



Batrachochytrium dendrobatidis and the Collapse of Anuran Species Richness and Abundance in the Upper Manu National Park, Southeastern Peru

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Abstract: *Amphibians are declining worldwide, but these declines have been particularly dramatic in tropical mountains, where high endemism and vulnerability to an introduced fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), is associated with amphibian extinctions. We surveyed frogs in the Peruvian Andes in montane forests along a steep elevational gradient (1200–3700 m). We used visual encounter surveys to sample stream-dwelling and arboreal species and leaf-litter plots to sample terrestrial-breeding species. We compared species richness and abundance among the wet seasons of 1999, 2008, and 2009. Despite similar sampling effort among years, the number of species (46 in 1999) declined by 47% between 1999 and 2008 and by 38% between 1999 and 2009. When we combined the number of species we found in 2008 and 2009, the decline from 1999 was 36%. Declines of stream-dwelling and arboreal species (a reduction in species richness of 55%) were much greater than declines of terrestrial-breeding species (reduction of 20% in 2008 and 24% in 2009). Similarly, abundances of stream-dwelling and arboreal frogs were lower in the combined 2008–2009 period than in 1999, whereas densities of frogs in leaf-litter plots did not differ among survey years. These declines may be associated with the infection of frogs with Bd. B. dendrobatidis prevalence correlated significantly with the proportion of species that were absent from the 2008 and 2009 surveys along the elevational gradient. Our results suggest Bd may have arrived at the site between 1999 and 2007, which is consistent with the hypothesis that this pathogen is spreading in epidemic waves along the Andean cordilleras. Our results also indicate a rapid decline of frog species richness and abundance in our study area, a national park that contains many endemic amphibian species and is high in amphibian species richness.*

Keywords: amphibians, elevational gradient, extinction, montane forest, Neotropics, threatened species, tropical Andes

Batrachochytrium dendrobatidis y el Colapso de la Riqueza de Especies y Abundancia de Anuros en el Parque Nacional del Manu, Sureste de Perú

Resumen: *Las poblaciones de anfibios están disminuyendo mundialmente, pero estas disminuciones han sido particularmente dramáticas en montañas tropicales, donde el endemismo y la vulnerabilidad a un patógeno fúngico introducido, *Batrachochytrium dendrobatidis* (Bd), están asociados con extinciones de anfibios. Muestreamos ranas en los Andes Peruanos en bosques montañosos a lo largo de un gradiente altitudinal pronunciado (1200–3700 m). Utilizamos técnicas de encuentros visuales para muestrear especies arbóreas*

y en quebradas y cuadrantes en la bojarasca para muestrear especies terrestres. Comparamos la riqueza de especies y la abundancia entre las estaciones lluviosas de 1999, 2008 y 2009. No obstante esfuerzos de muestreo similares, el número de especies (46 en 1999) disminuyó en 47% entre 1999 y 2008 y en 38% entre 1999 y 2009. Cuando combinamos el número de especies encontradas en 2008 y 2009, la disminución desde 1999 fue de 36%. Las disminuciones de especies arbóreas y que se reproducen en quebradas (una reducción de 55% en la riqueza de especies) fueron mucho mayores que las disminuciones de las especies terrestres (reducción de 20% en 2008 y 24% en 2009). De manera similar, las abundancias de ranas en quebradas y arbóreas fueron menores en el período combinado 2008–2009 que en 1999, mientras que las densidades de ranas en los cuadrantes de bojarasca no cambiaron en los años de muestreo. Estas disminuciones pueden estar asociadas con la infección de ranas con *Bd*. La prevalencia de *B. dendrobatidis* se correlacionó significativamente con la proporción de especies que estuvieron ausentes en los muestreos de 2008 y 2009 a lo largo del gradiente altitudinal. Nuestros resultados sugieren que *Bd* pudo haber llegado al sitio entre 1999 y 2007, lo cual es consistente con la hipótesis de que este patógeno se está dispersando en olas epidémicas a lo largo de las cordilleras Andinas. Nuestros resultados también indican una rápida disminución de la riqueza de especies y abundancia de ranas en nuestra área de estudio, un parque nacional que contiene muchas especies de anfibios endémicas y tiene una gran riqueza de especies de anfibios.

Palabras Clave: Andes tropicales, anfibios, especies amenazadas, gradiente altitudinal, extinción, bosque montano, Neotrópicos

Introduction

In addition to deforestation (Pineda & Halffter 2003; Galant et al. 2007) and climate change (Raxworthy et al. 2008), the pathogen *Batrachochytrium dendrobatidis* (*Bd*) is associated with amphibian declines throughout the world (Berger et al. 1998; Briggs et al. 2005; Lips et al. 2006). These declines have been particularly severe in tropical mountains, where species have intrinsically higher probabilities of extinction because their ranges are restricted geographically (McDonald & Brown 1992).

The hypothesis that *Bd* is an emergent disease is supported by the fungus' low genetic diversity (Morehouse et al. 2003), poor correlation between fungal genotypes and geography (Morgan et al. 2007), and epidemic level of infection and dramatic effect on naïve amphibian populations (Berger et al. 1998; Vredenburg et al. 2010). *B. dendrobatidis* may have spread along the Andean cordilleras in three epidemic waves from two sites where it was introduced in the late 1970s or early 1980s, one in southwestern Ecuador and the other in the Venezuelan Andes (Lips et al. 2008). One wave spread south and west of Venezuela, one spread north from Ecuador, and the third spread south from Ecuador. Lips et al. (2008) believe the systematic spread of *Bd* through areas with suitable climates, geography, and host populations is analogous to an epidemic wave, in which a virulent pathogen spreads through naïve host populations. The rate of movement of the wave toward the Peruvian Andes was between 37 and 202 km/year. If *Bd* is spreading in an epidemic wave and there are no other introduction points of *Bd* in the Andes, *Bd* should have already reached southern Peru or will reach it by 2025. Chytridiomycosis in Peru was first recorded in 1999 in a population of *Atelopus patzensis* (Venegas et al. 2008) approximately 600 km south of the presumed introduction point in Ecuador (Lips et al. 2008). Seimon et al. (2007) detected *Bd* in

two species of high-elevation frogs in southern Peru in 2002 and linked the appearance of this pathogen to declines in abundance. Therefore, we know that *Bd* has been affecting montane frogs in Peru since at least 1999. Moreover, Ron (2005) found the eastern slopes of the Peruvian Andes fell within the predicted distribution of the fundamental niche of *Bd*.

We sought to examine whether amphibian species richness and abundance changed from 1999 to 2009 in a large national park in the Tropical Andes in the context of previous declines of Neotropical amphibian faunas. In addition, we considered whether *Bd* is spreading in the central Andes in epidemic waves. We compared our results with those of other studies of frogs conducted in Neotropical mountains. In particular, we examined similarities in groups of species, land-cover associations, and reproductive modes among species that declined at other sites. Because previous work suggests that epidemic waves of chytridiomycosis resulted in major declines in frogs in northern South America, we assessed the potential influence of chytridiomycosis on amphibian declines in our study area. We hypothesized that the arrival of *Bd* in southern Peru and *Bd* infection of frogs is associated with declines in abundance and extirpations during our study period (1999–2009). Our approach allowed us to evaluate whether *B. dendrobatidis* may be driving declines of amphibian species richness and abundance in a protected area with minimal anthropogenic disturbance.

Methods

Study Sites

The study sites were on the eastern slopes of the Cordillera de Paucartambo, Cusco, in the drainage basin

of the Río Kosñipata, southern Peru. The Kosñipata Valley lies within Manu National Park and its buffer zone (a legally recognized area, where land use should be compatible with the objectives of the adjacent national protected area in Peru). The park covers 17,163 km² of Amazonian lowlands and Andean mountains between 300 and 4020 m in elevation and protects most of the Manu River watershed in the upper Madre de Dios basin. Manu National Park was established in 1973 and designated a UNESCO Reserve of the Biosphere in 1977 and a World Heritage Site in 1987. It is Peru's second largest national park and is part of an area with a high concentration of species richness and endemism (Tropical Andes hotspot; Myers et al. 2000). Our study sites were along the Paucartambo–Shintuya road, where we surveyed montane forests, montane scrub, and high-Andean grasslands along a steep elevational gradient from 1200 m (San Pedro, 13°03'16"S, 71°32'45"W) to 3700 m (Acjanaco, 13°11'56"S, 71°37'03"W).

We inferred maximum and minimum air temperatures along the elevational gradient by regressing the corresponding daily average temperatures between 2001 and 2006 from four weather stations operated by the Servicio Nacional de Meteorología e Hidrología. Regression coefficients were high ($R^2 = 0.99$, $p = 0.003$), and regression slopes were similar (maximum temperature: -0.59 °C/100 m; minimum temperature: -0.50 °C/100 m) to the value expected from adiabatical cooling (average defined by the International Civil Aviation Organization is 0.65 °C/100 m). We used data from these weather stations to compare temperature and precipitation patterns during January and February of 2008 and 2009. We calculated the differences between the amounts of precipitation, average minimum and maximum temperatures in 2008 and 2009 and the 2001–2009 averages for January–February (Supporting Information). These calculations showed that 2008 and 2009 did not have unusually high or low temperatures or amounts of precipitation. January and February were slightly wetter in 2008 and slightly drier in 2009 than the 2001–2009 average. Minimum and maximum temperatures in both seasons were slightly colder than the 2001–2009 average.

Sampling Techniques for Frogs

We sampled terrestrial frogs in 298 10 × 10 m leaf-litter plots (Scott 1976) during the day, and we sampled arboreal and riparian frogs in 69 visual-encounter surveys (visual surveys) (Crump & Scott 1994) at night. Results from leaf-litter plots provided data on the density (number of frogs/100 m²) of terrestrial breeding species. We established four leaf-litter plots every 100 m of elevation between 1200 and 3700 m (i.e., four plots in each elevational class). In 2008 only two plots were sampled in

the 1200-m elevational class. We searched for frogs by lifting logs, rocks, moss, and leaf litter within a plot. Sampling effort was 100 plots in 1999, 98 plots in 2008, and 100 plots in 2009 (374, 421, and 430 h of sampling, respectively). Most plots were on steep slopes with abundant understory vegetation, which made the installation of fences impractical. Therefore, we marked the boundaries of all plots with measuring tape, and we sampled plots by starting at the lower edge and moving upslope.

We designed visual surveys to sample as great an elevational range as possible and to capture most of the species of aquatic, riparian, and arboreal frogs. We report relative abundances as the number of individuals captured per hour of sampling. Steep topography impeded randomization of sampling location, and surveys were restricted to areas along the road or along trails that could be safely accessed at night. Because we used the same road in all survey years, we are confident that the data we collected can be compared without much bias due to location. We visually searched for frogs in all habitat types in the study area, between 18:00 and 24:00 and at elevations between 1200 and 2800 m. We did not use visual surveys above 2800 m because we determined previously that this technique does not work well above 2800 m.

We conducted almost twice the number of visual surveys in 1999 as we did in 2008 or 2009. Therefore, we pooled survey data from 2008 and 2009 and compared the combined data (hereafter 2008 and 2009 combined) with data from 1999 (Supporting Information). Combining visual surveys from 2008 and 2009 improved the probability of detecting different sets of species in our surveys relative to sampling within a single season. The overall sampling effort and the effort exerted in each elevational class we sampled were higher in the combined 2008 and 2009 period than in 1999. Thus, in the most recent surveys the probability of observing individuals was higher.

We conducted a mark-recapture study along a montane stream at 1980 m (13°06'12"S, 71°34'14"W) for eight nights in February 1999 and for eight and three nights, respectively, in 2008 and 2009. We conducted this survey to validate results from visual surveys that indicated decadal changes in the species richness and abundance of riparian frogs. We identified, measured, and marked (toe clipping) captured frogs and released them at their place of capture. We exclude unidentified specimens from the analyses. Voucher specimens of species that appeared to be new or that could not be identified were collected and were deposited at the Museo de Historia Natural, Universidad Nacional de San Marcos, American Museum of Natural History, Musée d'Histoire Naturelle de la Ville de Genève, Museum of Vertebrate Zoology, University of California at Berkeley, and Senckenberg Naturhistorische Sammlungen Dresden.

Species Richness Estimates

To determine whether species richness changed between the 1999 survey and the surveys in 2008 and 2009, we calculated sample-based rarefaction curves using the Mau Tao method in EstimateS 8.0 (Colwell 2009). The sample was the total number of individuals found across all elevations. To compare species richness, we truncated sample size to the smallest number of individuals shared by all surveys. We computed rarefaction curves for leaf-litter plots and visual surveys separately. We included data from all leaf-litter plots in our comparison of rarefaction curves for 1999 and 2009. When comparing 1999 with 2008, we reduced sample sizes for 1999 from four to two leaf-litter plots for the 1200-m elevational class.

Batrachochytrium dendrobatidis Infection

We swabbed the skin (Hyatt et al. 2007) of all frogs we sampled in the Kosñipata Valley in 2008 and 2009 to determine whether individuals were infected with Bd. We also captured and swabbed the skin of frogs from Abra Huallahualla (3100–4550 m; 13°34'32"S, 71°08'26"W) and Abra Malaga (3300–4050 m, 13°08'16"S, 72°18'05"W) in March 2008.

We used a real-time polymerase chain reaction (PCR) assay on material collected on swabs to detect Bd and quantify the level of infection (Boyle et al. 2004). This assay uses genetic markers specific to Bd and allowed us to quantify the genetic material in the sample. The assay compares the sample to a set of standards and calculates a genomic equivalent for each sample (zoospore equivalent or Z_{swab}). Dry swabs were stroked across the frog skin in a standardized way: five strokes on each side of the abdominal midline, five strokes on the inner thighs of each hind leg, and five strokes on the foot webbing of each hind leg (total of 30 strokes/frog). Tadpoles were swabbed with 10 strokes on the mouthparts. We followed DNA extraction and real-time PCR methods of Hyatt et al. (2007) and Boyle et al. (2004), except that we analyzed single-swab extracts once instead of three times (Kriger et al. 2006; Vredenburg et al. 2010). The real-time PCR technique for Bd uses DNA extracts from swabs that are diluted 80-fold during extraction and PCR. Thus, to estimate Z_{swab} , we multiplied the genomic equivalent values generated during the real-time PCR by 80.

To calculate Bd prevalence (proportion of swabbed frogs infected with Bd), we categorized swabs as Bd positive if $Z_{\text{swab}} > 0$ and Bd negative if $Z_{\text{swab}} = 0$. We estimated prevalence along the elevational gradient with a generalized linear model of logistic regression with binomial errors for prevalence data grouped in elevational classes of 100 m. We used the same logistic regression model to evaluate whether the proportion of missing species along the elevational gradient was related to Bd prevalence. We defined missing species as the number of species absent from each 100-m elevational class in

2008 and 2009 but present in 1999 (leaf litter and visual surveys combined).

Results

Species Richness

We recorded 48 species in nine families of frogs (all surveys combined; Supporting Information). We observed fewer species in 2008 (25 species, 47% fewer) and 2009 (29 species, 38% fewer) than in 1999 (46 species), despite similar sampling effort and period (January–March). When we combined data from 2008 and 2009, the number of observed species (30) was still 36% lower than in 1999. Among relatively species-rich families of frogs, declines in species richness were greatest in Bufonidae, Centrolenidae, and Hylidae (all aquatic-breeding). The number of species in terrestrial-breeding Strabomantidae was similar between 1999 and 2008 and 2009 combined (Supporting Information). We found two *Telmatobius* species (Ceratophryidae) and four species of poison-dart frogs (Aromobatidae, Dendrobatidae) in 1999 and none of these species in 2008 and 2009.

Leaf-litter plots had fewer species in 2008 and 2009 than in 1999 (Fig. 1a), on the basis of sample-based rarefaction curves. For example, for a sample size of 225 individuals, the estimated species richness was 22 species in 1999 (same number when only two plots were included in estimates for the 1200-m elevational class), 17 species in 2008 (24% fewer species), and 18 species in 2009 (20% fewer). The number of species detected in visual surveys was much lower in 2008 and 2009 combined than in 1999 (Fig. 1b). For a common sample size of 585 individuals, the estimated species richness was 36 species in 1999 and 16 species in 2008 and 2009 combined (55% fewer species). Separate rarefaction curves for 2008 and 2009 were almost identical to the rarefaction curve for 2008 and 2009 combined.

Traits of Missing Species

Declines in abundance of frogs with aquatic life stages were greater than declines of marsupial (family Hemiphractidae) and terrestrial-breeding (Strabomantidae) frogs (Fisher exact probability test, $p < 0.01$ for surveys in 2008, 2009, and 2008 and 2009 combined). Among species missing in 2008 and 2009 combined, 63% of the species lay eggs in streams and 87% have aquatic tadpoles, but in 1999 only 33% of species lay eggs in streams and 49% of species have aquatic tadpoles (Supporting Information). When we examined missing species on the basis of reproductive mode (following Wells 2007), 37% of missing species have mode 2 reproduction (eggs in flowing water, tadpoles feed in streams), 25% have mode 20 (terrestrial nests, tadpoles carried to water), 13% have mode 25 (eggs on leaves over water,

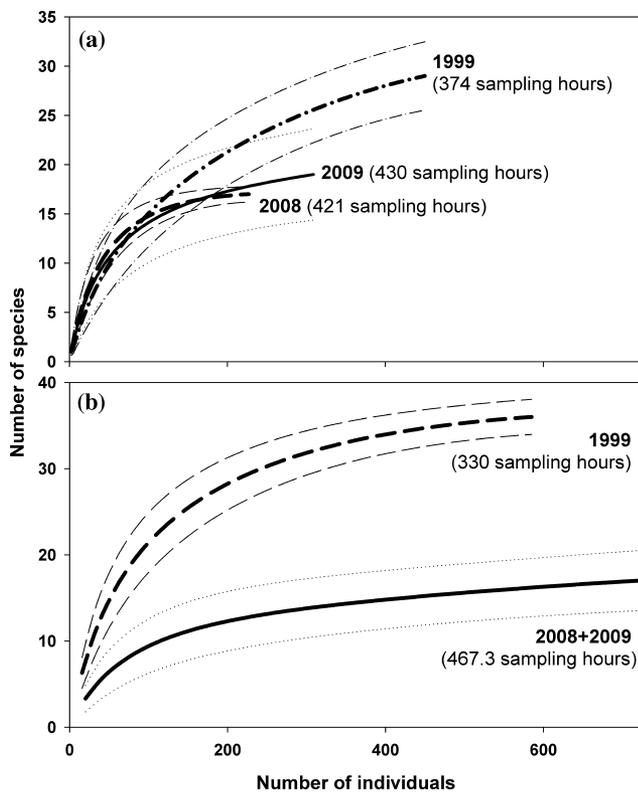


Figure 1. Sample-based rarefaction curves for frogs surveyed in (a) 10×10 m leaf-litter plots in 1999 and 2009 and (b) nocturnal visual encounter surveys in 1999 and 2008 and 2009 combined (2008 + 2009) (dotted and dashed lines, 95% CI).

tadpoles feed in streams), and 6% each have modes 1 (eggs in still water, tadpoles in pond), 23 (terrestrial nest, direct development), 30 (foam nest in burrow, tadpoles feed in pond after flooding), and 37 (marsupial frogs, direct development). Among species that were still present but had become rare (fewer than five individuals), two stream-dwelling frogs that were common (over 25 individuals) in 1999, *Rhinella leptoscelis* and *Hyloscirtus armatus*, were only observed in either 2008 or 2009. Three direct-developing species were missing in 2008 and 2009 combined, two of which were rare in 1999.

Frog Abundances

Densities of terrestrial frogs did not vary over time (Fig. 2a; $F_{2,15} = 1.45$, $p = 0.26$). By contrast, the relative abundance of frogs detected in visual surveys decreased between 1999 and 2008 and 2009 combined (Fig. 2b; Wilcoxon rank sum test, $W = 1164.5$, $p < 0.01$) for each 400-m elevational class. The sample distributions of the two survey periods differed as well (Kolmogorov-Smirnov test, $D = 0.64$, $p < 0.01$) because relative abundances of frogs in visual surveys in 1999 were skewed to the left and had a much larger range than in 2008 and 2009 combined. Relative abundances also differed between sur-

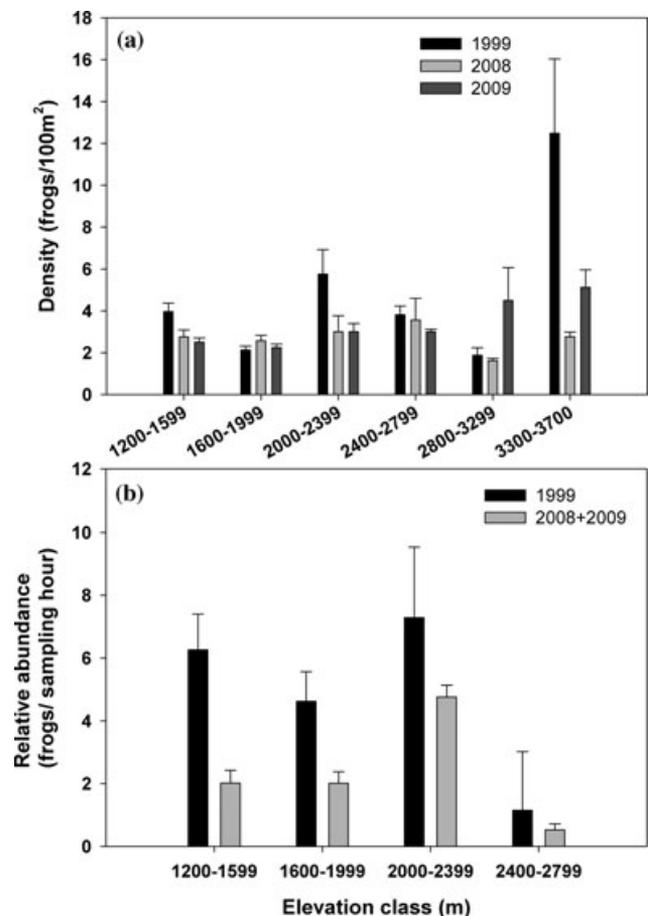


Figure 2. Mean relative abundance of frogs in (a) 10×10 m leaf-litter plots in 1999, 2008, and 2009 and in (b) nocturnal visual encounter surveys in 1999 and 2008 and 2009 combined (2008 + 2009) (lines above bars, standard errors of the mean).

vey periods when abundance was analyzed separately for each elevational class (1200–1599 m, Wilcoxon rank sum test, $W = 191$, $p < 0.01$; 1600–1999 m, $W = 49.5$, $p = 0.04$; 2000–2399 m, $W = 33$, $p = 0.01$; 2400–2799 m, $W = 40.0$, $p = 0.02$).

Capture-Mark-Recapture Study

In the mark-recapture study (stream at 1980 m) in 1999, we marked 45 individuals of eight species and four families of frogs in eight visits (Supporting Information). We recaptured eight individuals once, two individuals twice, and five individuals more than twice. The overall recapture rate was 38%; individuals of three *Pristimantis* species had a >45% recapture rate. In 2008 and 2009 we found no frogs at the mark-recapture site in 11 visits.

Elevational Distribution of Bd

We analyzed skin-swab samples from 1344 frogs captured at the Kosñipata Valley at elevations between 1200 and

3700 m. The overall disease prevalence was 10.1%. Between 1250 and 3700 m (Table 1), 16 species were infected. Prevalence decreased as elevation increased in 2008 (logistic regression with binomial errors, $p < 0.001$) but not in 2009, when Bd was detected in frogs at high elevations (Fig. 3). Prevalence was higher in stream-dwelling frogs (four species, 33.7%) than in direct-developing (nine species, 8.1%) and marsupial frogs (three species, 12.1%; $G = 41.4$, $df = 2$, $p < 0.01$). Juveniles of the stream-dwelling *H. balzani* had the highest prevalence and infection intensity (Z_{swab}). The proportion of missing species increased as Bd prevalence increased along the elevational gradient (Fig. 4) in 2008 ($p < 0.001$), 2009 ($p < 0.001$), and 2008 and 2009 combined ($p < 0.001$).

We detected Bd in other montane forests and high-Andean grassland sites around Cusco (Table 1). Most infected frogs were individuals of the aquatic-breeding species *Pleurodema marmoratum* and *Telmatobius marmoratus*.

Discussion

Our results show there has been a dramatic decline in species richness of amphibians in our study area over the past 10 years. Stream-breeding species were disproportionately represented among the missing taxa in 2008 and 2009 combined, which is consistent with observations in other Neotropical (Lips 1998) and Australian (Laurance et al. 1996; Williams & Hero 1998) sites where the species richness and abundance of frogs have declined or frogs have been extirpated. Declines in these other areas are unequivocally linked to the introduction of Bd to naïve amphibian populations (Berger et al. 1998; Lips et al. 2006). The decline in the relative abundances of stream-breeding frogs we observed mirrored the decline in species richness. This result suggests that decreases in abundance, either gradual or sudden, lead to decreases in anuran species richness, which is similar to observations at other Neotropical sites (Lips et al. 2003).

Our findings show that amphibians are declining, as elsewhere, in Manu National Park and areas surrounding the park. Bustamante et al. (2005) found that species richness of amphibians in the Ecuadorean Andes has been declining over 20–30 years. Consistent with our results, they documented declines in species richness among stream-dwelling species and species with aquatic larval stages, but not in direct-developing species. Bustamante et al. (2005) surveyed seven sites. Each site was surveyed over a shorter time, had smaller elevational range and area, and was exposed to heavier human disturbance than our sites.

In Manu National Park we found Bd in both stream- and terrestrial-breeding frogs. Disease prevalence was greatest among recent metamorphs of the stream-breeding *H.*

balzani, the only treefrog species that did not decline in abundance between 1999 and 2009. One individual of the most common centrolenid frog was infected with Bd. Two species in the family Centrolenidae have not been seen since 1999 despite intensive searches in 2008 and 2009 (Supporting Information). Prevalence of Bd was greater than 20% for several species of terrestrial-breeding frogs that did not decline in abundance during our study. Our findings suggest that these species might have low levels of mortality following Bd infection and that they might act as reservoirs for Bd. Observations from 1999 (Supporting Information) indicate that species of *Pristimantis* inhabit riparian areas in addition to leaf litter on the forest floor. *Pristimantis* species could become infected when using riparian areas, carry Bd from riparian to terrestrial areas, and possibly reintroduce Bd into riparian areas. Alternatively, Bd may persist in moist leaf litter (Puschendorf et al. 2006).

Our results show that the geographic and elevational distribution of Bd extends to southeastern Peru, and this extent is consistent with Lips et al. (2008) hypothesis that Bd is spreading in a wave southward from the hypothetical introduction site of Bd in southwestern Ecuador. Given the paucity of data from intermediate locations along the Peruvian Andes (von May et al. 2008), however, it is difficult to exclude alternative hypotheses. One alternative is that there were additional introduction sites. Cusco receives more visitors than any other tourist destination in Peru, and frogs originating from many streams, ponds, and lakes of the Bolivian-Peruvian Altiplano are sold live in the city for human consumption (Angulo 2008). Catenazzi et al. (2010) found high Bd prevalence in *Telmatobius* frogs sold at the main market in Cusco and hypothesize that the frog trade could facilitate the spread of Bd over large areas in southern Peru.

The apparent disappearance of the two species of *Atelopus* at our study site is not surprising because many species in this genus have declined or disappeared throughout the Andes (Ron et al. 2003; La Marca et al. 2005), and local declines of these species have been linked to chytridiomycosis (Venegas et al. 2008). *A. tricolor*, which we did not observe, was recorded in our survey area in 1991. The disappearance of this species before 1999 raises the possibility that Bd was present in the region before 1999. Another genus known to be especially prone to decreases in abundance (Merino-Viteri et al. 2005) and vulnerable to Bd (Barrionuevo & Mangione 2006; Seimon et al. 2007) is *Telmatobius*. We observed the two *Telmatobius* species in 1999, but not in 2008 or 2009. One male of *Telmatobius* sp. (an undescribed species) was last seen in July 2007 (Supporting Information). This species was collected in 1972 (voucher specimens at Kansas University) at a lower elevation (1700 m) than during our survey in 1999 (2200 m) and our observation in July 2007 (2800 m). As for *A. tricolor*, the extirpation of the *Telmatobius* species from lower

Table 1. Frog species infected with *Batrachochytrium dendrobatidis* during the rainy seasons (January–March) of 2008 ($n = 639$) and 2009 ($n = 695$ frogs) at Kosñipata and other montane locations around Cusco, Peru.^a

Location and species	Infected 2008			Infected 2009		
	n	%	mean Z_{swab} (SE), range	n	%	mean Z_{swab} (SE), range
Kosñipata Valley (1250–3700 m)						
<i>Bryophryne cobpites</i>						
juveniles	3	-	-	4	-	-
adults	30	-	-	16	25.0	33002.4 (32993.1), 0.6–131981.6
<i>Gastrotheca antoniiocoai</i>						
juveniles	-	-	-	3	-	-
adults	-	-	-	3	33.3	0.7
<i>Gastrotheca excubitor</i>	4	-	-	11	18.2	3788.4, 28.7–7548.0
<i>Gastrotheca ocoai</i>						
juveniles	1	-	-	3	25.0	29051.2
adults	2	-	-	4	-	-
<i>Hyalinobatrachium bergeri</i>	8	12.5	63.1	17	-	-
<i>Hypsiboas balzani</i>						
tadpoles	4	-	-	4	100.0	42954.2 (28718.7), 39.4–125803.2
juveniles	7	85.7	173.6 (357.7) 0.0–897.0	12	83.3	20174.4 (11860.9), 14.6–119700.0
adults	7	57.1	0.6 (1.2), 0.0–2.4	13	53.8	222.94 (142.4), 2.5–1039.2
<i>Hyloscirtus armatus</i>	-	-	-	1	100.0	17.0,
<i>Noblella</i> sp.	7	28.6	0.3 (0.2), 0.1–0.4	14	-	-
<i>Pristimantis danae</i>						
juveniles	56	21.4	0.2 (0.3), 0.0–1.2	53	5.7	1957.7 (1186.3), 0.3–20696.0
adults	116	20.7	42.8 (107.8), 0.1–426.0	91	11.0	6899.0 (6898.5), 0.6–9692.0
<i>Pristimantis pharangobates</i>						
juveniles	8	-	-	38	2.6	15300.8
adults ^b	87	4.4	66.8 (127), 0.1–257.3	46	2.2	0.1
<i>Pristimantis platydactylus</i>						
juveniles	2	-	-	6	16.7	5091.2
adults	3	-	-	7	14.3	3556.0
<i>Pristimantis salapatium</i>						
juveniles	10	10.0	0.3	5	-	-
adults	6	-	-	10	10.0	26.7
<i>Pristimantis toftae</i>						
juveniles	12	-	-	12	8.3	512.8
adults	47	27.7	16.5 (41.9), 0.0–139.5	43	16.3	2983.4 (2827.8), 0.2–19931.2
<i>Pristimantis</i> sp. ^c	3	33.3	0.4	1	-	-
<i>Psychrobrynella usurpator</i>						
juveniles	6	-	-	14	-	-
adults	42	-	-	96	9.4	2172.3 (2166.0), 0.3–19500.0
<i>Psychrobrynella</i> sp. ^d						
juveniles	13	-	-	11	-	-
adults	37	-	-	34	2.9	6223.2
<i>Rhinella inca</i>	2	-	-	2	100.0	67.6, 16.1–119.2
Marcapata Valley & Abra						
Abra Huallahualla (3100–4550 m), 2008 only						
<i>Bryophryne zonalis</i>						
juveniles	4	25.0	974.60			
adults	8	-	-			
<i>Pleurodema marmoratum</i>						
juveniles	11	9.1	39.48			
adults	16	-	-			
<i>Telmatobius marmoratus</i>						
tadpoles	22	63.6	4.7 (9.1), 0.1–33.9			
juveniles ^e	2	100.0	1.0 (1.4), 0.0–2.0			
adults	2	-	-			
Abra Malaga (3300–4050 m), 2008 only						
<i>Pleurodema marmoratum</i>	8	12.5	0.44			

^aSpecimens are adults unless otherwise indicated. Z_{swab} is an estimate of the genomic equivalent of *Bd* zoospores for each sample.

^bOne male found dead, $Z_{swab} = 257.3$.

^cJuvenile frogs, most likely *P. toftae*.

^dUndescribed species, referred to as *Psychrobrynella* sp. "P" in Supporting Information.

^eOne juvenile found dead, $Z_{swab} = 2.0$.

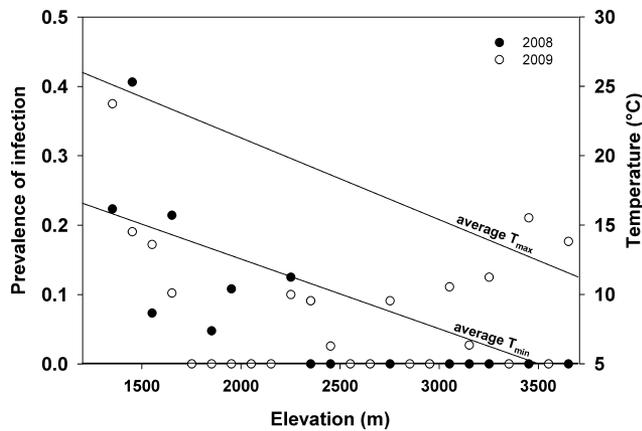


Figure 3. Prevalence of infection (proportion of sampled frogs infected) with *Batrachochytrium dendrobatidis* in frogs in 2008 (solid circles) and 2009 (open circles) and average minimum and maximum temperatures within the elevational gradient (1200–3700 m) sampled at the Kosñipata Valley.

elevations could indicate arrival of Bd before 1999 or the effects of other stressors on these populations.

Climate change has been proposed as a driver of amphibian declines (Pounds et al. 1999; Carey & Alexander 2003). Recent hypotheses suggest a synergistic effect of Bd outbreaks and climate change (Pounds et al. 2006; Bosch et al. 2007; Rohr & Raffel 2010), in which either the pathogen was endemic but not infecting frogs before climate changed or areas currently occupied by frogs

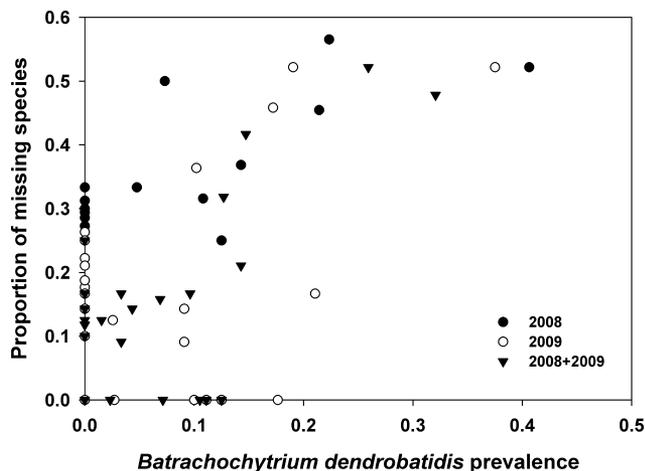


Figure 4. Proportion of missing frog species (species found in 1999 but absent from the 2008 or 2009 surveys) as a function of *Batrachochytrium dendrobatidis* prevalence for each 100-m elevational class within the elevational gradient sampled in the Kosñipata Valley (1200–3700 m) during the rainy seasons (January and February) of 2008, 2009, and 2008 and 2009 combined ($n = 1334$ frogs tested for Bd).

were unfavorable for Bd before climate changed (Seimon et al. 2007). Even as a synergistic driver, however, the influence of climate change on declining amphibian populations remains controversial (Rachowicz et al. 2005; Lips et al. 2008; Rohr et al. 2008). *B. dendrobatidis* is already found or has the potential to spread under a wide range of environmental temperature and moisture conditions (Walker et al. 2010).

In southern Peru Bd infects frogs in four different inter-Andean and Amazonian river drainages (Sibinacocha, Umasbamba, Marcapata, and Kosñipata) at elevations between 1250 and 5440 m and in ecosystems ranging from montane forests to high-elevation grasslands (Seimon et al. 2007). Precipitation at these locations varies widely because the montane forest at Kosñipata and other sites on the eastern slopes of the Andes receive far more precipitation than high-elevation Andean sites in the Peruvian-Bolivian Altiplano. Given that Bd occurs in a wide range of land-cover types and microclimates, it is unclear how climate change would increase the rate of spread or the virulence of such an opportunistic pathogen in frog populations. For example, Seimon et al. (2007) found that frogs colonized deglaciated areas and thus extended their elevational distribution and probably increased their abundance. A recent decline in abundance is linked to the arrival of Bd at this study site (Seimon et al. 2007) and not to any climatic anomaly associated with climate change.

B. dendrobatidis is implicated in the dramatic decrease of anuran species richness and abundance in our study area between 1999 and 2009. This pattern is consistent with a high number of extirpations that rapidly followed the arrival of Bd in other montane Neotropical sites (Lips et al. 2006). The percentage of species absent from recent surveys increased as the prevalence of Bd increased along the elevational gradient. Furthermore, the prevalence of Bd infection and infection intensity (Z_{swab}) in surviving stream-breeding frogs were high. The declines in species richness and abundance of frogs we observed occurred in a well-protected national park and in areas in which human land use has not changed substantially over the past decade. We believe there are grounds for serious concern and that an emphasis on understanding the dynamics of disease-driven extinction processes should be rigorously pursued so that the spread of this novel pathogen can be contained.

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Supporting Information

Weather data (Appendix S1), sampling effort for visual surveys (Appendix S2), species and number of frogs observed (Appendix S3), and composition of an anuran montane stream community in 1999 (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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