



Original research article

# The demography of *Atelopus* decline: Harlequin frog survival and abundance in central Panama prior to and during a disease outbreak



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## ABSTRACT

Harlequin frogs (Bufonidae: *Atelopus*) are a species-rich genus of Neotropical toads that have experienced disproportionately severe population declines and extinctions caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). The genus *Atelopus* is of high conservation concern, but relatively little is known about the population dynamics and life history of the majority of species. We examined the demography of one population of *Atelopus zeteki* and two populations of *A. varius* in central Panama using three to six years of mark-recapture data collected prior to and during an outbreak of *Bd*. We estimated male survival probabilities prior to the arrival of *Bd* and sex-specific population sizes for these three populations using state-space Bayesian population models. Prior to the arrival of *Bd*, monthly apparent survival probabilities were higher for *A. varius* males than for *A. zeteki* males, and recaptures among years were low in both species. Abundance of both species varied over time and declined rapidly after the arrival of *Bd*. Male densities were generally greater than female densities, though female densities were higher or equivalent to males after the arrival of *Bd*. Estimates of survival and abundance over time may be explained by differences in the use of stream habitat by the two sexes and three populations, both during and between breeding seasons. These estimates provide key baseline population information that can be used to inform reintroductions from captive assurance colonies and studies of extant *Atelopus* populations as part of conservation and management programs.

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## 1. Introduction

Harlequin frogs (Bufonidae: *Atelopus*) are among the most threatened amphibian taxa in the world (La Marca et al., 2005), and are emblematic of tropical amphibian declines caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, *Bd* (Lips et al., 2006, 2008; Crawford et al., 2010). At least 40 of 97 described species have disappeared in the past 20 years, with three species listed as extinct (IUCN, 2014), 82 species listed as Endangered or Critically Endangered (IUCN, 2014), and only 10 stable species (La Marca et al., 2005; Lips et al., 2008). Declines have been particularly severe at elevations above 1000 m, although lower elevation populations have also been affected. Species have disappeared from throughout the range of this large genus, from Costa Rica and Panama to Colombia, Ecuador, Venezuela and Peru (La Marca et al., 2005). The

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alarming declines in this genus have prompted biologists to conduct extensive surveys of habitats within the genus' range to determine the presence of remnant populations in areas affected by *Bd*, identify new populations, and initiate monitoring efforts for remaining populations. Despite the attention to population declines in these species, we know relatively little about their natural history and population dynamics. Because of this, studies of *Atelopus* population dynamics are a critical missing component of ongoing research and recovery programs.

Past studies of *Atelopus* species have focused on general distribution patterns, ecology, and behavior. Species in this large genus are quite similar in their general ecology (Löfatters, 1996; Savage, 2002). These toads are typically small to medium-bodied, with bright and contrasting aposematic coloration (Wells, 2007). They are typically diurnal and found on stream banks or on rocks in the stream where they breed during the dry season or year-round. Territoriality and aggression by males of certain species have been well-documented (Crump, 1988; Lindquist and Hetherington, 1996), and males appear to exhibit high site fidelity (Crump, 1986). In two recent studies, researchers have quantified population parameters for a remnant *Atelopus cruciger* population in Venezuela (Lampo et al., 2012), and a rediscovered *Atelopus spumarius* population in Ecuador (Tarvin et al., 2014). These estimates provide a baseline for comparing demographic rates to other South American *Atelopus* species. Analyses such as these can provide information on demographic rates in rediscovered *Atelopus* populations where *Bd* has been present (Tarvin et al., 2014), as well as on demographic responses to *Bd* outbreaks across species, populations, and time periods (Lampo et al., 2012). Additionally, they can help with designing monitoring programs, and can provide critical demographic information to inform captive breeding programs and potential reintroduction efforts in critically endangered species. For example, understanding natural population densities may be useful for determining how many individuals to reintroduce into stream habitat, and estimates of survival from *Bd*-free and infected populations provide an important basis with which to compare survival in captive and reintroduced populations of interest.

Capture–recapture studies provide a powerful design for estimating survival and population size in amphibian populations. By tracking individuals, researchers can estimate survival, recruitment, and other population parameters of interest while accounting for probability of detection, where raw counts of individuals are corrected for imperfect detection (Schmidt et al., 2002; Funk et al., 2003; Mazerolle et al., 2007). Detection probabilities are rarely constant over time, so indices of population size like visual counts often do not capture true population dynamics (Lampo et al., 2012; Guimarães et al., 2014). Capture–recapture studies are relatively rare in the amphibian literature (Mazerolle et al., 2007), and are particularly infrequent in studies of amphibian populations in tropical systems (Guimarães et al., 2014). However, their importance in Neotropical amphibian ecology has been recognized and several recent papers have estimated survival and abundance parameters from capture–recapture data in the past ten years (Ryan et al., 2008; Murray et al., 2009; Longo and Burrowes, 2010; Lampo et al., 2012; McCaffery and Lips, 2013; Cole et al., 2014; Tarvin et al., 2014). In tropical studies, capture–recapture analyses of amphibian populations have led to a greater understanding of the natural population dynamics of several species, including baseline survival estimates, density estimates, and population growth rates (e.g., McCaffery and Lips, 2013). Furthermore, they have contributed to our understanding of how habitat disturbance and climate may impact species demography (Cole et al., 2014), provided insight into the demographic mechanisms of decline due to *Bd* (Ryan et al., 2008; Murray et al., 2009), and yielded information on survival rates in populations persisting with disease (Murray et al., 2009; Lampo et al., 2012).

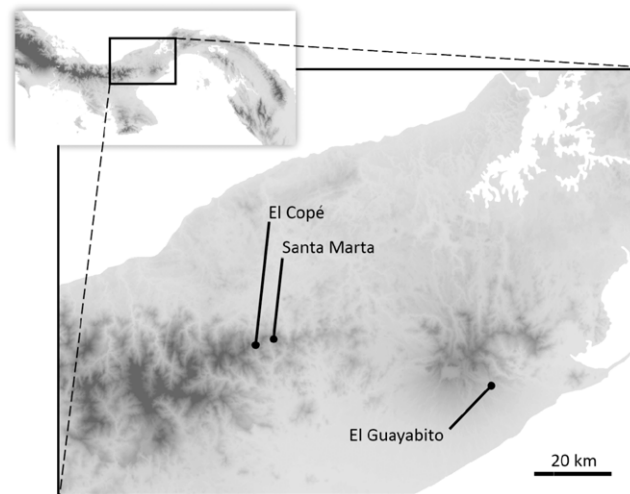
We examined the abundance and survival of two species of *Atelopus* found in central Panama prior to and during an outbreak of *Bd*. *Atelopus varius* is a species that is native to Costa Rica and Panama, and was historically found in moist lowland and montane rainforest localities along fast-flowing, high gradient streams (Savage, 2002). The species experienced dramatic population declines and disappearances across its range, starting in northwestern Costa Rica in the 1980s and moving into central Panama by the 2000s. In 2008 it was listed as Critically Endangered on the IUCN Red List (Pounds et al., 2010). In recent years, a few individuals of *A. varius* have been found in several areas where they were presumed extirpated following the arrival of *Bd*, including sites in Costa Rica (González-Maya et al., 2013) and Panama (Hertz et al., 2012; Perez et al., 2014). *Atelopus zeteki*, also known as the Panamanian golden frog, is endemic to a small area of central Panama, where it is typically found in low to middle elevation dry and moist forest habitat (Zippel et al., 2006). It has experienced dramatic declines over the past 15 years as *Bd* has moved from west to east through Panama, and is also listed as Critically Endangered on the IUCN Red List (Lips et al., 2010). It is considered extinct in the wild (Hertz et al., 2012). Both species are being maintained and bred in several zoos for eventual reintroduction (Zippel, 2002).

Between 1999 and 2006, we collected capture–recapture data for three populations of these two species as part of larger projects addressing other research questions. Because of the need for demographic data for ongoing conservation and reintroduction programs we analyzed these two datasets together and present results to maximize possible application by practitioners. Our aims for this study were to: (1) estimate survival probabilities in male *Atelopus* prior to the outbreak of *Bd*; (2) estimate abundance of male and female *Atelopus* prior to and during the disease outbreak; and (3) determine whether the three populations differed in survival and abundance patterns.

## 2. Methods

### 2.1. Study areas and field methods

We studied *Atelopus varius* in two neighboring areas in central Panama (Fig. 1). The two sites were both located in the 25,000 ha Parque Nacional G. D. Omar Torrijos, located in Coclé Province. We considered each site to be a separate population.



**Fig. 1.** Map of three study locations in central Panama.

At the first site (hereafter ‘El Copé’), the population was monitored along four 200-m permanent stream transects. Three transects were located on smaller streams that were tributaries to a larger stream. All four transects were located at approximately 700 m elevation in tropical moist forest. Surveys of the entire amphibian community have taken place at El Copé annually since 1999 as part of ongoing research (e.g., Lips et al., 2006; Crawford et al., 2010). Capture–recapture studies of *A. varius* began in 2000 to better understand the demography and natural history of this and other focal species. Transects were surveyed approximately weekly between May and August (7–8 times per year) from 2000 to 2004 (primary survey periods), with some variation in survey effort among years and transects due to differences in personnel and other research activities. *Bd* was first detected at the site in September 2004 (Lips et al., 2006). Data from diurnal and nocturnal surveys on all transects were aggregated for this analysis to maximize the number of captures. Field assistants surveyed the stream transect by slowly walking upstream and capturing animals by hand on rocky substrate in the stream or in surrounding vegetation. Each individual was measured (length and weight), sexed, and released at the point of capture. Animals were identified by unique dorsal spot patterns, which were recorded by hand for all individuals during each capture session. Most individuals captured were males, but many females were seen over the years. However, females could not usually be individually identified due to a lack of dorsal pattern, so we were not able to estimate survival or density for females at this site.

The second *A. varius* monitoring site was located 4.5 km from the first study site along two tributaries near Santa Marta, at approximately 500 m elevation in tropical moist forest (hereafter ‘Santa Marta’). This population was monitored along four 200-m transects (except in 2003, when only two transects were surveyed). Transects were surveyed 4–5 times during each of five primary survey periods: August 2003, February 2004, July 2004, February 2004, and July 2005. All surveys were diurnal, and survey effort varied among years due to differences in personnel and other research activities. July and August surveys occurred during the wet season, while February surveys were during the dry, primary breeding season. *Bd* arrived between the July 2004 and February 2005 primary survey periods (Lips et al., 2006). As with the El Copé population, individuals were captured by hand, and were weighed, measured, and sexed before being returned to their point of capture. Individuals at this site were given a unique toe-clip combination upon first capture (up to three toes clipped per frog), which was recorded upon subsequent capture. All frogs were also photographed so that unique dorsal markings could be used as a secondary method of individual identification.

Finally, we also studied an *A. zeteki* population located along a 3 km stretch of river near El Guayabito, located at approximately 300 m elevation in Panama Province in a lowland dry forest (hereafter ‘El Guayabito’; Fig. 1). Three 200-m transects were surveyed five times during five primary survey periods, all conducted during the dry season: January 2004, December 2004, January 2005, December 2005, and January 2006. All surveys were diurnal, and corresponded with the species’ breeding season, which occurs from early December to late January. At this site, *Bd* arrived between the January 2005 and December 2005 primary survey periods (Richards-Zawacki, 2010). The field sampling protocol was identical to that for Santa Marta. At both Santa Marta and El Guayabito, both males and females were captured, and data were analyzed separately for each species and sex.

## 2.2. Survival and abundance analysis

We used a hierarchical modeling framework for all analyses. We chose this framework because it explicitly accounts for different sources of variation in ecological data (e.g., separating observation error from process variance), and because it can more easily accommodate uneven sampling effort, small datasets, and complex models than can other methods (Royle

and Dorazio, 2008; Kery and Schaub, 2012). In this approach, the capture–recapture data are described as the result of two processes. The state process is unknown and represents the true, ecological process of the system, while the observation process refers to the observed data, that is, whether or not each individual was seen during each time period (Royle and Dorazio, 2008; Kery and Schaub, 2012). The observation process is conditional on the state process, and the state process is estimated as part of the analysis.

We selected population models that best reflected the sampling design for the populations and allowed for comparison among populations. We used Cormack–Jolly–Seber (CJS) models to model survival for all three populations across primary survey periods. We used open Jolly–Seber (JS) population models to estimate density at El Copé and El Guayabito, because the populations were not closed to additions and deletions within each primary survey period. At El Copé, secondary surveys (i.e., weekly) within each primary survey period (i.e., annual) were conducted over multiple months so populations were open to additions and deletions during this window. At El Guayabito, individuals had very low recapture rates despite the narrow  $\sim 1$  week sampling window, suggesting that individuals did not stay on the stream for long. Furthermore, observed illegal collecting during the primary sampling periods affected closure in this population (CLRZ, pers. obs.). We used closed population models to estimate density within each primary survey period at Santa Marta. We estimated demographic parameters separately for each population, and describe our models below. For each population, we combined capture data from all surveyed transects in that population for analysis.

### 2.2.1. Male survival

For all three populations, we used Cormack–Jolly–Seber (CJS) models to estimate survival across primary sampling periods. For these models, we collapsed capture histories within each primary survey period to indicate whether an individual was seen at any point during a given primary survey period. We did this for the male datasets only: females were rarely captured in more than one sampling session, so we were unable to estimate female survival. For both populations of *A. varius*, we only estimated male survival prior to the arrival of *Bd*, as we had too few captures to estimate survival after the pathogen arrived.

For these models, the state process referred to the unknown true state of the individual at each time period, where  $z_{i,t} = 1$  if the individual  $i$  was alive at time  $t$  and  $z_{i,t} = 0$  otherwise. This was estimated as part of the analysis. The observation process referred to whether we observed each individual over time and was conditional on the state process:  $y_{i,t} = 1$  indicated that individual  $i$  was seen at time  $t$  and  $y_{i,t} = 0$  indicated that it was not seen. This was the raw capture data for each population. The capture probability for each individual and time period was denoted by  $p_{i,t}$ . Finally, we defined a vector  $f$  where  $f_i$  represented the occasion at which individual  $i$  was first encountered. The state of an individual at first encounter ( $z_{i,f_i}$ ) is 1 with probability 1, since that individual was alive with certainty. Subsequent to the first capture, states were generated using Bernoulli trials. Survival probability from  $t - 1$  to  $t$  for individual  $i$  was denoted by parameter  $\varphi_{i,t}$ . The following model described the state process.

$$z_{i,t} | z_{i,t-1} \sim \text{Bernoulli}(z_{i,t-1} - 1, \varphi_{i,t-1}). \quad (1)$$

The observation process is conditional on the state process and was described by the following model:

$$y_{i,t} | z_{i,t} \sim \text{Bernoulli}(z_{i,t} p_{i,t}). \quad (2)$$

Here, when  $z_{i,t} = 0$ , then  $y_{i,t} = 0$  with probability 1; otherwise,  $y_{i,t}$  is a Bernoulli trial with parameter  $p_{i,t}$ . These equations specify models where both survival and detection probability vary over time. We introduced constraints to specify different model structures, and estimated survival ( $\varphi$ ) and detection probability ( $p$ ) for each study population. We developed four models where survival and detection probabilities were either constant (.) or varied over time ( $t$ ): (1) both survival and detection probability were constant ( $\varphi(.)p(.)$ ), (2) survival varied over time and detection probability was constant ( $\varphi(t)p(.)$ ), (3) survival was constant and detection probability varied over time ( $\varphi(.)p(t)$ ), and (4) both survival and detection probability varied over time ( $\varphi(t)p(t)$ ). We compared estimates from these four models using the Deviance Information Criterion (DIC, Spiegelhalter et al., 2002), where the model with the smallest DIC value indicates the model that would best predict a replicate dataset with the same structure. To standardize our survival estimates across all populations and allow for ease of comparison to other *Atelopus* studies, we estimated monthly survival probabilities for each population.

### 2.2.2. Male and female abundance

For both open (El Copé and El Guayabito) and closed (Santa Marta) abundance models, we used parameter-expanded data augmentation as part of the modeling process when estimating population size (Royle and Dorazio, 2008, 2012; Kery and Schaub, 2012). This method was developed for Bayesian MCMC models to accommodate technical challenges in the MCMC algorithm related to the dimension of the parameter vector for population size. For this technique, we augmented the data in each model with a large number of all-zero capture histories,  $M$ : these are potential unobserved individuals in the system. We then analyzed this reparameterized version of the original model, which essentially works as a modified occupancy model. The estimated population size ( $N$ ) lies between the observed capture history,  $n$ , and the augmented capture history  $M$ .

For El Copé *A. varius* and El Guayabito *A. zeteki* populations, we used Jolly–Seber (JS) models to estimate abundance within each primary survey period. JS models are open capture–recapture models that estimate recruitment into a population as

well as survival by analyzing the complete capture-history. The zeros in a capture history before the initial capture of an individual were included in the analysis, and indicated that the individual had not yet recruited into the population, or was in the population but had not yet been detected (Kery and Schaub, 2012). Survival was estimated from the period after the initial capture. We formulated the models as restricted dynamic occupancy models (Royle and Dorazio, 2008; Kery and Schaub, 2012). By using the whole capture history, we estimated the population size as a derived parameter from the recruitment and survival processes. This was denoted by  $N_s$ , and referred to the total number of individuals ever alive during each primary survey period (Schwarz and Arnason, 1996). A certain fraction of these individuals were already alive and present in the study area when we began the study, and all remaining individuals entered at some point during the study. The probability of any individual entering the population at occasion  $t$  was  $b_t$ , and was called the entry probability. The number of individuals entering the population at time  $t$  was then denoted as  $B_t = N_s * b_t$ .

For the restricted occupancy formulation of the JS model, individuals could be in three possible states: not yet entered, alive, or dead. The transitions between these states were the ecological processes of entry (immigration or recruitment) and survival, which were estimated as part of the model. The state process was governed by two equations. In these equations,  $z_{i,t}$  was a binary variable indicating whether an individual in the augmented data matrix  $M$  was in the true population or not at time  $t$ . The parameter  $\gamma_t$  described the probability that an individual in  $M$  would transition from “not yet entered” to “alive” at time  $t$ . The initial state of an individual,  $i$ , at time  $t = 1$  was:

$$z_{i,1} \sim \text{Bernoulli}(\gamma_1). \quad (3)$$

Subsequent states were determined by the survival of individuals already in the population ( $z_{i,t} = 1$ ) and the entries of new individuals ( $z_{i,t} = 0$ ):

$$z_{i,t+1} \sim \text{Bernoulli}\left(z_{i,t}\varphi_{i,t} + \gamma_{t+1}\prod_{k=1}^t(1 - z_{i,k})\right). \quad (4)$$

The observation process was conditional on the state process and was modeled in the same way as in the survival analysis. From these models, we were able to determine the population size ( $N_s$ ) for each primary survey period in each study population. Because study transects differed in total length among populations, we standardized all abundance estimates as the number of individuals seen per 100 m for ease of comparison. We estimated abundance for both males and females at El Guayabito, but we were unable to estimate female abundance at El Copé because females were not individually marked.

As with the survival models, we estimated four models for each primary survey period, sex, and study population: (1) both survival and detection probability were constant ( $\varphi(\cdot)p(\cdot)$ ), (2) survival varied over time and detection probability was constant ( $\varphi(t)p(\cdot)$ ), (3) survival was constant and detection probability varied over time ( $\varphi(\cdot)p(t)$ ), and (4) both survival and detection probability varied over time ( $\varphi(t)p(t)$ ). Time and detection probability were modeled as a random effect. We compared estimates from these four models using DIC.

For *A. varius* at Santa Marta, we used closed population models to estimate abundance within a primary survey period. We believed these models were appropriate for this site, because of the narrow sampling window at each time period (typically <1 week) and the high site fidelity of the species, where individuals were typically seen multiple times over each 5-day survey period. In these models, we assumed that populations were closed to additions (recruitment or immigration) or deletions (mortality or emigration) within each time period. We examined the following models:  $M_0$  (detection probability is constant),  $M_t$  (detection probability varies over time), and  $M_b$  (detection probability varies depending on whether an individual was captured before or not; i.e., trap response; Otis et al., 1978). We compared estimates from these three models using DIC and estimated abundance separately for males and females, and for each primary survey period.

### 2.3. Model implementation

All model parameters were estimated in a Bayesian framework. We combined our described models with prior distributions for each parameter. We used uninformative prior distributions for all of our parameters. Specifically, we used uniform (0, 1) distributions for survival, entry, and detection probabilities; and uniform distributions (0, 10) for the standard deviation of the normal distributions used to explain extra variation in the logit of survival and detection probability in the models where we estimated time-specific survival and/or detection probabilities. All models were fit using the programs R (R Development Core Team, 2004) and JAGS (Plummer, 2003). For the CJS models, we estimated 35,000 samples, discarding the first 20,000 as burn-in, and ran three separate chains for each analysis, thinning samples by three to result in 15,000 samples. For the JS models, we estimated 14,000 samples for each model, discarding the first 5000 as burn-in, and ran three separate chains for each analysis, thinning samples by three to result in 9000 samples. For the closed population models, we estimated 33,000 samples for each model, and discarding the first 3000 as burn in. Again, we ran three separate chains for each analysis, and thinned samples by three to result in 30,000 samples. These numbers of samples for the different models were sufficient to ensure that the scale reduction factor (R-hat) was <1.01, indicating that the models had converged.

**Table 1**

Summary of capture effort at the three study areas, including number of unique individuals captured at each sampling period with total number of captures in parentheses. Dashes indicate that populations were not sampled during that period. Horizontal lines indicate when *Bd* arrived on each stream.

Date	Study site and species				
	El Copé	Santa Marta		El Guayabito	
	<i>A. varius</i>	<i>A. varius</i>		<i>A. zeteki</i>	
	Male	Male	Female	Male	Female
6–8/2000	22 (29)	–	–	–	–
6–8/2001	27 (56)	–	–	–	–
6–8/2002	42 (99)	–	–	–	–
6–8/2003	73 (142)	14 (30)	2(2) <sup>a</sup>	–	–
1–2/2004	–	85 (182)	17 (20)	149 (213)	64 (96)
6–8/2004	66 (119)	59 (179)	6 (12)	–	–
12/2004	–	–	–	210 (358)	77 (86)
1–2/2005	–	0 (0)	10 (15)	87 (126)	74 (109)
7/2005	–	4 (5) <sup>a</sup>	0 (0)	–	–
12/2005	–	–	–	121 (212)	41 (48)
1/2006	–	–	–	102 (115)	94 (117)
Total	196 (445)	127 (396)	34 (49)	648 (1024)	383 (456)

<sup>a</sup> Too few individuals to estimate population parameters.

### 3. Results

At El Copé, 196 male *A. varius* individuals were captured a total of 445 times over the five primary survey periods (Table 1). Twenty-eight males were captured two years in a row, three males were caught over three years, and one male was caught over five years. At Santa Marta, 123 male *A. varius* individuals were captured a total of 396 times over the five primary sampling periods. Thirty-four females were captured a total of 49 times over the same periods. Twenty-nine males were captured over two primary survey periods (6 months apart), and 3 males were captured three times (spanning one year). Only one female was captured during two separate sampling primary survey periods (6 months apart). At El Guayabito, 648 male *A. zeteki* individuals were captured a total of 1024 times over the five primary sampling periods, and 383 females were captured a total of 456 times. Recapture rates between sampling periods were low at El Guayabito, even within one breeding season: 34 of 210 males (16%) captured in December 2004 were seen in January 2005, and 14 of 210 males (7%) seen in December 2005 were captured in January 2006. Only 9 males (1% of total males) were seen in both December 2004/January 2005 and December 2005/January 2006.

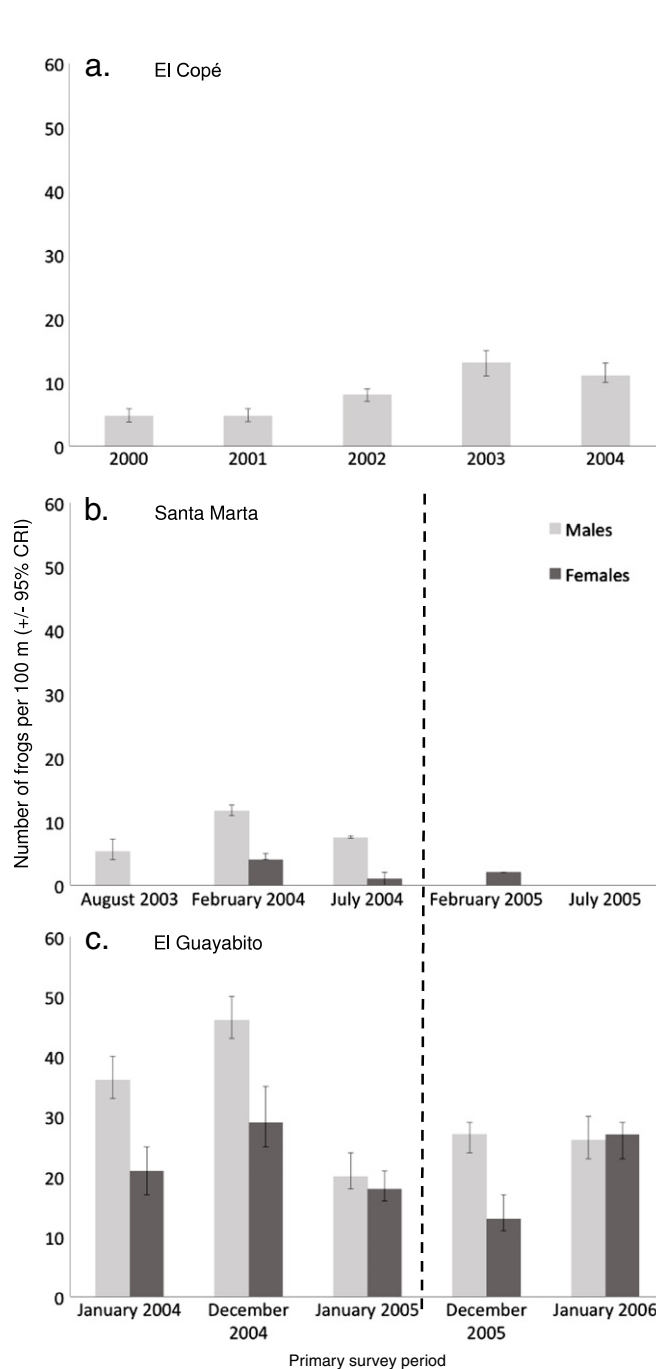
#### 3.1. Male survival

For *A. varius* at both El Copé and Santa Marta, the model with the lowest DIC had constant survival but detection probabilities that varied over time. For *A. zeteki* at El Guayabito, the model with the lowest DIC had survival that varied over time and constant detection probability. There were no competing models for any of the populations (<10 DIC units from the top model).

Mean monthly apparent survival varied between the two species, with *A. varius* showing higher survival probabilities than *A. zeteki*. The mean monthly apparent survival rate for *A. varius* at El Copé was 0.88 (0.84–0.92 95% Credible Interval; hereafter CRI), and detection probabilities ranged from 0.32 (0.03–0.76 CRI) to 0.74 (0.42–0.97 CRI) over time. Similarly, mean monthly apparent survival rate prior to the arrival of *Bd* for *A. varius* at Santa Marta was 0.85 (0.80–0.93 CRI), with detection probabilities ranging from 0.61 (0–1.0 CRI) to 0.90 (0.50–1.0 CRI) over time. In contrast, mean monthly survival varied over time for *A. zeteki* at El Guayabito. Mean monthly survival was 0.82 (0.75–0.89 CRI) from January to December 2004, 0.32 (0.16–0.77 CRI) from December 2004 to January 2005, 0.72 (0.62–0.80 CRI) from January to December 2005, and 0.43 (0.13–0.94 CRI) from December 2005 to January 2006. Mean detection probability for this population was 0.30 (0.14–0.67 CRI), which was generally lower than what we estimated for *A. varius*.

#### 3.2. Male and female abundance

At El Copé, male densities of *A. varius* ranged from 5 to 13 individuals per 100 m over the period from 2000–2004 (Fig. 2(a)). Densities were highest on the main stream transect in all years, and most individuals captured in this population were found on the main stream rather than the tributary streams. After the arrival of *Bd* in September 2004, the numbers of frogs at El Copé decreased rapidly over a period of two months, densities could not be estimated, and only four live male *A. varius* were seen on transects after November 2004. We were unable to estimate female densities because females could not be uniquely identified in most cases. For this population, models with constant survival and detection probabilities had the lowest DIC in 2000, 2001, and 2002. Models with constant detection probability and time varying survival had the lowest



**Fig. 2.** Mean population densities (number of frogs per 100 m) with 95% credible interval for males (light gray) and females (dark gray) in the three *Atelopus* populations in each primary sampling period: (a) *A. varius* at El Copé, (b) *A. varius* at Santa Marta, and (c) *A. zeteki* at El Guayabito. We were unable to estimate female population size at El Copé. Vertical dashed lines indicate when *Bd* arrived in each population.

DIC in 2003 and 2004, followed by the constant model (Table S1, Appendix A). Models within 10 DIC units of the top model had nearly identical posterior distributions for population density (Table S1, Appendix A).

At Santa Marta, densities of male *A. varius* were similar to those at El Copé (Fig. 2(b)). Densities of male frogs ranged from 5 to 12 individuals per 100 m before the arrival of *Bd*. Densities dropped to <1 individual per 100 m within a few months after *Bd* arrived. Prior to the arrival of *Bd*, density of frogs was higher during the dry season (February 2004) than during the wet season survey periods. Female densities ranged from 0 to 4 individuals per 100 m (Fig. 2(b)). Very few females were seen during the wet season sampling periods (August 2003 and July 2004), with higher densities during the dry season breeding

survey periods. For all primary survey periods, female densities were lower than male densities. The model with constant detection probability ( $M_0$ ) had the lowest DIC in all survey periods and for both sexes, except for females in February 2004, where the model with time-varying detection probability ( $M_t$ ) had the lowest DIC (Table S1, Appendix A). Models within 10 DIC units of the top model had nearly identical posterior distributions for density (July 2004 and February 2005 females; Table S1, Appendix A).

Male densities of *A. zeteki* at El Guayabito were the highest of the three populations, ranging from 21 to 46 individuals per 100 m (Fig. 2(c)). Male densities were higher in the two 2004 primary survey periods than in the remaining survey periods. Female densities were also higher for *A. zeteki* at El Guayabito than for *A. varius* at Santa Marta. These densities ranged from 13 to 29 individuals per 100 m (Fig. 2(c)), and were generally lower than male densities for equivalent survey periods. For males, models with constant detection and survival probabilities had the lowest DIC in all sampling periods but December 2004 and January 2005, where the model with constant detection probability and time-varying survival had the lowest DIC. For females, models with constant detection and survival probabilities had the lowest DIC in all survey periods (Table S1, Appendix A).

#### 4. Discussion

We present a demographic comparison of three *Atelopus* populations monitored prior to and during a chytridiomycosis outbreak in central Panama. We found that pre-decline detection, abundance, and survival of adults varied among species, with *A. zeteki* showing higher estimates of abundance and lower estimates of survival compared to the two *A. varius* populations. We hypothesize that differences in demography resulted from differences in both natural history and environments among the populations. After the arrival of *Bd*, we documented dramatic declines in population densities in both species. We provide an important baseline for future studies of recovering populations or for planned reintroductions of *Atelopus* ([http://www.cbsg.org/sites/cbsg.org/files/documents/PGF\\_WorkshopFinalReport\\_22July2014.pdf](http://www.cbsg.org/sites/cbsg.org/files/documents/PGF_WorkshopFinalReport_22July2014.pdf)).

##### 4.1. Male survival

Estimated differences in male survival between the *A. varius* populations and the *A. zeteki* population may be a result of differences in behavioral, life history, or environmental characteristics between the two species. The *A. zeteki* population studied here belongs to a lowland dry forest evolutionary significant unit (ESU) within *A. zeteki* that exhibits unusual behavior even within this species (Richards and Knowles, 2007). This population exhibited explosive breeding, coming to the stream only during a short, intense pulse during the dry season, while other *A. zeteki* and *A. varius* populations had more protracted breeding seasons. We hypothesize that this behavioral difference may explain the lower estimates of survival for *A. zeteki*, as males leaving the population were unlikely to be seen again, whereas *A. varius* males tended to hold territories during the wet and dry seasons. Low male survival rates between December and January may reflect emigration of individuals off the stream rather than true mortality, since the survival models we used were unable to separate death from emigration. Illegal collecting of individuals on the stream may also have influenced the lower survival estimated in *A. zeteki*: one of us (CLRZ) observed illegal collectors at El Guayabito on multiple occasions. The removal of individuals during the breeding season, both during and between capture sessions, may have contributed to the low apparent survival, especially between the December and January sampling periods during the breeding seasons. Finally, low apparent survival estimates in the final two survey periods for *A. zeteki* may have been caused by the appearance of *Bd*. Because of the rapid loss of adult *Atelopus* during chytridiomycosis epidemics (e.g., Lips et al., 2006), we were unable to model changes in survival as a result of *Bd*.

Our data indicate that different *Atelopus* species, occurring across a large geographic range, may exhibit similar demographic and life history characteristics, and this information may be useful for designing monitoring and conservation plans for other species of *Atelopus*. Both *A. varius* populations had stable and similar monthly apparent survival rates prior to the arrival of *Bd*. These survival estimates were similar to those estimated in *Bd*-infected populations of *Atelopus cruciger* in Venezuela (Lampo et al., 2012) and *A. spumarius* in Ecuador (Tarvin et al., 2014). Our mean monthly apparent survival estimates for *A. varius* at El Copé and Santa Marta were similar to those estimated for *A. cruciger* (0.94 [0.90–0.96 CI]) and *A. spumarius* (0.88), and credible intervals overlapped substantially. This is particularly interesting since both of these latter populations are persisting with *Bd*. In contrast, apparent survival for *A. zeteki* during the pre-disease period was lower than what has been measured for other *Atelopus* populations, suggesting population- or species-specific differences, or differences in habitat use after the breeding season.

Low recapture rates and high site fidelity of both males and females among primary survey periods (which were separated by 1–9 months) suggest high population turnover and localized population dynamics, both of which are relevant to conservation actions for these species. *A. varius* is generally thought to hold consistent breeding territories (Crump, 1986, 1988), and our study supports this observation: individuals of both species were often captured several times at the same location on the stream within a season. While individuals could move off of the study transects between sessions to other locations in the streams, less than 1% of individuals in the three populations changed transects over the course of the study. Low recapture rates among primary periods suggest that only a few individuals survive a full year after reproduction, which could influence reintroduction efforts. A combination of high site fidelity and high population turnover suggests that reintroduction and monitoring efforts should occur at a very local population scale and at high frequency, and that multiple



reintroduction events may be necessary to establish populations experiencing high turnover, depending on the reproductive success of reintroduced populations.

#### 4.2. Male and female abundance

Despite temporal variation in abundance in the three populations, we demonstrate large differences in male and female density between the two species that should be considered in conservation plans. Densities of *A. varius* at Santa Marta were higher during the breeding (dry) season than during the wet season for both males and females, suggesting an increase in site attendance during the breeding season. For equivalent periods (wet season 2003 and 2004), Santa Marta had lower densities of *A. varius* males than El Copé, which may be due to differences in the local environment. Two of the stream transects at Santa Marta were bordered by pastureland rather than forest, which may have affected abundance and dispersal distance of frogs at these sites. The high densities seen at El Guayabito reflected the unique breeding behavior of this population. Densities of males at El Guayabito were higher in 2004 than in subsequent survey periods. The decline seen in later periods may be due in part to the arrival of *Bd*, though we cannot separate these effects from other factors in the stream environment. Differences in natural male and female densities can be incorporated into captive breeding and reintroduction plans, as can the large differences in densities measured between the two species.

A comparison of density estimates with other studies suggests that elevation may contribute to differences in population density among species. The densities of male frogs estimated for the low elevation (120–220 m a.s.l.) *A. cruciger* population (28–47 frogs per 100 m; Lampo et al., 2012) was higher than what was seen for either mid-elevation (500–700 m a.s.l.) population of *A. varius* (Fig. 2(a), (b)), and more similar to densities seen at El Guayabito for *A. zeteki* (Fig. 2(c)), another low elevation (300 m a.s.l.) site. Densities for *A. spumarius* (9–18 frogs per 100 m; Tarvin et al., 2014) were similar to the densities we measured for *A. varius*, but lower than what we saw for *A. zeteki*. The *A. spumarius* population is located in high elevation (1000 m a.s.l.) tropical forest ecosystem. The lower densities of *Atelopus* seen at mid- to high elevation sites compared to low elevation sites could contribute to reintroduction and monitoring programs: lower elevation sites may be able to support higher densities of frogs than high elevation sites. This pattern, if upheld, could influence how many individuals might be reintroduced at various elevations and inform what densities to expect in stable monitored populations.

#### 4.3. Effects of *Bd* on demography

*Atelopus* populations declined dramatically in all three populations within six months after *Bd* was first detected, but showed differences in the initial response to *Bd*. For *A. varius*, *Bd* was first detected in September 2004 at El Copé (Lips et al., 2006), and arrived at Santa Marta between the August 2004 and February 2005 sampling periods. In contrast, *Bd* appeared at El Guayabito prior to the December capture session in 2005. This is consistent with the wave-like progression of *Bd* through Panama (Lips et al., 2006). Despite *Bd* being detected in December of 2005 (14% prevalence) and January of 2006 (47% prevalence) in this population, large numbers of males and females were caught in these time periods. Both the life history of these two species and the habitat conditions of the sites may explain this difference. First, *Bd* has been most devastating to species that occupy stream habitats year round (Lips et al., 2006), because it is thought to persist longer and be transmitted more rapidly in the cooler and moister habitats along streams (Piotrowski et al., 2004; Rowley and Alford, 2007; Ryan et al., 2008). Frogs from our two *A. varius* populations – and males of these populations in particular – spent both the wet and dry season occupying territories on the stream, and this may have contributed to their early decline, along with the rapid increase in disease in these species once infected (DiRenzo et al., 2014). The low recapture rate for male *A. zeteki* in January 2006 suggests that animals were beginning to die by that period, and ten dead *Bd*-positive individuals were found on the stream in that survey period (Richards-Zawacki, 2010). Females coming to the stream during that time period may have been exposed to *Bd* for the first time, as noted for earlier decline sites in western Panama (Lips, 1998, 1999).

Finally, differences in environmental conditions at the two sites may have affected how populations initially responded to the arrival of *Bd*. The slower decline of *A. zeteki* may have been due to the warm, dry conditions less conducive to *Bd* growth and survival at that low elevation site (Richards and Knowles, 2007). In a laboratory study, frogs exposed to *Bd* under dry conditions and at warmer temperatures lived longer than in cooler temperatures and wetter conditions (Bustamante et al., 2010). Other studies have found *Bd* prevalence to be very low in tropical dry forest locations (Zumbado-Ulate et al., 2014) and at sea level (Flechas et al., 2012), suggesting these are areas that may serve as potential climatic refuges from *Bd*. Finally, the *A. cruciger* population persisting with *Bd* is also located in a low elevation dry tropical forest (Lampo et al., 2012), and habitat conditions may explain the ability for this population to remain stable in the presence of *Bd*. Unfortunately, we were unable to monitor the *A. zeteki* population in the months after *Bd* was detected and prevalence in the population increased, but no animals have been detected at this site during biannual surveys from 2012 to 2015, suggesting that the population is likely extinct. The possibility for *Atelopus* populations to persist with endemic *Bd* in warmer or drier environmental conditions should be considered in conservation and reintroduction plans.

### 5. Conclusions

Frogs in the genus *Atelopus* are among the most threatened amphibian taxa in the world, and require numerous urgent conservation actions, basic ecological monitoring, and captive breeding programs. Understanding natural densities, sex

ratios, and survival rates are crucial to developing captive breeding and reintroduction programs that mimic and are informed by natural population processes. Our analysis provides salient information on the life history of two critically endangered species, *Atelopus varius* and *A. zeteki*. We report differences in survival probabilities and density among the three populations and compare them to other *Atelopus* populations, showing similarities in demography between our pre-decline populations and remnant populations persisting with *Bd*. Knowing the range of values seen for these population parameters both pre- and post-*Bd* can inform the success of captive breeding and reintroduction programs by providing estimates that can be compared to monitored populations.

Our study and analyses can further inform monitoring protocols for any surviving or reintroduced *Atelopus* populations. Despite the differences in our field sampling protocols among the three populations, we estimated similar demographic parameters for both *A. varius* populations and found intriguing similarities in demographic parameters between our study populations and other published demographic rates for *Atelopus* (Lampo et al., 2012; Tarvin et al., 2014). However, we suggest that a robust design (Pollock, 1982) sampling approach would provide the strongest inference in future studies, where density can be estimated during secondary periods of population closure (i.e. 3–5 days of repeated sampling) and survival can be estimated across primary survey periods (i.e., 1–3 months apart). Surveys could take place year-round, or be focused on the breeding season when captures of males and females are likely to be the highest.

Finally, we recommend that monitoring programs should include the monitoring of potential variables that might affect population dynamics, such as disease prevalence and intensity, climate (i.e. temperature and moisture), amphibian community composition, and characteristics of the stream itself (e.g., quantification of breeding territories and other factors). Monitoring of remnant *Atelopus* populations as well as captive populations will allow us to understand and compare basic life history and demographic characteristics of these vulnerable species, and to document changes in population characteristics as part of programs aimed at protecting and restoring populations of these species.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2015.07.003>.

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