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Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis

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ABSTRACT

We conducted a mark-recapture study of three subpopulations of *Craugastor punctariolus* at a mid-elevation site in central Panama between 1999 and 2005. The study spans a period over which the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) was absent from the site, invaded the site and decimated all amphibian populations, and now persists. We quantified natural demographic parameters prior to and during an event of mass mortality due to chytridiomycosis caused by *Bd*. Prior to the event of mass mortality, all three subpopulations of *C. punctariolus* were large (19–68 animals/200 m), showed a stable age-size distribution, and had high survival. All age-size classes of this species co-occurred on boulder clumps along streams, and adults showed high site fidelity and were likely territorial. Following detection of *Bd* at this site in late September 2004, four dead *C. punctariolus* were found infected with *Bd* and all three subpopulations completely disappeared from this site within 2 months. The association of all age-size classes with microhabitats appropriate for survival and growth of *Bd* likely contributed to the rapid and severe degree of decline of this species at this site. These data provide insight into the patterns and mechanisms of decline within a species due to *Bd*.

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1. Introduction

Approximately 43% of amphibian species are declining (Stuart et al., 2004) and several species have gone extinct (Wilson and McCranie, 2003; La Marca et al., 2005; Lips et al., 2006). The Neotropics are home to more than half of the world's 6000 described amphibian species (Frost et al., 2006), with high species endemism at middle and upper elevations (Duellman, 1999). It is these middle and upper elevation amphibian communities that have declined most severely (e.g. Lips et al., 2004; Stuart et al., 2004). Declines in upland areas of the Neo-

tropics and Australia have been especially severe and geographically and taxonomically broad, with approximately half an amphibian assemblage being extirpated within a year, and an 80% reduction in the abundance of the remaining species (Pounds et al., 1997; Williams and Hero, 1998; Lips et al., 2003; Stuart et al., 2004; Bustamante et al., 2005; La Marca et al., 2005).

Two Neotropical clades that exemplify the montane, riparian amphibian decline scenario are the genus *Atelopus* (La Marca et al., 2005) and the *Craugastor rugulosus* species group (Campbell and Savage, 2000; McCranie and Wilson, 2002; Lips,

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1999; Lips et al., 2004, 2006). *Atelopus* declines have been well documented, in part because they are colorful, diurnal frogs that are easy to observe (Löfthors, 1996). In a comprehensive review of 113 species of *Atelopus*, La Marca et al. (2005) found that all species that occurred above 1000 m had declined and 75% of those species had completely disappeared. Less well known has been the decline and disappearance of the *C. rugulosus* species group, a clade of drab, nocturnal stream frogs found predominantly in upland areas from Mexico to Panama. Twenty-six (79%) of the 33 species in this clade are critically endangered, endangered, or vulnerable (IUCN et al., 2008), and the best available data suggest that this entire group has experienced severe population declines throughout Central America (i.e. Campbell, 1998; McCranie and Wilson, 2002; Savage, 2002; Lips et al., 2003, 2004). Only two populations are currently known to occur at historic abundances, *Craugastor ranoides* in the low elevation dry forest habitat on the Santa Elena Peninsula of Costa Rica (Puschendorf et al., 2005), and low to mid-elevation populations of *C. punctariolus* between El Valle de Anton and the Panama Canal watershed (R. Ibáñez, pers. comm.).

In many cases, amphibian die-offs and declines have been attributed to chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (*Bd* hereafter). These declines are non-random with respect to timing (Laurance et al., 1996; Lips et al., 2006; Kriger and Hero, 2006), physical habitat (Ron, 2005), and ecology of affected species (Williams and Hero, 1998; Lips et al., 2003; Murray and Hose, 2005), with species most closely associated with riparian habitats in cool moist forests at greatest risk. Not all populations respond to the same degree or at the same rate following the introduction of *Bd* to a population (e.g. Brem, 2006; Lips et al., 2006; Rowley and Alford, 2007b). Differences in species responses within a community have been attributed to differences in susceptibility (Daszak et al., 2000), micro-environmental conditions (e.g. Brem, 2006), and population connectivity (e.g. Robertson et al., 2008).

In order to understand the mechanisms involved in population fluctuations, it is necessary to know the abundance of each sex and age class and the rates of change of demographic parameters. Long-term data sets (e.g. Alford and Richards, 1999; Houlahan et al., 2001) based on mark-recapture techniques (Pollock et al., 1990; Alford and Richards, 1999; Schmidt, 2003; Muths et al., 2003; Grafe et al., 2004) can provide estimates of population abundance, survival, dispersion, sex ratio, and age structure necessary to detect population trends. A long-term data set of multiple demographic units (e.g. male, female, and juvenile subunits) can indicate whether groups respond uniformly to environmental perturbations (Stearns, 1976; Crouse et al., 1987; Collins and Storer, 2003; Grafe et al., 2004). For example, in Costa Rica and Panama many amphibian populations have declined to less than 20% of typical abundances and have shown no subsequent recovery in the past 10 to 15 years (e.g. Lips et al., 2003, 2006). This pattern suggests that many adults are affected, that no reproduction occurs, or that all stages are affected rapidly and simultaneously. Capture-recapture studies have been conducted on various amphibians (e.g. Richter and Seigel, 2002; Muths et al., 2003; Schmidt, 2003; Scherer et al., 2005), although rarely for tropical anurans (but see Funk et al., 2003; Grafe et al., 2004).

Here we describe the natural population dynamics and habitat use of three subpopulations of *Craugastor punctariolus* over a 6 year period and document changes in demographic parameters following the arrival of *Bd* at the site in 2004. We estimated the amount of time from the first detectable decline in survival to the time when the population completely disappeared from our study area. We also include data related to the basic natural history of this poorly known endangered species because it has declined precipitously throughout its range and is likely to go extinct in the near future.

2. Methods

2.1. Study area

We studied *C. punctariolus* in the 25,000 ha Parque Nacional G. D. Omar Torrijos H., El Copé, Coclé Province, Panama (8°40' N, 80° 37' 17" W; 600–900 m; Fig. 1). The park is situated at the eastern end of the Serranía de Tabasará, or Cordillera Central of Panama, where it straddles the continental divide. It is primarily tropical moist forest, with the greatest precipitation on the Atlantic slope and drier conditions on the Pacific slope. We measured daily rainfall and air temperature with a rain gauge and max–min thermometer respectively. Mean annual rainfall was <3500 mm with a pronounced dry season between December and March, and average daily temperatures taken on the exposed continental divide ranged between 19 and 26 °C (Lips et al., 2006). Survey transects were located at approximately 700 m elevation along three spring-fed headwater streams. Quebrada Cascada and Loop Stream are first order streams that drain into the slightly larger Río Guabal on the Atlantic versant (Fig. 1). All three streams were similar in structure and microenvironments with swift flowing water and characterized by deep pools alternating with riffles and runs; channel substrates consisted of sand, cobble and boulder. The banks of the streams were generally steep and consisted of mud, clay, or exposed bedrock covered with leaf litter and overhung with dense vegetation.

2.2. Study species

C. punctariolus belongs to the *rugulosus* clade, and is endemic to Central Panama (Fig. 1). It inhabits the Pacific slope from Cerro Colorado in Veraguas Province eastward to El Valle de Antón, Coclé Province, and is expected to occur on the Atlantic slope in that same region (Campbell and Savage, 2000). Males of *C. punctariolus* are distinguished from females by their grayish nuptial thumb pad and larger tympanum diameter (63–92% of eye length versus 37–44% in females). Females have no nuptial pad and are larger than males (79 mm snout-to-vent length (=SVL), versus 53 mm in males; Campbell and Savage, 2000). We were first able to reliably distinguish between males and females midway through the 2002 field season, so all comparisons between the sexes are based on 2002 and 2003 data only. We were not able to determine the reproductive season of this species because we saw only one amplexic pair, and because the thick ventral disk and integument prevented us from determining whether females were gravid. Hayes (1985) observed a female *Craugastor angelicus*, a

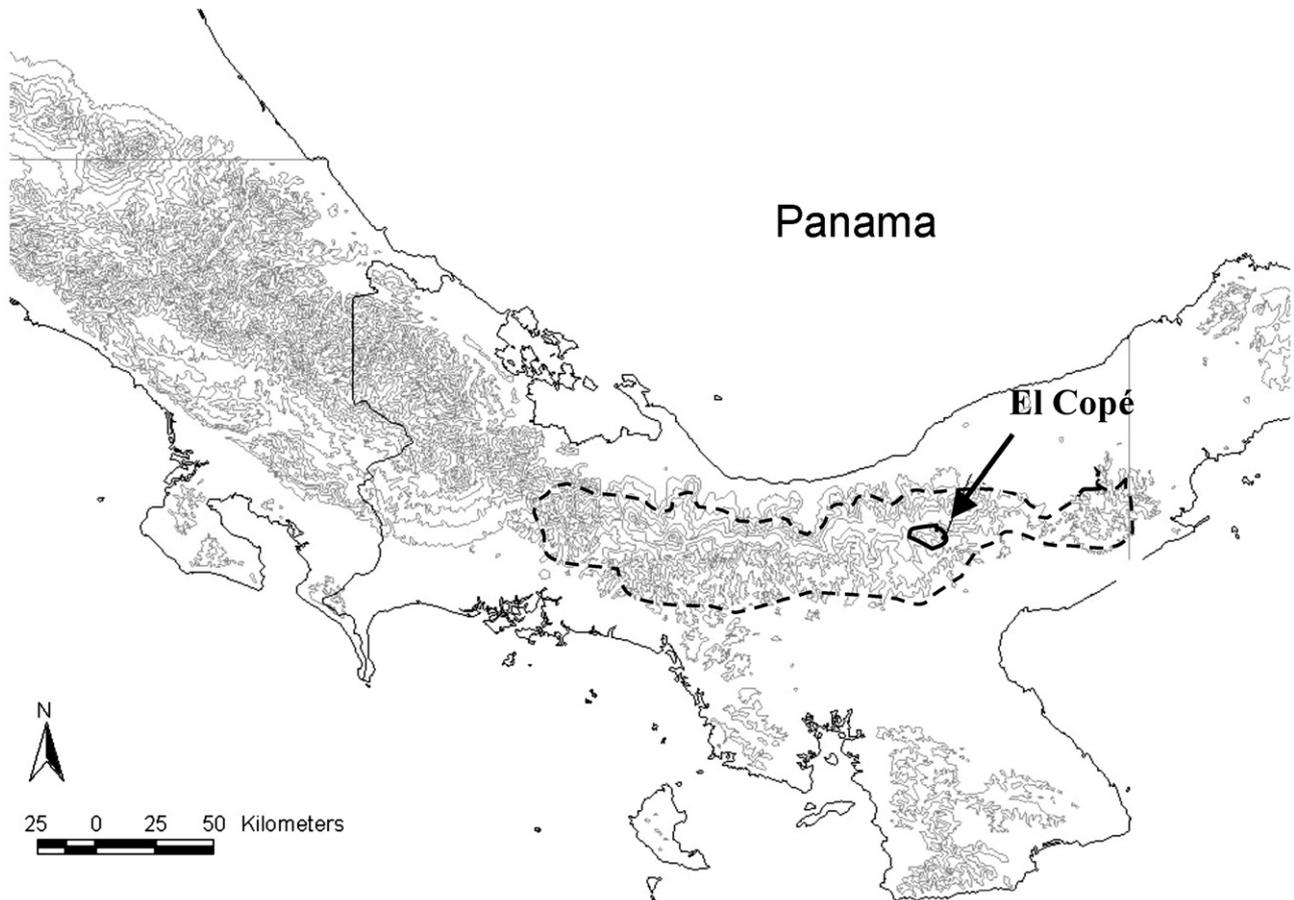


Fig. 1 – Map of Central Panama showing the location of Parque Nacional Omar Torrijos (solid line), El Copé, Coclé Province and the range of *Craugastor punctariolus* (dashed line).

closely related species, excavating a nest in a sandy bank along a stream in Monteverde, Costa Rica. Very little ecological information exists on this species, or others in this clade, although they likely have similar ecologies (Campbell and Savage, 2000; K. Lips, pers. obs.). During previous surveys we observed that adults occurred on boulders along stream channels at night, that they retreated to crevices of large boulders, leaf litter or other debris along stream courses during the day, and that hatchlings were found on gravel and cobble stream banks during diurnal transects.

2.3. Age and sex specific habitat use

Amphibian surveys have been conducted on three terrestrial transects and four stream transects at El Copé from 1999 until 2007 as part of an ongoing research project by KRL. Each 200 m stream transect was flagged at 20 m intervals (Fig. 2), with two or more surveyors slowly walking and scanning all substrates from the water's edge to the top of the stream bank for all species of amphibians and reptiles. Between 1999 and 2007 all transects were monitored with the same methodology, but changes in personnel and funding resulted in unequal survey efforts. We used five distinct sampling regimes that differed in duration or frequency, with the result that some statistical analyses are not possible (i.e. comparing mark-recapture data among all sampling periods). The

first period was represented by four 3-month survey periods (May–August) from 1999 to 2002, during which we surveyed each stream transect twice weekly (one nocturnal and one corresponding diurnal survey). The second period corresponded to 11 diurnal and 11 corresponding nocturnal surveys between May and August 2003 that were combined into 11 sampling units. Period three represented monthly surveys (one nocturnal and one corresponding diurnal survey that were combined into one sampling period) conducted between August 2003 and September 2004. Period four included surveys conducted between 1 October 2004 and 14 January 2005 at 1–3 day intervals during the period of amphibian mass mortality (Lips et al., 2006). The fifth period included sporadic surveys conducted between February 2005 and September 2006 following the mass mortality event.

We captured individual *C. punctariolus* by hand and processed them. We recorded SVL (mm), mass (g), sex (for 2002–2003 captures only), meter location on transect, perch type (microhabitat), and perch height over water (cm) for the first capture of an individual or its first capture for the season. We assigned each adult frog a unique toe clip combination that did not involve cutting the innermost digit of the hands, which are used in amplexus (Heyer et al., 1994). We measured SVL with dial vernier calipers accurate to 0.1 mm and mass with 10 g or 50 g Pesola® spring scales. To decrease

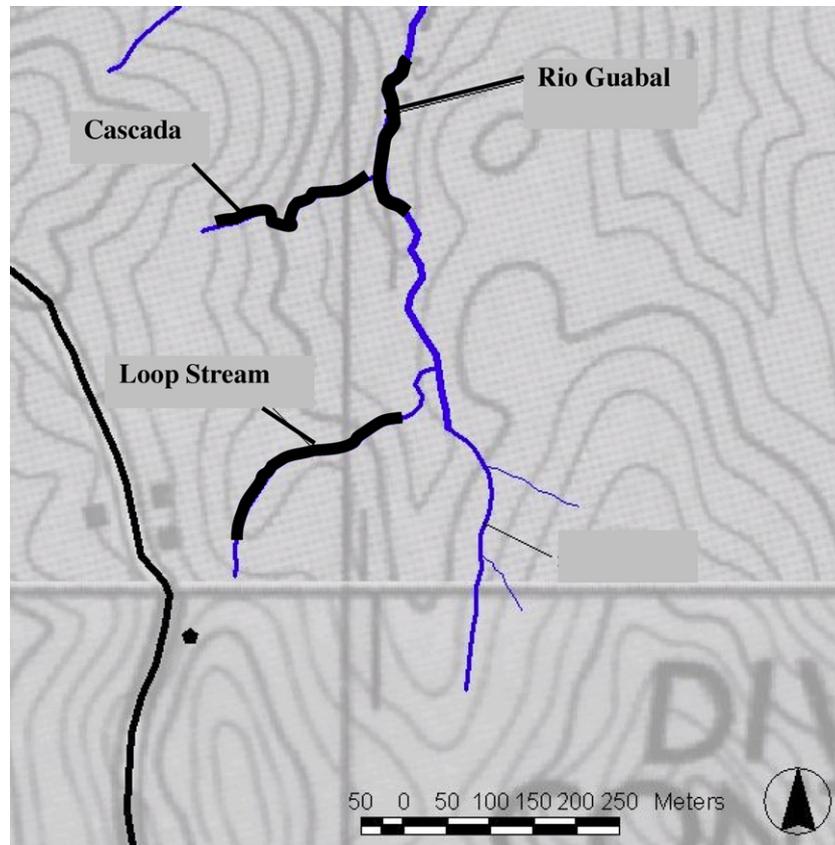


Fig. 2 – Map of the three study transects, Rio Guabal, Quebrada Cascada, and Loop Stream, in Parque Nacional Omar Torrijos. The 200 m transects are noted by the thick black lines. The layout shows how the study system was interconnected and how frogs could potentially move among transects.

handling time, we did not measure either SVL or mass for recaptured animals. Table 1 provides a summary of survey efforts, number of animals encountered, marked, and recaptured for each of the years of the study.

We followed established methods for species of *Eleutherodactylus* (Stewart and Woolbright, 1996) to assign frogs to discrete age classes based on body size and secondary sex characters to identify sexually mature individuals. Body size and age are correlated in most amphibians, and although

there may be variance within age classes (Halliday and Verrill, 1988; Platz and Lathrop, 1993), and we used three discrete size classes.

We quantified substrate composition of each transect to determine whether these frogs were associated with particular habitat types. We used a modified Wentworth scale (Allan, 1995) to visually estimate the percent coverage of each 1 m section of each 200 m transect as either boulder/bedrock, cobble/pebble/gravel, or silt/sand in July 2003. We used a one-way analysis of variance (ANOVA) to compare the percent substrate composition among the three study transects. In 2003, we also measured the wetted width of the stream channel and estimated the percent of stream channel habitat represented by pools (deep, low water velocity), riffles (shallow, high velocity, broken water surface), and runs (intermediate in depth and velocity; Allan, 1995).

To determine whether spatial distribution of *C. punctariolus* captures was random or clumped along the stream transects, we tested for goodness-of-fit (SAS, 2001) to a Poisson distribution at 1 and 5 m intervals (Ludwig and Reynolds, 1988) for all captures on each transect for the period 1999–2003. To determine whether different age-classes were partitioning habitat, we compared the number of captures of each sex-age class (adult males, adult females, juveniles) among the three substrate types in a 3 × 3 χ^2 contingency table (Ludwig and Reynolds, 1988). We calculated linear selection indices for each transect (Strauss, 1979) in which the selection index (*L*)

Table 1 – Sampling effort (number of surveys and hours) and captures (total, marked individuals, recaptured individuals) of *Craugaster punctariolus* along three stream transects at Parque Nacional Omar Torrijos, between 1999 and 2005

Year	Total surveys	Total hours	Total captures	Marked individuals	Recaptures (% of total)
1999	11	28	36	15	2 (6)
2000	14	28	60	25	6 (10)
2001	21	65	159	66	22 (14)
2002	24	71	294	102	47 (16)
2003	46	81	492	162	109 (22)
2004	29	86	166	62	44 (27)
2005	33	36	0	–	0 (0)
Total	178	395	1207	432	230 (59)

was calculated as $L = r_i - p_i$, where r_i represents the relative proportion of the number of captures in a habitat type (i), and p_i the relative proportion of habitat type (i) in the transect. The index ranges from -1.00 (an abundant but avoided habitat) to $+1.00$ (an extensively used but rare habitat); values of 0 indicate a habitat used in proportion to its abundance (Frissel and Lonzarich, 1996). We also investigated vertical partitioning by comparing perch height between adults and juveniles and between males and females for 2002–2003 captures.

2.4. Sex and age ratio

To determine the distribution of the three age-sex classes for all transects we compared the distribution of SVL of captures made during 1999–2002 with a χ^2 test: size class 1 (juveniles) <33 mm; size class 2 (adult males and females) 34–54 mm; and size class 3 (females) >55 mm for 2001–2003. For adults captured during 2002–2003, we corrected for potential differences in detection probability between adult males and females by subtracting the transect-specific male capture probability from the transect-specific female capture probability, then multiplying that sum by the number of males observed during the corresponding survey, and finally, adding that product to the number of males observed on the corresponding survey. We then calculated the overall population sex ratio (number of males/number of adults), the sex ratio for each transect, and the nightly sex ratio and compared differences with χ^2 tests.

2.5. Movement

For each captured individual, we recorded the frog's linear position in meters along each transect and determined the distance between the two farthest captures. We recorded the difference for all captures between 1999 and 2005, and we also differentiated between males and females using the 2002–2003 data. We compared the up-down stream direction moved between males and females with a Mann-Whitney U -test and compared the distance moved with a one-way ANOVA. We tested for skewness of the intra-capture distances (e.g. Lowe, 2003) to determine whether animals showed a bias in movement direction. If a frog moved to a different transect, we assumed it traveled along the streambed (i.e. not overland) because all *C. punctariolus* observed to date have been found within 2 m of the streambed (K. Lips, unpublished data).

2.6. Survival

We used the Cormack–Jolly–Seber (recaptures only) model in program MARK to estimate 7 day apparent survival ($\hat{\phi}$, the probability an individual lives and remains on the study area between the intervals i and $i + 1$) and detection probability (\hat{p} , the probability an individual is alive and available for capture at time i) for two periods: 18 May–31 July 2003, and 26 September 2003–18 October 2004. Temporal variation in parameter estimates can detect the early stages of a decline or identify the timing and rate of population recovery. Apparent survival is a minimum estimate of survival because mortality and emigration events are indistinguishable. During the

first period, time intervals between sampling periods varied from 4 to 13 day ($\bar{x} = 7.1$ days) and during the second period time intervals between sampling periods varied from 14 to 51 day ($\bar{x} = 29.4$). For both periods, we standardized the time between sampling periods to 7 day using the “set time intervals” option in program MARK. Because the length of time between sampling periods varied among streams, we analyzed captures from each stream separately. We used Akaike's Information Criterion for small sample size (AICc) to determine which model best fit the data. We tested fit of the global model, a model that allowed $\hat{\phi}$ and \hat{p} to vary by sex and time interactively, by using the parametric bootstrap procedure in program MARK (Burnham and Anderson, 2002). In cases where more than one model competed well (i.e. the AIC score was within 4 points of the most parsimonious model) we used the model averaging procedure in program MARK to estimate the weighted average parameter point estimate and unconditional standard error and 95% confidence intervals from all models within four AIC points of the most parsimonious model (Burnham and Anderson, 2002). We multiplied mean 7 day survival estimates from the 26 September 2003–18 October 2004 time-period for each transect by the 52nd power to estimate apparent annual survival. We only used data from this time-period because survival likely varied during different periods of the annual cycle and the second time-period extended over a 13 month period incorporating all periods of the annual cycle. Furthermore, we used the mean of the three transects because we assumed variation in point estimates among transects was due to sampling variance and not true biological variance among transects, thus the mean of those point estimates better estimated true survival.

2.7. Abundance

The width of the transects varied due to variability in the width of the stream and visibility at any given point. This affected the data structure and prevented us from using a formal model to estimate abundance (e.g. Jolly–Seber model). Therefore, we used an *ad hoc* method of estimating abundance by dividing the total number of individuals observed on an individual transect by the probability of catching an individual during any of the individual surveys during the survey period. We estimated the transect-specific probability of observing an individual during any of the individual surveys with the equation:

$$P_i = 1 - \prod_{s=1}^n (1 - p_s)$$

where, P_i = the transect-specific probability of observing an individual during any of the individual surveys, n = the number of surveys during a survey period for each transect, and p_s = the detection probability estimated for surveys using the previously mentioned survival analysis in program MARK. This *ad hoc* method precludes us from estimating standard errors of our point estimates of abundance and is biased low due to mortality between survey periods, but provides an indication of the prevalence of the species on our transects.

2.8. Ability to detect demographic changes following invasion by Bd

We detected the first dead frog on 4 October 2004, although observations of other species indicated that amphibian abundance had began declining on riparian transects by 4 September 2004 (Lips et al., 2006). This suggests that frogs were infected and dying for at least a month prior to finding the first dead individual. To determine if we could detect a decline in survival rate before *C. punctariolus* began to show a measurable population decline, we ran a series of models in which we sequentially allowed survival to vary during one or more survey periods. We started with a model that allowed survival to differ during the last time-period while holding survival constant among the remaining time-periods. We then compared that to a model that allowed survival to differ between the last two time-periods while being held constant for the remaining time-periods. We continued this in a stepwise approach to the last five time-periods.

3. Results

3.1. Age and sex specific habitat use

The study transects differed slightly in habitat type and composition: Quebrada Cascada had the highest percentage of boulder/bedrock (49.5 ± 1.3%) followed by Río Guabal (37.8 ± 0.9%) and Loop Stream (30.8 ± 0.7%). Río Guabal had the highest percentage of cobble/pebble/gravel (33.5 ± 0.7%) followed by Loop Stream (30.2 ± 0.59%) and Quebrada Cascada (23.8 ± 0.7%). Loop Stream had the highest percentage of fine material (38.9 ± 0.6%) followed by Río Guabal (28.7 ± 0.6%) and Quebrada Cascada (26.7 ± 0.6%). Each transect also differed in stream channel characteristics: Quebrada Cascada had 81.5% riffle, 18.5% run, and 4.8 m wetted width; Río Guabal had 71.5% riffle, 28.5% run, and 6.7 m wetted width; and Loop Stream had 90% riffle, 10% run, and 5.4 m wetted width.

C. punctariolus captures were significantly aggregated on boulder/bedrock habitats, as indicated by χ^2 tests and coefficients of dispersion greater than 1, on all three transects at both 1 m and 5 m intervals (Table 2). Age-size classes differed

in habitat use ($\chi^2 = 14.12$, $df = 2$, $P < 0.001$). Captures of juveniles were divided between boulder/bedrock (44%) and cobble/pebble/gravel (50%) with 5% on sand/silt substrates, while 65% of adults were captured on boulder/bedrock substrate, 20% in cobble/pebble/gravel, and 15% on silt/sand substrate. Males and females did not differ in their use of substrates; most captures of each sex occurred on boulder/bedrock (74% of female, 70% of male; $\chi^2 = 15.7$, $df = 4$, $P < 0.004$), with 20% of female captures and 25% of male captures on cobble/pebble/gravel, and the remaining captures on sand/silt (6% of female, 5% of male). Adult males ($L = 0.30$) and females ($L = 0.26$) chose boulder/bedrock habitat and avoided cobble/pebble/gravel (males: $L = -0.12$, females: $L = -0.16$), while juveniles chose cobble/pebble/gravel on all streams ($L = 0.45$), and all age-sex classes avoided sand/silt substrates (males: $L = -0.58$, females: $L = -0.59$, juveniles: $L = -1.00$).

Individuals partitioned vertical space on boulder clumps by body size (ANOVA $df = 2$, $P < 0.001$); the smallest frogs occupied the lowest perches (average perch height $\bar{x} = 25.4 \pm 4.2$ cm), medium-sized frogs occupied medium height perches ($\bar{x} = 62.8 \pm 3.3$ cm), and the largest frogs occupied the highest perches ($\bar{x} = 88.2 \pm 5.6$ cm). Larger adults ($\bar{x} = 68.2 \pm 4.2$ cm) perched higher than juveniles ($\bar{x} = 28.4 \pm 6.6$ cm; ANOVA $df = 1$, $P < 0.001$), and larger females ($\bar{x} = 81.79 \pm 7.28$ cm; one-way ANOVA $df = 5$, $P < 0.01$) perched higher than smaller males ($\bar{x} = 58.37 \pm 6.2$ cm).

3.2. Sex and age ratio

Females were 26% larger than males (ANOVA $df = 2$, $P < 0.001$), with female SVL ranging between 34.12–78.92 mm ($\bar{x} = 56.9 \pm 1.12$ mm). Male SVL ranged between 34.02–56.08 mm ($\bar{x} = 46.11 \pm 5.14$ mm) and juvenile SVL ranged between 10.0–33.9 mm ($\bar{x} = 23.3 \pm 6.5$ mm). All frogs >55 mm SVL were females, although male and female SVL overlapped in the 34–55 mm range. Size class structure for the combined transects was relatively constant over the period 2001–2004, with the proportion of adults comprising 70%, 64%, 79%, and 82% of the total marked population for each of the 4 years, respectively. Frogs in size class 2 were most abundant and represented 54%, 41%, 55%, and 43% of the total population for each of the four years, respectively. In 2003 when size class was further subdivided by sex, size class 2 consisted of 82% males, whereas size class 3 was comprised of 92% females (see Fig. 3).

In general, males and females occurred in equal numbers among nights, transects, and over the entire study (Fig. 2). During the 2003 field season, we captured males more often (179 captures) than females (147 captures), and the three transects differed in the total number of males and females captured, ($\chi^2 = 5.1$, $df = 1$ $P < 0.02$). Between 18 May–31 July 2003 we marked 67 adult frogs (37 male, 30 female; 55% males) on Quebrada Cascada, 56 (36 male, 20 female; 64% males) on Río Guabal, and 18 (11 male, 7 female; 61% males) on Loop. The nightly sex ratio, defined as the ratio of males to all adults captured on a given night, was even for all transects combined ($\chi^2 = 1.16$, $df = 2$, $P < 0.5$). Both Loop Stream ($\chi^2 = 1.0$, $df = 2$, $P < 0.3$) and Quebrada Cascada ($\chi^2 = 0.63$, $df = 2$, $P < 0.4$) had even nightly sex ratios, while Río Guabal had a male bias ($\chi^2 = 4.6$, $df = 2$, $P < 0.03$).

Table 2 – Captures of *Craugastor punctariolus* were aggregated on each transect as indicated by χ^2 test and coefficients of dispersion (CD) greater than 1 on all three transects at both 1 m and 5 m intervals

Transect	\bar{Y}	χ^2	CD
Loop Stream			
1 m	0.69	4.2	5.9
5 m	3.50	66.4	18.9
Río Guabal			
1 m	1.90	6.6	3.3
5 m	9.50	70.4	7.3
Quebrada Cascada			
1 m	1.9	5.2	2.7
5 m	9.6	60.7	6.2

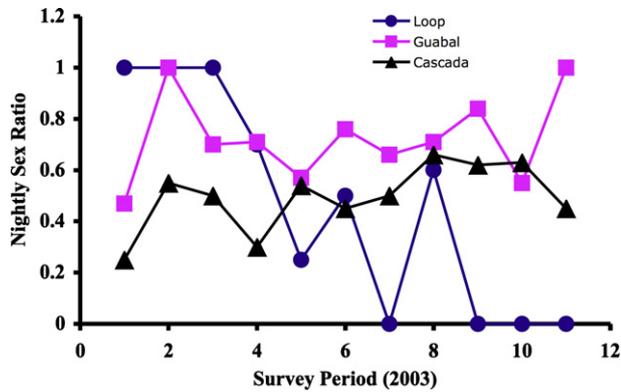


Fig. 3 – Nightly sex ratio (NSR) for each transect during the 2003 sampling season. NSR is the proportion of marked males to marked males and females. NSR varied per survey, but overall was 1:1 ($\chi^2 = 1.16$, $df = 2$, $P < 0.5$).

3.3. Movement

Most *C. punctariolus* individuals moved short distances, with 83% of 384 total movements less than 25 m (range = 0–504 m; median = 5 m; $\bar{x} = 27.1 \pm 77.8$ m). Nineteen frogs marked on one transect moved to another transect, moving on average 329.6 ± 139 m (range = 104–504 m). Quebrada Cascada gained nine frogs and lost two, Río Guabal gained four frogs and lost eleven, and Loop Stream gained five and lost five. Fig. 2 shows the stream connectivity and potential travel routes of the frogs that changed transects.

When we excluded the 19 inter-transect movements, the average frog movement dropped to 10.8 ± 18.1 m (range = 0–163 m, median = 5 m); 25% (92) of these movements were <1 m and 55% (203) were < 5 m. Of those <5 m long, 169 oc-

curred within the same field season, 33 occurred after 1 yr, and two occurred after 2 years at the same location. All movements <1 m were frogs moving within the same boulder clump.

Males and females did not differ in distance (ANOVA, $df = 1$, $P > 0.4$) or direction moved (ANOVA $df = 1 = 0.02$, $P > 0.3$), nor were there any differences in these factors among transects (ANOVA, $df = 2$, $P > 0.5$). Adults showed no directional bias in movement, and had a mean displacement of 0.1 ± 15.2 m (skewness -0.3 ; range = +32 – –40 m, median = 0).

3.4. Survival

For all analyses of data collected from 18 May to 31 July 2003 the deviance of the global model was within the lowest 50% of 1000 bootstrap iterations, indicating the global model fit the data (Burnham and Anderson, 2002). The model allowing apparent survival to vary by sex was the most parsimonious model only for the frogs captured on Río Guabal with males having slightly higher survival than females, while the most parsimonious model from frogs captured on both Quebrada Cascada and Loop Stream held survival constant between sexes (Table 3). Four models were within 4 AIC points of the most parsimonious model for Quebrada Cascada and Loop Stream, thus we used the model averaging component of program MARK to provide the average point estimates and unconditional standard errors and 95% confidence intervals of the parameters from the 4 models. For the Río Guabal transect the model that allowed survival to vary by time competed well (Table 3), but survival could only be estimated for 3 of the 10 time intervals indicating our data set was inadequate to support this model, so we excluded this model from the model averaging process. Estimates of 7 day survival were similar among streams during this period (Table 4).

Table 3 – Ranking of models used to estimate apparent survival of *Craugastor punctariolus* from 18 May–31 July 2003

Transect	Model	AICc	Δ AICc	AICc weight	K	Deviance
Cascada	ϕ (.) ρ (.)	409.9	0.0	0.48	2	265.4
	ϕ (.) ρ (s)	411.2	1.3	0.25	3	264.6
	ϕ (s) ρ (.)	412.0	2.1	0.17	3	265.4
	ϕ (s) ρ (s)	413.0	3.1	0.10	4	264.4
	ϕ (t) ρ (.)	417.8	7.9	<0.01	11	253.4
	ϕ (t) ρ (t)	423.4	13.5	<0.01	19	238.9
Loop	ϕ (.) ρ (.)	102.1	0.0	0.50	2	77.3
	ϕ (.) ρ (s)	103.6	1.5	0.23	3	76.4
	ϕ (s) ρ (.)	103.9	1.8	0.20	3	76.6
	ϕ (s) ρ (s)	106.1	4.0	0.07	4	76.3
	ϕ (t) ρ (.)	128.6	26.5	<0.01	11	74.1
	ϕ (t) ρ (t)	176.0	73.9	<0.01	19	63.3
Guabal	ϕ (s) ρ (.)	302.9	0.0	0.36	3	190.3
	ϕ (.) ρ (.)	303.1	0.2	0.33	2	192.7
	ϕ (t) ρ (.)	303.2	0.34	0.23	11	172.1
	ϕ (.) ρ (s)	304.2	1.3	0.19	3	191.6
	ϕ (s) ρ (s)	305.0	2.2	0.12	4	190.3
	ϕ (t) ρ (t)	318.1	15.2	<0.01	19	165.0

Survival (ϕ) and capture probability (ρ) was held constant in models indicated by (.), varied by sex in models indicated by (s), and varied by week as indicated by (t). For each transect models are listed from most to least parsimonious based on Akaike's Information Criteria (AICc) for small sample size and K indicates the number of parameters in the model.

Table 4 – Point estimates, standard errors, and 95% confidence intervals of 7 day apparent survival (ϕ) and detection probability (ρ) for three subpopulations of *C. punctariolus* for the period 18 May–31 July 2003

Transect/sex	ϕ (SE)	95% CI	ρ (SE)
Cascada/male	0.872 (0.035)	0.785–0.927	0.304 (0.046)
Cascada/female	0.869 (0.034)	0.787–0.922	0.327 (0.048)
Loop/male	0.884 (0.069)	0.673–0.966	0.226 (0.075)
Loop/female	0.904 (0.081)	0.601–0.983	0.254 (0.088)
Guabal/male	0.941 (0.037)	0.813–0.982	0.210 (0.037)
Guabal/female	0.898 (0.056)	0.727–0.967	0.198 (0.045)

Point estimates and variance for the Quebrada Cascada and Loop Stream were derived using the model averaging component of program MARK by calculating the weighted average of all models that were within 4 AIC points of the most parsimonious model from Table 1. For the Río Guabal transect the model that allowed survival to vary by time competed well (Table 1), but the small data set prevented survival from being estimated for all but 3 of the 10 time intervals indicating our data set was inadequate to support the model, thus, we excluded this model from the model averaging process.

For the survey period of 26 September 2003–18 October 2004, we captured and marked 44 frogs on Quebrada Cascada, 20 frogs on Loop Stream, and 27 frogs on Río Guabal. Survival and detection probability were similar among streams (Table 5). The 95% confidence intervals of 7 day survival point estimates for the period prior to a detectable decline in survival overlapped with those from the period of 18 May–31 July 2003, although point estimates that included the entire annual cycle are slightly higher (Tables 4 and 5). We estimated the mean 7 day survival of the three transects as 0.96 and annual survival as 0.12.

Table 5 – Point estimates, standard errors, and 95% confidence intervals of 7 day apparent survival (ϕ) and detection probability (ρ) for three subpopulations of *C. punctariolus* for the period 26 September 2003–18 October 2004

Transect	Model	ϕ (SE)	95% CI	ρ (SE)
Quebrada Cascada	Sept.–Aug.	0.990 (0.013)	0.960–1.000	0.105 (0.031)
Loop Stream	Sept.–Aug.	0.952 (0.027)	0.864–0.984	0.160 (0.076)
Río Guabal	Sept.–Jun.	0.935 (0.042)	0.789–0.982	0.126 (0.072)

Point estimates and variance were derived using the model averaging component of program MARK by calculating the weighted average of all models that were within 4 AIC points of the most parsimonious model from Table 3.

3.5. Abundance

We estimated the probability of observing an individual during at least one survey period (P) to be 98.4% for Quebrada Cascada, 92.1% for Río Guabal, and 95.1% for Loop Stream. Using these estimates of detection probability, we estimated 68 individuals were present on Quebrada Cascada, 61 individuals were present on Río Guabal, and 19 individuals were present on Loop Stream during the period of 18 May–31 July 2003.

For the survey period of September 2003–October 2004, we estimated the probability of observing an individual during any of the 14 individual surveys (P) to be 78.4% for Quebrada Cascada, 86.0% for Río Guabal, and 92.3% for Loop Stream. Using these detection probabilities, we estimated 56 individuals were present on Quebrada Cascada, 31 individuals were

Table 6 – Comparison of models in which survival was held constant among all surveys except for the last month, then all surveys except the last 2 months, then all surveys except for the last 3 months, continuing up to the last 5 months for three subpopulations of *C. punctariolus* between 26 Sept. 2003 and 18 Oct. 2004

Transect	Model	AICc	Δ AICc	AICc weight	K	Deviance
Cascada	ϕ (last 2 months) ρ (.)	168.3	0.0	0.69	2	100.2
	ϕ (last 4 months) ρ (.)	171.9	3.6	0.11	3	101.7
	ϕ (last 3 months) ρ (.)	172.0	3.7	0.11	3	101.7
	ϕ (last 5 months) ρ (.)	173.7	5.5	0.04	3	103.5
	ϕ (last month) ρ (.)	174.0	5.7	0.04	2	106.0
	ϕ (.) ρ (.)	178.3	10.1	<0.01	2	110.3
Loop	ϕ (last 2 months) ρ (.)	58.5	0.0	0.59	2	50.2
	ϕ (last month) ρ (.)	60.5	2.1	0.21	2	52.2
	ϕ (.) ρ (.)	62.2	3.8	0.09	2	53.9
	ϕ (last 5 months) ρ (.)	64.5	6.1	0.03	3	53.7
	ϕ (last 4 months) ρ (.)	64.7	6.2	0.03	3	53.8
	ϕ (last 3 months) ρ (.)	64.7	6.2	0.03	3	53.9
Guabal	ϕ (last 4 months) ρ (.)	48.0	0.0	0.54	2	30.3
	ϕ (last 3 months) ρ (.)	50.0	2.0	0.20	2	32.3
	ϕ (last 5 months) ρ (.)	51.7	3.7	0.09	3	31.5
	ϕ (last 2 months) ρ (.)	51.7	3.7	0.09	2	33.9
	ϕ (last month) ρ (.)	52.6	4.7	0.05	2	34.9
	ϕ (.) ρ (.)	53.2	5.2	0.04	2	35.5

Survival was held constant over the entire period (.), or allowed to vary during the last months as indicated in parentheses to determine when a measurable decline in survival occurred due to the infection of *Batrachochytrium dendrobatidis*. For each transect models are listed from the most to least parsimonious based on Akaike's Information Criteria (AICc) for small sample size and K indicates the number of parameters in the model.

present on Río Guabal, and 22 individuals were present on Loop Steam.

3.6. Detection of demographic changes following invasion by *Bd*

The deviance of the global model was in the lowest 50% of each of the bootstrapped models indicating the global model fit the data for each analysis. The most parsimonious model for Quebrada Cascada and Loop Stream indicated a detectable decrease in survival beginning in August 2004, while the most parsimonious model from Río Guabal indicated a detectable decrease in survival beginning in June 2004 (Table 6). Although we were able to determine the period during which survival changed using the model selection process (Table 6), lack of data prevented us from estimating apparent survival during the final sampling periods for which survival was determined to have decreased (Table 5). This was likely due to the high mortality that was first observed in October 2004 and which produced a 100% population decline in this species within 3 months (Lips et al., 2006).

4. Discussion

4.1. Age and sex specific habitat use

We found that all population subunits of *C. punctariolus* lived along stream courses; and in approximately 550 survey hours we did not record a single *C. punctariolus* on any terrestrial transect (K.R. Lips, unpublished data). We found that individuals aggregated on boulder piles and waterfalls (Witters, 2002) and that they remained at these sites predictably among years. At night, we found individuals of *C. punctariolus* on boulders, while during the day we encountered adults and subadults sleeping in the interstitial spaces of boulder piles. Within a boulder pile *C. punctariolus* partitioned vertical space by body size, suggesting inter-specific competition for the best perches (e.g. Inger and Greenberg, 1966; Miyamoto, 1982; Werner and Gilliam, 1984).

The linear nature of streams may promote local competition among individuals for patchily distributed resources such as boulders, and may also result in high site fidelity of adults. We found limited movement and high site fidelity with most individuals moving less than 5 m over two or more years. Phylogenetically related frogs, *Eleutherodactylus coqui* and *Eleutherodactylus johnstoneii* exhibited philopatry to diurnal retreat sites and nocturnal calling sites (Woolbright, 1985; Ovaska, 1992). Other tropical montane stream frogs exhibit site fidelity (e.g. Crump and Pounds, 1989; Lips, 1995; Robertson et al., 2008), perhaps because they require patchily distributed oviposition or retreat sites (Wells, 2007).

Patchy habitat and host aggregative behaviors (e.g. amplexus, breeding, territorial defense) can promote contact among individuals and can facilitate density-independent disease transmission (Begon et al., 2002; Ezenwa, 2004; Rowley and Alford, 2007b). Retreat sites likely provided cool and moist microhabitats, protection from scavengers and predators, and refuge from floods (e.g. Lemckert and Brassil,

2000). These cool, permanently wet microhabitats may also serve as a refuge for *Bd* (e.g. Lips et al., 2006; Rowley and Alford, 2007b), and could have promoted the rapid decline of this species. The endangered Australian stream frogs, *Mixophyes iterates* and *Litoria nannotis* are ecologically similar to *C. punctariolus* and have shown similar responses to environmental perturbations. *Mixophyes iterates* exhibited philopatry to daytime retreats, over-wintering sites, and oviposition sites (Lemckert and Brassil, 2000). *L. nannotis* exhibited philopatry to moist retreat sites in riparian boulder piles, and most captures occurred within the stream. Populations of *L. nannotis* are susceptible to *Bd* and have declined more than populations of sympatric species that spend less time in contact with water, less time in contact with other frogs, or less time in streams (Rowley and Alford, 2007b).

Although most *C. punctariolus* moved very little over the length of the study, a few made long distance movements. The smaller sympatric glassfrog, *Centrolene prosoblepon*, had a higher rate of immigration/emigration that produced genetic homogeneity in these same headwater streams (Robertson et al., 2008). As in *C. prosoblepon*, *C. punctariolus* has an extended (>6 years) adult lifespan and restricted movements, but moved among streams, suggesting that subpopulations living on our three transects likely comprise one population (e.g. McCullough, 1996). If true, population connectivity will be critical for dispersal, recolonization, and the persistence of headwater stream populations of *C. punctariolus* because of their exclusive use of a patchily distributed habitat within the stream system. We recommend forested buffer zones as an important management tool for conserving Neotropical stream frogs.

We found little evidence that survival or detection probability differed between males and females in our study. Point estimates of 7 day apparent survival for our study were similar among streams with 95% confidence intervals overlapping substantially, suggesting that each transect represented a subpopulation of a larger population and/or that each subpopulation responded to similar environmental conditions. This population structure is further supported by the analyses of individual movements (see above).

We estimated annual survival at 12%, which is within the range of estimates for tropical frogs representing a diverse range of life histories (e.g. Brown and Alcalá, 1970; Kluge, 1981; Galatti, 1992; Lips, 1995; Stewart, 1995; Robertson, 2002). However, none of those studies used mark-recapture analyses and thus they could not make strong inferences about demographic parameters (Wood et al., 1998; Biek et al., 2002). Our analyses were also limited because of uneven survey efforts but we detected differences in the population responses of *C. punctariolus* to invasion of *Bd* as compared to the temperate toad, *Anaxyrus (Bufo) boreas*. Pre-decline survival estimates of this toad were in the range of 0.5–0.8 and population estimates between 120 and 250 males on their study area (Muths et al., 2003). *Bd* invaded this population but toad survival and abundance declined slowly over 3 years, as compared to the 100% decline of three subpopulations of *C. punctariolus* within 4 months.

Of all the species at El Copé, *C. punctariolus* showed the fastest and most severe population response to the arrival

of, and infection by *Bd* (K. Lips, unpublished data). We believe that this was the result of a combination of factors described herein. First, all population subunits lived year-round on boulder piles in waterfall splash zones, a habitat well suited for survival of *Bd* (Piotrowski et al., 2004; Rowley and Alford, 2007b). Second, these same boulders tested positive for *Bd* during the epidemic (Lips et al., 2006), and frogs that shared the same boulder pile were likely to spread the disease to each other through direct or indirect contact. Third, because frogs were philopatric and all age and sex classes co-occurred in the same microhabitat, frequency-dependent *Bd* transmission likely occurred within these aggregations such that all stages were affected simultaneously. This demographically broad transmission reduced any chance of rescue from unaffected demographic groups (de Castro and Bolker, 2005; Rowley and Alford, 2007a).

The last living *C. punctariolus* was observed 29 October 2004, which coincided with the peak of the die-off and decline of the entire amphibian community (Lips et al., 2006). Surveys have continued through 2007 with no additional observations of *C. punctariolus*, indicating that this population has been extirpated. Only four dead *C. punctariolus* were found during the die-off despite intensive searches along transects where 148 animals were estimated to occur, likely explained by the fact that these animals expired while they occupied their diurnal retreat sites in boulder piles.

We found no evidence that habitat use, sex ratio, or age-size structure changed prior to declines in October 2004, although survival rates had declined by August 2004 for Loop Stream and Quebrada Cascada, and by June 2004 for Río Gual; these declines occurred over 3 month prior to detection of the first dead frog, and over 2 month prior to the initiation of the decline on 4 September 2004 (Lips et al., 2006). This suggests that either the fungus was present at low levels but undetected, that this species was infected earlier than other species, and/or that other factors were contributing to declines in survival.

Mark-recapture parameter estimates such as these may provide more power to detect declines and conduct sensitivity analysis (Funk et al., 2003), and may instigate conservation actions (e.g. Gagliardo et al., 2008). Changes in survival or detection probability can indicate perturbations in a population undetected by surveys. Further studies of declining populations should incorporate multiple methods of analyzing and detecting changes in populations to better understand transmission routes, disease dynamics, and mechanisms of population decline.

The natural spread and long-term persistence of *Bd* at a site renders *in situ* conservation measures such as habitat protection, surveys, and monitoring ineffective (Zippel et al., 2006). *Ex situ* conservation measures such as captive breeding and population supplementation (Mendelson et al., 2006) may be the only option to prevent the immediate extinction of this species and other *rugulosus* Group species. *C. punctariolus* is a species of highest conservation priority in Panama, and in 2006 animals were collected from the wild to establish captive breeding populations (Poole, 2007). These animals are alive and breeding in captivity in Panama and in the United States (J. R. Mendelson pers. comm.). Captive breeding is not a solution to *Bd*, but it al-

lows for future releases of animals should effective treatments be discovered.

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