

Evaluating multiple causes of amphibian declines of Ecuador using geographical quantitative analyses

Pablo A. Menéndez-Guerrero and Catherine H. Graham

P. A. Menéndez-Guerrero (pamenendez@puce.edu.ec), Museo de Zoología QCAZ, Escuela de Ciencias Biológicas, Pontificia Univ. Católica del Ecuador, Apartado 17-01-2184 Quito, Ecuador. – C. H. Graham, Dept of Ecology and Evolution, Stony Brook Univ., 650 Life Sciences Building, Stony Brook, NY 11784-5245, USA.

Amphibians are declining at alarming rates worldwide; however, the causes of these declines remain somewhat elusive. Here we evaluated three major threats implicated in declines of populations and disappearance of Ecuadorian amphibians: chytridiomycosis, climate change, and habitat loss. We assessed spatial patterns of these key threats to Ecuadorian amphibians using a multi-species database of endemic frogs along with information on the pathogen's distribution and environmental requirements, species sensitivity to climate change (indirectly based on species geographical distribution and ecological properties) and habitat loss. Our results show that amphibians display a non-random pattern of extinction risk, both geographically and taxonomically. Further, climate change, chytridiomycosis, and their synergistic effects, are likely currently exerting the greatest impact on amphibians in Ecuador, while habitat loss does not seem to be causing precipitous declines. The most threatened species under the IUCN extinction risk categories are exactly those that appear to be the most affected by these threats. By examining multiple potential causes of amphibian threat level in a spatially explicit framework our study provides new insights about what combination of factors are most important in amphibian declines in a tropical diversity hotspot. Further, our approach and conclusions are useful for studying declines in other regions of the world.

Amphibian populations across the globe are declining at an unprecedented rate (Wake 1991, Stuart et al. 2004, Pounds et al. 2006, Sodhi et al. 2008, Collins 2010), with Neotropical species among the most affected (Stuart et al. 2004). The causes of these declines are still matter of research and debate, and might act alone, or in concert to create a complex web of stresses (Kiesecker et al. 2001, Blaustein and Kiesecker 2002, Whitfield et al. 2007, Bielby et al. 2008, Rohr et al. 2008, Collins 2010). Declines in Neotropical regions have most often been associated with three major potential causes: climate change, an emerging disease called chytridiomycosis and habitat loss (Lips et al. 2005, Hof et al. 2011). However, to date, few studies have simultaneously evaluated the relative impact of these three threats on amphibian populations. Additionally, previous Neotropical studies have generally been limited in scope, either because they have evaluated a small number of species (Lampo et al. 2006, Lips et al. 2006, Pounds et al. 2006, Bustamante et al. 2010) or focused on a restricted, and often environmentally homogeneous, geographic region (Puschendorf et al. 2006, Kolby et al. 2010, Catenazzi et al. 2011). To thoroughly examine the major threats to amphibians requires evaluation of multiple species with distinct levels of extinction risk across a variety of habitats. Here we evaluate the main potential causes of amphibian declines in

the environmentally heterogeneous country of Ecuador, where the amphibian fauna is the third most diverse in the world (Ron et al. 2011a). We use a comprehensive multi-species database of Ecuadorian amphibians to examine how the predicted spatial distribution patterns of chytridiomycosis, habitat destruction, and climate characteristics relate to the amphibian International Union for the Conservation of Nature (IUCN) threat categories (IUCN 2010).

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, has been implicated in the declines, and sometimes mass die-offs, of amphibian populations in Neotropical regions and around the world (Daszak et al. 2003, Lips et al. 2006, Skerratt et al. 2007, Fisher et al. 2009, James et al. 2009). However, several other factors such as climate change (Donnelly and Crump 1998, Pounds et al. 1999, 2006, Rödder et al. 2010) and habitat destruction (Cisneros-Heredia et al. 2010) may also be directly and indirectly responsible for declines. For instance, Ron et al. (2003) suggested that unusual weather conditions may have played an important role in the decline of jambato toad, *Atelopus ignescens*, an endemic species to the Ecuadorian Andes. Cisneros-Heredia et al. (2010) attributed the critically endangered status of the Ecuadorian glassfrog *Cochranella mache* to extensive deforestation in western Ecuador.

Further, chytridiomycosis, climate change and habitat fragmentation may act in concert to decimate amphibian populations (Kiesecker et al. 2001, Pounds et al. 2006, Bielby et al. 2008, Rödder et al. 2010). For instance, if susceptibility to disease is influenced by on-going changes in temperature and precipitation, as has been suggested by several authors (Bosch et al. 2007, Bustamante et al. 2010, Kielgast et al. 2010, Longo et al. 2010, Rohr and Raffel 2010), then it is critical to evaluate the relationship between climate conditions, prevalence of disease and observed declines. Likewise, habitat degradation may stress amphibian species making them more susceptible to disease (but see Becker and Zamudio 2011). Nonetheless, the lack of studies that simultaneously evaluate the relative importance of these different threats on amphibian populations is marked, especially in Neotropical regions.

Ecuador provides an ideal setting to study amphibian declines for several reasons. First, Ecuador is environmentally heterogeneous; elevation ranges from sea level to over 6000 m a.s.l. resulting in strong temperature and precipitation gradients. This heterogeneity provides the opportunity to evaluate amphibians under a range of environmental conditions. Second, Ecuador is among the most biodiverse countries in the world with 513 known amphibian species (Ron et al. 2011a). Approximately 31% of the Ecuadorian amphibian species have been red-listed (updated from Ron et al. 2011b), and are considered threatened with local extinction. Many of these species (42%) are only known from Ecuador. Finally, there has been extensive research conducted on amphibians in Ecuador making it possible to analyze different factors that might be influencing population declines. Population declines in amphibians in Ecuador have been reported since the early 1990s (Vial and Saylor 1993, Coloma 1995, 1996, 2002, Lötters 1996, Stebbins and Cohen 1997, Coloma et al. 2000, 2007); however, the main identified causes of these declines are a matter of debate. Here we used spatial explicit modeling to evaluate key threats – disease, climate change, habitat destruction – which endanger Ecuadorian amphibian species. Specifically, we conducted a series of multivariate statistical analyses and environmental niche models to assess the relationship between amphibian extinction risk (based on the IUCN categories), distribution of the chytridiomycosis and environmental variables. We also evaluated if habitat loss was associated with variation in species extinction risk. We predicted that the overlap in environmental conditions between frog distributions and chytridiomycosis occurrence will be greater for the most endangered frog species than for species not currently threatened. Further, we predicted that species with small range size and small and marginal niches will tend to be red-listed since they may have small population sizes and were likely more vulnerable to recent past climate change in Ecuador. Finally, species that have lost the largest area of suitable habitat should be most threatened.

Material and methods

Data

Occurrences of frogs infected by *B. dendrobatidis* were obtained from literature (Ron et al. 2011b). In total we had

10 unique occurrence locations of chytridiomycosis (Supplementary material Appendix 1, Table A1-1), one of which is in the lowlands (i.e. <1000 m a.s.l., McCracken et al. 2009). We used information of lowland occurrences of fungus only from endangered amphibians. We made this decision because the fungus has been associated with host mortality in highlands where it seems to become more pathogenic because of lower temperatures (Daszak et al. 2003), and, in Ecuador, most declines have occurred in the highlands (Ron et al. 2011b). Given that *B. dendrobatidis* has a broad environmental niche, the inclusion of lowland records from non-endangered species will not help us understand the climatic conditions of the disease in Ecuador.

Distributional data for 109 endemic frog species (n = 1000 unique occurrence records) were taken from the collection of Museo de Zoología, Pontificia Univ. Católica del Ecuador (QCAZ) and published records (Supplementary material Appendix 1, Table A1-2). These species represent about 51% of the total endemic species currently described for Ecuador (Ron et al. 2011a) and belong to seven genera: *Atelopus* (15 species), *Pristimantis* (65, formerly *Eleutherodactylus*), *Telmatobius* (3), *Allobates* (3), *Colostethus* (1), *Epipedobates* (2) and *Hyloxalus* (20, the species from the final 4 genera were formerly *Colostethus*, thus hereafter called as ‘former *Colostethus*’). While data were not available for all endemic species, our database included species from all Ecuadorian bioregions and the three genera with the highest number of endemic species (i.e. *Pristimantis*, *Hyloxalus* and *Atelopus*) are well represented; therefore they should provide a reasonable representation of endemism and extinction risk of Ecuadorian amphibians. Species names and taxonomic arrangements followed Frost (2011). Species were assigned IUCN Red List categories of extinction risk: critically endangered (CR); endangered (EN); vulnerable (VU); near threatened (NT); least concern (LC) and data deficient (DD), following an updated version of categories for all Ecuadorian amphibian species by Ron et al. (2011b). Occurrence data were georeferenced using digital maps and a suite of electronic resources: Alexandria Digital Library (<<http://middleware.alexandria.ucsb.edu/client/gaz/adl/index.jsp>>), Fallingrain (<www.fallingrain.com/world>) and Google Earth (<<http://earth.google.com/>>). We also consulted the original collectors to verify locations when possible. All occurrences were mapped and checked for accuracy by PAM using range information from the Global Amphibian Assessment Project (IUCN et al. 2008) and expert opinion (L. A. Coloma checked *Atelopus*, Dendrobatidae, and *Telmatobius* and S. R. Ron checked *Pristimantis*).

Environmental data included a series of interpolated climatic variables, a digital elevation layer and a digital vegetation map. The 19 climatic variables were obtained from WorldClim database (Hijmans et al. 2005, <www.worldclim.org>). These GIS coverages are generated through interpolation of average monthly temperature and precipitation values and represent biologically meaningful variables for characterizing species ranges (Nix 1986). The temporal resolution of climatic variables is 50 yr and the spatial resolution is 30 arc-second (i.e. ~1 km² resolution). Given that these climate data are somewhat outdated and

potentially of variable quality in montane areas, we also evaluated the robustness of our results using a new Ecuadorian bioclimatic model created by Ministerio del Ambiente del Ecuador (MAE climate model 2011; Supplementary material Appendix 2). The elevation layer was obtained from the Shuttle Radar Topography Mission (SRTM, Farr et al. 2007), and modified from the native 90 m resolution to a 1 km² spatial resolution in order to maintain consistency with other environmental layers. The digital vegetation map of Ecuador was a simplification of Sierra's (1999) Landsat based map (1:500000) done by Ron et al. (2011b). It delineates 10 major biogeographic regions in continental Ecuador. We also used Sierra (1999) to generate a binary map of degraded (disrupted by agricultural and other human activities) and non-degraded areas. While we realize that Sierra's landcover map is based on Landsat imagery from a single date, it was the only map available at the time of these analyses and should provide a rough estimate of how habitat loss might influence conservation status of Ecuadorian amphibians. Further, our species occurrence records date from 1980 to 2002 which coincides with some of the largest deforestation events, especially in the Andes (FAO 2007).

Environmental niche modeling

We used the maximum entropy method (MaxEnt ver. 3.3.1, Phillips et al. 2006) to generate predictive models of species distribution. This method is appropriate for presence-only occurrence data and performs well compared to other modeling techniques (Elith et al. 2006, Hernandez et al. 2006). The approach consists of estimating the most uniform distribution (maximum entropy) across the study area subject to the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its empirical average (Phillips et al. 2006). We used MaxEnt with the default settings including 500 iterations, a threshold value of 0.00001, regularization multiplier of 1, employment of the auto features, 10000 maximum number of background points and a cumulative probability output. Of the 109 species in our database, 66 had equal or greater than five unique occurrences. While acknowledge that five occurrences is a low number for modeling, all of our models were checked by experts (L. A. Coloma and S. R. Ron) and those that did not conform to known ranges of the species were not used (i.e. four species). Finally, we compared the global predictive model of the potential distribution of *Batrachochytrium dendrobatidis* from Ron (2005) with our model to evaluate the completeness of our niche estimate and a possible underestimation of chytrid potential distribution due to the small sample size (i.e. 10 unique occurrence locations; Supplementary material Appendix 3).

Given that the 19 Worldclim variables are highly correlated, we reduced the number of variables by constructing a global correlation tree based on all pixels within Ecuador using UPMGA in BioMapper 4.0 (Hirzel et al. 2007). We selected those variables that were not highly correlated ($r < 0.85$) and have previously been identified as important for modeling species ranges (L. A. Coloma and S. R. Ron

pers. comm., Menéndez-Guerrero unpubl.). We choose eight climatic variables: annual mean temperature, mean monthly temperature range, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, precipitation of warmest quarter, and precipitation of coldest quarter. In MaxEnt if variables are close to a uniform distribution they are not weighted heavily in the model or are not used at all. As a result most models used only a subset (4–5) of variables. Given that we are modeling in the same geographic region (sampling the same background for all models), not extrapolating across space or time, and not drawing inference based on what variables are used in the model, we let MaxEnt find the best possible model using the eight variables (Elith et al. 2011).

For models built with equal or greater than 25 points (nine species) we split the original occurrence data set into two sets: a training set with 75% of the occurrence sites, which were randomly selected, and a testing set with the remaining 25%. We evaluated these models using the binomial test and the area under the receiver operating characteristic curve (AUC, DeLong et al. 1988, Elith et al. 2006). We acknowledge recent criticisms of this method by Lobo et al. (2008) and Peterson et al. (2008) that the value of AUC is influenced by the prevalence of the species modelled relative to the total extent of the study area (all current discrimination and calibration measures will be subject to this limitation). To partly address this issue we use a limited background, Ecuador, which has an area of 256370 km² and includes all of the environmental conditions where our study species are found but does not contain large areas where no species are present. For models with < 25 points (57 species), we used a jackknife validation (also called 'leave-one-out') developed by Pearson et al. (2007) for small sample size. Model performance was assessed based on its ability to predict the single occurrence that is excluded from the 'training' dataset using the p-value program provided by Pearson et al. (2007). All models were also vetted by expert opinion (see above). To transform the maps of relative suitability for a given species into presence/absence maps we used MaxEnt's maximum training sensitivity plus specificity threshold criterion, which performed well in previous studies (Liu et al. 2005, Jiménez-Valverde and Lobo 2007) and in initial testing this threshold provided predictions that corresponded to the known distribution of the species.

Overlap of environmental conditions of pathogen and host

To characterize the environmental space of chytridiomycosis, we generated 4000 random points within our predicted, thresholded chytridiomycosis environmental niche model and extracted values for 19 climatic variables and altitude. Environmental values were also extracted for all unique species occurrence records. We conducted principle component analyses (PCA) using raw environmental variables and variables where the influence of altitude was removed to visualize overlap of environmental conditions between species extinction risk categories, genera and chytridiomycosis.

Given that both temperature and precipitation variables are highly correlated with altitude it is difficult to evaluate the independent contribution of these two factors. To remove the effect of altitude on climatic variables we performed a multivariate regression of 19 climatic variables with altitude as the independent variable. This analysis provided unstandardized residuals for each climatic variable. Analyses were performed with and without species records of *Pristimantis* because species in this genus have direct development (Pough et al. 2003) and are less likely to be affected by chytridiomycosis than those species whose larval development occurs in water bodies (Lips 1998). All multivariate analyses were conducted in SPSS ver. 14.0 (SPSS 2005).

We used Morisita's overlap index C_m (Hurlbert 1978), a statistical measure of dispersion of individuals in a population, to analyze the overlap between the environmental niche of chytridiomycosis and each of the 66 amphibian species for which we had equal or greater than five unique locality data. We used annual mean temperature and precipitation taken from thresholded MaxEnt models as integrative variables on which to compare the niches and compute their overlap. Given that these two climate variables are highly correlated with the rest of variables they should provide a good approximation of overlap between environmental conditions characterizing the distribution of chytridiomycosis and each amphibian species. Analyses were done in Biomapper 4.0. (Hirzel et al. 2008), a program that has been widely used to compare species-specific ecological niche parameters (Buckland and Elston 1993, Manel et al. 1999, Vayssières et al. 2000). To evaluate if differences in overlap between species and chytridiomycosis among extinction risk categories were statistically significant, we performed a non-parametric multivariate analysis of variance (npMANOVA), based on Bray–Curtis distances and 10 000 permutations, in which extinction risk categories were the multiple nominal independent variables and Morisita's overlap indexes were dependent variables. Data deficient species were not included in this analysis. To evaluate differences between pairs of categories, we performed post-hoc multiple comparison tests by using the Bonferroni corrected approach.

The analyses above were based on overlap in environmental conditions of chytridiomycosis and amphibian species across Ecuador; however most threatened species occur in the Andes. To further investigate the association between chytridiomycosis distribution, climatic variables and variation in species' extinction risk for this region we redid the above analyses but with PCA analyses using the environmental rasters themselves, instead of occurrence points. As above we controlled for the effect of elevation by running multivariate linear regression of the altitude raster on the 19 climate variables to obtain residuals. We then ran a PCA using these altitude-free rasters from Ecuadorian Andes to obtain a reduced dimensionality map of environmental conditions in the Andes. Principal components rasters were classified using the Jenks Natural Breaks method, which identifies break points that best group similar values and maximize the differences between classes (Jenks 1967). We obtained three climatic classes which corresponded to areas with less, equal and more precipitation

relative to that expected based on their altitude. We then calculated the geographic overlap between each climatic class, the chytridiomycosis model and the percentage of critically endangered species records that fell in each climatic class. Regression analyses were performed in ArcInfo Workstation 9.0 (ESRI 2005) and raster-based PCA analyses were conducted using Biomapper 4.0. (Hirzel et al. 2008).

Environmental niche properties as predictors of species sensitivity to climate change

Environmental niche properties (position and breadth) of species were calculated based on thresholded MaxEnt models (i.e. 66 species) and compared to IUCN extinction risk level. We used the ecological niche factor analysis (ENFA, Hirzel et al. 2002) to quantify niche position, defined as the deviation of a species' optimum conditions from the conditions in the study area, in our case the country of Ecuador. ENFA, like PCA, summarizes all predictor variables (in this case 19 worldclim variables) into a few uncorrelated and standardized factors (Hirzel et al. 2002), the first of which describes niche position, referred to by Hirzel et al. (2002) as marginality. Marginality values usually range from zero (the species tends to live in average conditions) to one (the species tends to live in extreme conditions), although these usually can exceed 1 (Hirzel et al. 2002).

To estimate niche breadth we calculated the Hurlbert's niche breadth index B' (Hurlbert 1978) along both annual mean temperature and annual precipitation variables. B' varies between zero (corresponding to specialized species) and one (corresponding to generalist species). We used a non-parametric multivariate analysis of variance (npMANOVA, Anderson 2001) to determine whether descriptors of species niches (position and breadth) were different among extinction risk categories. Extinction risk categories were the multiple nominal independent variables and the niche properties were dependent variables. The npMANOVA was followed by post hoc multiple comparison analyses using the Bonferroni corrected approach (Rice 1989). Finally, because by definition, species with larger ranges have wider niches, as niches are inferred from the geographical range, we examined the relationship between environmental niche properties and geographic range size. We ran linear regressions analyses between marginality index and range size, B' along temperature and range size and B' along precipitation and range size. These regressions allowed us to investigate if environmental niche properties were important predictors of extinction risk after the effect of our model based range size estimate was removed from the analyses.

Habitat degradation and extinction risk categories

To estimate the relationship between habitat loss and extinction risk in amphibians we determined the percentage of the records of all 109 species from each extinction risk category that fell in either remnant natural or degraded areas. We also calculated the reduction in geographic range size due to habitat degradation for 66 amphibian species for

which MaxEnt models were created. To determine if differences in range reduction among different extinction risk categories were statistically significant, we performed a Kruskal–Wallis test followed by Mann–Whitney post hoc multiple comparison analyses with Bonferroni correction (Sokal and Rohlf 1995).

Analyses of combination of threats

Finally, we combined the data sets from the amphibian threats into a single analysis. In order to evaluate the effect of chytridiomycosis, climate change and habitat loss simultaneously on Ecuadorian amphibians, we conducted a principle component analysis (PCA) using Morisita's overlap index (C_m) on temperature and precipitation, Hurlbert's niche breadth index (B') along temperature and precipitation, marginality values and percentage of reduction in geographic range size due to habitat degradation as variables for 66 amphibian species for which MaxEnt models were created. This analysis was followed by Kruskal–Wallis tests and by Mann–Whitney post hoc multiple comparison analyses with Bonferroni correction (Sokal and Rohlf 1995) to determine if differences in PC I scores and PC II scores among different extinction risk categories were statistically significant (Supplementary material Appendix 4).

Results

Environmental niche model of chytridiomycosis

The predictive performance of the chytridiomycosis distribution model was high (AUC = 0.89, jackknife success rate = 0.6, $p < 0.001$). The distributional area of chytridiomycosis encompassed 33 103 km² in the Ecuadorian Andes with suitable regions generally above 2000 m a.s.l. (Fig. 1). The distribution of chytridiomycosis in Ecuador was characterized by low values for both temperature and precipitation relative to climatic conditions across Ecuador. The mean annual temperature ranged between -5.6°C and 16.1°C ($\bar{X} = 9.03^\circ\text{C}$, $\text{SD} = 2.86$) and annual precipitation ranged from 381 to 1828 mm ($\bar{X} = 949$ mm, $\text{SD} = 219.18$). About 34% of the region predicted by the chytridiomycosis model was in governmental and private protected areas, including Parque Nacional Sangay, Reserva Ecológica Cayambe-Coca, Parque Nacional Llanganates, protected forests in the Paute River Basin, Reserva de Producción Faunística Chimborazo, and Reservas Ecológicas Antisana, Los Illinizas, and Cotacachi-Cayapas. Further, about 60% of the predicted distribution of chytridiomycosis occurs in relatively pristine habitats. The model predicts chytridiomycosis in 4 of the 10 biogeographic, including approximately 40% of western montane forests, 90% of

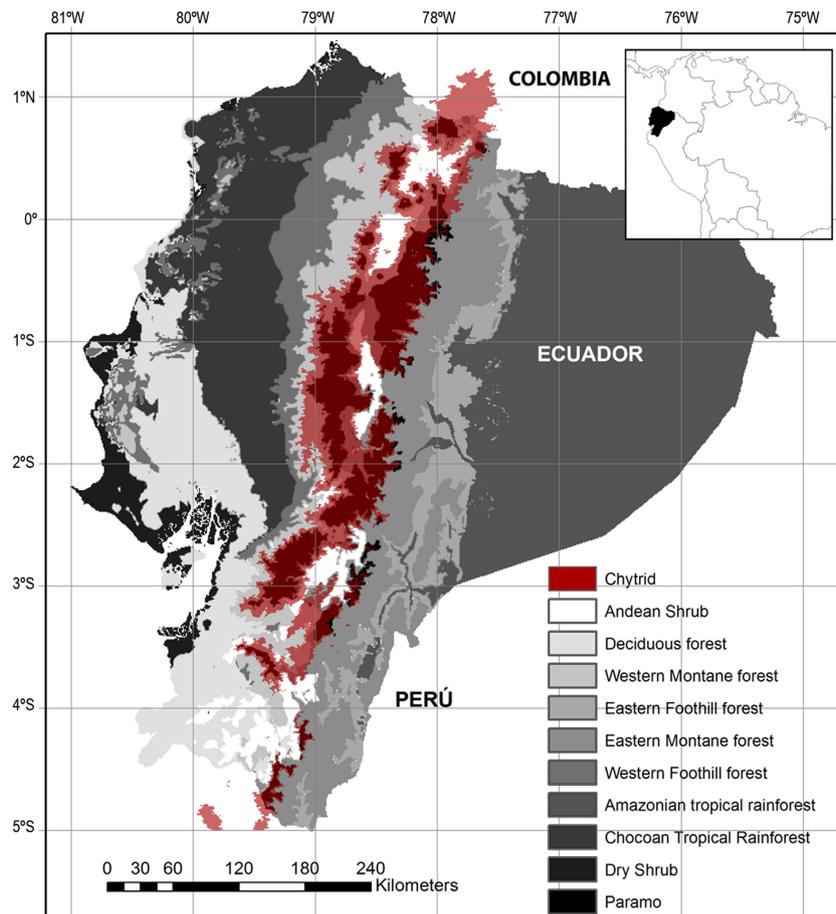


Figure 1. Predicted distribution of the niche of chytridiomycosis in red. The model is projected across Ecuador's biogeographic regions based in Sierra's (1999) land-cover classification.

paramo, 20% of Andean shrub and 15% of eastern montane forest areas (Fig. 1). Regions with low predicted suitability (i.e. cumulative probability between 10 and 54) included the western lowlands below 1200 m a.s.l., such as the western Andean foothills and Chococoan tropical rain forests, deciduous forests and dry shrubs.

To evaluate the model made with the Ecuador occurrence data, which may only represent part of the environmental niche of the species given that chytridiomycosis occurs globally, we compared our model to the global model of Ron's (2005). There was a 95% overlap in the distribution predicted by these two models in Ecuador suggesting that we have likely adequately captured the distribution of chytridiomycosis with our model (Supplementary material Appendix 3, Fig. A3).

Overlap of environmental conditions of pathogen and host

The first axis (41.4% variation explained) of the PCA that included raw variables described extreme or limiting temperatures and elevation (Fig. 2a). The following variables were important in defining axis 1: maximum temperature of warmest month, annual mean temperature, mean temperature of wettest quarter and mean temperature of warmest quarter (Supplementary material Appendix 1, Table A1-3). The second PCA axis (18.9% of the variation explained) was based on precipitation, primarily precipitation during driest periods and coldest quarter (Supplementary material Appendix 1, Table A1-3). Occurrences of critically endangered species and points derived from the chytridiomycosis

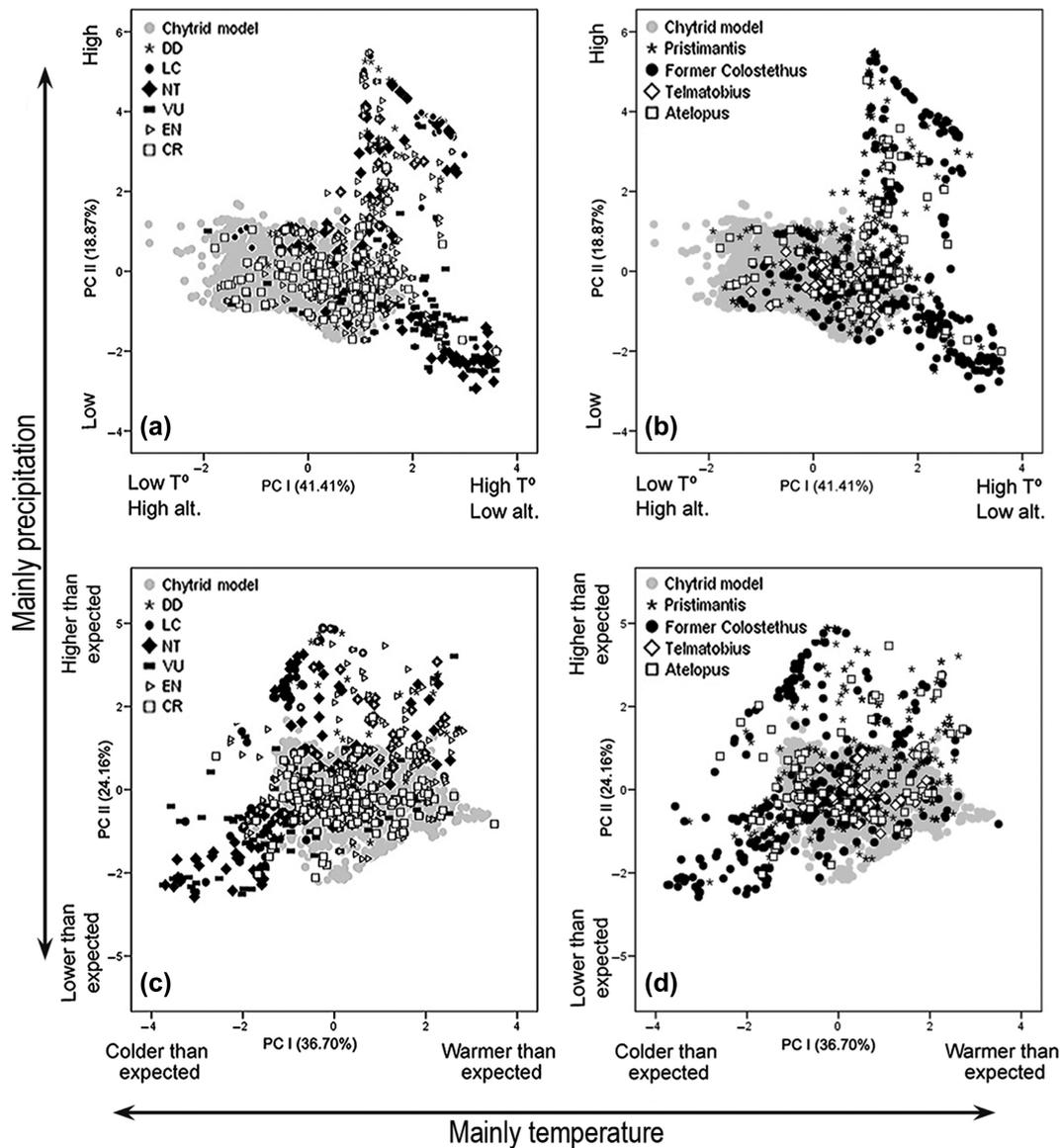


Figure 2. Axes I and II from principal components analyses (PCA) based on 19 environmental variables at 4000 random points within the predicted, thresholded chytridiomycosis environmental niche model in Ecuador (gray circles). All unique species amphibian records (1000) in Ecuador were also included in the PCA (black and white symbols). (a) and (b) PCA conducted using raw environmental variables and altitude, latitude and longitude; (c) and (d) PCA conducted using variables where the influence of altitude was removed; (a) and (c) species occurrence records classified based on IUCN Red List (critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT), least concern (LC), data deficient (DD)); and (b) and (d) species occurrence records classified by genera.

environmental niche tended to occur in high elevation, cool areas, as well as in dry areas (Fig. 2a). Eighty-nine percent of the species records in the critically endangered category overlapped with the chytridiomycosis environmental niche model, whereas 53 and 51% of those under endangered and vulnerable categories, respectively, fell in the chytridiomycosis envelope. There was an overlap of 35 and 19% between the chytridiomycosis area and species in the near threatened and least concern categories, respectively. Occurrences of *Atelopus* (77%) and *Telmatobius* (91%) genera showed higher overlap with the chytridiomycosis niche model than those of former *Colostethus* (46%) and *Pristimantis* (50%, Fig. 2b). As expected, given that species of the genus *Pristimantis* are direct developers and likely not strongly impacted by chytrid, the degree of overlap between the less threatened (i.e. vulnerable, near threatened and least concern) species and chytridiomycosis niche model decreased when this genus was removed from the PCA (Supplementary material Appendix 1, Table A1-3). Eighty-nine percent of the species occurrence records in the critically endangered category, 62% in endangered and 33% in vulnerable categories overlapped with the chytridiomycosis niche model. Frog species in the near threatened and least concern categories did not overlap with the environmental conditions of the fungus (Supplementary material Appendix 1, Fig. A1-a).

When we used the residuals of the multivariate regressions between altitude and climatic variables in a PCA the first axis (36.7% variation explained) was mainly defined by temperature variables: maximum temperature of warmest month, temperature annual range and mean diurnal range (Fig. 2c, Supplementary material Appendix 1, Table A1-3). This axis showed an inverse pattern relative to the first PCA axis generated from raw environmental variables; the most threatened species' occurrences (i.e. critically endangered) and chytridiomycosis model points tended to be in areas that were either warmer or similar to that expected based on their altitude (Fig. 2c). The second PCA axis (24.2% variation explained) was defined by precipitation variables, mainly precipitation of driest month, precipitation of driest quarter and precipitation of coldest quarter (Supplementary material Appendix 1, Table A1-3). Along this axis, most critically endangered species' occurrences and the chytridiomycosis model tended to be in areas close to the expected precipitation conditions (Fig. 2c). Ninety four percent of the records under the critically endangered category overlapped with the chytridiomycosis niche model, whereas 72% of those in endangered, 62% in vulnerable, 52% in near threatened and 36% in least concern categories fell in the chytridiomycosis environmental envelope. When occurrence records were classified in genera we obtained a similar pattern to previous analysis where the effect of altitude was not removed (Supplementary material Appendix 1, Table A1-3). Former *Colostethus* (56%) and *Pristimantis* (68%) genera once again presented lower overlaps than those of *Atelopus* (81%) and *Telmatobius* (98%, Fig. 2d). Finally, when records from *Pristimantis* genus were removed, 94% of records in critically endangered category overlapped with the fungus niche model (Supplementary material Appendix 1, Fig. A1-b).

Seventy-four, 34 and 25% of those in endangered, vulnerable and near threatened categories, respectively, fell in the chytridiomycosis space. No least concern records overlapped with the chytridiomycosis distribution.

The amount of overlap between the species and chytridiomycosis models calculated using Morisita's overlap index (C_m) computed on both annual mean temperature and annual precipitation dimensions conformed to the PCA results. For annual mean temperature, critically endangered species showed the highest mean overlap for the C_m index ($\bar{X} = 0.66$, $SD = 0.29$). Species under endangered ($\bar{X} = 0.34$, $SD = 0.35$), vulnerable ($\bar{X} = 0.23$, $SD = 0.32$), near threatened ($\bar{X} = 0.19$, $SD = 0.27$) and least concern ($\bar{X} = 0.17$, $SD = 0.39$) categories exhibited less overlap with chytridiomycosis model. For annual precipitation, critically endangered species also presented the highest mean of C_m ($\bar{X} = 0.78$, $SD = 0.29$), followed by vulnerable ($\bar{X} = 0.44$, $SD = 0.42$), endangered ($\bar{X} = 0.40$, $SD = 0.33$), near threatened ($\bar{X} = 0.27$, $SD = 0.32$) and least concern species ($\bar{X} = 0.18$, $SD = 0.30$). Results from npMANOVA indicate that there were differences among extinction risk categories for these environmental niche-overlap indexes ($F = 2.08$, $p < 0.05$). Multiple comparison analyses indicated that critically endangered species differed from all categories for both precipitation and temperature (all $p < 0.05$), though were only marginally different than endangered species ($p = 0.07$).

PCA analyses based on the raster maps of the Ecuadorian Andes further refined the relationship between temperature, precipitation, chytridiomycosis and the distribution of endangered frogs. The first PCA axis (38.5% variation explained) was mostly correlated with temperature variables, mainly maximum temperature of warmest month, annual mean temperature and mean temperature of coldest quarter (Supplementary material Appendix 1, Table A1-3). The second axis (30.5% variation explained) was mainly described by annual precipitation as well as precipitation of driest quarter and precipitation of driest month (Supplementary material Appendix 1, Table A1-3). The map based on the first axis did not yield a clear geographic pattern associated with temperature. However, the map based on the second axis showed clear patterns; areas with equal or less precipitation than expected are primarily located in western slopes of Andes as well as in some inter-montane valleys. Wetter regions than expected for their altitude are located mainly in the eastern Andean slopes of Ecuador (Fig. 3a, b). Only in the areas with equal or less precipitation than expected do the majority (83%) of critically endangered records occur (i.e. 58% in areas with less precipitation and 25% in those close to the expected precipitation conditions for their altitude). Remarkably, just 17% of critically endangered records of all analyzed species occur in wetter areas than expected (Fig. 3a). Further, 42% of the chytridiomycosis model overlapped with drier areas than expected for their altitude, 33% overlapped with areas that did not deviate from expected precipitation conditions. Only 25% of the model overlapped with areas wetter than expected (Fig. 3b). In sum, regions that were drier or closer to the expected precipitation conditions for their altitude, contained most of the critically endangered species' occurrences.

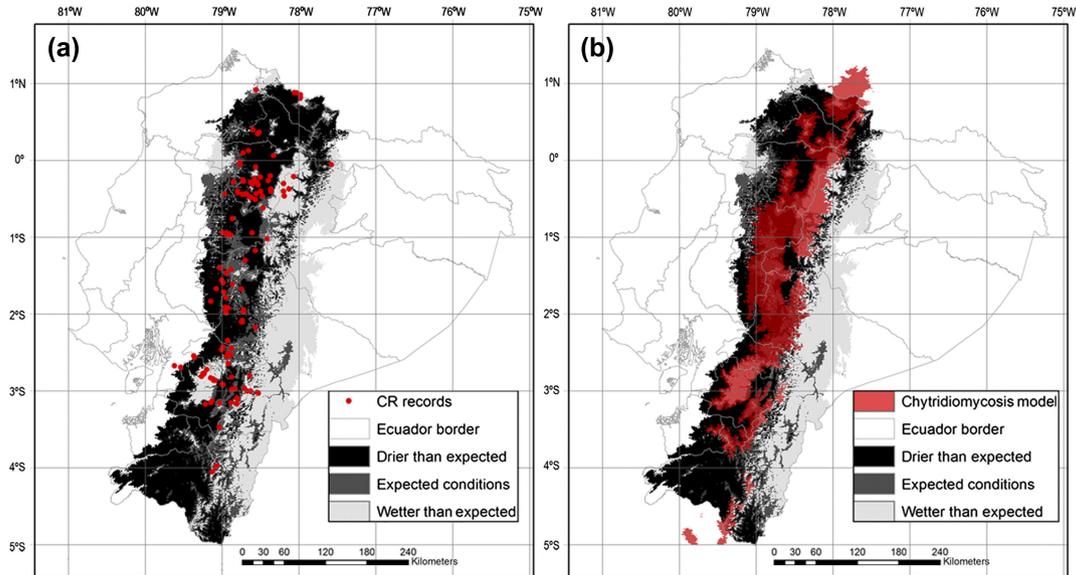


Figure 3. Map of principal component II from a principal component analysis (PCA) using 19 altitude-free environmental rasters from Ecuadorian Andes. Areas with less precipitation than expected are black colored, areas with equal precipitation than expected are gray darker colored, whereas wetter regions than expected for their altitude are light gray colored. (a) Records from critically endangered (CR) species are shown in red; (b) chytridiomycosis model is shown in red.

These same areas also appear to have better climatic conditions for chytridiomycosis. These results are qualitatively consistent with those obtained when using the MAE climate information instead of the Worldclim data (Supplementary material Appendix 2, Fig. A2).

Environmental niche properties as predictors of species sensitivity to climate change

The non-parametric multivariate analysis of variance (npMANOVA) showed that there is an overall difference among risk categories with regard to the three environmental niche properties (i.e. marginality and niche breadth based on temperature and precipitation, $F = 2.49$, $p < 0.05$). Post hoc multiple comparison analyses indicated that only critically endangered species differed from the rest categories (all $p < 0.05$). Critically endangered species were the most marginal species ($\bar{X} = 3.07$ for marginality) and the most specialized, both on temperature ($\bar{X} = 0.13$ for B') and

precipitation ($\bar{X} = 0.24$ for B'). Least concern species occur in environmental conditions nearer to the mean of those available in Ecuador ($\bar{X} = 1.80$ for marginality) and occur in a broader array of climate conditions across their ranges especially in terms of temperature ($\bar{X} = 0.34$, Table 1).

The regression between marginality and range size was negative and statistically significant ($R^2 = 0.1$, $F = 4.09$, $p < 0.05$, Fig. 4c). There were positive, significant, relationships between Hurlbert's niche breadth index B' calculated on temperature and range size ($R^2 = 0.4$, $F = 48.48$, $p < 0.001$, Fig. 4a) and B' based on precipitation and range size ($R^2 = 0.2$, $F = 0.18$, $p < 0.001$, Fig. 4b). The plot of marginality against range size reveals that approximately 90% of critically endangered species were more marginal than expected in relation to their geographic range size (Fig. 4c). Likewise, in both plots of niche breadth against range size the most critically endangered species (~80%) tend to have more environmentally restricted niches than expected in relation to their geographic range size (Fig. 4b, c).

Table 1. Environmental niche properties (position and breadth) of species in different threat categories. Means, standard deviations and maximum and minimum of each threat IUCN category (n = 66) for marginality, niche breadth on temperature (T) and niche breadth on precipitation (P). Ecological niche factor analysis (ENFA, Hirzel et al. 2002) was used to quantify marginality (niche position). Niche breadth along temperature and precipitation are based on the Hurlbert's niche breadth index B' (Hurlbert 1978).

IUCN Red List category	Marginality	Niche breadth B' on T	Niche breadth B' on P
Critically endangered	3.0726 (0.832) (1.102–4.045)	0.1328 (0.073) (0.062–0.308)	0.2425 (0.157) (0.130–0.631)
Endangered	2.343 (0.846) (1.164–4.202)	0.1605 (0.126) (0.040–0.538)	0.3231 (0.188) (0.079–0.716)
Vulnerable	2.4275 (0.953) (1.556–4.380)	0.2732 (0.207) (0.049–0.599)	0.3868 (0.113) (0.173–0.538)
Near threatened	2.03 (0.595) (1.377–3.051)	0.214 (0.152) (0.097–0.553)	0.386 (0.200) (0.158–0.724)
Least concern	1.81 (0.873) (0.822–3.363)	0.337 (0.177) (0.128–0.544)	0.2854 (0.198) (0.114–0.624)

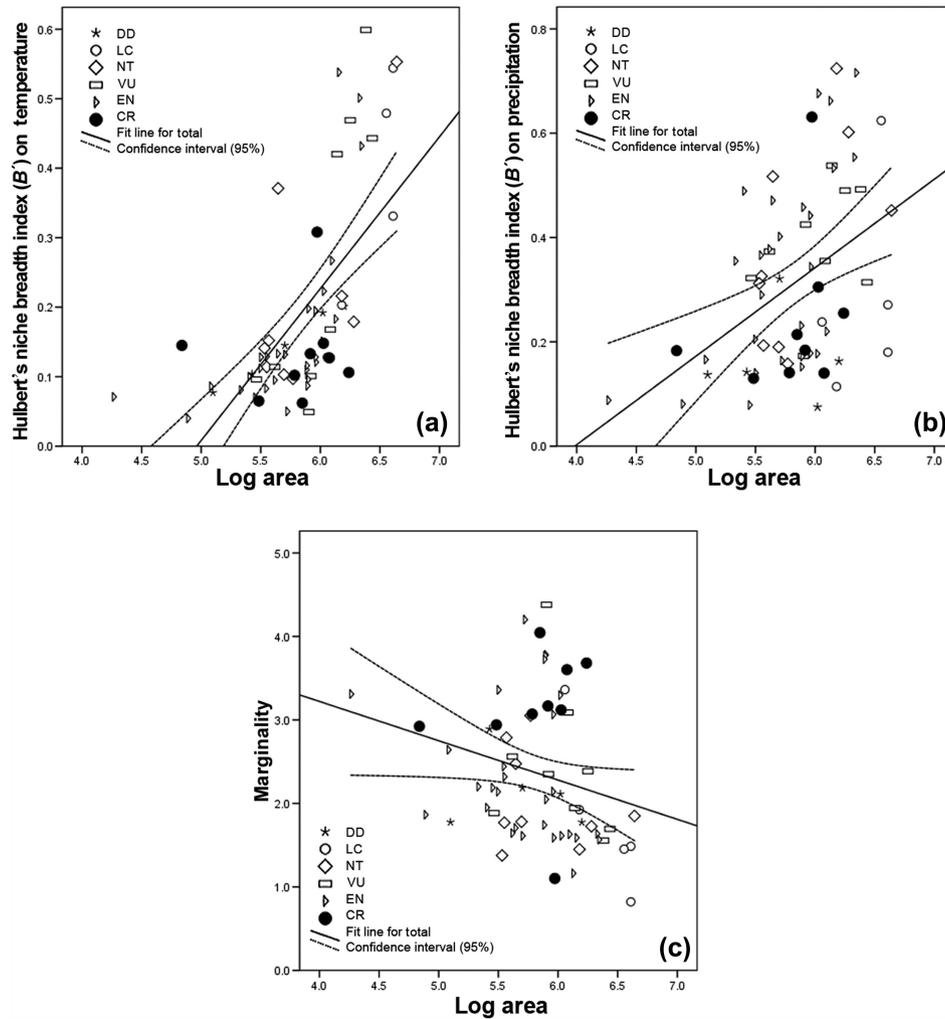


Figure 4. Relationship between geographic range size and environmental niche properties (position and breadth) of 66 amphibian species. Species (symbols) were classified under some category of threat according to the IUCN Red List. (a) Range size vs niche breadth along temperature based on the Hurlbert's niche breadth index B' ; (b) range size vs niche breadth along precipitation based on the Hurlbert's niche breadth index B' ; and (c) range size vs marginality.

Habitat degradation and extinction risk categories

Thirty four percent of critically endangered occurrences and 35% of endangered occurrences were in natural vegetation, but surprisingly, lower extinction risk categories had fewer occurrences in these areas (18.23% vulnerable, 32.48% near threatened and 29.00% least concern). Our analyses of habitat reduction from deforestation of individual species ranges indicated that vulnerable species showed the highest percent of reduction in geographic ranges due to habitat degradation ($\bar{X} = 66.96$), followed by critically endangered ($\bar{X} = 43.55$), endangered ($\bar{X} = 43.53$), near threatened ($\bar{X} = 34.43$) and least concern ($\bar{X} = 26.25$) species. Although there were overall differences in the reduction of geographic range among all risk extinction categories (Kruskal–Wallis $\chi^2 = 10.92$, $p < 0.05$), post hoc multiple comparison analysis indicated that only vulnerable and least concern categories differed on the percent of habitat degradation ($p < 0.05$; Supplementary material Appendix 1, Table A1-4).

Analyses of combination of threats

Results from the combined analysis were consistent with our previous results (Supplementary material Appendix 4). The most endangered species tend to overlap more with chytridiomycosis model and have more marginal and more restricted climate niche breadth than the less endangered species. Additionally, this analysis showed that habitat loss does not seem to be the major determinant of amphibians threat (IUCN Red-Listed; for more details see Supplementary material Appendix 4, Table A4-1, 2, Fig. A4).

Discussion

Chytridiomycosis niche model

Our niche model suggests that the highest suitability for the chytridiomycosis occurs in montane regions of the Ecuadorian Andes, especially above 2000 m a.s.l. This is

consistent with niche models of the potential distribution of the chytrid developed over larger geographical regions (Ron 2005, Rödder et al. 2009). The areas with the highest suitability for *B. dendrobatidis* are the montane biogeographic regions that contain the country's most diverse amphibian faunas such as forests on the eastern and western slopes of the Andes (Ron et al. 2011b). Most of the Ecuadorian lowlands were predicted to be relatively unsuitable for the occurrence of the chytridiomycosis; however, a recent study reported the occurrence of *B. dendrobatidis* on an Ecuadorian lowlands species at an elevation of about 300 m a.s.l. (McCracken et al. 2009). According to a review of the conservation status of Ecuadorian amphibian species by Ron et al. (2011b), there is no evidence that these, or other lowland, frog populations have suffered declines.

Environmental variables and chytridiomycosis as correlates of amphibian threat risk

Most critically endangered species and species belonging to the *Atelopus* and *Telmatobius* genera occur in regions that our model characterized as highly suitability for *B. dendrobatidis*. These results suggest an environmental and geographical association between critically endangered species and chytridiomycosis that is consistent with hypotheses implicating the pathogen in the extinction of amphibians (Ron et al. 2003, Merino-Viteri et al. 2005, Pounds et al. 2006, Lips et al. 2008, Menéndez-Guerrero et al. 2008, Lötters et al. 2009, Murray et al. 2011). Temperature and altitude partly explain this association, with both pathogen and endangered species occurring in cooler and higher environments than those species in lower threat categories. This pattern is concordant with laboratory studies in which *B. dendrobatidis* grows best and is most pathogenic to frogs in cool conditions (Berger et al. 1998, 2004, Longcore et al. 1999, Piotrowski et al. 2004). Other experimental studies have reported that amphibians exposed to *B. dendrobatidis* live significantly longer at warmer temperatures than at cooler ones (Andre et al. 2008, Bustamante et al. 2010). In addition, amphibian population declines and disappearances are concentrated in montane areas of the tropics (Young et al. 2001), and Ecuador is no exception. The high and cool montane biogeographic regions in Ecuador (i.e. Andean shrub, paramo, eastern montane forest and western montane forest) contain the highest proportion of species either endangered or critically endangered (Ron et al. 2011b). This indicates that temperature, including mean temperature or temperature variability (Raffel et al. 2006, Alford et al. 2007, Whitfield et al. 2007, Rohr and Raffel 2010), may be an important factor in disease emergence.

When we controlled for the effect of altitude our results showed that both chytridiomycosis and critically endangered species mainly occur in warmer areas than expected in relation to their altitude (but still cooler than the average climate conditions of Ecuador). This suggests that past temperature increases in Ecuador, such as those caused by anthropogenic global warming, may have had a greater impact on species occurring in these environmental conditions than those occurring in colder areas than

expected. Species inhabiting warmer temperatures often are closer to their upper thermal tolerance limits than species inhabiting colder temperatures (Compton et al. 2007, Deutsch et al. 2008, Tewksbury et al. 2008). Temperatures outside of a species' thermal optimum will cause physiological stress and potentially suboptimal immunity in amphibians which could lead to greater susceptibility to diseases, such as chytridiomycosis (Raffel et al. 2006). In fact, mean annual temperature in tropical Andes shows an increase of 0.7°C over the past seven decades (1939–2006, Vuille et al. 2008), and temperature increases as large as 5.6°C have been recorded in some sites in Ecuador (Ron et al. 2003, Merino-Viteri et al. 2005).

When we focused our analyses on the Ecuadorian Andes, the region with the highest concentration of critically endangered species, annual precipitation emerges as an important correlate of amphibian threat level. Critically endangered species mainly occur in drier areas than expected in relation to their altitude, and, just as with temperature, these amphibians might be more affected by decreases in rainfall over the past decades than species occurring in wetter sites than expected. These species also may be at the limit of their dehydration tolerance (Araújo et al. 2006). There has been a significant decrease in total annual precipitation in the Ecuadorian Andes during the 1970s to 1998 (Fig. 5); a period when most of the Neotropical amphibian declines occurred (Pounds et al. 2006). Previous studies have shown that the increasing length of dry periods (Stewart 1995, Pounds et al. 1999, Burrowes et al. 2004) or overall decreases in annual precipitation (Pounds and Crump 1994) may have caused the decline and probable extinction of several species of amphibians and may be even more significant than increases in temperature. The impact of reduced water availability might be particularly important for species occurring in areas that are already under hydrological stress, such as the drier Andean areas identified in this study. Additionally, there is a positive correlation between amphibian species' dehydration tolerance and their degree of terrestriality (Feder and Burggren 1992) that might explain why most of the critically endangered species of Ecuador are water dependent (i.e. species from genera *Atelopus*, *Hyloxalus* and *Telmatobius*). Chytridiomycosis has also been documented to be more prevalent and severe under dry conditions (Woodhams and Alford 2005), suggesting the existence of complex interactions among amphibians, chytrid and droughts that may lead to population crashes (Longo et al. 2010, but see Kriger 2009). This would be consistent with our results that regions drier or with the same expected precipitation conditions, where most of the critically endangered species' records occur, appear to have better climatic conditions for chytridiomycosis. On the other hand, some studies have also shown that chytridiomycosis outbreaks might be more likely to occur under wet conditions (Piotrowski et al. 2004, La Marca et al. 2005, Bustamante et al. 2010). This lack of agreement suggests that the interaction between precipitation and disease will likely prove to be habitat-specific. For instance, droughts events in the Andes may have a different effect on disease prevalence than droughts in Australia or Central America. While our findings do not directly test hypotheses about the relationship

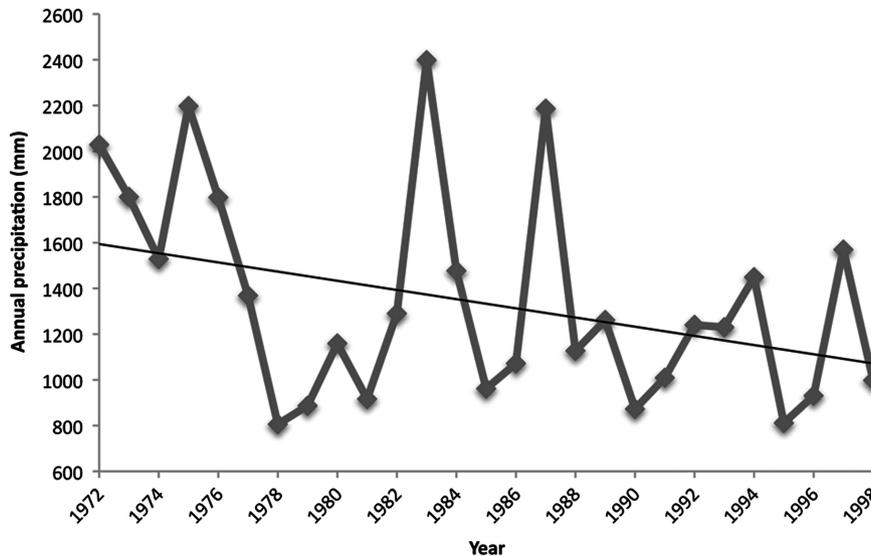


Figure 5. Mean annual precipitation in the Ecuadorian Andes between 1970s and 1998. There has been a significant decrease in total annual precipitation in the Ecuadorian Andes during this period ($R^2 = 0.15$, $F = 4.73$, $p < 0.05$). Precipitation values are from a monthly climate dataset by ~ 0.2 degree grid for 1901–2002 that were provided by R. Hijmans.

between climate change, chytridiomycosis and the decline of Ecuadorian frogs, they do suggest that synergistic effects between environmental conditions and fungus disease may explain current patterns of amphibian extinction risk.

Environmental niche properties as predictors of species sensitivity to climate change using niche modeling

Climate change is expected to be a major driver of biodiversity loss in the 21st century and extinction risk under climate change is expected to depend, in part, on biological susceptibility of species. Theoretical and modeling studies suggest that extinction risk may depend not only on severity of threat, but also on species' ecological and geographical characteristics (Thuiller et al. 2004, Broennimann et al. 2006), where marginal species (those with requirements which do not match the mean climate conditions of a given geographic region) may be more susceptible to climate change than species with requirements close to the mean climate conditions of a given region (Swihart et al. 2003). Similarly, species with a larger climate niche breadth (i.e. generalist species) should tolerate climate changes better than specialist species (Brown 1995). Our results suggest that climatic niche properties of species may be good surrogates for their conservation status (extinction risk based on IUCN red list), and hence may be good predictors of species' vulnerability to recent climate change. The most endangered species (especially critically endangered) were the most marginal and have restricted environmental niche breadth. Although range size (which is often positively correlated with environmental niche breadth and negatively with marginality) has consistently emerged as a major determinant of extinction risk in amphibians (Cooper et al. 2008, Sodhi et al. 2008, Lee and Jetz 2011), our results show that critically endangered species have greater than expected

marginality and environmental specialization given their range size. These findings suggest that range size alone might not be the best GIS-based correlate for extinction risk, and that niche-based environmental factors should also be considered. Further, these results are consistent with our other results and indicate that recent climate changes in the Ecuadorian Andes might have caused some frog population declines.

Habitat degradation correlates of extinction risk categories

According to our prediction, if habitat degradation influences extinction risk, we would expect to see a greater proportion of occurrences for critically endangered and endangered species occurring in degraded areas than occurrences for species in the near threatened and least concern categories. Further, we would expect that threatened species' ranges would have lost a greater percentage of habitat in comparison to less threatened species. Our results do not implicate habitat conversion as a cause of amphibian decline, though we cannot rule out the possibility that changes in vegetation structure at the local scale (i.e. those not captured by our relatively coarse grain vegetation map) influence distribution patterns of amphibians. Instead, our findings further support the idea that other processes, notably disease and climate change, threaten rapidly declining species (Lips et al. 2006, 2008, Pounds et al. 2006, Murray et al. 2011). For instance, Ron et al. (2011b) found a significant relationship between the percentage of remaining natural habitat and the residuals from the regression between the percentage of threatened species and mean temperature in different biogeographic regions of Ecuador. Their results showed that habitat loss was a good predictor of the proportion of Ecuadorian endangered species only after the effect of temperature was removed in the analysis.

At a global geographic scale, Sodhi et al. (2008) showed that small ranging species that experienced high habitat loss and, marked seasonality in temperature and precipitation tended to be Red-Listed by IUCN. Taken together our research suggests that while habitat loss may be a crucial factor affecting biodiversity decline (Bickford et al. 2008, Cooper et al. 2008) and it may have contributed to amphibian declines of many species in Ecuador (Cisneros-Heredia et al. 2010), habitat loss alone cannot explain the rapid and enigmatic declines that have occurred since the mid of 1980s.

There are several potential limitations of our approach. First, we used a correlative model based on where a species currently occurs and as such only estimate the realized niche. This may influence our estimation of the potential distribution of a species and species niche characteristics. Detailed physiological knowledge of the fundamental niche of the study species may help alleviate this problem but was not available, but certainly should be considered in the future. We assume that the realized niche provides sufficient information to learn about how niche parameters and distribution relate to amphibian declines. Second, we have limited occurrence data for modeling; if environmental conditions across the range of species are not sufficiently sampled models can be poor (Menke et al. 2009). While we attempted to obtain a complete and accurate dataset for all species, data for amphibians in Ecuador are limited. Given this limitation we conducted multiple analyses both with MaxEnt models and occurrences grouped according to threat and genus. Third, the climate data we used are created by extrapolation from weather stations which can be somewhat problematic in topographically complex regions. Finally, we did not conduct independent evaluation of our models by going to the field, as funding for this was not available. While we acknowledge these limitations we believe that the analyses and data we present provide new insights into the role of multiple drivers in amphibian declines and point to future research that could further elucidate mechanisms decimating amphibian populations.

Further study on amphibian declines requires manipulative experimentation to identify the underlying ecological and evolutionary causes of amphibian responses to climate change and emerging diseases. Additionally, given that the effects of threats can be expected to vary both spatially and across species (Murray et al. 2011), broader geographic coverage, especially in environmentally complex regions with high species diversity are needed to make better inferences across entire taxa and biogeographic regions and thus fully understand the issue of amphibian declines.

Acknowledgements – We thank Luis A. Coloma (former QCAZ curator) and Santiago R. Ron (current QCAZ curator) for allowing us to extract presence data from the amphibian database of Museo de Zoología at the Pontificia Univ. Católica del Ecuador (QCAZ) and for checking the accuracy of maps. We also extend our particular thanks to Sofía V. Carvajal-Endara, Luis A. Coloma, Santiago R. Ron, Windsor E. Aguirre, Pilar A. Hernandez, E. Jorge Velásquez-Tibará, Liliana M. Dávalos, and the editor, Miguel Araújo, for helpful discussions and comments on earlier versions of this paper. Additionally, we wish to thank Santiago R. Ron for making available his global predictive model of the potential distribution of the chytrid fungus for further comparisons. María del Pilar Rodríguez, Belén Baus, Paulina Álava and Cristina Félix helped on several

spatial analyses and georeferencing localities. Ailín Blasco-Zúñiga generously helped in editing figures. This research was supported by NASA New Investigator Grant to CG. The final stages of this paper were funded to QCAZ by Conservation International-Ecuador throughout the project ‘Identificación de prioridades para la conservación de anfibios ecuatorianos mediante el uso de sistemas de información geográfica’.

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Supplementary material (Appendix E7877 at <www.oikosoffice.lu.se/appendix>). Appendix 1–4.