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RESEARCH ARTICLE

Dusky Gopher Frog (*Lithobates sevosus*) Repatriation at a Reintroduction Site Through Zoo-Led Captive-Release Efforts

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ABSTRACT

Captive-release programs are an increasingly popular conservation strategy to combat wild extinctions. However, it is critical to determine if translocating animals from captive colonies ("source populations") leads to the establishment of new wild populations that are both stable and self-sustaining. To fill this knowledge gap, we provide a case study from the dusky gopher frog (*Lithobates sevosus*) reintroduction program to serve as an example for other critically endangered amphibians. In this study, we provide quantitative information on the reintroduction and survivorship of zoo-bred individuals that are released into the wild. This unique opportunity is the culmination of close to 20 years of collective efforts across multiple agencies. By taking advantage of the key monitoring window shortly after initial releases, we can formally declare the first successfully reintroduced, breeding population of dusky gopher frogs founded solely from a captive-bred colony.

1 | Introduction

The loss of biodiversity has been accelerating, triggering the Earth's entrance into its sixth mass extinction (Barnosky et al. 2011; Pimm et al. 2014). Globally, more than 42,100 species are threatened with extinction (IUCN 2023), with an estimated one million species at risk of extinction within the next few decades (Bongaarts 2019). Since 1900, 198 extinctions of vertebrates have been formally recorded, which is roughly one hundred times higher than the natural baseline rate of extinction (Ceballos et al. 2015). In response, a number of methods have been employed to mitigate or prevent further species declines, such as habitat preservation (Wilson et al. 2016), poaching prevention (Tasirin et al. 2021), invasive species management (Tingley et al. 2017), community engagement (Schuttler et al. 2019), keystone species conservation (Delibes-Mateos et al. 2007), and reintroductions (Fritts et al. 1997).

Reintroductions, sometimes referred to as conservation translocations, serve as a management tool for mitigating population and species declines (Berger-Tal, Blumstein, and Swaisgood 2020; Griffith et al. 1989; Seddon 2010). Translocations can involve moving animals from one location to another, head-starting in situ before the move, or animals that are bred in captivity and later released into the wild. In the latter case, translocations combine ex situ husbandry, historical, geographical, and genetic expertize with the in situ needs of the species (Adams et al. 2023; Steiner et al. 2024). However, despite an increase in translocation efforts in recent years (Bubac et al. 2019), few studies have quantified the success of these efforts in situ at both early and later stage benchmarks (but see Castellón et al. 2022). This is of particular concern because, within vertebrate taxa, approximately 69.8% of translocation efforts fail at population establishment within the first 4 years (Bubac et al. 2019).

The definitions of what a successful population establishment entails are nearly as numerous as the number of species that have had translocation efforts. Translocations can be evaluated differently in terms of population size and time scale of the assessment. Some translocation efforts consider successful establishment as having a certain number of recaptures in the months immediately following release (Castellón et al. 2022), while others look at population sizes over several years (Chambert et al. 2022). Some define population establishment as having documented recruitment of a second generation (Hinkson et al. 2020), while still others quantify the establishment of a metapopulation based on the resulting genetic diversity (United States Fish and Wildlife Service [USFWS] 2015). Although assessments may differ based on the life history of the species, it is clear that some form of post-release monitoring is needed to assess translocation efforts. In particular, there is a lack of long-term, post-release monitoring in amphibians (Bubac et al. 2019), pointing to the need for studies to quantify these efforts.

Globally, nearly half of amphibian species are threatened with extinction (IUCN 2023). Regardless of the extinction scenario imposed, simulations show amphibians as one of the taxa most impacted by these risks (Toussaint et al. 2021). Globally, there are currently 816, 1265, and 795 species of amphibian listed as vulnerable, endangered and critically endangered, respectively (IUCN 2023). Within these species, the dusky gopher frog (*Lithobates sevosus*) is considered one of most endangered anuran species in the United States (IUCN 2023), with only one remaining population consisting of roughly 100–200 adults in the last population estimate (Baillie and Butcher 2012; United States Fish and Wildlife Service [USFWS] 2015). In recent decades, efforts have been made to expand the wild population through head-starting and translocation of wild-caught individuals, as well as habitat management (Thurgate and Pechmann 2007; United States Fish and Wildlife Service [USFWS] 2015). Captive colonies were established in 2001 across a network of Association of Zoos and Aquariums (AZA) accredited zoos and captive-release efforts began in 2017 (Reichling et al. 2022; United States Fish and Wildlife Service [USFWS] 2015). This reintroduction effort provides a unique opportunity to study the re-establishment of a critically endangered species and in a new, naive population in its historical range.

Herein, we conducted in situ surveys of dusky gopher frogs to quantify the effectiveness of these reintroduction efforts in a population established solely with captive-release individuals (Figure 1). Specifically, we used drift fence surveys, burrow scoping, aquatic visual surveys, and terrestrial visual encounter transect surveys over the course of 6 months to determine which methodologies at this translocation site would provide the best rates of detection, and whether they would detect breeding or recruitment. We hypothesized that certain survey methods would be more cost-effective and better suited for shorter survey time frames, which is particularly critical for captive-release programs that are unable to support long term

FIGURE 1 | (A) Aquatic survey of dusky gopher frog eggs, with drift fence in the background. (B) Egg clutch with hatchlings of dusky gopher frogs at Ward Bayou Wildlife Management Area. (C) Juvenile dusky gopher frog inside of a gopher tortoise burrow detected using burrow scope. (D) Juvenile dusky gopher frog at the entrance of a gopher tortoise burrow. [Color figure can be viewed at wileyonlinelibrary.com]

follow-up studies in the field. Post-translocation monitoring of this species is critical to determining success and quantifying the usefulness of reintroduction beyond the point of release (Roznik and Reichling 2020).

2 | Methods

2.1 | Study Site and Species

Dusky gopher frogs are a critically endangered frog endemic to fire-maintained, sandy uplands along the central Gulf Coast of the United States (Richter and Jensen 2005). This open-canopy habitat is dominated by longleaf pine (*Pinus palustris*) as well as grassy, herbaceous understories (Kirkman and Jack 2017). Adult frogs spend the majority of their lives underground in stump holes and burrows dug by gopher tortoises or small mammals, migrating only seasonally to isolated, temporary ponds to breed (Richter et al. 2001). By 2001, their populations had declined sharply, due primarily to habitat degradation, with only two known breeding populations remaining in Jackson and Harrison counties in Mississippi (United States Fish and Wildlife Service [USFWS] 2015). Consequently, the species was federally listed as Endangered in 2001 (United States Fish and Wildlife Service 2001), and a federal recovery plan was created to preserve existing populations and establish new ones through translocation and reintroduction (United States Fish and Wildlife Service [USFWS] 2015).

2.2 | Captive-Breeding and Release

Between 2017 and 2022, 4306 captive-bred individuals were released across two designated ponds at Ward Bayou Wildlife Management Area (WBWMA) within the historical range of the species in Mississippi (Table 1). Translocated releases were comprised of zoo-bred and head-started offspring, either via in vitro fertilization (IVF) or natural breeding (Supporting Information S1: Table S1). While IVF was the main method of breeding in earlier years, natural amplexus in outdoor, artificial pond enclosures took over as the main source of captive-bred individuals from 2020 onward (Reichling et al. 2022).

After oviposition, dusky gopher frog offspring were either reared to a minimum of 10 days post-oviposition and released as tadpoles or held and reared through metamorphosis in captivity and released as juveniles (Supporting Information S1: Table S2). Individuals were hard-released into two reintroduction ponds (Gil's and Mayhaw Pond) in WBWMA, either in the pond, pond edges, or burrows (Table 1). WBWMA is located 15 miles north of Pascagoula, along the Pascagoula River basin in Jackson County. The area has 13,234 acres of mostly bottom-land hardwoods with about 700 acres of upland pines. The area is managed by the Mississippi Department of Wildlife, Fisheries, and Parks and the U.S. Army Corp of Engineers (USACE) for both game and non-game wildlife.

2.3 | Post-Release Surveys

Gil's Pond and the surrounding upland forested areas at WBWMA were monitored monthly, from February to June 2022, using four survey methods, including aquatic egg surveys, terrestrial visual encounter surveys, drift fence surveys, and gopher tortoise burrow surveys. Each monthly survey period lasted for 5–6 consecutive days. Within this period, aquatic egg mass and tadpole surveys were conducted at Gil's Pond on two separate, non-consecutive days, between 12:00 and 15:30 h to ensure optimum visibility. In addition to these systematic surveys, opportunistic egg mass surveys were conducted after periods of heavy rain between January and April at Mayhaw and Gil's Pond. Visual encounter surveys were conducted along five, 100 m transects in the upland longleaf pine forest. Terrestrial transects were haphazardly selected in the upland pine forested areas, and ranged from roughly 80–286 m from the edges of Gil's Pond. Each transect was surveyed twice per night between 19:00 h and 23:00 h for four nights each month. For drift fence surveys, four 40 m-long drift fences were installed on each of the four cardinal sides of Gil's Pond. Drift fence was constructed with temporary wildlife fencing by Animex Fencing (AMX-40). Pitfall traps constructed with 10-L buckets were installed every 8 m on both sides and at the ends of the drift fence. Each bucket was equipped with a large sponge to prevent the desiccation of trapped animals and 8–10 holes were drilled in the bottom of the bucket for drainage. Drift fences and pitfall traps were surveyed at 2-h intervals between 17:00 and 23:00 h for four nights each month. For gopher tortoise burrow surveys, previously identified gopher tortoise burrows were surveyed once per month using an adult tortoise camera scoping system from Environmental Management Services. Surveys began at a minimum of 4 h before sunset and concluded by sunset. Burrows were assessed to a maximum depth of 7.8 m, given the length and structure of the scope.

TABLE 1 | Captive-bred and zoo-reared dusky gopher frog reintroduced to Ward Bayou Wildlife Management Area.

Year	Juvenile released	Tadpoles released	Total released	Breeding method
2017	82	0	82	In vitro fertilization
2018	351	0	351	In vitro fertilization
2019	667	0	667	In vitro fertilization
2020	1946	1260	3206	In vitro fertilization; natural amplexus
2021	1018	2570	3588	Natural amplexus
2022	281	0	281	Natural amplexus

3 | Results

Surveys were conducted at WBWMA from February to July 2022. Each monthly survey consisted of two observers. Across the 6-month period, 12 h of aquatic surveys were conducted over 12 days, 80 h of terrestrial visual encounter surveys were conducted over 24 nights, and 72 h of drift fence surveys were conducted over 24 nights. A total of 44 burrows were surveyed monthly, with a total of 18 days of surveys across the study period.

Of the four survey types employed, dusky gopher frogs were located using aquatic and burrow scoping surveys (Figure 1). While adult frogs have been observed in the vicinity of the pond (*pers. comm.* Allison Bogisich and Sinlan Poo) and upland habitats (*pers. comm.* Todd Cotterman) at the reintroduction site, we did not observe any frogs during our visual encounter or drift fence surveys. Three egg masses were located in March in Mayhaw Pond through opportunistic aquatic surveys, which was consistent with the period that egg masses were found at the reintroduction site in past years (Table 2). Seven dusky gopher frogs were found to be occupying six gopher tortoise burrows (Table 3). One of the burrows occupied by a dusky gopher frog was also found to be actively used by a gopher tortoise.

4 | Discussion

This study establishes the first longitudinal assessment of the dusky gopher frogs following 6 years of reintroductions. Conducting monthly post-release surveys, we report findings of eggs and juvenile frogs in a wild population established solely from captive-bred and released individuals. The presence of frogs was detected primarily through burrow surveys, with no detection using drift fences or terrestrial visual encounter surveys. These findings highlight the importance of substantial post-release monitoring to assess the efficacy of reintroduction programs and provide much-needed insight into the recovery of this critically-endangered species.

Finding juveniles occupying burrows in the upland forest area indicates recent, successful translocation survivorship of captive-reared individuals previously released as metamorphs or tadpoles. Importantly, these findings indicate that juvenile frogs are able to traverse the upland habitat and find refuge in their expected fossorial habitat. Movement patterns provide key insight into the habitat requirements of a species, including the type and amount of habitat necessary for survival and reproduction (Kenward, Walls, and Hodder 2001; White and Garrott 2012). In spotted salamander (*Ambystoma maculatum*) and American toad (*Anaxyrus americanus*) juveniles, habitat use has been linked to higher survivorship (Rothermel and Semlitsch 2002). For Ngahere geckos (*Mokopirirakau* 'southern North Island') that are reintroduced, both hard and soft-released geckos occupied their known arboreal habitat post-release, which is a key indicator of post-release survivorship (Yee, Monks, and Bell 2022). For reintroduced captive-bred Cabot's tragopan pheasants (*Tragopan caboti*), untrained cohorts selected lower elevations for their habitat, dominated by bamboo, whereas the trained tragopans selected habitats more typical of wild populations (Liu et al. 2016). The inability to find and utilize the appropriate habitat within an environment resulted in a more than 60% difference in post-release survival of trained individuals compared to their untrained counterparts (Liu et al. 2016). Similarly, in dusky gopher frogs, the ability of juveniles to find appropriate shelter and refugia is associated with a higher survival rate (Roznik and Reichling 2020). In light of these studies that point to the importance of post-release habitat use, our findings serve as an encouraging sign for the reintroduced dusky gopher frog population at the WBWMA.

TABLE 2 | Number of dusky gopher frog egg masses found at reintroduction ponds at Ward Bayou Wildlife Management Area.

Year	Gil's Pond	Mayhaw Pond
2021	1	2
2022	1	0
2023	0	3

The detection of egg masses at WBWMA for 3 consecutive years (Table 2) is evidence of natural reproduction from sexually mature adult frogs that were previously released as juveniles. In addition to our surveys, three incidental sightings of adult dusky gopher frogs occurred between 2020 and 2021 (*pers comm.* Allison Bogisich, Sinlan Poo, Todd Cotterman). Natural reproduction and recruitment are used as key indicators of a self-sustaining population. For instance, reproduction in a reintroduced population of African wild dogs (*Lycaon pictus*) over multiple breeding seasons has been used as a barometer for the successful re-establishment (Bouley et al. 2021). Similarly, long term reintroduction programs of the California condors (*Gymnogyps californianus*) have noted the first fledglings produced by reintroduced birds and keep track of the number of their wild-born offspring (Walters et al. 2010). In black-footed ferrets (*Mustela nigripes*), reproduction by reintroduced individuals has been a key factor in wild population recovery (Santymire et al. 2014). In our particular case, the detection of dusky gopher frogs across different life stages and the observation of natural recruitment can be seen as early signs of a new population that is starting to take shape.

Given the utilization of different habitats across amphibian life stages, gaining a better understanding of these differences by employing an array of survey methods can lead to more appropriate and effective ways of habitat and population management. The four survey methods in the current study were selected to determine whether passive capture methods or active surveys would prove the most effective. Moreover, we chose to focus on detecting individuals outside of the breeding season in upland long-leaf pine habitat, which have rarely been examined in the past. Survey method evaluation is imperative for species that occupy different niches across life stages (Rothermel and Semlitsch 2002) and for the detection of rare and endangered species with low detection rates (Tanadini and Schmidt 2011). This is especially true for amphibians, which generally have smaller ranges and relatively complex habitat requirements. Without a systematic comparative survey, historical confirmation bias from certain methods can hinder our

TABLE 3 | Dusky gopher frogs found at reintroduction site at Ward Bayou Wildlife Management Area via gopher tortoise burrow surveys.

Date	Burrow ID	Time found	Life stage	Depth found (m)
2022-03-07	113	15:40	Juvenile	3.6
2022-04-06	47	14:59	Juvenile	3.6
2022-05-05	111	15:03	Juvenile	4.8
2022-05-05	A	14:53	Juvenile	3.7
2022-05-05	112	15:12	Juvenile	4.6
2022-06-01	112	15:01	Juvenile	4.2
2022-06-29	9	16:22	Juvenile	4.5

understanding of the presence and abundance of some species for many decades (Devan-Song et al. 2021).

In the case of dusky gopher frogs, low detection rate coincides with the need for more attention in this critically-endangered species. Furthermore, for reintroduced populations, there is a need to distinguish between translocated individuals and their wild-produced offspring to assess the success of recovery efforts (Semlitsch 2002). For the current study, we were able to determine a generational difference and confirm recruitment through the detection of eggs and juvenile frogs, which were presumed to be offspring of captive-released individuals due to their respective life stages at time of detection, the seasonal timing of these detection events relative to dates of translocation events that occurred at this reintroduction site, and the age at which individuals of this species begin reaching reproductive maturity. The extirpation of this species within the range of the release site (United States Fish and Wildlife Service [USFWS] 2015) also served as an indication that these offspring were the result of reproduction from captive-released individuals. However, individual identifiers, such as passive integrated transmitter (PIT) tags, or DNA samples hold great potential not only for tracking the genetic profile of individuals released into the wild but also for long term heredity and pedigree comparisons.

By conducting post-release monitoring, we can provide greater context to this reintroduced population's larger population structure and demographics. More specifically, we are able to document the presence and breeding in this captive-release population over several years. The egg masses and individuals detected provide us with an approximate age demographics for the population. The estimated minimum age at maturity is between 6 and 8 months for males and 2–3 years for females (Richter and Seigel 2002). With the more substantial releases of juvenile frogs beginning in 2019 (Table 1), we'd expect a larger cohort of wild females to reach reproductive maturity around 2021–2022, which coincides with our observations of the first evidence of wild breeding in 2021 (Table 2).

In other natural, wild populations of dusky gopher frogs, the presence of breeding was consistent across years, but the number of egg masses produced and juveniles surviving was highly variable (3–130 egg masses, 0–2488 juveniles; Richter et al. 2003). This has been attributed to the fluctuating and unpredictable hydroperiods in the region (Crawford et al. 2022; Richter et al. 2003). This high variation in reproductive output is not atypical and has been observed in closely related species, such as the Carolina gopher frog (*Lithobates capito*) (Crawford et al. 2022; Semlitsch, Gibbons, and Tuberville 1995). In contrast, the fact that ponds at the WBWMA reintroduction site have had adults return to breed for 3 consecutive years speaks to the suitability and habitat management of the ponds and surrounding ecosystem and its ability to support this nascent population.

Long term efforts to monitor translocated amphibian species are crucial to their successful re-establishment in the wild. In addition to the case we present here, a number of recovery programs have made significant strides to document the impacts of their efforts following captive-releases. For instance, release efforts for the Chiricahua leopard frog (*Rana chiricahuensis*), led by the Phoenix Zoo, have observed a fourfold increase in site occupancy and breeding records between 2007 and later observations in 2016 and 2021 (Harris et al. 2022). The St. Louis Zoo's captive-breeding and release program for the Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) has released 10,000 hellbenders since 2008 and documented the first wild clutch sired by a captive-released individual in 2023 (Ettling et al. 2017; Saint Louis Zoo 2022; Wheeler 2023). Consistent, long term financial investment is needed for these ongoing, captive-breeding and reintroduction programs. Along with our findings, a growing body of evidence demonstrates the capability of zoos and other agencies to produce new self-sustaining populations in the wild.

The challenges for the dusky gopher frog recovery program are emblematic of the problems that many other captive-release or translocation efforts face, namely, finding ways to define and quantify successful post-release population establishment. The observed increase in dusky gopher frog site occupancy and breeding after 6 years of release, the presence of nascent metapopulations in two of the five species recovery range blocks across Mississippi, Alabama, and Louisiana (Hillard et al. 2023) and the collaborative management of critical habitat are significant benchmarks of progress. As our findings show, the long-term persistence of dusky gopher frogs at this reintroduction site, and in Mississippi as a whole, still requires intensive monitoring and management. The recovery program described here highlights the substantive contributions zoos can make in support of regional species conservation. While significant challenges remain, thoughtful, sustained partnerships can lead to the success of these conservation efforts.

Author Contributions

Allison Bogisich and Sinlan Poo conceived of the study. Allison Bogisich, Ana Karen Candia, Jessica Cantrell, Cassandra Collins, Steven B. Reichling, and Sinlan Poo collected the data. Allison Bogisich analyzed the data. Allison Bogisich and Sinlan Poo wrote the initial manuscript. All authors edited and approved the final manuscript.

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Ethics Statement

All procedures were approved by Animal Care and Use Committees (Memphis Zoo Approval 17-109), the Mississippi Department of Fish and Wildlife (Permit 0908211, 0310232), and the U.S. Fish and Wildlife Services (Permit ES171493).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available upon request and USFWS review.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.