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POTENTIAL CAUSES FOR AMPHIBIAN DECLINES IN PUERTO RICO

Patricia A. Burrowes[1,3], Rafael L. Joglar[1], and David E. Green[2]

[1]*Department of Biology, University of Puerto Rico, P.O. Box 23360, San Juan, PR 00931-3360, USA*
[2]*U.S.G.S. Geological Survey, National Wildlife Health Center, Madison, WI 53711-6223, USA*

Abstract: We monitored 11 populations of eight species of *Eleutherodactylus* in Puerto Rico from 1989 through 2001. We determined relative abundance of active frogs along transects established in the Caribbean National Forest (El Yunque), Carite Forest, San Lorenzo, and in the vicinity of San Juan. Three species (*Eleutherodactylus karlschmidti*, *E. jasperi*, and *E. eneidae*) are presumed to be extinct and eight populations of six different species of endemic *Eleutherodactylus* are significantly declining at elevations above 400 m. Of the many suspected causes of amphibian declines around the world, we focused on climate change and disease. Temperature and precipitation data from 1970–2000 were analyzed to determine the general pattern of oscillations and deviations that could be correlated with amphibian declines. We examined a total of 106 tissues taken from museum specimens collected from 1961–1978 and from live frogs in 2000. We found chytrid fungi in two species collected at El Yunque as early as 1976, this is the first report of chytrid fungus in the Caribbean. Analysis of weather data indicates a significant warming trend and an association between years with extended periods of drought and the decline of amphibians in Puerto Rico. The 1970's and 1990's, which represent the periods of amphibian extirpations and declines, were significantly drier than average. We suggest a possible synergistic interaction between drought and the pathological effect of the chytrid fungus on amphibian populations.

Key words: Amphibian declines; Caribbean; Climate change; Chytrid fungi; Disease; *Eleutherodactylus*; Puerto Rico

Population declines have occurred in at least four endemic anuran species on the island of Puerto Rico (Joglar, 1998; Joglar and Burrowes, 1996). The species are the Puerto Rican crested toad, *Bufo* (= *Peltophryne*) *lemur*, and three *Eleutherodactylus* (*E. karlschmidti*, *E. jasperi*, and *E. eneidae*). In spite of repeated efforts to find them in the past 10 yr, the latter three species have not been observed in Puerto Rico since 1976, 1981, and 1990, respectively, and are presumed extinct (Burrowes and Joglar, 1991; Joglar, 1998; Joglar and Burrowes, 1996). Common denominators in the decline of Puerto Rican anurans are high elevation and ecological specialization, such as restriction to stream or bromeliad habitats (Joglar, 1998; Joglar and Burrowes, 1996). Long-term (12 yr) monitoring identified population declines and disappearances (Joglar and Burrowes, 1996), but mortality events (i.e.,"die-offs") or malformation of individuals were not observed.

Among the known and suspected causes of amphibian population declines are human-related habitat deterioration (Fellers and Drost, 1993; La Marca and Reinthaler, 1991; Lips, 1998; Wake and Morowitz, 1991; Welsh and Ollivier, 1998), increased ultraviolet-B radiation (Blaustein et al., 1994; Keisecker and Blaustein, 1995; Middleton et al., 2001; Pahkala et al., 2002), climate change (Alexander and Eischeid, 2001; Heyer et al., 1988; Ingram, 1990; Pounds and Crump, 1994;

[3] Correspondence: e-mail, pburrowe@upracd.upr.clu.edu

Pounds et al., 1999; Stewart, 1995; Weygoldt, 1989), pollution by airborne contaminants, herbicides and pesticides (LeNoir et al., 1999; Stallard, 2001), introduction of exotic species (Bradford et al., 1993; Gillespsie, 2001; Lawler et al., 1999), and disease (summarized by Carey et al., 1999; Muths et al., 2003). In recent years, herpetologists working in six continents (Africa, Australia, South America, Central America, North America, and Europe) have linked amphibian declines among certain montane species to the presence of a chytridiomycete fungus in the skin (R. Speare and L. Berger, personal communication). Longcore et al. (1999) isolated a new chytrid fungus pathogenic to frogs and named it *Batrachochytrium dendrobatidis*. Here, we present results of an on-going study to determine the incidence of chytrid fungi among eight species of Puerto Rican *Eleutherodactylus*, based on individuals collected in 2000, and on museum specimens collected as early as 1960.

In addition to diseases, global climate change has received much attention in relation to amphibian declines in the past two decades (Beniston et al., 1997; Colón, 1983; Donnelly and Crump, 1998; Knutson et al., 1998; Parrilla et al., 1994; Pounds et al., 1999; Scatena, 1998; Still et al., 1999). Current rates of temperature change, especially in montane areas, may exceed the ability of plants and animals to adapt (Carey et al., 2001). Because amphibians are potential bioindicators (Wake and Morowitz, 1991; Welsh and Ollivier, 1998; Weygoldt, 1989), herpetologists concerned with the phenomenon of amphibian declines around the world are investigating relationships between their decline and climate change (Alexander and Eischeid, 2001; Donnelly and Crump, 1998; Pounds et al., 1999; Stallard, 2001). Analysis of weather station data for Puerto Rico has shown an upward trend in temperature over the last 100 yr, and below average precipitation in the 1980's and early 1990's (Alexander and Eischeid, 2001). Colón (1983) found that 1970–1979 was the driest decade of the past century in Puerto Rico, with an average decrease in annual rainfall of 18%. The time frame mentioned in these climate studies (1970–1990) coincides with the period of amphibian declines in Puerto Rico (Joglar, 1998; Joglar and Burrowes, 1996). The majority of anurans endemic to Puerto Rico are within *Eleutherodactylus*, which lay terrestrial eggs that develop directly into froglets. Although the included species do not depend on aquatic habitat for reproduction, they do require relatively cool, moist environments for rehydration and to avoid desiccation of terrestrial egg clutches. Subtle climatic changes that result in warmer, drier conditions are potential stressors for various aspects of the population biology of the *Eleutherodactylus* fauna.

In this paper we: (1) report on the current status of the Puerto Rican anuran species for which we have 12 yr of population density data; (2) describe localized climatic patterns in a tropical montane rain forest; (3) analyze climate patterns for potential stress of increased temperature or decreased rainfall during the past 30 yr; and (4) report the presence of a chytrid fungus and other disease agents among endemic Puerto Rican frog species. Although Puerto Rican amphibian declines have been well documented (Burrowes and Joglar, 1991; Joglar and Burrowes, 1996), few causes had been identified.

Materials and Methods

Status of Amphibians

During 1989–2001, we conducted extensive fieldwork to document population levels of Puerto Rican amphibians and to evaluate predictors of populations fluctuations. Since 1988, approximately 3400 h have been spent searching for three endemic species (*Eleutherodactylus karlschmidti*, *E. jasperi*, and *E. eneidae*) that had not been observed since 1976, 1981, and 1990, respectively. We surveyed, by day and night, all known localities and adjacent areas where habitat seemed suitable for these three species in the Caribbean National Forest (hereafter referred to as El Yunque), the Carite-Guavate State Forest area (including forested areas in Cayey, Jájome alto), and Aguas Buenas caves (see Joglar, 1998 for geographical distribution of all species). Depending on the type of terrain, transects of various lengths (20–100 m) were established to monitor 11 extant populations of eight other species at five different localities. Transect surveys were conducted biweekly from 1990–1992 and monthly thereafter. Surveys consisted of walking transects by night during which frogs were counted, identified to species, and categorized by sex and age; the exception was *E. gryllus* and *E. unicolor*, for which acoustic surveys were performed. Relative abundance of frogs was calculated as a ratio of the total number of individuals seen active over the area of the transect sampled (length × 3 m wide). In addition, three other species (*E. richmondi*, *E. wightmanae*, and *E. hedricki*) were monitored at least once per month by determining their presence or absence in sites within their historical geographical range. Population fluctuations were examined by performing time-series trend analysis of the mean relative abundance over time (years), then using a simple linear regression to calculate if time was a significant predictor of the variation of anuran abundance in the Puerto Rican forests. Only adult individuals were considered in all the analyses of population data. If juveniles were included in the analyses, then no significant trends were observed and population fluctuations appeared erratic. Inclusion of juveniles introduces a sampling error associated with the difficulty in finding juveniles in the field and/or because juvenile abundances vary seasonally.

Description of Study Sites

We used the following census sites. The Elfin Forest site at El Yunque is a lower montane wet forest in the Holdridge system (Ewel and Whitmore, 1973). Our transects are set at an altitude of 850 m near the Mount Britton Tower (18° 18′ 2.9″ N; 65° 47′ 35.5″ W). The Palo Colorado Forest at El Yunque is part of the subtropical wet forest association (Ewel and Whitmore, 1973) (its name is related to the abundance of the native tree [*Cyrilla racemiflora*]). Our transects were located at an elevation of 661 m, near the field station of the University of Puerto Rico (18° 18′ 5.8″ N; 65° 47′ 7.4″ W). The Carite-Guavate forests are mostly in the subtropical wet forest life zone, except for the highest peaks that are lower montane wet forest (Ewel and Whitmore, 1973). The transects were at an altitude of 600 m (18° 5′ 28.7″ N; 66° 1′ 51.6″ W), but we visited sites within an elevational range of 400–750 m in search for *E. jasperi*. Although a large part of this forested area is protected as a state park, the land is fragmented by private homes and is thus more susceptible to human influence than El Yunque. Lowland habitats were within the metropolitan area of San Juan. The altitudinal range in these areas is 0–200 m, and the habitat included pasture, secondary growth forest with an abundance of exotic plant species, and urban landscape.

Disease Diagnosis

To determine the occurrence of chytrid fungus and other potential diseases in endemic species of Puerto Rican anurans, we examined 106 individuals histologically. Preserved specimens collected from 1961–1978 were obtained from the University of Kansas Natural History Museum (39 specimens), University of Puerto Rico at Mayagüez (13 specimens), and the private collections of Richard Thomas and Rafael Joglar (17 specimens). We also performed histology diagnoses on skin rolls and cultured organ tissues of 37 individuals captured between 500 and 1000 m at El Yunque in 2000. Because introduced species are considered possible vectors of diseases (Leighton, 1995; Lips, 1999), we examined skin tissue and organs from individuals of *Bufo marinus*, a species introduced to Puerto Rico around 1920. Eight species were studied, including seven endemic *Eleutherodactylus* (*E. coqui*, *E. portoricensis*, *E. eneidae*, *E. gryllus*, *E. karlschmidti*, *E. jasperi*, *E. richmondi*) and *B. marinus*. The live anurans included toads *B. marinus*, *E. coqui*, and *E. portoricensis*.

Live frogs were shipped via overnight courier or hand carried to the National Wildlife Health Center in Madison, Wisconsin, for examination. Upon receipt, individuals were weighed, sedated with tricaine methanesulfonate (MS-222), and dissected. Once the general health condition of the animal was assessed, we obtained samples from internal organs for tissue cultures. We focused on isolation and/or identification of infectious agents that have been associated with amphibian population declines and mortality events in North America. These included, but were not limited to, chytrid fungus (Chytridiomycota), ranaviruses (Iridoviridae), clamydia, and helminths (including acanthocephalans). Skin from hind limb digits ("toes") and the pelvic patch on the abdomen were examined histologically from all individuals. From the 37 live anurans examined, we analyzed visceral organs from 31 (83%), virus cultures from 36 (97%), and bacterial cultures from 27 (73%).

Climate Change

The relationship between climate and Puerto Rican amphibian declines has been explored recently by Alexander and Eischeid (2001) and Stallard (2001). Both studies found that although fluctuations in temperature and precipitation were warmer and drier during the period of amphibian declines, they were not beyond the range of normal variability and, thus, cannot be considered a direct cause of decline. Alexander and Eischeid (2001) used satellite data from the entire island, and Stallard (2001) analyzed data from eastern Puerto Rico. Herein, we analyze climate data obtained from ground-based weather stations in the vicinity of the transects where we recorded amphibian population densities for the past 10–12 yr. Our data were collected to profile the relationship of local climate and amphibian population fluctuations.

Climate data were obtained from the National Oceanic and Atmospheric Administration (NOAA). For El Yunque, we analyzed daily temperature and precipitation from Pico del Este Station (18° 16′ N, 65° 46′ W, 1051 m elevation) for the years between 1970–2000. At the Carite-Guavate Forest, where the endemic *E. jasperi* were last observed in 1981, the only data set available from the Guavate weather station (18° 07′ N, 66° 04′ W, 780 m elevation) was precipitation from 1970–1994. Total monthly precipitation, as well as yearly and monthly averages of precipitation and temperatures, were calculated from daily readings. We used minimum daily temperature data in all analyses because Puerto Rican *Eleutherodactylus* are active at night. Time series and regression analysis were used to determine trends in temperature and precipitation over the last 30 yr. We performed a Runs Test to check for randomness in the distribution of precipitation through time; graphic analyses of departures from the mean (anomalies) were used to distinguish years that potentially were stressful. Climate conditions were considered potentially stressful when they were above or below the 95% confidence interval of the mean. In cases where weather data appeared grouped (similar for consecutive years), a Kruskal-Wallis test for equality of medians among groups was performed. Stewart (1995) found that anuran declines in 1984–1989 at El Yunque (El Verde field station), were correlated with the distribution of rainfall and not total monthly precipitation. Thus, we tabulated the number of consecutive days without rain per year for El Yunque (Pico del Este Station) during the period of 1970–2000. We followed Stewart (1995) in considering $\leq$3.0 mm of rainfall as a dry day and defined dry periods as time lapses with five or more consecutive days with $\leq$3.0 mm of rainfall. Finally, because El Niño Southern Oscillation Events have been associated with climatic changes hypothesized to be responsible for amphibian declines in the Costa Rican mountains (Pounds and Crump, 1994), we compared climatic patterns between El Niño and non-El Niño years in Puerto Rico. All statistics were performed with Minitab version 12 (1998) at $\alpha = 0.05$.

Results

Status of Amphibians

In spite of intensive efforts to find *E. karlschmidti*, *E. jasperi*, and *E. eneidae*, these species have not been found since 1976, 1981, and 1990, respectively, and are presumed extinct. Other researchers have also searched for, but not found, these three species (Moreno, 1991). Populations of *E. richmondi* and *E. wightmanae* that were abundant in the late 1970's through 1982 at the UPR Biological Station at El Yunque (661 m) disappeared from this site in 1987 and 1991, respectively. These two species and *E. locustus* have not been observed at higher elevations (850 m) at the Elfin forest of El Yunque since 1991. An analysis of the long-term population data for species active in transects at the Elfin forest of El Yunque varies by species when compared to relative abundances from 1990–1996 (Joglar and Burrowes, 1996): *Eleutherodactylus unicolor* continues to be stable; populations of *E. coqui* and *E. portoricensis* have increased; and the arboreal bromeliad-dweller, *E. gryllus*, continues to decline (Fig. 1). In the Palo Colorado Forest, approximately 200 m lower than the Elfin Forest of El Yunque, populations of *E. coqui* and *E. portoricensis* are still declining (Fig. 1).

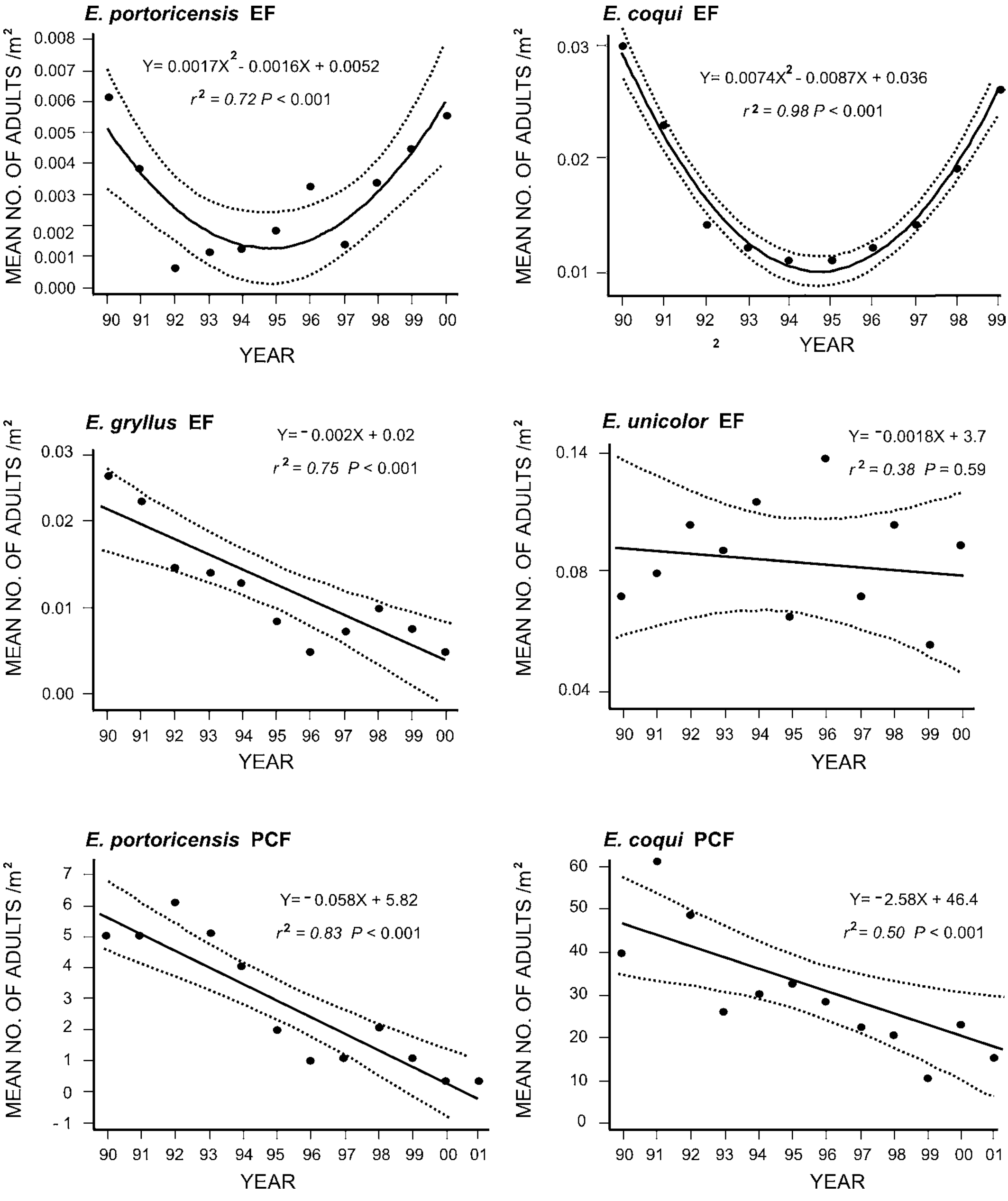

FIG. 1.—Relative abundance over time of six different populations of four species of *Eleutherodactylus* at two localities in El Yunque, Puerto Rico: Elfin Forest (EF); and Palo Colorado Forest (PCF).

Amphibians appear to be stable at low elevations and at mid-elevations (up to 400 m) in forested areas that have not been conserved as national parks. We found no trend in abundances of *E. coqui*, *E. cochranae*, and *Leptodactylus albilabris* in the lowlands (San Juan, approximately 0 m), and the cave-dwelling frog, *E. cooki*, remains stable in San Lorenzo (300 m). Quantitative population data are not available for *Bufo lemur*, the endemic Puerto Rican toad.

TABLE 1.—Summary of species examined and diagnosed diseases during an on-going study to determine potential illnesses involved in declining amphibian populations from Puerto Rico. The numbers in parentheses indicate the number of frogs. With the exception of the chytrid fungus, all diseases or parasites were found in cultures from live frogs collected in 2000.

Species	No. of specimens examined	Collection dates	Localities	Diseases or parasites
Bufo marinus	8	1961 (1)	El Yunque	*Salmonella* (3)
		1978 (1)	El Verde	Amoebic enteritis (2)
		2000 (6)	Humacao Wildlife Reserve	Polyoma-live virus in liver (2)
				Trematodes in intestine (3)
				Gastric granulomas due to *Physocephalus*-like nematodes (1)
				Myxozoans in gall bladder (1)
				Protozoan cysts in spleen (1)
Eleutherodactylus coqui	28	1978 (9)	El Yunque	Chytrid fungi (*Batrachochitrium dentrobatidis*) in skin (2)
		2000 (19)	El Yunque (13)	*Rhabdias* lung-worms (7)
			Río Abajo Forest (6)	Pinworms (8)
				Acanthocephalans (2)
				Gastric granulomas due to *Physocephalus*-like nematodes (6)
E. portoricensis	20	1977 (5)	El Yunque	*Rhabdias* lung-worms (1)
		1978 (3)	El Yunque	Pinworms (2)
		2000 (12)	El Yunque	Acanthocephalans (2)
				Gastric granulomas due to *Physocephalus*-like nematodes (1)
E. eneidae	17	1976 (3)	El Yunque	Gastric granulomas due to *Physocephalus*-like nematodes (1)
		1961 (8)	El Yunque	
		1974 (4)	Toro Negro	
		1976 (1)	Toro Negro	
		1979 (1)	Toro Negro	
E. gryllus	6	1965	El Yunque	None
E. karlschmidti	10	1961 (3)	El Yunque	
		1962 (1)	El Yunque	
		1963 (5)	Guavate Forest	
		1976 (1)	El Yunque	Chytrid fungi (*B. dentrobatidis*) in skin (1)
E. locustus	7	1976	El Yunque	None
E. richmondi	5	1975 (2)	El Yunque	None
		1982 (3)	Toro Negro	
E. jasperi	5	1974	Carite Forest	None

Disease Diagnoses

The most significant finding was the occurrence of a pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (Longcore et al., 1999), in the skin of two preserved *E. coqui* collected January 1978, in El Yunque at 500 m and in the last known specimen of *E. karlschmidti* also collected at El Yunque in 1976 (Table 1). Chytrid fungus was found in the skin of the pelvic patch, toes and ventral thighs of specimens. In the skin of infected frogs, we saw sloughing epidermal skin and three layers of chytrids with sporangia and discharge tubes infiltrated in the stratum corneum.

No viruses were isolated in cultures, and there was no histological evidence of virus infections in any *Eleutherodactylus* (Table 1). Two (33.3%) live *B. marinus* had subtle abnormalities in their livers that were suggestive of a novel virus infection. Two isolates of the pathogenic bacterium, *Salmonela aberdeen* and *S. miami*, were found in the livers of three of five live *B. marinus*. Numerous other gram-negative bacilli were isolated from the cloacae of the toads and *Eleutherodactylus*, but these bacilli were considered nonpathogenic components of normal gut flora in anurans. A variety of protozoan, acanthocephalan, and helminthic parasites were found in the anurans (Table 1), but none was considered a heavy infection or a likely cause of mortality. The two parasitic infections that caused the most concern were intestinal amoebiasis in *B. marinus* only, and unidentified species of acanthocephalan worms in the stomachs and intestines of *E. coqui*, and *E. portoricensis* (Table 1). Because of the propensity for acanthocephalan worms to penetrate and perforate the wall of the stomach and intestine, infections by this parasite may cause secondary, life-threatening bacterial infections (gastritis, enteritis, and peritonitis). Some anurans in our study (Table 1) had mild infections by "lungworms" (*Rhabdias* sp.), oxyurids ("pinworms,"), and unidentified intestinal trematodes ("flukes,"). No evidence of gross morphological problems in the gonads was observed.

Climate Change

General description.—Analysis of mean monthly temperature and rainfall data from 1970–2000 revealed a few generalizations for El Yunque (Pico del Este Station) (Fig. 2A–B). First, January–April are characterized as being cool and dry, with temperatures of 14.5–15.5 C and total monthly precipitation <300 mm. Second, May is a transition month between the cool/dry season and the warm/wet season, when temperatures rise ($\bar{x} \pm$ SD = 16.58 ± 0.68 C) and total precipitation achieves the first peak ($\bar{x} \pm$ SD = 467.1 ± 221.6 mm). Third, summer and fall months (June–November) are the warmest with mean temperatures between 17.5–18.0 C, and increasing precipitation that peaks again in October and November with mean total monthly rainfall ranging between 460–494 mm. Fourth, December is a transition month between the warm/wet and the cool/dry seasons, with milder temperatures ($\bar{x} \pm$ SD = 16.7 ± 0.75 C), and decreased total precipitation ($\bar{x} \pm$ SD = 393 ± 171.4 mm). The rainfall pattern in Carite-Guavate Forest is similar to El Yunque, although total precipitation is lower than in El Yunque for every month, and the May peak is not as high (Fig. 2C). The mean total monthly precipitation per year is 205.3 mm

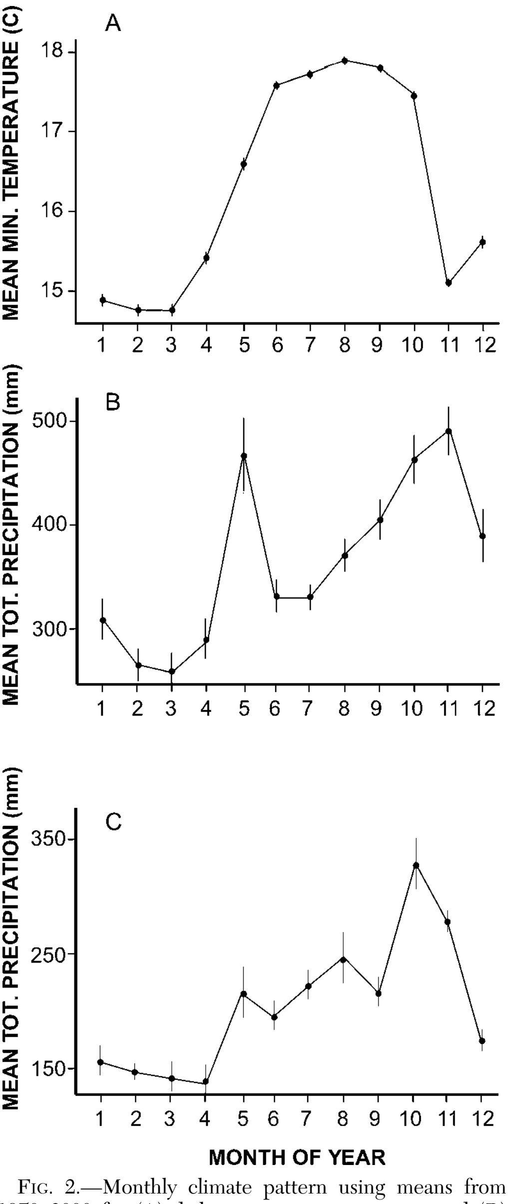

FIG. 2.—Monthly climate pattern using means from 1970–2000 for (A) daily minimum temperature and (B) total precipitation at El Yunque (Pico del Este Station); (C) mean total precipitation per month from 1970–1994 at the Carite-Guavate Forest (Guavate Station). Solid circles with extending lines represent means ± SE.

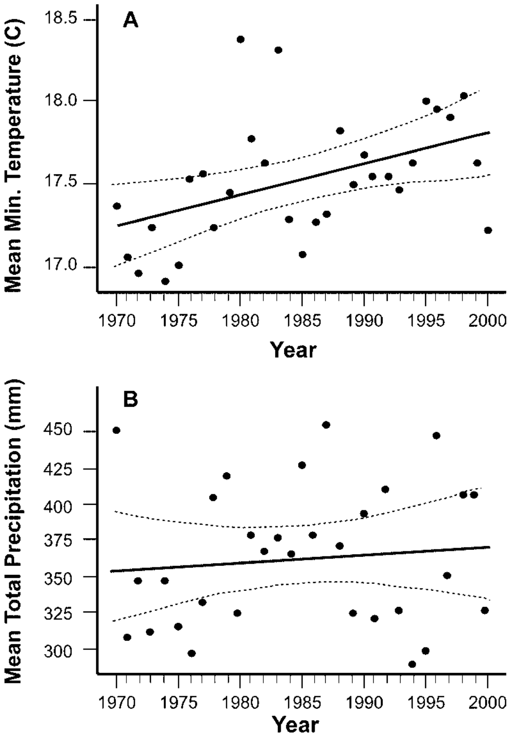

FIG. 3.—(A) Mean minimum daily temperature per year showing a significant warming trend (Y = 0.02X + 17.18, $F = 6.96$, $P = 0.013$, $r^2 = 0.29$), and (B) mean total monthly precipitation per year (ns) at El Yunque, Puerto Rico.

(± SD = 59.4) and the years of 1979–1983 were distinguished as a significantly drier than consecutive years before (1970–1978) or after (1984–1994) (Kruskall Wallis $H = 6.69$, df = 2, $P = 0.03$).

Climate analyses.—The period of 1970–2000 encompasses anuran abundance (1970–1974), when three species were no longer observed (1976, 1981, 1990), and when populations of other species declined (1991–present). A regression of minimum daily temperature per month over time revealed that temperature increased significantly each month from May–October (Y = 0.02X + 17.18, $F = 6.96$, $P = 0.013$, $r^2 = 0.29$), which corresponds to the warm/wet season and peak of amphibian activity in Puerto Rico (Fig. 3A). Time series analysis showed a greater but nonsignificant variation in total precipitation than in temperature over time (Fig. 3B). Because May is the onset of the rainy season, it represents a critical month for amphibian biology in Puerto Rico. Rainfall in May showed a clustered series of precipitation anomalies (Runs Test, $P = 0.003$) not seen in any other month (Fig. 4A). A Kruskal-Wallis test for equality of medians between these periods was significant ($H = 18.79$, df = 2, $P < 0.01$) and provided similar Z values (Z = 2.15 and 2.16) for the two dry periods. An average of nine dry periods (lapses of five or more consecutive days with ≤3 mm of rainfall) per year occurred at El Yunque from 1970 to 2000 (Fig. 4B). The years of 1974, 1976–1977, 1981, 1987, 1989, 1991–1992 and 1994 had 11 or more dry periods, which is above the 95% confidence interval. El Niño years were not associated with significant changes in temperature or precipitation for a given year. However we did find a significant difference in the mean number of dry periods between El Niño and non-El Niño years (ANOVA: $F_{1,\,30} = 11.13$, $P = 0.003$), suggesting that El Niño was associated more frequently with events of drought in Puerto Rico ($\bar{x}$ ± SD = 13 ± 3 dry periods).

DISCUSSION

Three species of anurans (*E. karlschmidti*, *E. eneidae* and *E. jasperi*) have disappeared from Puerto Rico in the past 26 yr. Populations of another six species of *Eleutherodactylus* (*E. locustus*, *E. richmondi*, *E. gryllus*, *E. wightmanae*, *E. portoricensis* and *E. coqui*) are declining in parts of El Yunque, one of the best protected forests on the island. Among the diverse potential pathogens found in the 106 anurans examined (Table 1), *B. dendrobatidis* is of most concern because it can be lethal to anurans (Berger et al., 1998), and it has been associated with die-offs and extirpations in many parts of the world. Our analysis of weather data provides insight on how climate patterns acting synergistically with disease agents, may be associated with amphibian declines in Puerto Rico.

Yearly activity and reproduction of *Eleutherodactylus coqui* at El Yunque is highly correlated with the increase in temperature and precipitation described in Fig. 3A–B (Joglar and Burrowes, 1996; Stewart and Pough, 1983; Townsend and Stewart, 1994), suggesting that these tropical frogs are sensitive to subtle climate changes. Precipitation is especially important because amphibians lose

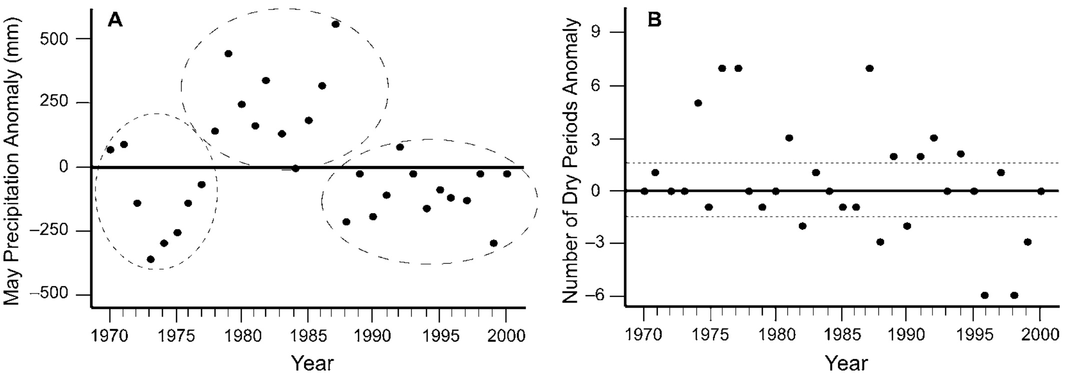

FIG. 4.—(A) Anomalies in total precipitation in May. The line corresponds to the mean over 30 yr, $\bar{x}$ = 468.6 mm, 221.6 SD, the dotted circles indicate significant clusters. (B) Anomalies in the number of dry periods per year (the line corresponds to the mean over 30 yr, $\bar{x}$ = 9.1 periods, 2.11 SD) at El Yunque Puerto Rico.

water through their skin, and their kidneys are unable to conserve water by producing concentrated urine (Shoemaker et al., 1992). The *Eleutherodactylus* species in Puerto Rico are mesic-adapted, terrestrial species that rely on the condensation of moisture on vegetation to rehydrate (Van Berkum et al., 1982). Pough et al. (1983) found that, during dry nights, males of *E. coqui* assume water conservation postures that allow them to reduce evaporative water loss. After five days of drought, however, juveniles die (Stewart, 1995), while adult males remain in their retreat sites and miss opportunities to feed, reproduce, and defend their territories (Pough et al., 1983). Extension of the dry season is another aspect of climate change that has been suggested to affect tropical amphibians (Donnelly and Crump, 1998) and has been associated with the extinction of *B. periglenes* in Costa Rica (Crump et al., 1992). A reduction of the May rainfall peak (Fig. 4A) results in an extension of the dry season in Puerto Rico that may stress *Eleutherodactylus* species, affecting their reproductive activity and recruitment. An increase in the frequency of dry periods and prolonged dry seasons during the mid 1970's and the 1990's (Fig. 4A–B) potentially are associated with the extinction of *E. karlschmidti* by 1976 and the declines of other *Eleutherodactylus* thereafter (Fig. 1).

Accurate population data for *E. jasperi* before it disappeared in 1981, does not exist. During 1973–1975, G. Drewry (unpublished) estimated the population to be between 1000–2000 individuals (Diaz, 1984). The last known location for *E. jasperi* was an 100-km^2 area south of Cayey, comprising parts of the Carite-Guavate Forest. This species fits the pattern described for montane amphibians with small, genetically homogeneous populations that tend to be narrowly adapted to the temperature and humidity regime of their altitudinal range (Brattstrom, 1970). Significantly drier years from 1979–1983 may have had severe consequences for this small ovoviviparous frog that was restricted to bromeliads and dependent on the water accumulated in the axils.

The mechanism by which cutaneous chytridiomycosis kills adult frogs is not yet known. The thickening of the stratum corneum in response to the inflammatory reaction caused by the chytrid fungus may decrease efficient absorption of water, especially when it infects the pelvic patch, an important area for rehydration in most anurans (Berger et al., 1998). During consecutive days without rain at El Yunque, *E. coqui* depends on the absorption of dew through the pelvic patch to survive (Pough et al., 1983). Thus, frogs infected by chytrid fungus on their pelvic patch would be more vulnerable during dry periods than at times when they can obtain water directly from rainfall. The presence of this pathogen in Puerto Rico at approximately the same time that anuran populations declined and extirpations began, and when the climate was significantly drier than average, offers a likely etiology for the declines and disappearances of the amphibians. We hypothesize that drought-stressed *Eleutherodactylus* species that become infected by chytrids are more likely to die from the disease due to their inability to uptake water.

In conclusion, we propose the "climate-linked epidemic" hypothesis postulated by Pounds and Crump (1994) as the best explanation for the declines of amphibians in El Yunque, Puerto Rico. This hypothesis suggests that, when climate change creates suboptimal temperature and humidity regimes, amphibians suffer a negative impact on their behavior and energy budget. As a consequence, populations tend to move from a dispersed to a clumped distribution which makes them more vulnerable to disease. The earliest record of chytrid fungus affecting amphibians at El Yunque is 1976, which is one of the driest years in the past three decades. We hypothesize that in the years 1974–1978 the *Eleutherodactylus* species that became stressed for water tended to move to more humid microhabitats where the chytrid fungus was likely to be found. Frogs that became contaminated with the fungus were unable to rehydrate efficiently during consecutive days without rain, died, and spread the disease to nearby neighbors. In the case of *E. karlschmidti*, a stream dweller, clumping along shallow pools of water in nearly dried-out streams could have caused the rapid disappearance of the species after being abundant until 1974 (Joglar, 1998). Carey (1993) suggested that environmental changes responsible for amphibian declines do not need to be severe. The unusually dry years and the warming trend in El Yunque, Puerto Rico, most likely represent sublethal climatic fluctuations to the anurans. However, these changes acting synergistically with other factors such as increased concentration of pollutants (Stallard, 2001) or a clumped distribution, could stress amphibians sufficiently to compromise their immune systems (Carey and Bryant, 1995). Subsequent infection with a pathogen, like the chytrid fungus, is likely to result in death. Recovery of populations of *E. coqui* and *E. portoricensis* only at the Elfin Forest of El Yunque in the last five years, represents an unclear population effect (Fig. 1). Davidson et al. (2003) showed that susceptibility to chytridiomycosis may vary within a species. Our current research on the population, ecological, seasonal, and ontogenetic correlates to chytrid infections in Puerto Rico may shed light to our understanding of the biology of this pathogen and its relation to amphibian declines.

Resumen

Hemos estudiado 11 poblaciones de ocho especies de *Eleutherodactylus* en Puerto Rico desde 1989 hasta 2001. Estimamos abundancia relativa de ranas activas en transectos establecidos en el Bosque Nacional del Caribe (El Yunque), el Bosque de Carite-Guavate, San Lorenzo y áreas bajas en la vecindad de San Juan. Tres especies, *E. karlschmidti*, *E. jasperi* y *E. eneidae*, se presumen extintas y ocho poblaciones de otras seis especies de *Eleutherodactylus* están disminuyendo significativamente en elevaciones mayores a 400 m. De los varios factores que se consideran causantes de las declinaciones de anfibios alrededor del mundo, nos concentramos en cambios climáticos y enfermedades. Analizamos datos de temperatura y precipitación entre los años 1970–2000, para determinar el patrón general de variaciones, y las desviaciones inusuales que estuvieran asociadas a las declinaciones de anfibios. Examinamos tejido de 106 especímenes de museos colectados entre 1961–1978 y de ranas vivas que capturamos en 2000. Encontramos el hongo quítrido en dos especies de El Yunque colectadas entre 1976 y 1978, representando este, el primer informe de quitridiomicósis para el Caribe insular. Análisis de datos climatológicos reflejaron una tendencia significativa al aumento de temperatura y una asociación entre años con períodos de sequía extendidos y las declinaciones de anfibios. La década de los 70 y los 90, que representan los períodos de declinaciones de anfibios en Puerto Rico, fueron significativamente más secas. Sugerimos una posible interacción sinergística entre la sequía y la patogenicidad del hongo quítrido en los anfibios.

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Appendix I

List of preserved specimens examined for chytrid fungi and other potential diseases. Specimens were borrowed from KU = University of Kansas Natural History Museum, RUM = University of Puerto Rico–Recinto Universitario de Mayagüez, RT = Richard Thomas' private colletction; RLJ = Rafael L. Joglar's private collection. Specimens positive for chytrid fungi are indicated with an asterisk (*).

Species	Sex	Catalog number	Collection date	Locality in PR	Elevation (m)
Bufo marinus	F	KU 180385	2 Jan 78	2 Km NE of Rt 960 on Rt 186 in Caribbean National Forest	NR
B. marinus	F	KU 264224	19 Apr 61	3 miles S of El Verde	NR
Eleutherodactylus coqui	F	KU 180414	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180415*	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	F	KU 180516	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180422	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180423	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180424	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180438*	5 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	M	KU 180439	5 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. coqui	F	KU 180440	5 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
Eleutherodactylus eneidae	F	KU 278155	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	F	KU 278156	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	ND	KU 278157	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	ND	KU 278158	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	ND	KU 278159	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	F	KU 278160	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	F	KU 278161	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	ND	KU 278162	15 May 61	Area Recreo La Mina, 11.8 km, S of Palmer	NR
E. eneidae	ND	RT 6461	1976	12 km S Mameyes, El Yunque	NR
E. eneidae	F	RT 2220	1974	Toro Negro Forest, Central Cordillera	NR
E. eneidae	ND	RT 2221	1974	Toro Negro Forest, Central Cordillera	NR
E. eneidae	ND	RT 2223	1974	Toro Negro Forest, Central Cordillera	NR
E. eneidae	ND	RT 2224	1974	Toro Negro Forest, Central Cordillera	NR
E. eneidae	ND	RUM 6311	1979	El Yunque	NR
E. eneidae	ND	RUM 6542	1976	El Yunque	NR
E. eneidae	ND	RUM 6543	1976	El Yunque	NR
E. eneidae	ND	RUM 6544	1976	El Yunque	NR
Eleutherodactylus gryllus	ND	KU 279282	26 July 65	10.3 km E of La Pica	1185
E. gryllus	ND	KU 279283	26 July 65	10.3 km E of La Pica	1185
E. gryllus	ND	KU 279286	8 July 65	13.7 km N of Sabana Grande	NR

APPENDIX I

Continued.

Species	Sex	Catalog number	Collection date	Locality in PR	Elevation (m)
E. gryllus	ND	KU 279287	8 July 65	13.7 km N of Sabana Grande	NR
E. gryllus	ND	KU 279288	8 July 65	13.7 km N of Sabana Grande	NR
E. gryllus	F	KU 279289	17 Mar 65	10.6 km SE of Villa Perez	1030
Eleutherodactylus karlschmidti	F	KU 281243	15 May 61	Area Recreo La Mina, 11.8 km S of Palmer	NR
E. karlschmidti	F	KU 281244	15 May 61	Area Recreo La Mina, 11.8 km S of Palmer	NR
E. karlschmidti	ND	KU 281294	10 Jun 63	Bosque de Guavate, 8 km from Las Cruces	630
E. karlschmidti	ND	KU 281295	10 Jun 63	Bosque de Guavate, 8 km from Las Cruces	630
E. karlschmidti	M	KU 281332	16 May 61	12 km S of Palmer, on path to El Yunque	NR
E. karlschmidti	F	KU 281350	3 Sep 62	2.2 km SW of Sabana	300
*E. karlschmidti**	ND	RT 3689	1976	Hwy 191 near El Toro, El Yunque	NR
E. karlschmidti	ND	RUM 6004	~1963	El Yunque, PR	NR
E. karlschmidti	ND	RUM 6805	~1963	El Yunque, PR	NR
E. karlschmidti	ND	RUM 6806	~1963	El Yunque, PR	NR
Eleutherodactylus portoricensis	ND	KU 180537	29 Dec 77	Humacao, 10 km S of Palmer on Rt 191, El Yunque	455
E. portoricensis	ND	KU 180538	29 Dec 77	Humacao, 10 km S of Palmer on Rt 191, El Yunque	455
E. portoricensis	M	KU 180539	29 Dec 77	Humacao, 10 km S of Palmer on Rt 191, El Yunque	455
E. portoricensis	ND	KU 180540	29 Dec 77	Humacao, 10 km S of Palmer on Rt 191, El Yunque	455
E. portoricensis	M	KU 180541	29 Dec 77	Humacao, 10 km S of Palmer on Rt 191, El Yunque	455
E. portoricensis	M	KU 180542	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. portoricensis	F	KU 180543	1 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
E. portoricensis	F	KU 180544	5 Jan 78	Humacao, Big Tree Trail, Caribbean National Forest	500
Eleutherodacatylus jasperi	ND	RUM 6429	1974	Carite Forest, Cayey	NR
E. jasperi	ND	RUM 6430	1974	Carite Forest, Cayey	NR
E. jasperi	ND	RUM 6431	1974	Carite Forest, Cayey	NR
E. jasperi	ND	RUM 6436	1974	Carite Forest, Cayey	NR
E. jasperi	ND	RUM 6439	1974	Carite Forest, Cayey	NR
Eleutherodactylus richmondi	ND	RT 2157	1975	El Yunque	NR
E. richmondi	ND	RLJ B-1	1982	University of Puerto Rico Field House, El Yunque	NR
E. richmondi	ND	RLJ FNA32	1982	University of Puerto Rico Field House, El Yunque	NR
E. richmondi	ND	RLJ FNA56	1982	University of Puerto Rico Field House, El Yunque	NR
E. richmondi	ND	RUM 2309	1975	El Yunque	NR
Eleutherodactylus locustus	ND	RT 6454	1976	El Yunque	NR
E. locustus	ND	RT 6455	1976	El Yunque	NR
E. locustus	ND	RT 6456	1976	El Yunque	NR
E. locustus	ND	RT 6457	1976	El Yunque	NR
E. locustus	ND	RT 6458	1976	El Yunque	NR
E. locustus	ND	RT 6459	1976	El Yunque	NR
E. locustus	ND	RT 6460	1976	El Yunque	NR