A male Variable Harlequin Frog (Atelopus varius) is shown in a natural, dark, leafy environment. The frog has a bright orange body with several dark brown spots. A small, green and black radiotransmitter is attached to its waist using a thin, clear surgical tube and a cotton thread. The frog is perched on a dark, textured surface, possibly a log or a branch. The background is filled with dark, brown leaves and twigs, creating a dense, naturalistic setting.

Reintroducing captive-bred Variable Harlequin Frogs (*Atelopus varius*) back into the wild

By Brian Gratwicke, Roberto Ibáñez & Blake Klocke.

Male frog with a 0.3 g radiotransmitter attached to its waist using soft surgical tubing and cotton thread attachment.

Executive summary

We conducted the first-ever release trials of 560 surplus-bred *Ateolopus* at two sites in Panama to understand the transition of captive animals back into the wild. The goals were to improve post-release monitoring methods to understand dispersal, sources of mortality, habitat selection and disease dynamics in both the released frogs and the existing amphibian community. We found that mark-recapture methods were not very effective, even when using large numbers (ca 500) animals, because animals quickly dispersed outside of the resurvey transect areas, making recapture probability less than 5%. Using radiotransmitters, we documented dispersal and predation by ants, spiders, whip-scorpions and snakes (*Erythrolampus epinephelus*). Due to the high cost (\$180) and short battery life (21 days) of the transmitters, we anticipate that mesocosms will be an important tool to monitor frogs in future reintroductions. However, disadvantages of mesocosms are that frogs cannot escape ants or snakes that find their way in to the cages, and cannot select their own habitats and microhabitats. Some released frogs quickly became infected with the chytrid fungus and their disease loads rapidly increased to lethal levels within a few weeks. We found no evidence that the releases substantially increased Bd prevalence in the native frog community. We will use climate data collected from this work to begin evaluating potential climatic refuges from the disease that regularly exceed the maximum temperature threshold for the amphibian chytrid fungus as potential release sites. This grant was exceedingly important in providing the funds to maximize our learning opportunities at this critical stage in our project development.

Objective 1: We will conduct the first-ever experimental release trial of 300-400 captive-bred *Atelopus varius* at two sites in the Donoso region of Panama where populations have declined significantly because of the amphibian chytrid fungus.

In January 2018, we conducted the first ever release trials of 500 *Atelopus varius* at a site in the Donoso region in Panama, in July 2018 we conducted a second release trial of 58 *Atelopus limosus* in the Mamoni Valley. This progress report showing our results and data analysis to date. We will be preparing a publication in a peer-reviewed journal as a post-project deliverable once all data analysis is complete.

Release Trial 1

We successfully released 500 adult *Atelopus varius* into the field at one field site, exceeding our stated goals for that species. We have additional surplus animals, but wanted to fully evaluate this trial and select a second site in the region before proceeding with a modified release strategy, following adaptive management principles. Click on this link to see a video update from the field.

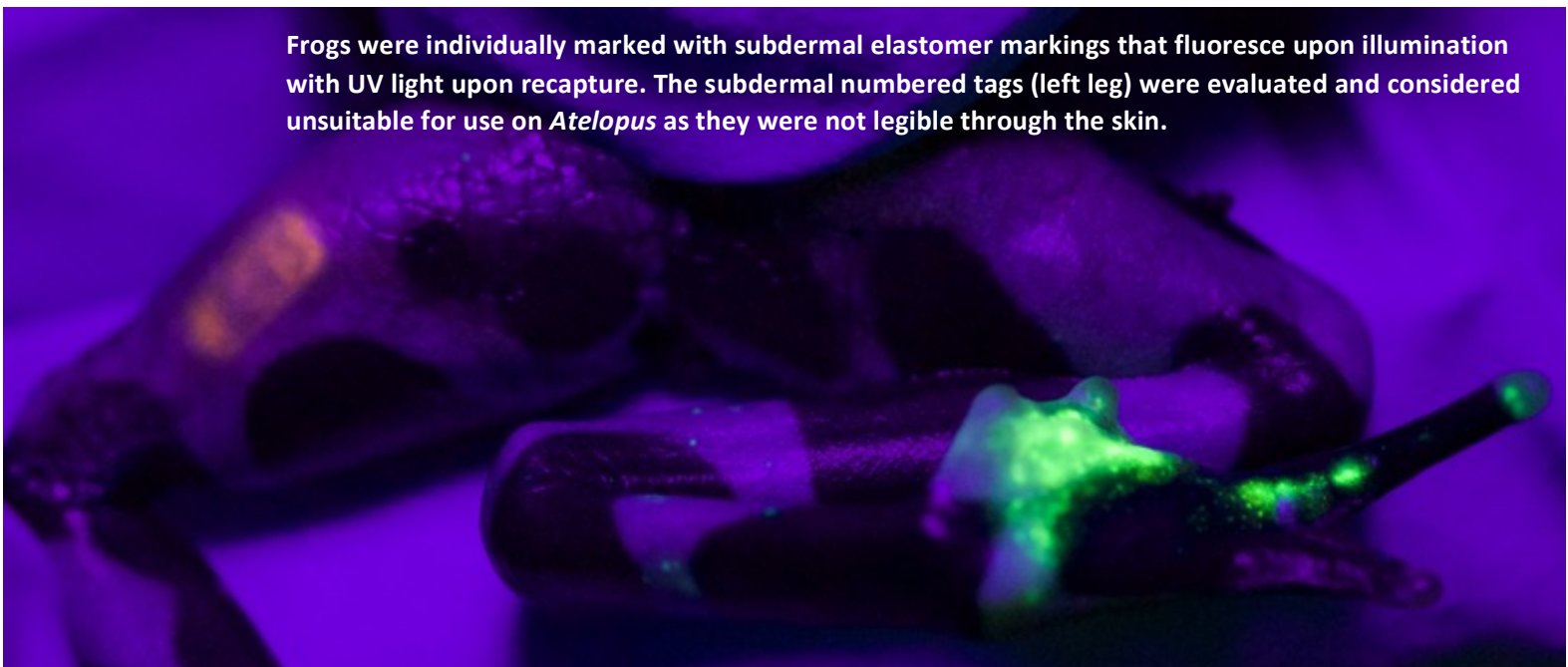
<https://video.nationalgeographic.com/video/news/00000161-0b2b-d70d-a7fd-8b3b8a760000>

Release Trial 2

We selected a second release site in the Mamoni Valley for logistical reasons, so that we could more easily monitor the released animals. The species used was *Atelopus limosus* as this is the species native to that site. Because of our poor recapture rates of animals without radiotracking devices, the second release focused on just 58 individuals using methods where recaptures could be enhanced either by radiotracking or by holding animals in mesocosms. In July 2018 we released 28 frogs that were tracked with radiotransmitters for 36 days, and 30 animals held in mesocosms for 50 days. The idea being that we would be able to better evaluate sources of mortality (through radiotracked animals) and disease (in mesocosms). Click this link to see a video update from the field

<http://amphibianrescue.org/2018/08/13/update-from-release-trial-research-at-centro-mamoni/>

Frogs were individually marked with subdermal elastomer markings that fluoresce upon illumination with UV light upon recapture. The subdermal numbered tags (left leg) were evaluated and considered unsuitable for use on *Atelopus* as they were not legible through the skin.



Objective 2: We will conduct mark-recapture resurveys to estimate survival and disease prevalence in released animals. We will radio track a small subset of 8 male and 8 female animals on a daily basis for 60 days to estimate dispersal, sex-specific habitat selection, and non-disease related mortality.

Release Trial 1

We tagged 500 frogs using a combination of elastomer tags and toe-clipping and released them along a single 150m stream transect and conducted recapture surveys on the stream transect and 2 adjacent terrestrial transects for 3 consecutive days post release, and 30 days post-release. This continued for 6 months. We failed to detect any of these released animals more than 30 days post-release.

We radio-tagged a subset of 30 frogs for a 21-day radiotracking study. Frogs dispersed much more widely than predicted, and we think it may have been connected to the exceptionally heavy rains associated with our release trial period. We decided to increase the numbers of frogs and reduce the total time monitoring them (compared to our original stated objective) in order to reduce the chances of transmitter abrasion injuries to the frogs.

Overall, we found that frogs dispersed far outside of our re-survey area, after 21 days, just 16% of the frogs remained within the area where our resurvey efforts could detect them. This is critical information to learn at this release trial stage, because in order to recapture frogs to evaluate reintroduction success, we have to be able to monitor them. This indicates that a new approach to post-release monitoring will be needed, alternatively use of mesocosms and a soft-release stage could reduce dispersal tendencies of frogs released from captivity into the wild. We will continue to analyze our data and design future releases to account for these factors.

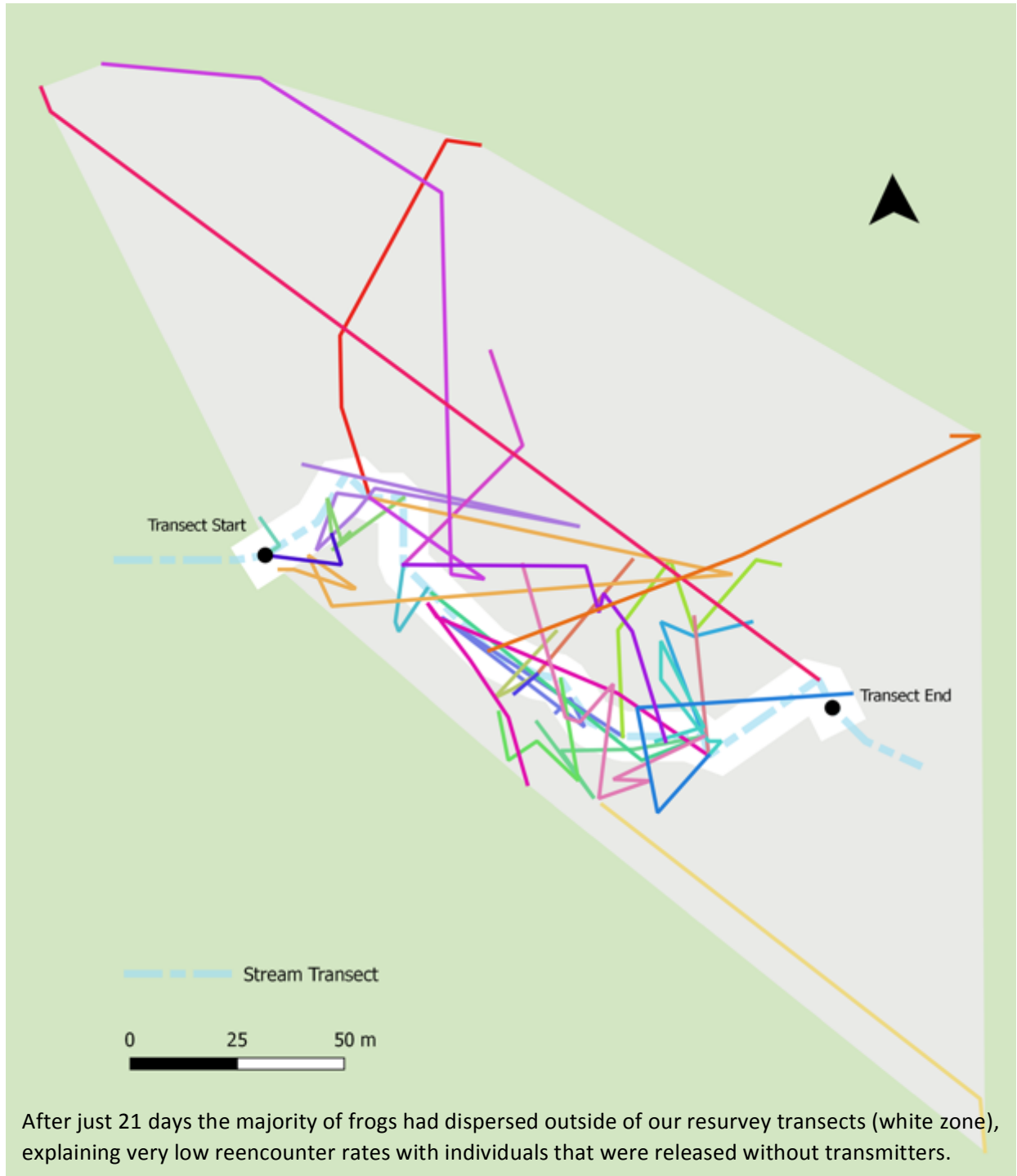
Release Trial 2

A second release trial using *Atelopus limosus* tracked 26 frogs for 36 days (two transmitters per frog). The additional frog radiotracking work was facilitated by additional grants that this MBZ grant helped to leverage.



Atelopus limosus, one of 26 animals tracked for 36 days post-release in the Mamoni Valley.

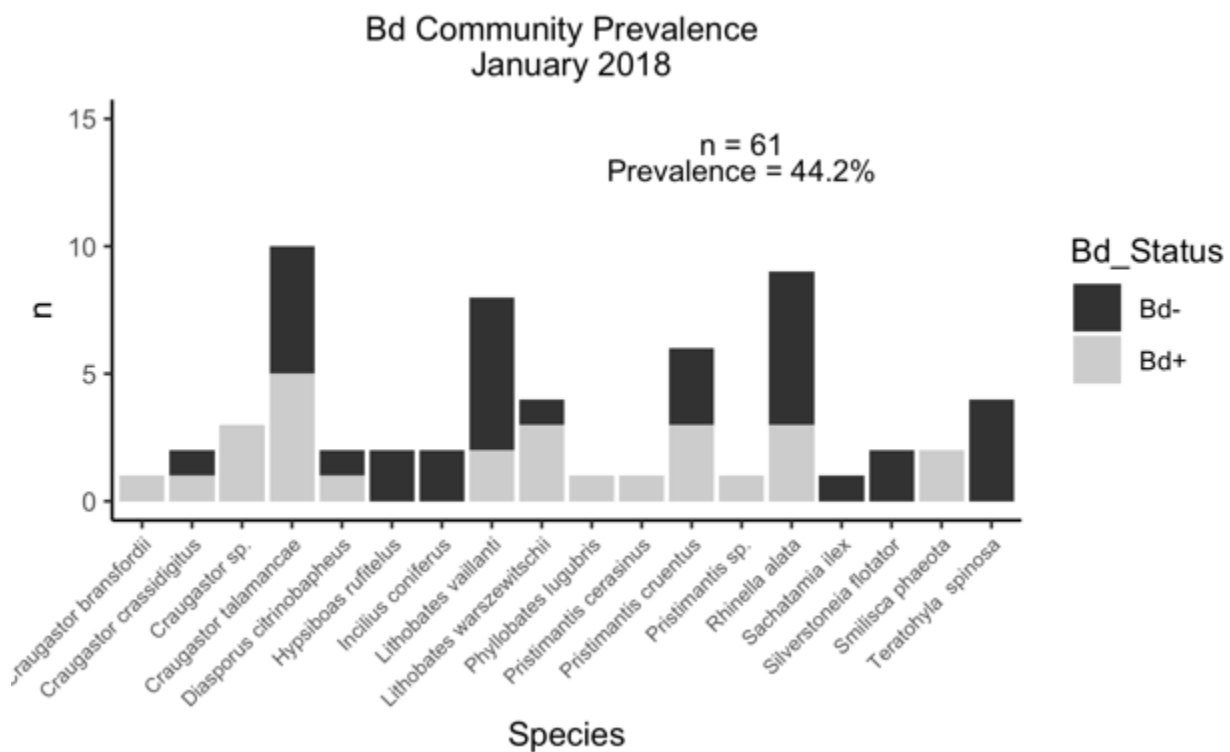
Map of individual *A. varius* dispersal at the Donoso release site.



Objective 3: We will collect baseline amphibian disease-prevalence information from the pre-existing community and monitor climatic conditions at each site. Data from this release trial will immediately inform demographic models of frog population persistence and decline in an adaptive management framework that guides future reintroduction efforts.

Release Trial 1

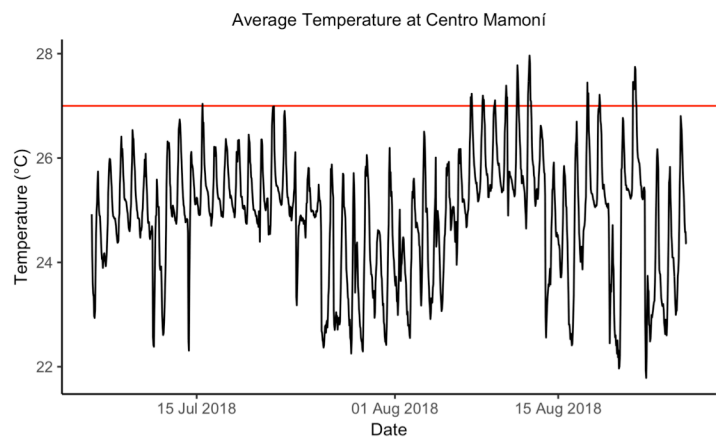
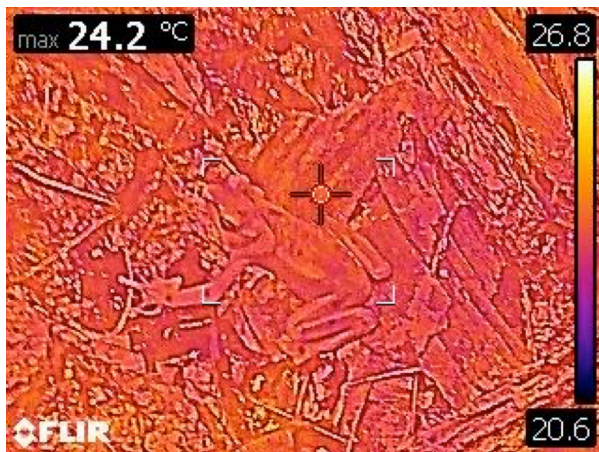
We set up pre-release monitoring transects of the native amphibian community using diurnal and nocturnal transects at the release site, and a second near-by stream, to evaluate the impact of the release on disease and community composition in the pre-existing frog community. We found an exceptionally high pre-existing disease prevalence in the frog community of 44%. In addition our native frog surveys recovered dead and dying frogs infected with the chytrid fungus.



We are still processing swabs from the remaining 6 months of community surveys to see if release of our 500 *A. varius* impacted Bd prevalence in the release stream, compared to the control stream. Clearly this high disease prevalence is not an ideal disease situation to conduct a release trial with susceptible species and may explain the lack of recaptures after 1 month. We did not manage to get any longer-term recapture swab data from *Atelopus* to look at disease dynamics in the released population of frogs, but over the short term recaptured frogs were turning up Bd positive.

We set up 14 mesocosms to monitor individual frogs over a 60-day post-release period to examine disease prevalence in a closely monitored cohort of frogs and production of defensive skin toxins. We are still analyzing results from that study with collaborators at Villanova University and Boston University.

At both sites we monitored frog body temperature on released frogs and set up temperature data loggers to measure ambient conditions. This will be critical data to link to hobo data-loggers that can then be used to model frog body temperature from downscaled global temperature data sets. Overlaid with chytrid suitability maps we plan to produce a country-wide map of potential climatic refuge areas from the disease that could be better release sites in the long term.

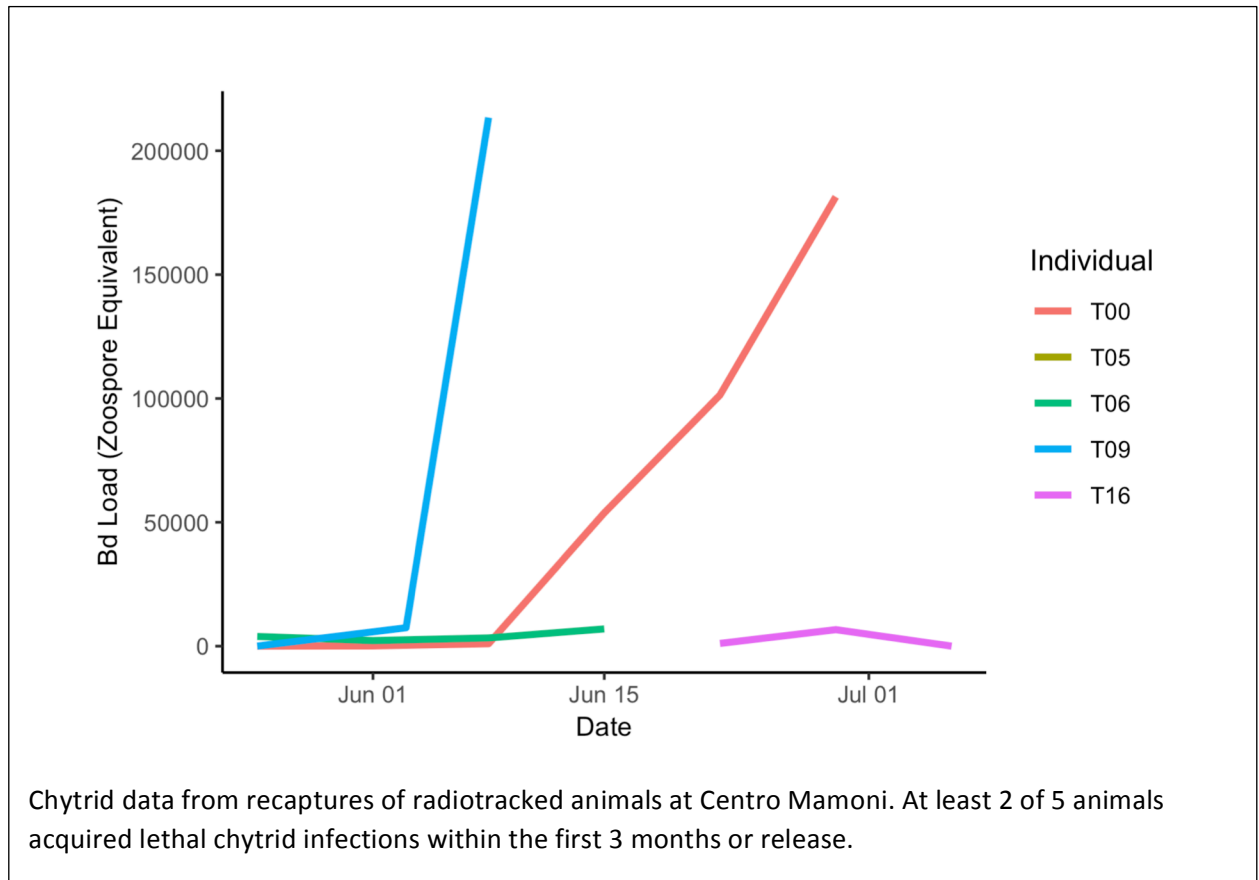


Frog body temperatures closely track the ambient temperatures (see thermal image). If frog body temperatures regularly exceed 27C there is a chance they could reduce or clear Bd infections, meaning that warmer places in *Atelopus* range have the potential to act as climatic refuges.

Release Trial 2

The before and after surveys of native amphibians did not find any significant increase in Bd prevalence in the native frog community after release of the trial animals which increased from 24.1% to 28.6%.

As far as disease goes, the release trial at Centro Mamoni found that radio-tracked did get infected with the chytrid fungus and loads rapidly grew to lethal levels in a matter of weeks, but not all frogs became infected right away.

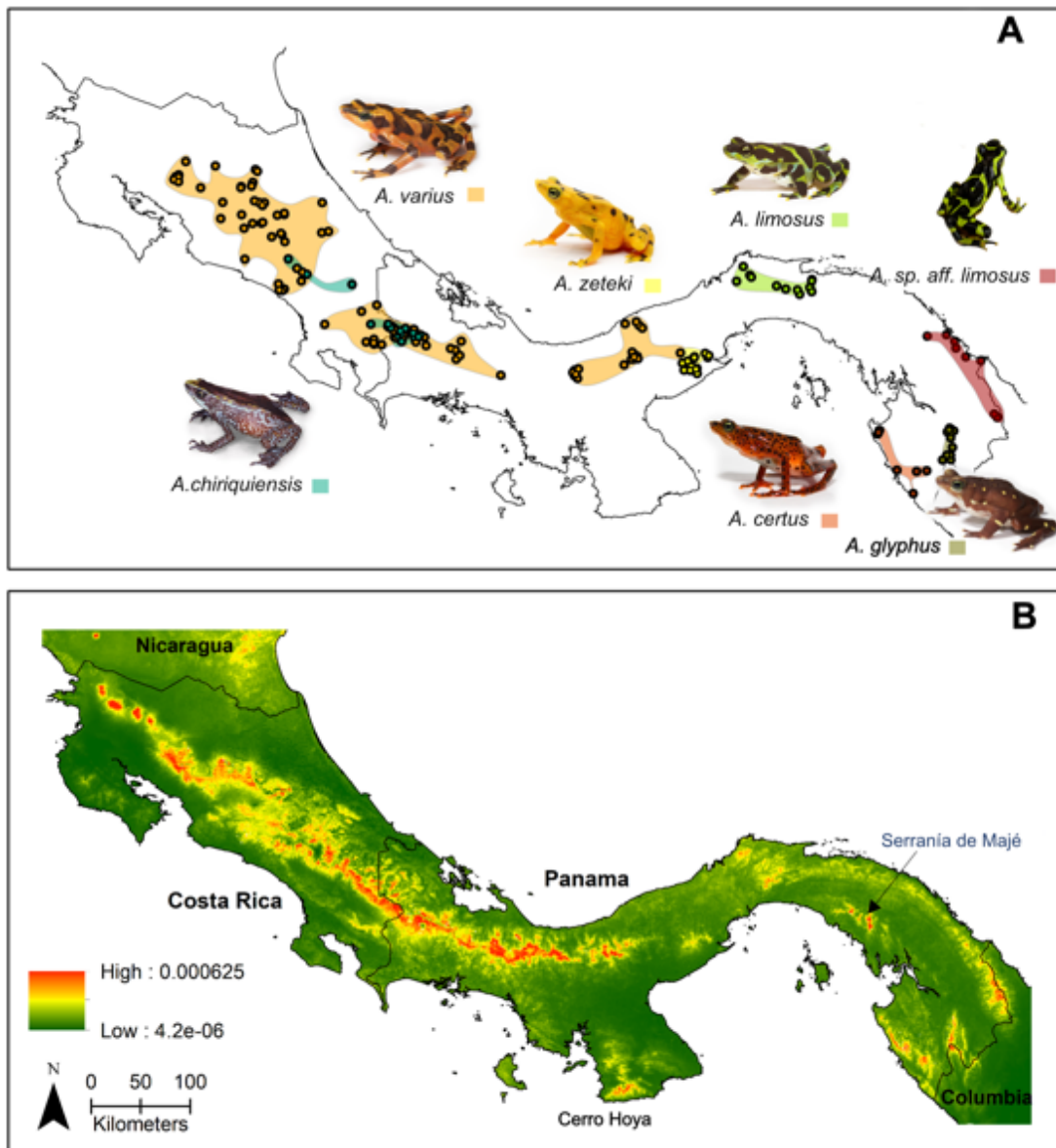


Release trial 2 documented detailed sources of mortality for radiotracked frogs. Released frogs were preyed by invertebrates including spiders, ants, snakes and whip-scorpions. One particular snake species *Erythrolampus epinephelus* is known to eat poison dart frogs and is likely immune to *Atelopus* tetrodotoxins was observed getting into the mesocosms to eat the frogs, and one radiotracked frog was found inside a snake (left). Clearly, we need to conduct research into how these

frogs regain their natural predator defenses, and understand if it is possible to 'arm' them in captivity with dietary supplements that may alter their toxicity and palatability to predators.

Objective 4: These preliminary release trials will be used as part of a larger long-term effort to restore *Atelopus* populations throughout Panama and evaluate whether microclimates at different sites have the potential to act as ‘climatic refuges’ from the amphibian chytrid fungus.

We have constructed a habitat suitability model for *Atelopus* based on historical distribution records (below) that will act as the foundation to overlay the chytrid suitability layer that is currently being compiled in order to identify climatic refuges. This first paper is in preparation for the journal Biological Conservation.



Below is the text of a press release we sent out relating to release trial 1 and some related media coverage.

PRESS RELEASE Jan 22 2018: Variable Harlequin Frog release trials begin in Panama

Once common along highland streams from western Costa Rica to western Panama, the variable harlequin frog is endangered throughout its range, decimated by a disease caused by the amphibian chytrid fungus. On Jan. 17, Smithsonian researchers released approximately 500 frogs at Cobre Panama concession site in Panama's Colon province as a first step toward a potential full-scale reintroduction of this species. This release trial is included in Cobre Panama's biodiversity conservation plan as an important part of their environmental commitments.

The variable harlequin frog, *Atelopus varius*, takes its name from the variety of neon colors—green, yellow, orange or pink—juxtaposed with black on its skin. In order to monitor the released frogs over time, 30 are wearing miniature radio transmitters. The scientific team also gave each frog an elastomer toe marking that glows under UV light to mark individuals as part of a population monitoring study.

“Before we reintroduce frogs into remote areas, we need to learn how they fare in the wild and what we need to do to increase their chances of survival in places where we can monitor them closely,” said Brian Gratwicke, international coordinator of the Panama Amphibian Rescue and Conservation project (PARC) at the Smithsonian Conservation Biology Institute. “Release trials may or may not succeed but the lessons we learn will help us to understand the challenges faced by a frog as it transitions from captivity into the wild.”

Variable harlequin frogs are especially sensitive to the amphibian chytrid fungus, which has pushed frog species to the brink of extinction in Central America. PARC brought a number of individuals into the breeding center between 2013 and 2016 as chytrid continued to impact wild populations.

“The variable harlequin frog is one of the closest relatives of *Atelopus zeteki*, Panama's iconic golden frog, another target species in our captive breeding program,” said Roberto Ibañez, PARC project director at the Smithsonian Tropical Research Institute in Panama. “We'll be monitoring the surrounding amphibian community and the climate at this site, and comparing this to the amphibian community at another, control site. This kind of intensive monitoring will help us to understand disease dynamics in relation to the release trials”

PARC hopes to secure the future for this and other endangered amphibians by reintroducing animals bred in captivity according to an action plan developed with Panama's Ministry of the Environment and the International Union for the Conservation of Nature (IUCN) and other stakeholders. “It took us several years to learn how to successfully breed these frogs in captivity,” said Ibañez. “As the number of individuals we have continues to increase, it provides new research opportunities to understand factors influencing survival that will ultimately inform long-term reintroduction strategies.”

The PARC project thanks Cobre Panama, National Geographic Society, **Mohammed bin Zayed Species Conservation Fund** and The WoodTiger Fund for their generous support.

PARC is a partnership between the Cheyenne Mountain Zoo, the Houston Zoo, the Smithsonian Tropical Research Institute, the Smithsonian Conservation Biology Institute and Zoo New England. It has two facilities in Panama: the Gamboa Amphibian Rescue and Conservation Center at STRI and the El Valle Amphibian Conservation Center at El Nispero. Combined, these facilities have a full-time staff caring for a collection of 12 endangered species.

Related media coverage:

- April [Frog reintroduction seeks chytrid-fungus answers](#) Eco Americas
- March 29 [What stops mass extinctions? Lessons from amphibian die-off in Panama](#) Science Daily
- Feb 2 [These Captive-Bred Frogs Are Facing Predators and the Chytrid Fungus to Make It in the Wild](#) Smithsonian.com
- Enero 23 [Liberan a 500 ranas en peligro de extinción como parte de un ensayo en Panamá](#) el Pais
- Jan 22 [Hundreds of Tiny Frogs Released on a Mission to Save Their Species](#) (Video) National Geographic
- Jan 18 [Frogs Tote Tiny Radios Released on a Mission to Save their Species](#) National Geographic
- Aug 29 [A Second Release Trial Advances The Goal of Reintroducing Endangered Frogs Into The Wild](#) Smithsonianmag.com

A full set of high-resolution field images can be found at this link: <https://flic.kr/s/aHskr2vzf4>

Presentations:

June 2018 Brian Gratwicke National Geographic Explorers Festival, Washington DC “Reintroducing captive-bred Variable Harlequin Frogs (*Atelopus varius*) back into the wild”.

November 2018 Blake Klocke 2nd International Wildlife Reintroduction Conference, Chicago “Reintroduction Conference Reintroduction efforts for two species of Panamanian Harlequin frogs (Genus: *Atelopus*) threatened by the amphibian chytrid fungus”.

Next Steps

We are still processing the large amount of data collected from these release trials. These will form the basis of a PhD thesis for Blake Klocke and will be prepared for publication in the coming year as a post-project deliverable. This critical funding has been transformational for our organization in conducting the first-ever releases. We were surprised to have so few recaptures so quickly after the releases, and these observations will force us back to the drawing board for devising post-release monitoring schemes. This funding enabled us to gather the critical information necessary to evaluate these releases and the observations necessary to improve in the future.

Future release trials will likely involve more radiotransmitting tracking studies and focus on evaluating dispersal differences between soft released frogs that are maintained in a mesocosm before-hand. Recapture methodology for non radio-tracked animals will likely have to involve some sort of unconstrained search method within the dispersal zone rather than being tied to transects. Dispersal zones will need to be determined through radiotransmission studies. Longer term mesocosm setups will probably be our best option for repeated capture and swabbing of animals, and experimentally evaluating the climatic refuge effects of different microclimates.

We are extremely grateful to The Mohamed bin Zayed Species Conservation Fund for their ongoing support of our amphibian conservation efforts in Panama.