

Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs

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ABSTRACT

Captive breeding programs are a valuable conservation strategy, particularly when integrated with research goals. Panamanian Harlequin frogs (genus *Atelopus*) serve as a case study for integrating captive breeding and research goals because they have experienced drastic chytridiomycosis-related declines and have large captive populations. Captive breeding efforts in Panama and the United States established secure ex-situ populations of *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*, and *A. zeteki*. *Atelopus chiriquiensis* is presumed to be extinct with no captive populations. The status of one undescribed species, *Atelopus* aff. *limosus*, has not been evaluated and no secure captive population has yet been established. Captive breeding efforts that produce a surplus of *Atelopus* are an important resource for research into collections management, disease mitigation, and adaptive management approaches for *Atelopus* reintroduction efforts. We reevaluated all Panamanian *Atelopus* species through the IUCN Redlist and compiled occurrence records for Panamanian *Atelopus* species to create a historical distribution map. We model *Atelopus* habitat suitability using Maxent and found annual mean air temperature to be the best predictor of *Atelopus* occurrence. The model will improve our knowledge of their likely spatial distribution and guide future conservation and reintroduction efforts. The recent proliferation of molecular tools, climate models, bio-banking, and reproductive technologies position us to address multiple applied and basic evolutionary questions such as: What factors cause differential disease outcomes? Do persisting populations have heritable traits associated with improved survivorship? Are there climatic refugia from disease? Ultimately, the answers to these questions will help us develop applied solutions and facilitate the reestablishment of self-sustaining wild populations.

1. Introduction

Amphibian populations are declining globally with more than one-third of evaluated species being listed as globally threatened (Stuart et al., 2004; Hoffmann et al., 2010; Olson et al., 2013). Threats to amphibians include habitat loss (Cushman, 2006), environmental contamination (Mann et al., 2009), global warming (Wake and Vredenburg, 2008), and human interference (La Marca et al., 2005). However, the disease chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) is a major threat that is decimating

amphibian populations worldwide (Berger et al., 2016). With no current tools to mitigate this threat, ex situ conservation programs have been set up to prevent imminent extinctions with the hope that species will eventually be reintroduced to the wild (Zippel et al., 2011).

Harlequin frogs in the genus *Atelopus* are among the most threatened amphibians in the world (Löters, 2007). Threats to *Atelopus* include habitat modification and collection for the pet trade, but Bd is the most pressing threat, responsible for catastrophic declines and disappearances throughout their range (La Marca et al., 2005; Berger et al., 2016). Panama has six described species of *Atelopus*: *A. certus*, *A.*

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chiriquiensis, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, and at least one undescribed species: *Atelopus* aff. *limosus* (Flechas et al., 2017). All of the described species are listed as Critically Endangered or Extinct by the IUCN (IUCN SSC Amphibian Specialist Group, in press). There is currently no realistic solution to remove or reduce the impact of *Bd* on *Atelopus* in Panama, making ex situ captive assurance colonies one of the only options to prevent their continued decline and extinction (Gratwicke et al., 2016).

Captive breeding is expensive and requires substantial effort, often with no clear end date, making fundraising a demanding task subject to donor fatigue. As a result, breeding programs are subject to the common criticism that funds should be prioritized elsewhere. However, the benefits of captive breeding go beyond simply preventing extinction. In the short-term, captive collections can prevent imminent extinction, and in the long-term, they can provide surplus animals for research. This can include research to improve collections management and sustainability, understand and mitigate disease, and increase the chances for successful reintroduction.

This paper uses Panamanian *Atelopus* as a case study for captive breeding as a conservation action. We chose to focus on *Atelopus* that occur in Panama because that is the focus of the Panama Amphibian Rescue and Conservation Project (PARC), which has been a leader in building captive assurance populations of amphibians in response to the threat of *Bd*. We discuss how captive breeding allows for research to improve collections management and sustainability, mitigate *Bd*, and reintroduce *Atelopus* populations. We summarize the conservation status of Panamanian *Atelopus* species both in the wild and in captivity. As part of the wild assessment, we update known historical distribution maps and model habitat suitability for *Atelopus* in Panama to inform conservation and reintroduction efforts. Finally, we discuss future research using captive populations that can move us towards science-based conservation solutions.

2. The role of captive breeding programs

Captive breeding and reintroduction are two priorities of the Global Amphibian Conservation Action Plan (Gascon et al., 2007). Of the > 7900 described amphibian species, 77 now have active captive breeding and reintroduction programs (Harding et al., 2016). These programs are often used to develop genetically viable, sustainable captive populations, as well as to grow and maintain suitable infrastructure and capacity to support those activities (Griffiths and Pavaudeau, 2008; Harding et al., 2016). Captive populations also serve as living ambassador animals with incredible power to engage audiences, and education programs are a critical element to captive breeding programs that help to build public support for the conservation of these species (Zippel et al., 2011).

In Panama, the first efforts to establish captive assurance populations of amphibians in response to the *Bd* threat began in 2001 when Panamanian golden frogs (*A. varius* and *A. zeteki*) were exported to U.S. zoos to be managed as part of a species survival program (Zippel, 2002). Later efforts built the physical infrastructure and staffing capacity needed to house additional at-risk species in Panama itself (Gratwicke and Murphy, 2016). A prioritization exercise examining 214 species of Panamanian amphibians found that *Atelopus* were among the species at highest risk for *Bd*-related extinctions and were expected to have the best chances of avoiding extinctions through captive breeding efforts (Gratwicke et al., 2016).

The Panama Amphibian Rescue and Conservation Project (PARC) is located at the Smithsonian Tropical Research Institute (STRI) in Panama. STRI hosts 1400 visiting scientists, students, and interns per year at six research stations around the country. The infrastructure and the ability to host visiting researchers uniquely positions the PARC project to integrate captive breeding and research goals, and access to *Bd*-susceptible animals is a critical resource for these goals. PARC holds captive assurance populations of 12 amphibian species, including five

Atelopus species (Box 1). The rapidly growing captive *Atelopus* populations at PARC have exceeded most of the captive management goals (Fig. 1, PARC 2017). Management options now include selective rearing of limited numbers of offspring, euthanizing surplus-bred juveniles, or allocating surplus frogs for research.

2.1. Research to improve collections management and sustainability

Many research priorities associated with captive collections are designed to help solve problems that affect the sustainability or improve the cost-effectiveness of captive rearing. For example, research on stress hormones helped to establish cost-effective group housing recommendations for *Atelopus* (Cikanek et al., 2014), while research on the causes of spindly leg syndrome generated new solutions for treatment of this lethal condition associated with captive populations (Ciani et al., 2018). Collection and treatment of large numbers of *Bd*-positive *Atelopus* from the wild as they were brought into captivity offered veterinarians a valuable chance to optimize disease screening and treatment protocols using a case-study approach (Baitchman and Pessier, 2013).

Genome resource banks and tissue collections of rare species are an invaluable resource. High-quality tissues are needed for genomic research that are not normally available for species on the brink of extinction (Comizzoli and Wildt, 2017). Hormone dosing methods can now be used to help improve representation of difficult-to-breed founding animals and to collect gametes to build cryopreserved genome resources that can safeguard against unintended genetic bottlenecks in captivity (Kouba and Vance, 2009). Cryopreservation methods for amphibians have not been perfected, but live amphibian offspring have been created from cryopreserved spermatozoa (Clulow and Clulow, 2016), and comprehensive biobanking protocols have been developed for amphibian species (Zimkus et al., 2018). Work has begun on cryopreserving tissue and sperm for all Panamanian *Atelopus*, though live offspring from frozen *Atelopus* sperm have not yet been produced (Della Togna et al., 2017). In the future, assisted reproduction technology could be applied to collect sperm from relict populations that may have survived the *Bd*-outbreak and introduce genotypes that may be resistant or tolerant to *Bd* into the captive populations.

2.2. Research into *Bd* management

Most instances of natural *Bd* infections in wild *Atelopus* populations have been associated with serious declines that ended in species non-detection (Table 1). As long as we have captive populations of susceptible *Atelopus* species, the incentive remains to continue working on finding a solution to the chytridiomycosis problem. We do not yet have a realistic solution to manage *Bd* in nature (reviewed by Garner et al., 2016), but a significant body of knowledge on the threat of *Bd* to *Atelopus* has been derived using surplus captive-bred animals. Captive *Atelopus* populations helped us understand that animals can tolerate infections for longer under warmer, drier condition (Bustamante et al., 2010) and that supershedding *Atelopus* do not predict higher infections in more tolerant co-housed species (DiRenzo et al., 2018). They have allowed research that characterized genomic responses to infection showing that prior exposure to disease caused a vigorous immune response that was ultimately unsuccessful at controlling infection (Ellison et al., 2014; Ellison et al., 2015). Captive population research evaluated putative changes in *Bd* virulence illustrating the importance of laboratory passage and culture practices to prevent changes in virulence of pathogens in culture (Langhammer et al., 2013; Voyles et al., 2018). Research also assessed the effectiveness of behavioral (Sauer et al., 2018) and innate defenses (Voyles et al., 2018) against *Bd*, finding that captive *Atelopus varius* from colonies created pre-*Bd* had much less potent anti-*Bd* skin secretions than those that persist in the wild (Voyles et al., 2018).

We need more basic research to understand what influences

Box 1Conservation status of *Atelopus* in Panama.

Also see Fig. 2A for historical ranges of all species and Table 1 for information on *Bd* declines. The Amphibian Ark recommends collecting at least 20 pairs of each species as founders with the goal of 10 breeding pairs and growing the population to 300–500 individuals (Schad, 2008).

***Atelopus certus* - Status in the wild: Critically Endangered (IUCN, in press).** This species is endemic to the Darien region of Panama. *Bd* has not yet been detected in this species' range, but future population declines are projected based on declines observed for other species in this genus (La Marca et al., 2005). The last monitoring surveys in January 2016 recorded fewer frogs than expected, however, because no individuals were recorded as *Bd* positive at the time, it is uncertain whether this is due to infection with *Bd* or the drought-related to El Niño in 2016 (R.I., unpublished data). **Status in captivity: Secure.** The total living captive population is represented by 22 founders out of 28 individuals that were bred, and the captive population is about 350 adult animals.

***Atelopus chiriquiensis* - Status in the wild and captivity: Extinct (IUCN, in press).** This species was once considered locally abundant along streams near the border of Panama and Costa Rica but declined due to chytridiomycosis (Berger et al., 1998; Lips, 1999). There have been no known sightings of this species since 1996 (La Marca et al., 2005), and experts believe the species is Extinct (Gratwicke et al., 2016). No captive populations exist.

***Atelopus glyphus* - Status in the wild: Critically Endangered (IUCN, in press).** This species was once locally abundant in the Pirre range in Panama, but the first field observations of dead, *Bd*-positive frogs were reported in 2015 (M. Ponce pers. comm. October 2015). These appear to have been linked to marked population declines as the last survey in January 2018 recorded only a single *Bd*-positive individual over three days of searching (O. A. Garcés pers. comm. May 2018). **Status in captivity: Almost secure.** Of the 20 founders bred, only 18 have surviving captive offspring, requiring at least two more unrepresented founders to be bred to meet our minimum Amphibian Ark population goals. The total adult captive population is about 350 animals.

***Atelopus limosus* - Status in the wild: Critically Endangered (IUCN, in press).** This central Panamanian species has disappeared from many known localities since *Bd* was first detected in Chagres National Park in 2009 (R.I., unpublished data). Declines at higher elevation sites were very rapid while the declines at a lowland site, took place over five years and a few individuals were observed in 2015 (R.I., unpublished data). These sites have not been surveyed since 2016, so it is not known if remnant populations exist. In 2018, wild populations persisted at several sites within the Mamoni Valley but some individuals had heavy *Bd* infections (B. Klocke, pers. comm. May 2018). **Status in captivity: Secure.** Twenty-six individuals have been bred in captivity. Offspring from just 20 of those pairs survived to adulthood and make up the current captive population. The species shows geographic variants: some populations have a muddy brown coloration similar to the type-specimens of this species (Ibáñez et al., 1995), and others have a black and green coloration with a chevron-shaped black pattern on the dorsum. The captive founding population includes only this chevron variant.

***Atelopus varius* - Status in the wild: Critically Endangered (IUCN, in press).** This species' historical range stretches along the central cordillera of Costa Rica and Western Panama (Savage, 1972; Zippel et al., 2006). Most of the declines noted for this species occurred between 1987 and 2007. Since then, remnant populations have been rediscovered in Costa Rica (Ryan et al., 2005; Escobedo-Galván et al., 2013) and in Panama (Hertz et al., 2012; Perez et al., 2014; Voyles et al., 2018). The largest known population in Panama persists in the lowland Caribbean forests in the Donoso area but frogs there have a high *Bd* prevalence and are highly susceptible to chytridiomycosis (R.I., unpublished data). **Status in captivity: Secure.** A total of 24 founders are represented in captivity in Panama including 8 highland and 16 lowland-collected founders that are separately managed. Acquisition of founders from lowland areas in the Donoso area as recently as 2016 boosted the total founder populations for this species, but many of these animals still need to be bred. In addition to the Panamanian captive population, about 160 frogs descended from six highland founders exist in U.S. zoos (K. Barrett pers. comm 2018).

***Atelopus zeteki* - Status in the wild: Critically Endangered, Possibly extinct in the wild (IUCN, in press).** This species was found around the area of El Valle de Anton and Cerro Campana (Richards and Knowles, 2007). These populations have been in decline for decades due to habitat modification/loss and over-collecting for the pet trade. The first *Bd*-related declines were observed in 2005 near El Valle de Anton. The last wild animal was seen in 2009 (E. Griffith pers. comm. 2015). Surveys conducted once or twice each year between 2012 and 2017 at 4–6 historical sites in the El Valle area have not yet detected any persisting populations (C.Z. and J.V., unpublished data). **Status in captivity: Secure.** A total of 4 large-bodied upland founders are represented in the captive collection in Panama. The captive population in Panama is not regarded as secure from a genetic standpoint but it could be recovered through reimportation of U.S. blood-lines. > 1300 adult frogs descended from 32 individuals are managed by the Golden Frog Species Survival Program in the U.S. (Estrada et al., 2013; K. Barrett pers. comm. 2018). The sources of these captive populations include small-bodied lowland animals (12 founders) and larger bodied upland animals (20 founders) that are managed separately (Zippel et al., 2006; Estrada et al., 2013).

***Atelopus aff. limosus* - Status in the wild: Not Evaluated.** This population of *Atelopus* occurs in inaccessible areas of the Darien National Park (Cerro Tarcacuna) or indigenous Comarca areas where scientific sampling permits are challenging to obtain. Further exploration is urgently needed for this population which, if it is a new species, will likely be evaluated as Critically Endangered. The frogs differ in coloration from described *Atelopus* species, but somewhat resemble the chevron color variant of *Atelopus limosus*. Genetic and taxonomic work is needed to place this population in a phylogenetic context, and disease monitoring is needed to understand if *Bd* is impacting the population. **Status in captivity: Not secure.** This species is also known from the Colombian side of the border and two males exist in captivity at the Cali Zoo, but sustainable captive populations need to be established (Flechas et al., 2017; S. Flechas pers. comm. 2018).

amphibian microbial community structure and function before we can jump to applied solutions. One initial *Bd* mitigation approach that has been investigated for *Atelopus* is augmentation of the antifungal skin bacteria community to help prevent disease. Despite concerted research into *Atelopus* skin microbiomes (Flechas et al., 2012; Becker et al., 2014; Becker et al., 2015b; Rebollar et al., 2016), development of a probiotic disease mitigation protocol has been thwarted by difficulties in permanently altering *Atelopus* skin microbiomes (Becker et al., 2011; Becker et al., 2015a). While the findings of *Atelopus* microbiome studies have led to intriguing patterns of associations between disease and microbes (e.g. Becker et al., 2015b), difficulty controlling symbiotic microbial community composition is a common situation hindering probiotic applications in all systems (Yong, 2016).

2.3. Research into historic *Atelopus* distribution and reintroduction

A goal of PARC is reintroduction and reestablishment of wild *Atelopus* populations in Panama. One required step for reintroduction plans is understanding species' historic distributions and their habitat use (IUCN, 2013). We used historic occurrence data and known habitat requirements to improve distribution maps for all Panamanian *Atelopus* species (Fig. 2A). We are interested in habitat suitability for the genus *Atelopus*, so the locations for all seven species were combined into one *Atelopus* occurrence dataset for modeling. We made this decision because all seven species of *Atelopus* in Panama occupy similar habitats. In addition, we had different numbers of occurrence records for each species. The discrepancy in the number of records among species would

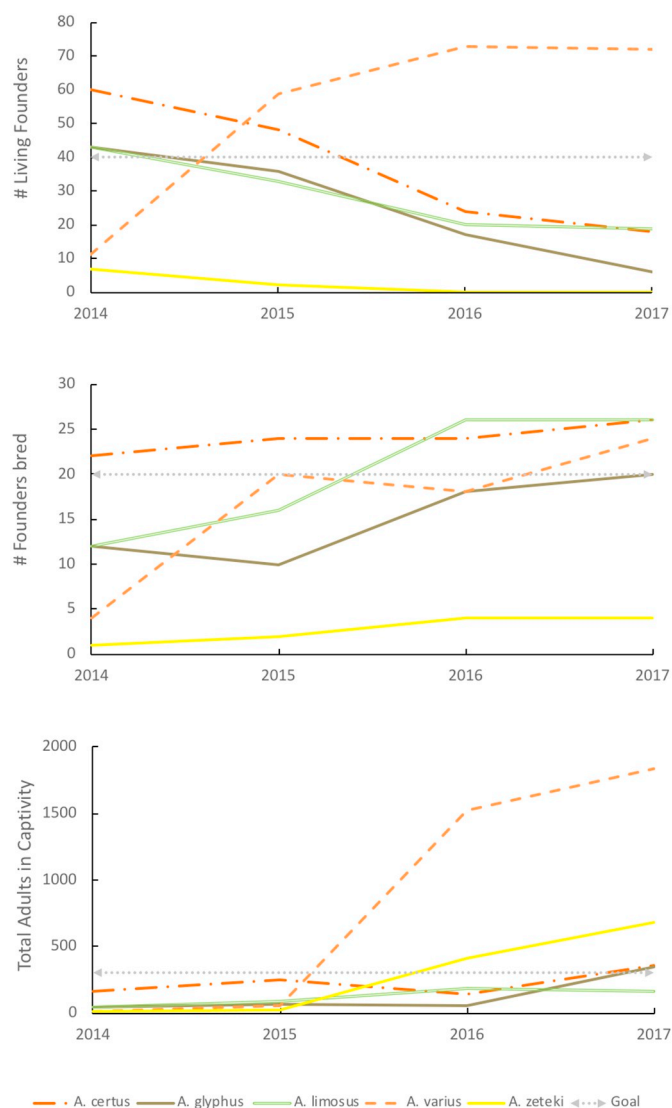


Fig. 1. Progress towards Amphibian Ark Captive Population Management Goals for each *Atelopus* species held in captivity in Panama between 2014 and 2017. The two species that are not represented in captivity are *A. chiriquiensis* and *A. aff. limosus*.

Table 1
2018 IUCN assessment of the conservation status of wild *Atelopus* in Panama.

Species	IUCN Red List status (In Press)	Bd/declines detected	Justification
<i>A. certus</i>	CR A3ce	Not yet detected, last survey 2016 (R.I., pers. comm.).	> 80% future declines predicted within 21 years (the next three generation lengths). Inferred from Bd-related declines observed in other high altitude <i>Atelopus</i> species in the same region.
<i>A. chiriquiensis</i>	EX	1993 Las Tablas, CR (Lips et al., 2003) 1994 Cerro Pando, PA (Berger et al., 1998)	This species has not been seen since 1996, despite 2 decades of intensive searches.
<i>A. glyphus</i>	CR A4ce	2015 (M. Ponce pers. comm. October 2015)	> 80% decline inferred in 10 years since first Bd-related declines 2015 that are projected to continue over 21 years (three generation lengths).
<i>A. limosus</i>	CR A4ce	2009 Chagres NP, PA (R.I., pers. comm.).	> 80% decline inferred in 10 years since first Bd-related declines 2009 that are projected to continue over 21 years (three generation lengths).
<i>A. varius</i>	CR A4ce	1986 San Ramón, CR (Puschendorf, 2003) 1987 Monte Verde, CR (Pounds and Crump, 1994) 1992 Rivas, CR (Puschendorf, 2003) 1997 Fortuna, PA (Berger et al., 1998) 2003 Santa Fe, PA (Brem and Lips, 2008) 2004 El Cope, PA (Lips et al., 2006; McCaffery et al., 2015)	> 80% decline of the known population estimated using a 21-year (3 generations) decline period window starting from 2002. Population size is unknown, but it is probably in the range of 250–2500 mature individuals remaining in the wild.
<i>A. zeteki</i>	CR A2ace	2005 El Valle de Anton, PA (Richards-Zawacki, 2010; McCaffery et al., 2015)	> 80% decline inferred in last 10 years (starting from 2008). Possibly Extinct in the wild. If any individuals remain in the wild it is unlikely that there are > 50.
<i>A. aff. limosus</i>	NE	Not detected by Flechas et al. (2012), but no recent Bd surveys have been conducted (R.I., pers. comm.).	Not Evaluated, but when it becomes taxonomically recognized would likely be listed as CR A3ce using a similar justification to <i>A. certus</i> .

have required different modeling methods for species with few records, and this could have resulted in less robust models. We used Maxent 3.4.1 to model habitat suitability for *Atelopus* in Panama and Costa Rica. Maxent is a maximum entropy method for modeling species' geographic distributions using environmental factors that has been shown to work well when only presence data is available (Phillips et al., 2006; Elith et al., 2006; Phillips et al., 2017). See online appendix for details on the data and methods used for the Maxent model, as well as discussion on the technical output.

The map produced by the Maxent model largely aligns with expectations based on our experience surveying for *Atelopus* in Panama (Fig. 2B). However, two areas of predicted suitable habitat that have been well-surveyed but found not to be occupied by *Atelopus* include Cerro Hoya on the Azuero peninsula and Serranía de Majé. These locations are disjunct from the central cordillera with unsuitable connecting habitat that may have been a biogeographic barrier to colonization (Fig. 2B). Taken together, the habitat suitability and distribution maps in Fig. 2 will help us identify areas of predicted suitability that have been poorly sampled due to inaccessibility and will allow us to prioritize potential survey, monitoring, and reintroduction sites.

The species distribution model revealed some taxonomic issues requiring attention. Firstly, the yet unnamed *Atelopus* aff. *limosus* should be investigated further and either described as a distinct species or recognized as a range extension for *A. limosus*, which would then occupy the entire Chagres Highlands-San Blas-Darien mountain range. Secondly, the uncommon sympatry between *A. chiriquiensis* and *A. varius* was based on morphological differences (Savage, 1972) but would benefit from data showing genetic distinctness as well, especially given that other morphologically distinct *Atelopus* species will readily hybridize in captivity (RI unpublished data). The Maxent model suggests that *A. varius* could have contiguous distribution throughout the central cordillera from San Jose in Costa Rica to El Copé in Panama (Fig. 1 B) but observed genetic differences between Costa Rican and Panamanian *Atelopus varius* (Richards and Knowles, 2007) may indicate that the unusually wide-ranging *Atelopus varius* is actually a species complex.

Our habitat suitability map could be used to identify locations where *Atelopus* may be persisting in climate refugia (Scheele et al. this volume). Climate refugia are areas where a host species can persist with the pathogen or where hosts persist outside of the potential distribution of the pathogen (Woodhams et al., 2011). Studies suggest that amphibians can use climate refugia to survive in areas with Bd by exploiting the fungus' sensitivity to temperature (Voyles et al., 2017) and moisture

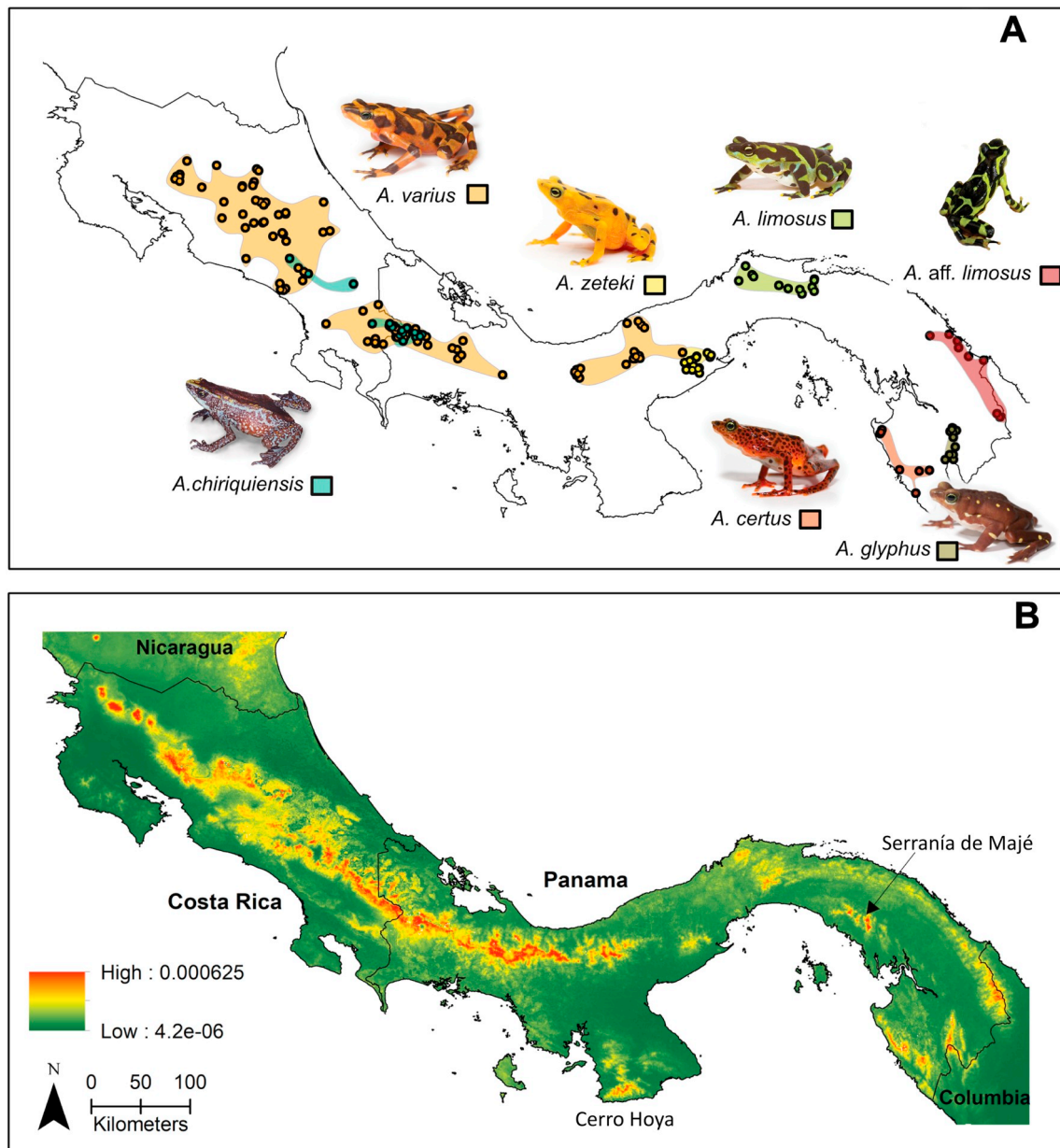


Fig. 2. A. Distribution map of Panamanian *Atelopus* species. Distributions were delineated using occurrence records combined with visual appraisal of habitat suitability. B. Maxent raw habitat suitability map for Panamanian *Atelopus*. Each cell's suitability value is proportional to the expected number of presences per unit area, with all values summing to 1. The values can be interpreted as the relative likelihood of occurrence. Photos courtesy S.V. Flechas, M. Guerra, B. Gratwicke.

(Berger et al., 2004). In Australia, *Litoria lorica* had disappeared from its known range, but a population was later found persisting with *Bd* infection in another drier area (Puschendorf et al., 2011). In Costa Rica, habitats that were marginal for *Bd* supported relict populations of frogs extirpated elsewhere (Puschendorf et al., 2009) and *Craugastor taurus* was rediscovered in an area that was drier and warmer than the species' historical habitat, despite a high *Bd* prevalence (Chaves et al., 2014). Rebollar et al. (2014) found that the disease burden of amphibians was lower in the lowlands of Panama. They hypothesized that this was because *Bd* did not grow or reproduce well in lowland climates, which allowed amphibian defenses to be relatively more effective (Rebollar et al., 2014). In addition, *Bd*-related mortality may be offset by recruitment (Lampo et al., 2017).

2.4. Future opportunities for research

Rödger et al. (2009) ran a Maxent model to predict global habitat

suitability for *Bd*. They found that three species of Panamanian *Atelopus* (*A. chiriquiensis*, *A. varius*, and *A. glyphus*) existed in areas of high predicted *Bd* suitability. However, chytridiomycosis-related declines of *Atelopus* in warmer, drier lowland areas tend to be slower than in higher elevation sites that are cooler and wetter (McCaffery et al., 2015). *Atelopus* populations once thought to be extinct, including *A. varius*, have been rediscovered in places that are marginally suitable for *Bd* (García-Rodríguez et al., 2012; Perez et al., 2014; Voyles et al., 2018). Future work will use the habitat suitability model we have presented for *Atelopus* combined with *Bd* suitability models using *Bd* prevalence and intensity to identify potential climate refugia that could be used as reintroduction sites. Captive breeding populations will be critical for testing the climate refugia hypothesis as it will require the release of animals in multiple unoccupied sites with different microclimates.

More information is needed for a full reintroduction effort and trial releases with surplus-bred frogs could provide valuable information. There is evidence that *Bd*-infected *Atelopus* release large numbers of *Bd*

zoospores into the environment (DiRenzo et al., 2014), though recent work indicates that this does not lead to higher infection intensity in other individual amphibians (DiRenzo et al., 2018) and may not cause an increase in *Bd* infection in the wild, a result that should be examined further in release trials. Other uncertainties with reintroduction are not necessarily related to *Bd*, and optimal reintroduction strategies depend on many variables and can vary with objectives (Canessa et al., 2014). Amphibians are generally thought to be better adapted for reintroduction efforts than other animals because of small body size, high fecundity, and hard-wired physiology and behavior (Griffiths and Pavajeau, 2008), though this does not necessarily apply to all species as life history traits are variable (Tapley et al., 2015). Trial releases could provide information on how well captive *Atelopus* would transition back into the wild, whether individuals recover their wild-type microbiome or toxicity, other sources of mortality, and which life stage has the highest probability of survival in relation to rearing costs. Trial releases can also provide information on the best release method (soft vs. hard release) to maximize persistence and help us to improve post-release monitoring methods that have notoriously low reencounter rates (Brannelly et al., 2016).

PARC has begun limited release trials with captive bred animals to begin researching these issues. Five hundred surplus *A. varius* and *A. limosus* were released in 2017 and 2018. The first trial evaluated holding frogs for 30 days in mesocosms (soft release) vs hard releases (direct introduction to the wild) but did not detect major differences in post-release survivorship (B. Klocke, A. Estrada and D. Medina, unpublished data). Released animals fitted with radio transmitters quickly dispersed out of the post-release monitoring area, resulting in low recapture rates of non-radiotracked animals and medium-term survivorship was not calculatable (B. Klocke, unpublished data). Therefore the deployment of mesocosms is more attractive to guarantee re-encounters with frogs over the medium term. While post-release monitoring is a difficult and resource-intensive exercise, it is essential to understand the fate of reintroduced animals for use in adaptive management frameworks (Canessa, this volume; Converse, this volume; Grant et al., 2017).

Captive populations could also be used in an alternative reintroduction approach. The intentional reintroduction of large numbers of animals to areas where *Bd* is present could allow natural selection to act upon many animals in the hopes that eventually there might be survivors. Given the significant number of individuals required, releases at the tadpole stage followed by intensive post-release monitoring would likely be the most cost-effective approach.

The recent discovery that recovering populations of Panamanian amphibians may have evolved more effective antifungal skin secretions (Voyles et al., 2018) offers the potential to translocate resistant genotypes to sites where they do not currently exist, but where suitable habitat exists (Mendelson et al. this volume). Captive populations will be an invaluable asset for breeding resistant frogs that can reduce *Bd* infections or tolerant frogs that can limit damage caused by infection (Rohr et al., 2010). Large numbers of frogs can be bred with minimal impacts to small source populations which otherwise could be severely impacted by removal of a small number of individuals. It is possible to collect and evaluate the effectiveness of anti-*Bd* skin secretions using a non-lethal standardized assay (Woodhams et al., 2006). Using skin secretions as an indicator of susceptibility could allow us to screen captive frogs and breed for resilience traits without exposing the animals to a pathogen, solving at least some of the ethical issues that might arise from large-scale assisted evolution trials involving disease exposure.

Another genomic approach is to identify genes associated with disease resistance or tolerance (eg. Savage and Zamudio, 2011) and to use marker-assisted breeding. Often, desirable traits are associated with large numbers of genes that each have small effects on the phenotype, which is why marker-assisted animal breeding has not been widely adopted. Technological advances now allow us to associate thousands of single nucleotide polymorphisms with desirable traits for genome selection, improving the prospects for wider adoption of this method

(Meuwissen et al., 2016). However, recent transcriptome studies found that desirable disease outcomes in one *Lithobates* species were associated with under (rather than over) expression of innate and acquired immune genes (Savage et al. pers. com.), suggesting that marker-assisted breeding for chytridiomycosis resistance may prove difficult.

Other potential approaches to mitigate the impact of *Bd* include hybridization with closely related resistant species that increase the frequency of adaptive alleles as a form of evolutionary rescue (Carlson et al., 2014) or through more direct genetic engineering. Both solutions have both been successfully applied to American chestnuts that were wiped out by the chestnut blight (Steiner et al., 2017). CRISPR/Cas 9 methods for genome editing have been successfully applied primarily to *Xenopus laevis* and *X. tropicalis* whose full genomes have been sequenced and are publicly available (Shigeta et al., 2016). Targeted gene disruption experiments are rapidly illuminating gene function in this model (Shigeta et al., 2016). As of 2018, the genomes of five anuran species have been sequenced and published (Edwards et al., 2018). Improvements in genome sequencing technology and assembly pipelines mean that many more, large amphibian genomes are likely to become available in the near future. It is conceivable that our understanding of the genetic basis for *Bd* resistance will improve to the extent that we might eventually be able to genetically engineer susceptible frogs to resist infection. While these cutting-edge solutions are exciting, the advances they offer are built on a foundation of basic research that involves established assisted breeding methods, reference genomes, differential gene expression studies and research into skin peptides. All of these basic research efforts will be greatly enhanced by integration with captive breeding efforts and access to genome resource banks that are only just being established as part of the Global Amphibian Conservation Action Plan (Gascon et al., 2007).

3. Conclusions

Observation is the first step in the scientific method and a hands-on approach may ultimately offer insights into reintroduction and other management solutions (Grant et al., 2017). *Bd* is an ongoing threat to amphibian populations in Panama, and without a way to mitigate the associated disease-related declines, reintroductions of amphibians are generally not recommended as they are likely to end in failure (Muths and McCallum, 2016). However, robust captive populations of *Atelopus* species in Panama offer a variety of opportunities to conduct research that improve our knowledge of the species' ecology and of *Bd* management. Captive collections serve two important conservation purposes – one immediate and one long-term. Immediately following the *Bd* epidemic, captive collections have prevented the extinction of some highly susceptible species. In the long-term, success in captive rearing has produced surplus individuals that can be used for research to improve conservation strategies and provide individuals for reintroduction. Our habitat suitability map for *Atelopus* could be used to identify potential refugia from *Bd* where *Atelopus* may be persisting or could be reintroduced. These living collections of animals are more than a simple insurance policy for threatened species, they are an important resource for research that could ultimately lead to the reestablishment of sustainable wild populations of these species. Acknowledgements

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Appendix A. Supplementary data

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References

- Baitchman, E.J., Pessier, A.P., 2013. Pathogenesis, diagnosis, and treatment of amphibian chytridiomycosis. *Veterinary Clinics: Exotic Animal Practice* 16 (3), 669–685 Sep 1.
- Becker, M.H., Harris, R.N., Minbiole, K.P., Schwantes, C.R., Rollins-Smith, L.A., Reinert, L.K., Brucker, R.M., Domangue, R.J., Gratwicke, B., 2011. Towards a better understanding of the use of probiotics for preventing chytridiomycosis in Panamanian golden frogs. *Ecohealth* 8 (4), 501–506. <https://doi.org/10.1007/s10393-012-0743-0>.
- Becker, M.H., Richards-Zawacki, C.L., Gratwicke, B., Belden, L.K., 2014. The effect of captivity on the cutaneous bacterial community of the critically endangered Panamanian golden frog (*Atelopus zeteki*). *Biol. Conserv.* 176, 199–206. <https://doi.org/10.1016/j.biocon.2014.05.029>.
- Becker, M.H., Walke, J.B., Cikanek, S., Savage, A.E., Mattheus, N., Santiago, C.N., Minbiole, K.P., Harris, R.N., Belden, L.K., Gratwicke, B., 2015a. Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. *Proc. R. Soc. B Biol. Sci.* 282(1805):20142881. [doi:https://doi.org/10.1098/rspb.2014.2881](https://doi.org/10.1098/rspb.2014.2881).
- Becker, M.H., Walke, J.B., Murrill, L., Woodhams, D.C., Reinert, L.K., Rollins-Smith, L.A., Burzynski, E.A., Umile, T.P., Minbiole, K.P., Belden, L.K., 2015b. Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal pathogen *Batrachochytrium dendrobatidis*. *Mol. Ecol.* 24 (7), 1628–1641. <https://doi.org/10.1111/mec.13135>.
- Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocumbe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., et al., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci.* 95 (15), 9031–9036.
- Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., et al., 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. Vet. J.* 82 (7), 434–439.
- Berger, L., Roberts, A.A., Voyles, J., Longcore, J.E., Murray, K.A., Skerratt, L.F., 2016. History and recent progress on chytridiomycosis in amphibians. *Fungal Ecol.* 19, 89–99. <https://doi.org/10.1016/j.funeco.2015.09.007>.
- Brannely, L.A., Hunter, D.A., Skerratt, L.F., Scheele, B.C., Lenger, D., McFadden, M.S., Harlow, P.S., Berger, L., 2016. Chytrid infection and post-release fitness in the reintroduction of an endangered alpine tree frog. *Anim. Conserv.* 19 (2), 153–162.
- Brem, F., Lips, K., 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Dis. Aquat. Org.* 81, 189–202. <https://doi.org/10.3354/dao01960>.
- Bustamante, H.M., Livo, L.J., Carey, C., 2010. Effects of temperature and hydric environment on survival of the Panamanian Golden frog infected with a pathogenic chytrid fungus. *Integr. Zool.* 5 (2), 143–153. <https://doi.org/10.1111/j.1749-4877.2010.00197.x>.
- Canessa, S., 2019. Adaptive management for amphibian recovery programmes: from theory to practice. *Biol. Conserv.* <https://doi.org/10.1016/j.biocon.2019.05.031>.
- Canessa, S., Hunter, D., McFadden, M., Marantelli, G., McCarthy, M.A., 2014. Optimal release strategies for cost-effective reintroductions. McCallum H, editor. *J. Appl. Ecol.* 51(4):1107–1115. [doi:https://doi.org/10.1111/1365-2664.12253](https://doi.org/10.1111/1365-2664.12253).
- Carlson, S.M., Cunningham, C.J., Westley, P.A., 2014. Evolutionary rescue in a changing world. *Trends Ecol. Evol.* 29 (9), 521–530.
- Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V.T., Ryan, M.J., 2014. Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Trop. Conserv. Sci.* 7 (4), 628–638. <https://doi.org/10.1177/194008291400700404>.
- Ciani, J.F.C., Guerrel, J., Baitchman, E., Díaz, R., Evans, M., Ibáñez, R., Ross, H., Klaphake, E., Nissen, B., Pessier, A.P., Power, M., Arlotta, C., Snellgrove, D., Wilson, B., Gratwicke, B., 2018. The relationship between spindly leg syndrome incidence and water composition, overfeeding, and diet in newly metamorphosed harlequin frogs (*Atelopus* spp.). *PLoS One* 13 (10), e0204314 (in press).
- Cikanek, S.J., Nockold, S., Brown, J.L., Carpenter, J.W., Estrada, A., Guerrel, J., Hope, K., Ibáñez, R., Putman, S.B., Gratwicke, B., 2014. Evaluating group housing strategies for the ex-situ conservation of harlequin frogs (*Atelopus* spp.) using behavioral and physiological indicators. *PLoS One* 9 (2), e90218. <https://doi.org/10.1371/journal.pone.0090218>.
- Clulow, J., Clulow, S., 2016. Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed. *Reprod. Fertil. Dev.* 28 (8), 1116. <https://doi.org/10.1071/RD15466>.
- Comizzoli, P., Wildt, D.E., 2017. Cryobanking Biomaterials from Wild Animal Species to Conserve Genes and Biodiversity: Relevance to Human Biobanking and Biomedical Research. *In: Biobanking of Human Biospecimens* Springer, Cham, pp. 217–235.
- Converse, S., 2019. Conservation outside of our control, and a rational, structured approach to research and management responses. *Biol. Conserv.* <https://doi.org/10.1016/j.biocon.2019.05.031>.
- Cushman, S.A., 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biol. Conserv.* 128 (2), 231–240. <https://doi.org/10.1016/j.biocon.2005.09.031>.
- Della Togna, G., Trudeau, V.L., Gratwicke, B., Evans, M., Augustine, L., Chia, H., Bronikowski, E.J., Murphy, J.B., Comizzoli, P., 2017. Effects of hormonal stimulation on the concentration and quality of excreted spermatozoa in the critically endangered Panamanian golden frog (*Atelopus zeteki*). *Theriogenology* 91, 27–35. <https://doi.org/10.1016/j.theriogenology.2016.12.033>.
- DiRenzo, G.V., Langhammer, P.F., Zamudio, K.R., Lips, K.R., 2014. Fungal infection intensity and zoospore output of *Atelopus zeteki*, a potential acute chytrid super-shedder. *PLoS One*. <https://doi.org/10.1371/journal.pone.0093356>.
- DiRenzo, G.V., Tunstall, T.S., Ibáñez, R., Longo, A.V., Zamudio, K.R., Lips, K.R., 2018. External reinfection of a fungal pathogen does not contribute to pathogen growth. *EcoHealth* 1–12. <https://doi.org/10.1007/s10393-018-1358-x>.
- Edwards, R.J., Tuipulotu, D.E., Amos, T.G., O'Meally, D., Richardson, M.F., Russell, T.L., Vallinoto, M., Carneiro, M., Ferrand, N., Wilkins, M.R., Sequeira, F., 2018. Draft genome assembly of the invasive cane toad, *Rhinella marina*. *GigaScience* 7 (9), giy095.
- Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., et al., 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29 (2), 129–151.
- Ellison, A.R., Savage, A.E., DiRenzo, G.V., Langhammer, P., Lips, K.R., Zamudio, K.R., 2014. Fighting a losing battle: vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3 Genes Genomes Genet.* 4 (7), 1275–1289. <https://doi.org/10.1534/g3.114.010744>.
- Ellison, A.R., Tunstall, T., DiRenzo, G.V., Hughey, M.C., Rebollar, E.A., Belden, L.K., Harris, R.N., Ibáñez, R., Lips, K.R., Zamudio, K.R., 2015. More than skin deep: functional genomic basis for resistance to amphibian chytridiomycosis. *Genome Biol. Evol.* 7 (1), 286–298. <https://doi.org/10.1093/gbe/evu285>.
- Escobedo-Galván, A.H., Wyatt, S.A., González-Maya, J.F., Schipper, J., Belant, J.L., Fischer, A., Hoepker, A., Cruz-Lizano, I., Cardenal, J., Castañeda, F., et al., 2013. Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphib-Reptil* 34 (4), 573–578. <https://doi.org/10.1163/15685381-00002910>.
- Estrada, A., Gratwicke, B., Benedetti, A., DellaTonga, G., Garrelle, D., et al., 2013. The Golden Frogs of Panama (*Atelopus zeteki*, A. varius): A Conservation Planning Workshop. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.
- Flechas, S.V., Sarmiento, C., Cárdenas, M.E., Medina, E.M., Restrepo, S., Amézquita, A., 2012. Surviving chytridiomycosis: differential anti-*Batrachochytrium dendrobatidis* activity in bacterial isolates from three lowland species of *Atelopus*. *PLOS One* 7 (9), e44832. <https://doi.org/10.1371/journal.pone.0044832>.
- Flechas, S.V., Blasco-Zúñiga, A., Merino-Viteri, A., Ramírez-Castañeda, V., Rivera, M., Amézquita, A., 2017. The effect of captivity on the skin microbial symbionts in three *Atelopus* species from the lowlands of Colombia and Ecuador. *PeerJ* 2017 Jul 31;5:e3594.
- García-Rodríguez, A., Chaves, G., Benavides-Varela, C., Puschendorf, R., 2012. Where are the survivors? Tracking relic populations of endangered frogs in Costa Rica. *Divers. Distrib.* 18 (2), 204–212. <https://doi.org/10.1111/j.1472-4642.2011.00862.x>.
- Garner, T.W.J., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., Weldon, C., Fisher, M.C., Bosch, J., 2016. Mitigating amphibian chytridiomycosis in nature. *Phil. Trans. R. Soc. B* 371 (1709), 20160207. <https://doi.org/10.1098/rstb.2016.0207>.
- Gascon, C., Collins, R.D., Moore, R.D., 2007. Amphibian Conservation Action Plan. Proceedings of the IUCN/SSC Amphibian Conservation Summit 2005. IUCN—the World Conservation Union, Gland, Switzerland.
- Grant, E.H.C., Muths, E., Katz, R.A., Canessa, S., Adams, M.J., Ballard, J.R., Berger, L., Briggs, C.J., Coleman, J.T., Gray, M.J., et al., 2017. Using decision analysis to support proactive management of emerging infectious wildlife diseases. *Front. Ecol. Environ.* 15 (4), 214–221. <https://doi.org/10.1002/fee.1481>.
- Gratwicke, B., Murphy, J.B., 2016. Amphibian conservation efforts at the Smithsonian National Zoological Park and Conservation Biology Institute. *Herp Review* 47 (4), 711–718.
- Gratwicke, B., Ross, H., Batista, A., Chaves, G., Crawford, A.J., Elizondo, L., Estrada, A., Evans, M., Garrelle, D., Guerrel, J., et al., 2016. Evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs. *Anim. Conserv.* 19 (4), 324–336. <https://doi.org/10.1111/acv.12249>.

- Griffiths, R.A., Pavaeau, L., 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conserv. Biol.* 22 (4), 852–861. <https://doi.org/10.1111/j.1523-1739.2008.00967.x>.
- Harding, G., Griffiths, R.A., Pavaeau, L., 2016. Developments in amphibian captive breeding and reintroduction programs. *Conserv. Biol.* 30 (2), 340–349. <https://doi.org/10.1111/cobi.12612>.
- Hertz, A., Lotzkat, S., Carrizo, A., Ponce, M., Köhler, G., Streit, B., 2012. Field Notes on Findings of Threatened Amphibian Species in the Central Mountain Range of Western Panama. vol. 6(2). pp. 22.
- Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., Chanson, J., Collen, B., et al., 2010. The impact of conservation on the status of the world's vertebrates. *Science* 330, 1503–1509. <https://doi.org/10.1126/science.1194442>.
- Ibáñez, R., Jaramillo, C.A., Solís, F.A., 1995. Una especie nueva de *Atelopus* (Amphibia: Bufonidae) de Panamá. *Caribb. J. Sci.* 31 (1–2), 57–64.
- IUCN, 2013. Guidelines for Reintroductions and Other Conservation Translocations. IUCN SSC Re-Intro Spec Group, Gland Switz Camb UK.
- IUCN SSC Amphibian Specialist Group, 2019. The IUCN Red List of Threatened Species. In press. www.iucnredlist.org.
- Kouba, A.J., Vance, C.K., 2009. Applied reproductive technologies and genetic resource banking for amphibian conservation. *Reprod. Fertil. Dev.* 21 (6), 719. <https://doi.org/10.1071/RD09038>.
- La Marca, E., Lips, K.R., Lotters, S., Puschendorf, R., Ibáñez, R., Rueda-Almonacid, J.V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., et al., 2005. Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37 (2), 190–201. <https://doi.org/10.1111/j.1744-7429.2005.00026.x>.
- Lampo, M., Señaris, C., García, C.Z., 2017. Population dynamics of the critically endangered toad *Atelopus cruciger* and the fungal disease chytridiomycosis. *PLoS One* 12 (6), e0179007. <https://doi.org/10.1371/journal.pone.0179007>.
- Langhammer, P.F., Lips, K.R., Burrows, P.A., Tunstall, T., Palmer, C.M., Collins, J.P., 2013. A fungal pathogen of amphibians, *Batrachochytrium dendrobatidis*, attenuates in pathogenicity with in vitro passages. *PLoS One* 8 (10), e77630. <https://doi.org/10.1371/journal.pone.0077630>.
- Lips, K.R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conserv. Biol.* 117–125.
- Lips, K.R., Green, D.E., Papendick, R., 2003. Chytridiomycosis in wild frogs from southern Costa Rica. *J. Herpetol.* 37 (1), 215–218.
- Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl. Acad. Sci.* 103 (9), 3165–3170.
- Lötters, S., 2007. The fate of the harlequin toads—help through a synchronous multi-disciplinary approach and the IUCN ‘amphibian conservation action plan’? *Zoosystematics Evol* 83 (S1), 69–73. <https://doi.org/10.1002/mmnz.200600028>.
- Mann, R.M., Hyne, R.V., Choung, C.B., Wilson, S.P., 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environ. Pollut.* 157, 2903–2927.
- McCaffery, R., Richards-Zawacki, C.L., Lips, K.R., 2015. The demography of *Atelopus* decline: harlequin frog survival and abundance in Central Panama prior to and during a disease outbreak. *Glob Ecol Conserv* 4, 232–242. <https://doi.org/10.1016/j.gecco.2015.07.003>.
- Mendelsohn III, J.R., Whitfield, S.M., Sredl, M.J., 2019. A recovery engine strategy for amphibian conservation in the context of disease. *Biol. Conserv.* <https://doi.org/10.1016/j.biocon.2019.05.025>. (This Volume). (X:XXX-XXX).
- Mewissen, T., Hayes, B., Goddard, M., 2016. Genomic selection: a paradigm shift in animal breeding. *Animal Frontiers* 6 (1), 6–14. <https://doi.org/10.2527/af.2016-0002>.
- Muths, E., McCallum, H., 2016. Why you cannot ignore disease when you reintroduce animals. In: Jachowski, D.S., Millsaugh, J.J., Angermeier, P.L., Slotow, R. (Eds.), *Reintroduction of Fish and Wildlife Populations*. Univ of California Press, pp. 217–244.
- Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., Garner, T.W.J., Weaver, G., Fisher, M.C., 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLoS One* 8, e56802. <https://doi.org/10.1371/journal.pone.0056802>.
- Perez, R., Richards-Zawacki, C.L., Krohn, A.R., Robak, M., Griffith, E.J., Ross, H., Gratwicke, B., Ibáñez, R., Voyles, J., 2014. Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphib Reptile Conserv* 8, 30–35.
- Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190 (3–4), 231–259. <https://doi.org/10.1016/j.ecolmodel.2005.03.026>.
- Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., Blair, M.E., 2017. Opening the black box: an open-source release of Maxent. *Ecography* 40 (7), 887–893. <https://doi.org/10.1111/ecog.03049>.
- Pounds, J.A., Crump, M.L., 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conserv. Biol.* 8 (1), 72–85.
- Puschendorf, R., 2003. *Atelopus varius* (harlequin frog) fungal infection. *Herpetol Rev* 34 (4), 355.
- Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., Alford, R.A., 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Divers. Distrib.* 15 (3), 401–408.
- Puschendorf, R., Hoskin, C.J., Cashins, S.D., McDonald, K.R., Skerratt, L.F., Vanderwal, J., Alford, R.A., 2011. Environmental refuge from disease-driven amphibian extinction: environmental refuge from extinction. *Conserv. Biol.* 25 (5), 956–964. <https://doi.org/10.1111/j.1523-1739.2011.01728.x>.
- Rebollar, E.A., Hughey, M.C., Harris, R.N., Domangue, R.J., Medina, D., Ibáñez, R., Belden, L.K., 2014. The lethal fungus *Batrachochytrium dendrobatidis* is present in lowland tropical forests of far eastern Panamá. *PLoS One* 9 (4), e95484.
- Rebollar, E.A., Hughey, M.C., Medina, D., Harris, R.N., Ibáñez, R., Belden, L.K., 2016. Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *ISME J* 10 (7), 1682.
- Richards, C.L., Knowles, L.L., 2007. Tests of phenotypic and genetic concordance and their application to the conservation of Panamanian golden frogs (Anura, Bufonidae). *Mol. Ecol.* 16 (15), 3119–3133. <https://doi.org/10.1111/j.1365-294X.2007.03369.x>.
- Richards-Zawacki, C.L., 2010. Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proc. R. Soc. B Biol. Sci.* 277 (1681), 519–528. <https://doi.org/10.1098/rspb.2009.1656>.
- Rödter, D., Kielgast, J., Bielby, J., Schmidlein, S., Bosch, J., Garner, T., Veith, M., Walker, S., Fisher, M.C., Lötters, S., 2009. Global amphibian extinction risk assessment for the Panzootic Chytrid fungus. *Diversity* 1 (1), 52–66. <https://doi.org/10.3390/d1010052>.
- Rohr, J.R., Raffel, T.R., Hall, C.A., 2010. Developmental variation in resistance and tolerance in a multi-host-parasite system. *Funct. Ecol.* 24 (5), 1110–1121.
- Ryan, M., Berlin, E., Gagliardo, R.W., Lacovelli, C., 2005. Further exploration in search of *Atelopus varius* in Costa Rica. *Froglog* 69, 1–2.
- Sauer, E.L., Fuller, R.C., Richards-Zawacki, C.L., Sonn, J., Sperry, J.H., Rohr, J.R., 2018. Variation in individual temperature preferences, not behavioural fever, affects susceptibility to chytridiomycosis in amphibians. *Proc. R. Soc. Lond. B Biol. Sci.* 285 (1885). <https://doi.org/10.1098/rspb.2018.1111>.
- Savage AE, Zamudio KR. 2011. MHC genotypes associate with resistance to a frog-killing fungus. *Proc. Natl. Acad. Sci.* 201106893. doi:<https://doi.org/10.1073/pnas.1106893108>.
- Savage, J.M., 1972. The harlequin frogs, genus *Atelopus*, of Costa Rica and western Panama. *Herpetologica* 28, 77–94.
- Schad, K., 2008. Amphibian population management guidelines. In: *Amphibian Ark Amphibian Population Management Workshop: 2007 December 10–11*. Amphibian Ark, San Diego, CA, USA.
- Scheele, B.C., Foster, C.N., Hunter, D.A., Lindenmayer, B.D., Heard, G.W., 2019. Beyond Mass Mortality: Ecological, Evolutionary and Management Implications of Endemic Chytridiomycosis. (This Volume). (X:XXX-XXX).
- Shigeta, M., Sakane, Y., Iida, M., Suzuki, M., Kashiwagi, K., Kashiwagi, A., Fujii, S., Yamamoto, T., Suzuki, K.I., 2016. Rapid and efficient analysis of gene function using CRISPR-Cas9 in *Xenopus tropicalis* founders. *Genes Cells* 21 (7), 755–771.
- Steiner, K.C., Westbrook, J.W., Hebard, F.V., Georgi, L.L., Powell, W.A., Fitzsimmons, S.F., 2017. Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. *New For.* 48 (2), 317–336.
- Stuart, S., Chanson, J., Cox, N., et al., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 80 (306), 1783–1786.
- Tapley, B., Bradfield, K.S., Michaels, C., Bungard, M., 2015. Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodivers. Conserv.* 24 (11), 2625–2646. <https://doi.org/10.1007/s10531-015-0966-9>.
- Voyles, J., Johnson, L.R., Rohr, J., Kelly, R., Barron, C., Miller, D., Minster, J., Rosenblum, E.B., 2017. Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. *Oecologia* 184 (2), 363–373. <https://doi.org/10.1007/s00442-017-3866-8>.
- Voyles, J., Woodhams, D.C., Saenz, V., Byrne, A.Q., Perez, R., Rios-Sotelo, G., Ryan, M.J., Bletz, M.C., Sobell, F.A., McLetchie, S., et al., 2018. Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science* 359 (6383), 1517–1519. <https://doi.org/10.1126/science.aaa4806>.
- Wake, D.B., Vredenburg, V.T., 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl. Acad. Sci.* 105 (Supplement 1), 11466–11473.
- Woodhams, D.C., Voyles, J., Lips, K.R., Carey, C., Rollins-Smith, L.A., 2006. Predicted disease susceptibility in a Panamanian amphibian assemblage based on skin peptide defenses. *J. Wildl. Dis.* 42 (2), 207–218.
- Woodhams, D.C., Bosch, J., Briggs, C.J., Cashins, S., Davis, L.R., Lauer, A., Muths, E., Puschendorf, R., Schmidt, B.R., Sheafor, B., et al., 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Front. Zool.* 8 (1), 8.
- Yong, E., 2016. I Contain Multitudes: The Microbes within us and a Grand View of Life. Harper Collins, NY (339 pp.).
- Zimkus, B.M., Hassapakis, C.L., Houck, M.L., 2018. Integrating current methods for the preservation of amphibian genetic resources and viable tissues to achieve best practices for species conservation. *Amphib Reptile Conserv* 12 (2), 27.
- Zippel, K., 2002. Conserving the Panamanian golden frog: Proyecto Rana Dorada. *Herpetol Rev* 33 (1), 11.
- Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., Martinez, C., Townsend, E., 2011. The amphibian ark: a global community for ex situ conservation of amphibians. *Herpetol. Conserv. Biol.* 6 (3), 340–352.
- Zippel, K.C., Ibáñez, R., Lindquist, E.D., Richards, C.L., Jaramillo, C.A., Griffith, E.J., 2006. Implicaciones en la conservación de las ranas doradas de Panamá, asociadas con su revisión taxonómica. *Herpetotrópicos* 3 (1), 29–39.