-115056>
- Suboski, M. D., & Templeton, J. J. (1989). Life skills training for hatchery fish: Social learning and survival. *Fisheries Research*, *4*, 343–352.
- Touchon, J. C., McCoy, M. W., Vonesh, J. R., & Warkentin, K. M. (2013). Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. *Ecology*, *94*(4), 850–860.
- Vimercati, G., Hui, C., Davies, S. J., & Measey, G. J. (2017). Integrating age structured and landscape resistance models to disentangle invasion dynamics of a pond-breeding anuran. *Ecological Modelling*, *356*, 104–116. <https://doi.org/10.1016/j.ecolmodel.2017.03.017>
- von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth laws. II). *Human Biology*, *10*(2), 181–213.
- White, K. S., Gregovich, D. P., & Levi, T. (2018). Projecting the future of an alpine ungulate under climate change scenarios. *Global Change Biology*, *24*(3), 1136–1149. <https://doi.org/10.1111/gcb.13919>
- Zilli, L., Schiavone, R., Zonno, V., Storelli, C., & Vilella, S. (2003). Evaluation of DNA damage in *Dicentrarchus labrax* sperm following cryopreservation. *Cryobiology*, *47*(3), 227–235. <https://doi.org/10.1016/j.cryobiol.2003.10.002>

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Poo, S., Bogisich, A., Mack, M., Lynn, B. K., & Devan-Song, A. (2021). Post-release comparisons of amphibian growth reveal challenges with sperm cryopreservation as a conservation tool. *Conservation Science and Practice*, e572. <https://doi.org/10.1111/csp2.572>