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Movement and survival of captive-bred *Limosa harlequin frogs (Atelopus limosus)* released into the wild

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The endangered *Limosa harlequin frog Atelopus limosus* has experienced significant chytridiomycosis-related declines, but has been successfully bred in captivity as part of the Panama Amphibian Rescue and Conservation Project. We conducted the first *Atelopus* release trial using 83 captive-bred *A. limosus*, and monitored individuals as they transitioned from captivity back into the wild at a site within their historic distribution with no extant population. We acclimated 23 animals to the environment prior to release by holding them in mesocosms for 30 days (soft release) and released a further 60 animals without prior acclimation (hard release). We radio-tracked a subsample of animals in each treatment group. We used a Bayesian mark-recapture analysis to integrate known fate data from radio-tracked individuals with data from resighted individuals that had unknown fates. For this analysis, we allowed survival to differ between release treatments (hard vs. soft) and assumed detection probability would differ between tracking methods. Per-survey detection probability was about 0.02 for individuals without radio transmitters vs. 0.88 for radio-tracked frogs. We observed that hard-released animals initially dispersed further than soft-released animals, and, despite the fact that 25% of the soft-released animals were *Batrachochytrium dendrobatidis* (Bd)-positive on release day, their estimated 30-day survival probability was 0.46 (vs. 0.31 for the hard-released animals). The insights from this release trial can be used in an adaptive management framework to improve and refine release methods that will inform the nascent field of amphibian reintroduction ecology.

KEYWORDS

captive-breeding, amphibian, mesocosm, release, radio telemetry, translocation

Introduction

The pathogenic amphibian chytrid fungi of the genus *Batrachochytrium* cause chytridiomycosis in amphibians; they are associated with the disappearance of 90 species and a >90% decline in the populations of 124 amphibian species (Scheele et al., 2019). In Latin America, Harlequin frogs (*Atelopus* spp.) have undergone dramatic population declines due to chytridiomycosis (La Marca et al., 2005; Scheele et al., 2019). Seven species of *Atelopus* from Panama are known: *Atelopus chiriquiensis*, *A. certus*, *A. fronterizo*, *A. glyphus*, *A. limosus*, *A. varius*, and *A. zeteki* (Lewis et al., 2019; Veseley and Batista, 2021). The Chiriquí Harlequin frog (*A. chiriquiensis*) has not been observed since 1996 (Lips, 1999) and the Panamanian golden frog (*A. zeteki*), the national symbol of Panama, has not been seen in the wild since 2009 (Lewis et al., 2019). Captive populations of the critically endangered *A. certus*, *A. glyphus*, *A. limosus*, *A. varius*, and *A. zeteki* have been established and maintained by the Panama Amphibian Rescue and Conservation Project (PARC), which prioritized species at risk of extinction from chytridiomycosis, with a goal to reintroduce at-risk species back into the wild (Gratwicke et al., 2016; Lewis et al., 2019).

Amphibian species from captive breeding programs used in reintroduction efforts are likely to face ongoing threats in the wild (Griffiths and Pavajeau, 2008). For instance, while chytridiomycosis is treatable in captivity (Brannelly et al., 2012; Baitchman and Pessier, 2013), it remains a significant challenge to mitigate in nature (Bosch et al., 2015; Garner et al., 2016). Some populations of *Atelopus* persist due to a high recruitment of offspring, but remain in a precarious state (Lampo et al., 2017; González-Maya et al., 2018). There is evidence that *A. varius* populations in Panama are developing resistance to *Batrachochytrium dendrobatidis* (Bd) (Voyles et al., 2018; Byrne et al., 2021), and rediscoveries of *Atelopus* populations once thought to be extinct may offer further insight into the genetic selection pressures that chytridiomycosis places on these populations (Jaynes et al., 2022).

Because the primary chytridiomycosis threat remains unsolved, the long-term goal of establishing viable populations of *Atelopus* in Bd-positive sites may seem out of reach. However, using captive populations in release trials may help to answer critical questions in reintroduction biology and will likely yield new observations that improve the outcomes of reintroductions and translocations for conservation purposes (Armstrong and Seddon, 2008; Jachowski et al., 2016). Optimizing release protocols can be achieved through a process of adaptive management by observing the effects of variables that may be taxon specific, such as developmental stage, location, season, group size, and acclimation (Morrison, 2015). For example, soft-release conditioning of animals is a costly but commonly deployed release protocol that can establish site fidelity and reduce mortality in some taxa, such as mammals, reptiles, and fish, but may increase stress and reduce success in other taxa, such as birds (Tetzlaff et al., 2019). Our goal was to understand how pre-release conditions (soft vs. hard release) affect dispersal and survivorship of captive-bred *Atelopus*. This is the first release trial conducted of any captive-bred *Atelopus* species, and direct observations of the animals' transition from captive

conditions to the wild provide a valuable learning opportunity to understand the challenges during this critical time.

Methods

Study site

The Mamóní Valley Preserve is located in Central Panama and is managed as part of the larger Mamóní Valley Conservation area, which has 1,500 ha of old-growth forest and 3,500 ha of watersheds that are undergoing restoration efforts following degradation from cattle and agriculture (Geoversity, 2020). *A. limosus* were collected here as a source population for the captive assurance colony at the Panama Amphibian Rescue and Conservation Project following the first Bd detection in the adjacent Chagres National Park in 2009 (Lewis et al., 2019). We selected a first-order perennial stream tributary to the Mamóní River for its accessibility, deep pools separated by riffles, and waterfalls in a mature secondary rainforest that does not have extant *Atelopus* populations. This site was about 5 km SE from the Madroño River where a small wild population of *A. limosus* persists.

Release trial

The aim of this study was to evaluate the movement and survival of 60 frogs that were held in the breeding facility and released directly into the wild (hard release) compared with 23 frogs that had been held for 30 days prior to release in mesocosms established in the wild (soft release). We used animals aged approximately 1 year derived from two clutches from founders collected at the Madroño River. ARC-Alim-F1-11 were descended from a male founder collected in 2010 and a female collected in 2011. ARC-Alim-F1-13 were derived from a male collected in 2010 and a female reared in captivity in 2013. The overall sex ratio was approximately even (37 female : 46 male). Frogs were individually marked with visible alphanumeric tags in accordance with the manufacturer's instructions [VI alpha; Visible Implant (VI) Tags standard size (1.2 mm × 2.7 mm), Northwest Marine Technology] (Heard et al., 2008; Pittman et al., 2008; Osbourn et al., 2011). We used a sterilized (alcohol-flamed) injector needle to insert the tag under the skin of each frog in the tibial region of the left hind leg rather than the dorsum, because it was less pigmented. We individually photographed the frogs' dorsal and ventral sides as a backup photographic identification reference. This proved necessary as the sub-cutaneous tags were difficult to read upon recapture, and we do not recommend them for future *Atelopus* studies. Soft-released animals were held individually in outdoor mesocosms (76 cm × 76 cm × 46 cm polythene mesh with 6 mm gaps) for 1 month prior to release (Estrada et al., 2022). We established three consecutive 80-m stream transects and released 7–8 "soft-release" and 20 "hard-release" *A. limosus* on each transect, placing one randomly selected frog every 5 m on either side of the stream at the transition from the dry to the rainy season on 17 May

2017. We conducted recapture surveys for the *A. limosus* and swabbed the existing frog community for Bd to estimate prevalence by conducting nocturnal and diurnal searches of the stream transects on weeks 4 and 8.

Bd swabbing

Frogs were swabbed for Bd following a standard protocol using an MW113 swab five times on each foot, 10 times on the belly, and 10 times on each thigh for a total of 40 passes (Kriger et al., 2006; Hyatt et al., 2007). Swabs were placed into 1.5-mL microcentrifuge tubes and stored in a -20°C freezer until processing. Swabs were extracted using Applied Biosystems PrepMan™ Ultra (Waltham, USA) following the manufacturer's protocol. For Bd detection, we used a real-time PCR assay following a standard protocol for Bd (Boyle et al., 2004) with some slight modifications. We used a Roche LightCycler® 96 System and 20 μL reactions consisting of 5 μL of sample and 15 μL of a master mix (Kriger et al., 2006; Hyatt et al., 2007; Estrada et al., 2022). We tested the samples in triplicate, along with five Bd-negative and one or two Bd-positive controls on every plate (Boyle et al., 2004; Kriger and Hero, 2007). Samples with two or three positive reactions were considered Bd positive. A few samples were repeated to confirm results. We quantified the infection load of Bd-positive samples based on the estimated number of zoospore equivalents using standards prepared from the JEL 423 Bd strain (Boyle et al., 2004; Estrada et al., 2022). We averaged the quantified load values and multiplied them by 100 to compensate for the extraction and dilution of the samples.

Radio tracking and recapture

We equipped 17 female *A. limosus* with radio transmitters prior to release (nine soft-release and eight hard-release frogs). We used

some of the smallest commercially available 0.31 g LB-2X transmitters with a 21-day battery life (Holohil Systems Ltd.) and attached transmitters to the largest females, which weighed approximately 3 g or more, to approximate the 10% radio transmitter-to-body-mass ratio traditionally observed in herpetological studies (Heyer et al., 1994; Altobelli et al., 2022). Surgical silicone tubing was used as a waistband with a fine cotton thread to mount the radio transmitters on the amphibians (Figure 1) (Bartelt and Peterson, 2000; Rowley and Alford, 2007; Pašukonis et al., 2014). The organic cotton thread eventually degrades and breaks, acting as an auto-release mechanism (Rowley and Alford, 2007; Pašukonis et al., 2014). We used a Biotracker radio receiver (Lotek, Ontario, Canada) to track *A. limosus* equipped with radio transmitters after release once per day during weekdays (total of 31 sessions). When an individual was located, we recorded coordinates using a Garmin GPS in WGS84. Fluorescent flagging tape with the individual's identification number was placed on the nearest vegetation as a starting point for the next radio-tracking period. Once a week we handled radio-tracked frogs to examine the condition of their skin where the silicone tubing contacted the frog, weighed and measured SVL, and swabbed them for Bd. The standard battery life of the transmitters is 21 days, with some variation, so we replaced radio transmitters every 18 days for a total radio-tracking duration of 54 days, after which the harness was removed and the frog released. If abrasion lesions were noted during the weekly swabbing and physical evaluation ($n = 5$ animals), the frog was removed from the study, the harness was removed, and the frog was placed in a plastic mouse cage and fed with wingless fruit flies. Abrasions were externally treated daily with silver sulfadiazine cream for 1 week, then frogs were released without any transmitter. Any non-radio-tagged *A. limosus* encountered during these radio-tracking surveys were identified or photographed and swabbed for incorporation into the mark-recapture estimates. The entire site was traversed during each radio telemetry survey, representing a continuous



FIGURE 1

Atelopus limosus female fitted with a 0.31-g LB-2X transmitter with 21-day battery life attached using organic cotton threaded through a 1–2 mm silicone tubing waistband to prevent abrasion.

unconstrained search for all released animals. We also conducted weekly dedicated stream transect surveys to find non-radio-tracked animals, and the animals found were identified, photographed, and swabbed for mark–recapture analysis.

Statistical analysis

Movement

We used the haversine formula to calculate the daily distance that the frogs covered, for which we had consecutive day locations recorded. However, the precision of the GPS readings (within 7.6 m, SD 1.98 m) was often not sufficient to distinguish the fine-scale daily movements of the frogs. Mean weekly distances covered were therefore calculated if locations were available for individual frogs at 7-day intervals. We used weekly rainfall data collected using an automated rain gauge placed at the station to examine distance covered in relation to rainfall. We tested the hypothesis that weekly distance covered was related to week, release type, or rainfall using a GLMMPQL model in the MASS package in R (Ripley et al., 2019). We selected this approach over a regular GLM to deal with an unbalanced design, as consecutive daily or weekly locations were not collected for all individuals, and to accommodate for repeated measures using individual frog ID as a covariate (Venables and Ripley, 2002). Frog tracks were mapped in ggplot2 using R.

Mark–recapture analysis

We estimated treatment-specific (hard vs. soft) survival probabilities by fitting a Cormack–Jolly–Seber model to individual encounter history data (Cormack, 1964; Jolly, 1965; Seber, 1965). We fit this model in a hierarchical Bayesian framework by specifying (1) a sub-model for the true state of each individual over time (alive or dead) and (2) a sub-model for the observation data (observed or not) (Royle, 2008; Kéry and Schaub, 2011). Our dataset integrated both radio-tagged individuals and VI-tagged individuals recaptured by chance in each of 31 radio-tracking survey occasions and four dedicated stream recapture surveys in the 58-day study period (total of 291 recaptures). We estimated standardized 30-day survival probabilities for both the hard- and soft-release treatment groups. Field observations indicate that radio-tracked individuals may have poorer survivorship than VI-tagged animals due to transmitter lesions, but for the purposes of this analysis we assumed survival did not differ between radio-tracked and VI-tagged individuals in order to estimate general survivorship for hard- and soft-released groups. We accounted for different detection probabilities associated with radio telemetry and visual resighting of individuals marked with visual implant tags but assumed, due to similarly low numbers of recaptures, that the detection probability of VI-tagged individuals did not differ between dedicated stream surveys and incidental observations during radio telemetry surveys. In cases of known mortality events ($n = 7$ animals), this additional information was incorporated by supplying the alive/dead state of the individual as observed data, rather than treating the state as a parameter to be

estimated for all surveys in which the individual was known to be dead. The lone exception was in the case of a single accidental mortality from a frog being stepped on, where observation data were “right-censored” by treating data as missing for all surveys after the mortality occurred to avoid biasing survival estimates. Observation data were also right-censored for four individuals from the date when radio transmitter signal was lost, with data treated as missing for all survey dates after the last detection of each individual.

We fit the model using the JAGS software run using the jagsUI package in the R program (Kellner, 2019; Plummer, 2020; R Development Core Team, 2021). We assigned uniform (0,1) priors for both detection and monthly survival probabilities. As is commonplace for most Bayesian analyses, JAGS performs model fitting using Markov chain Monte Carlo (MCMC) sampling by simulating probability distributions that are too complex to calculate mathematically. Probability distributions for model parameters were obtained by running three independent MCMC chains for 500,000 iterations each, discarding the first 250,000 iterations from each chain as “burn-in”, and thinning the remainder at a rate of 1 : 20 to obtain 37,500 samples from the joint posterior distribution (code provided in [Supplementary Material 2](#)).

Results

Release trial

Movement

After placing frogs alongside the stream, the animals mostly remained within 25 m of the stream, with a few, mostly hard-released individuals dispersing further ([Figure 2](#)). The release trial was started in May, which is considered the early rainy season in Panama, and dispersal appeared more pronounced after the onset of more frequent rainfall in June (BK, pers. obs.). However, there was no statistically significant relationship between dispersal distance and rainfall ([Table 1](#)). Released animals usually hid under cover, their aposematic colors became subdued, and they were very difficult to detect during re-surveys if they did not have radio transmitters. Just four re-encounters of VI-tagged frogs occurred on four dedicated stream transect surveys, and 23 re-encounters during 31 occasions spent searching for radio-tracked frogs (data in [Supplementary Material S1](#)). Hard-released frogs covered about 6 m further per week than soft-released animals [13.6 m (SD 9.1 m) vs. 7.7 m (SD 4.1 m), $p = 0.017$] ([Table 1](#), [Figure 3](#)), and soft-released frogs appeared to settle more in the immediate stream vicinity than hard-released frogs ([Figure 2](#)). Week was not a statistically significant predictor of covered distance; weekly distances covered by hard-released frogs began to decline in week 3, approximating those of soft-released frogs by week 5 ([Figure 3](#)). Bd status was not predictive of movement distance among the soft-released radio-tracked animals. The mean weekly distance covered was 7.5 m (SD 0.53 m) for Bd-positive frogs, vs. 7.9 m (SD 1.8 m) for Bd-negative frogs, two-sample t -test $p = 0.63$ ns.

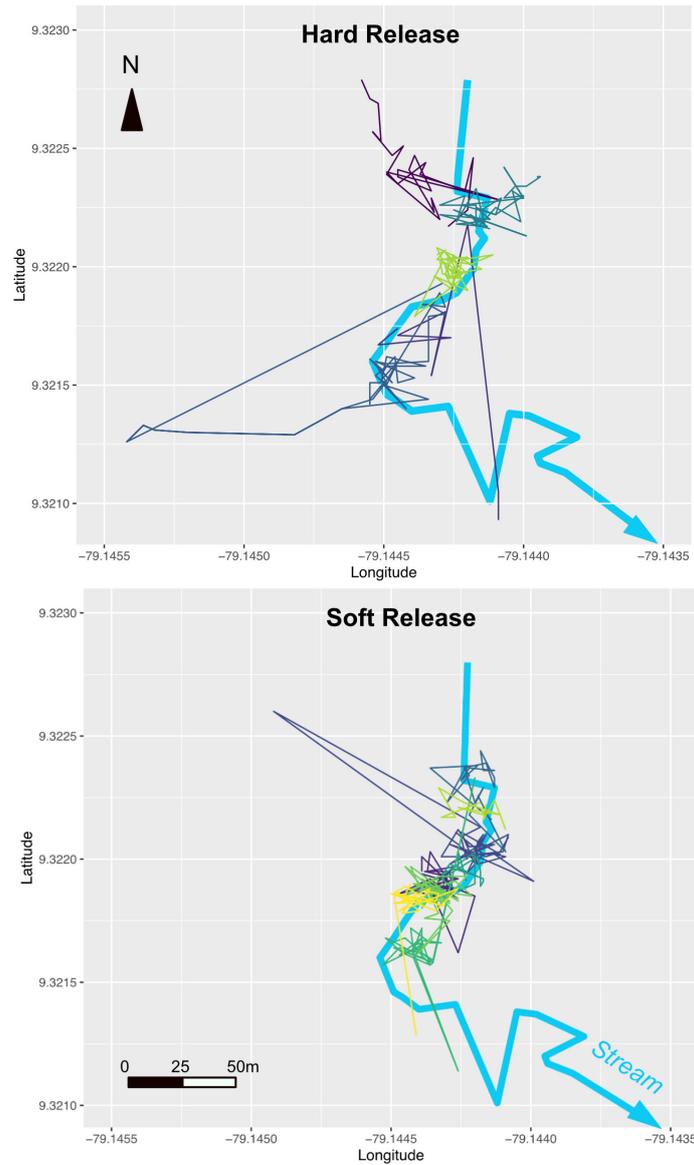


FIGURE 2 Tracks of individual hard-released animals (top) vs. soft-released animals (bottom) in relation to the release stream at the Mamoni Valley Preserve.

Survival

The individual fates of all radio-tracked animals are summarized in Table 2. Four of the frogs (25%) experienced abrasions from the radio transmitter and were removed from the experiment. All the abrasions were

detected during the third consecutive transmitter placement (between days 36 and 54). Four of the transmitters (25%) failed at some point during the tracking. Two of the frogs died from predation and one likely died from Bd. Only two were released into the wild following their final recapture at the end of the 58-day tracking period (Table 2).

TABLE 1 Main effects of a GLMMPQL analysis examining the effects of time, rainfall, and release type on weekly distance covered.

GLMMPQL [Weekly distance~ day + rainfall + release type, random = (frog D)]					
	Value	Standard error	DF	t-value	p-value
Intercept	15.39	2.69	36	5.7	0.000
Week	-0.56	0.65	36	-0.86	0.39
Rainfall	0.014	0.016	36	0.90	0.37
Release type	-6.768	2.398	11	-2.822	0.017*

* indicates statistical significance.

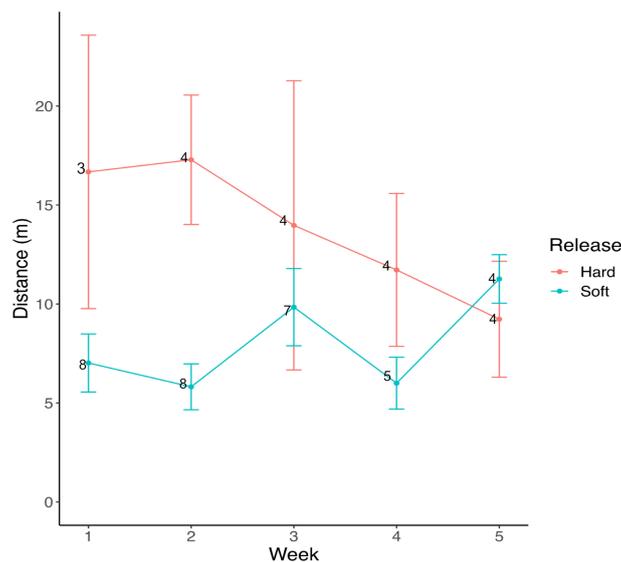


FIGURE 3

Mean weekly distances \pm SE covered by hard- vs. soft-released frogs. Numerals opposite the plotted means indicate numbers of individual animals for which weekly movement was observed.

We estimated per-survey detection probabilities of 0.024 (95% CRI: 0.014 to 0.04) for visual resighting of marked individuals and 0.88 (95% CRI: 0.84 to 0.92) for radio tagged individuals. Our low recapture rate of marked individuals led to a small sample size and considerable uncertainty in survival estimates. Monthly survival probability was estimated at 0.31 (95% CRI: 0.13 to 0.57) for individuals in the hard-release treatment and 0.46 (95% CRI: 0.23 to 0.72) for individuals in the soft-release treatment. The estimated difference in monthly survival (soft – hard) was 0.15 (95% CRI: –0.15 to 0.44), which equated to a posterior probability of 0.85 that individuals in the soft-release treatment had higher survival rates (Figure 4).

Bd

After 1 month in mesocosms, about 25% of the frogs were Bd positive (Table 3, Estrada et al., 2022). This prevalence of Bd was

similar to that observed in the stream frog community (24%–29%; Table 4) during our study. Smaller species of amphibians such as Dendrobatids or recent metamorphs of larger species were observed inside the mesocosms. Contaminated leaf litter potentially served as an additional source of infection. None of the hard-released animals were Bd positive at the time of release or upon subsequent recapture. One radio-tracked animal was found deceased with high Bd loads (6,950 zoospore equivalents) and two animals with transmitter-related abrasions died with high Bd loads (6,653 and 181,400 zoospore equivalents), with Bd likely contributing to the poor health and skin condition of these animals.

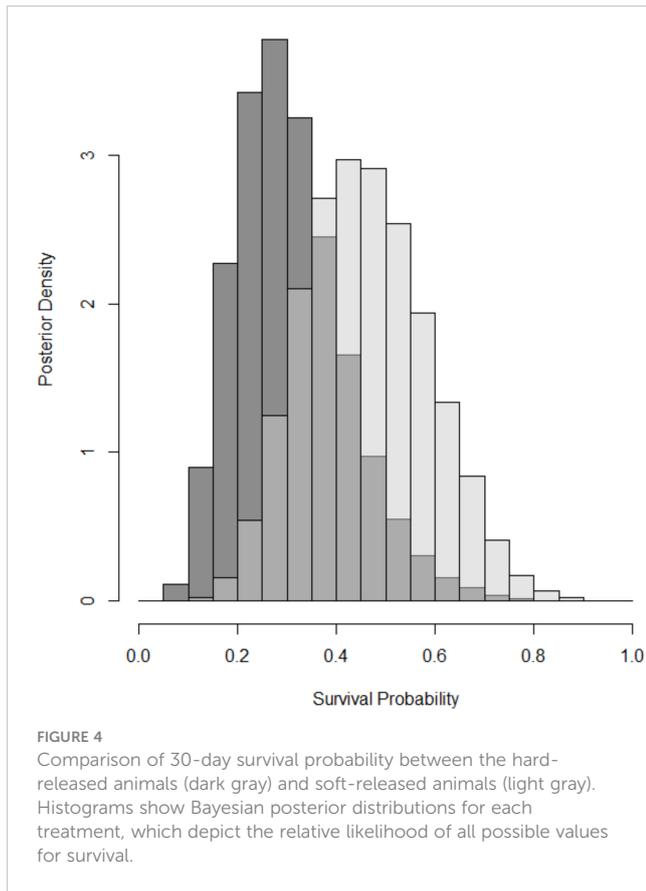
Discussion

We were surprised by the low re-encounter rates of non-radio-tracked animals in stream transects. Hence, using this recapture survey method alone in our study would have been insufficient for

TABLE 2 Summary of the fate of radio-tracked frogs.

Fate of frog		Hard release	Soft release
Survived	Released at end of radio tracking	2	
Unknown	Signal lost	2	2
	Transmitter dropped		2*
	Removed for abrasions and treated, then released	1	1
Died	Found dead, Bd positive		1*
	Predation [whip scorpion (<i>Phrynos</i> sp.), spider (<i>Trechalea</i> sp.)]	1	1
	Accidental mortality (trodden upon)	1	
	Removed for abrasions and treated, died in treatment	1	2*

Numbers and symbols in bold indicate Bd-positive animals. It is unclear whether the cause of death of the two Bd-positive frogs removed for abrasions was due to Bd or due to lesions caused by the transmitter. * indicates animals were Bd positive.



post-release monitoring. We determined that a release of 83 animals should be sufficient, as similar transect-based mark–recapture surveys had monitored wild *Atelopus* populations of 60–100 animals (Tarvin et al., 2014; McCaffery et al., 2015; Lampo et al., 2017). However, newly released captive animals are unlikely to exhibit the site fidelity observed in wild *Atelopus* (Crump, 1986; Tarvin et al., 2014), and translocated animals may be prone to dispersal (Knox and Monks, 2014; Linhoff and Donnelly, 2022).

To counter low recapture rates, we employed a hybrid mark–recapture analysis that used both radio telemetry data and re-

encounters of untracked animals, most of which were chance re-encounters during radio telemetry surveys. This approach allowed us to incorporate all redetection observations of animals into our mark–recapture model. Symptoms of heavy Bd infections include lethargy and the loss of righting reflex before death of the animal (Voyles et al., 2009); this could be a confounding influence explaining the reduced dispersal rates observed in the soft-released animals, as five out of nine soft-released radio-tracked animals were Bd positive by the end of the study and none of the hard-released animals was infected. However, there was no statistical difference in weekly distances covered between the soft-released Bd-positive and Bd-negative animals. Other studies with larger sample sizes noted that observed movement distances of *Litoria pearsoniana* were unrelated to Bd status (Murray et al., 2009). Although the probability of having a Bd infection in the soft-released cohort was much higher, there was likely a survival benefit from prior acclimation to the site in mesocosms. The observed reduction in survivorship of hard-released animals is likely connected to the increased dispersal distance observed for these animals, as increased activity and movement is associated with increased predation risk in amphibians (Chovanec, 1992; Skelly, 1994). In another study, a hard-released amphibian dispersed outside of its optimal habitat and died from desiccation (Linhoff and Donnelly, 2022).

While it is possible that these observed differences in survival are due to chance, these data will be useful to future practitioners, as our data point to some important potential benefits of a delayed release program similar to those observed in other species (Tetzlaff et al., 2019). For instance, a 2-week acclimation period had marked beneficial effects in Wyoming toads (Linhoff and Donnelly, 2022). Our observation that weekly dispersal distances were greater for hard-released frogs than for soft-released frogs for the first 3–4 weeks (Figure 3) suggests that 1 month might be a suitable acclimation period for *Atelopus*. A separately published study of these soft-released frogs during the 28-day period in the mesocosm found that skin bacterial communities were rewilded in approximately 3 weeks and the body condition approached that of wild counterparts in 28 days (Estrada et al., 2022), providing further biological support for a 3–4-week delayed release period.

Anecdotal observations of the fate of radio-tracked animals suggest that predation, Bd, and injury from radio transmitters all contributed to the low survivorship probability. Predation in released animals is likely to be higher than in their wild counterparts in part due to the fact that wild *A. limosus* are known to have a powerful neurotoxin in their skin, known as chiriquitoxin (Yotsu-Yamashita and Tateki, 2010; Pearson and Tarvin, 2022). These toxins are lost in captive settings (Daly et al., 1997) and the released animals probably lacked valuable anti-predator skin defenses. Mesocosms are also known to reduce the dispersal tendency of newly released animals (Knox and Monks, 2014; Tetzlaff et al., 2019; Linhoff and Donnelly, 2022) and may therefore reduce the chances of encountering a predator, such as a whip scorpion (*Phrynos* sp.) or a fishing spider (*Trechalea* sp.), both of which were directly observed preying on released animals. This reduced movement may account for the improved 30-day survivorship probability of soft-released animals.

TABLE 3 Summary of the Bd status of *Atelopus limosus* following release.

Week	Hard release		Soft release	
	Swabbed	Bd +	Swabbed	Bd +
0	60	0	23	6
1	6	0	10	4
2	5	0	9	3
3	5	0	6	3
4	4	0	7	2
5	4	0	4	3
6	4	0	4	2
8	3	0	2	1

TABLE 4 Bd prevalence in the natural stream frog community at release stream transects during weeks 4 and 8 of the study.

	Week 4			Week 8		
	Bd +	Total	%	Bd +	Total	%
<i>Colostethus panamansis</i>	6	18	33	10	18	56
<i>Craugastor fitzingeri</i>	0	1	0	1	5	20
<i>Craugastor optimus</i>	0	1	0			
<i>Craugastor</i> sp.	0	1	0			
<i>Diasporus diastema</i>	1	1	100	0	1	0
<i>Engystompops pustulosus</i>	0	1	0	2	8	25
<i>Pristimantis cerasinus</i>	0	1	0	0	1	0
<i>Pristimantis cruentus</i>	0	1	0	0	1	0
<i>Rhaebo haematiticus</i>	0	1	0	0	1	0
<i>Rhinella horribilis</i>				0	5	0
<i>Sachatamia ilex</i>				1	3	33
<i>Silverstoneia flotator</i>	0	3	0	0	6	0
Total	7	29	24	14	49	29

While radio transmitters were the most reliable way of tracking and understanding the fate of these animals, they occasionally failed or were dropped, and abrasion injuries became an issue after about 3 weeks of transmitter attachment. It is important to note that the three known mortalities potentially linked to abrasions (Table 2) could cause an underestimation in the survival rates compared with a release of only non-tracked individuals under similar conditions. These mortalities would not contribute to the higher survival estimates in the soft-release treatment, as two of these three mortalities occurred in the soft-release treatment. However, any differences in survival between VI-tagged and radio-tagged individuals do have the potential to increase variability in survival within the soft- and hard-released groups and may have reduced our power to detect the effects of release treatment. Abrasions were likely due to a loose waistband placement that allowed the transmitter to flip to a ventral position, requiring regular fit checks in the field (BK, pers. obs.). It is also possible that Bd, which disrupts skin function and causes skin flaking (Baitchman and Pessier, 2013), may have compounded the severity of transmitter-related skin abrasions.

In summary, we learned that soft-release strategies of just 3–4 weeks reduce dispersal and likely improve the survival of animals. The rapid dispersal of captive-bred animals quickly reduces the densities of animals, increases predation risk, and increases the total number of animals needed in a release trial to conduct effective post-release monitoring. Recapture probabilities for marked animals are very low using conventional survey methods, and radio tracking is the most reliable way to re-encounter frogs. However, transmitters should be snugly fitted to reduce the likelihood of injury to the animal. Due to GPS error, GIS

relocation has limitations for mapping fine-scale daily movements. Physical grids or mobile mapping applications to more precisely observe direct movements (McVey et al., 1981; Bartelt and Peterson, 2000; Pašukonis et al., 2018) will likely be needed to investigate territory establishment in *Ateolopus*. This release trial did not directly seek to establish a viable population of *A. limosus*, but our results will inform and improve monitoring and release methods for future studies and reintroduction efforts. Further intensively monitored release trials will be needed to provide knowledge that will be key to species recovery.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Smithsonian Tropical Research ACUC 2016-0311-2019.

Author contributions

BK, AE, DM, BG, EB, and RI conceived the research. BK, BG, RI, DM, AE, and LB obtained research funding and permits for the

field work. BK, MM, JG, ME, JB, AE, DM, BG, and RI conducted the field work. EB provided veterinary oversight. EI and RI analyzed swabs. BK, GC, and BG analyzed the data. LB, RI, EB, and BG supervised the project. BK and BG wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

This study received funding from a commercial entity, Holohil Systems Ltd. The funder was not involved in the study design; the collection, analysis, and interpretation of data; the writing of this article; or the decision to submit it for publication.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/famrs.2023.1205938/full#supplementary-material>

SUPPLEMENTARY MATERIAL S1

(1) *Atelopus* radiotelemetry data, (2) recapture data, and (3) Bd data.

SUPPLEMENTARY MATERIAL S2

R code for the Cormack–Jolly–Seber model.

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