Mass Mortality and Population Declines of Anurans at an Upland Site in Western Panama

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Abstract: *I report an episode of anuran mortality and decline in the Reserva Forestal Fortuna, Chiriquí, Panama. The symptoms of decline at this site include population reductions, the presence of dead or dying adults, and tadpole abnormalities. Streamside anurans were abundant and diverse in 1993–1995, were restricted to a few streams in December 1996–January 1997, and then became rare throughout the reserve in July–August 1997. Between December 1996 and January 1997, I found 54 dead or dying frogs belonging to 10 species, and 12% of tadpoles had abnormalities of the oral disc. In July–August 1997 I monitored nine streams 37 times and captured only six individuals, whereas 13 terrestrial surveys along five trails resulted in 18 captured individuals. No dead or dying animals were found during those two months, but 11% of tadpoles had mouthpart abnormalities. Necropsies revealed that 18 of 18 dead anurans had a fungal infection of the skin; because this fungus was the only infection shared among all dead frogs, I suggest that it killed them and contributed to the decline of these populations. The presence of mouthpart abnormalities during a period of adult mortality suggests that this symptom may also be linked to the fungus infection. Clinical signs of decline in the anurans of Fortuna are similar to those found in the anurans of Monteverde and Las Tablas, Costa Rica, and I hypothesize that this pathogen was involved in the declines at all three sites.*

Mortalidad Masiva y Disminuciones Poblacionales de Anuros en un Sitio Elevado del Oeste de Panama

Resumen: *Yo reporto un episodio de declinación y mortandad masiva en la fauna anfibia de Fortuna, Chiriquí, Panamá. Los síntomas de la declinacción incluyen reducciones en la población, mortandad o mortalidad de adultos y anormalidades en los renacuajos. Los anuros ribereños eran abundantes y diversos de 1993 a 1995, luego se restringían a pocos riachuelos entre diciembre 1996 y enero 1997, y después eran muy escasos en todas partes de la reserva en julio-agosto de 1997. También, encontré 54 ranas muertas o moribundas que pertenecen a 10 especies, y el 12% de los renacuajos tenían anormalidades en el disco oral. En julio-agosto 1997, efectué 37 inspecciones en nueve quebradas pero capturé solamente seis individuos, mientras en 13 recorridos terrestres por cinco senderos resultaban en 18 capturas. No encontré animales muertos o moribundos, pero el 11% de los renacuajos tenían anormalidades del disco oral. Las necropsias revelaban que 18 de los 18 muertos tenían una infección de un hongo en la piel. Como el hongo fue la unica infección entre todos las muertas, sugiero que eso mató las ranas y también contribuyó a la declinación en las poblaciones. El patrón de declinación de los anuros en Fortuna es similar al lo que he encontrado en Monteverde y Las Tablas, Costa Rica y sugiere que el patógeno estaba involucrado en las declinacciones en los tres sitios.*

Introduction

Amphibian populations have declined or disappeared around the globe (Weygoldt 1989; La Marca & Reinthaler 1991; Crump et al. 1992; Carey 1993; Drost & Fellers 1996; Laurance et al. 1996; Lips 1998). Widespread reports of amphibian declines are troubling because amphibians are indicator species sensitive to a variety of environmental contaminants (Bradford 1989; Colborn et al. 1993; Blaustein et al. 1994; Sparling 1995; Drury et al. 1995; Cunningham et al. 1996; Kiesecker & Blaustein 1997). Concern is greatest regarding declines with no

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apparent cause or in sites located in protected reserves, such as those from Costa Rica (Crump et al. 1992; Lips 1998), Brazil (Heyer et al. 1988; Weygoldt 1989), and Australia (Laurance et al. 1996), because these declines suggest the existence of global factors that ultimately could affect human welfare. I present another case of amphibian decline in a remote, protected reserve.

Determining whether reported declines from around the globe have some common cause or whether declines at each site result from independent agents has been difficult, especially because amphibians are susceptible to many stresses. To date, all unexplained declines in the tropics (and many in the temperate zone) have occurred in upland areas, suggesting that the agent is limited to uplands or that it has a synergistic interaction with some environmental condition(s) that varies with elevation (e.g., temperature, ultraviolet [UV-B] radiation, precipitation, wind patterns).

I describe a case of mass mortality of the riparian anurans of the Reserva Forestal Fortuna in western Panama, provide survey data showing reductions in populations of these species over a 4-year period, describe abnormalities of tadpole mouthparts, and provide evidence to suggest that investigations into pathogenic etiology is most likely to identify the specific causes of these amphibian declines.

Methods

Study Area

The Reserva Forestal Fortuna (8°43′30 N, 82°14′0 W), Chiriquí, Panama, is managed by the Smithsonian Tropical Research Institute and the Instituto de Recursos Hidraulicos y Electrificación (IRHE) (Fig. 1). This reserve was created to protect the watershed of the Río Chiriquí because of the hydroelectric dam recently completed by IRHE. In 1976 the Panamanian government organized a biological survey before construction of the dam, the transisthmian highway, and oil pipeline to provide baseline data on the climate, environmental conditions, and biodiversity of the site (Addames 1977).

The reserve is within Holdridge's (1982) premontane rainforest, with elevation ranging between 1000 and 2200 m. Average annual rainfall is about 4000 mm, with reduced amounts between February and May (Addames 1977), and cloud mist contributes 2–60% of the total water input (Cavalier et al. 1996).

Because amphibians are sensitive to low pH (Sparling 1995), I measured the pH of rainfall collected behind the field station seven times between 8 and 17 January 1997. I also measured the pH of each of seven survey streams one to nine times by collecting water samples during amphibian surveys and analyzing them within 6 hours of collection with a Hanna (model HI8314c) portable pH meter. I statistically compared the pH of rainwater to that of the streams with a two-sample t test to look for evidence of habitat acidification.

Surveys

Reasons for visiting Fortuna have changed over the years, so the sampling methodology (mark-recapture, visual encounter surveys, audio surveys) and the number, type, and identity of transects have varied. On the first visit a 3-day rapid assessment of amphibian diversity was conducted. In about 30 hours of searching along two streams, two trails, and the newly formed lakeshore, two researchers identified 35 species of amphibians by sight or by ear. I remained for another 3 months (September–November 1993) to study the breeding biology of *Hyla lancasteri* and monitored riparian amphibians along a few nearby streams, but I did no terrestrial transects.

Following anuran declines at Las Tablas, Costa Rica, in 1993–1994 (Lips 1998), I began monitoring riparian and terrestrial amphibians at Fortuna to establish baseline population data. Additional transects were added every year so that the number of transects increased from two streams in 1993 to 49 in July–August 1997, for a total of 159 surveys (133 riparian and 26 terrestrial) along nine streams and five trails. I calculated Pearson product-moment correlations between capture rates and year to identify changes in amphibian abundance. I began each riparian transect just upstream from where the highway and pipeline crossed the stream. I utilized the existing trail system of the reserve for terrestrial transects, which were maintained by periodic clearing of the undergrowth by IRHE personnel.

Monitoring of all transects involved one or two people slowly walking and scanning the bank, emergent rocks, and overhanging vegetation for adult amphibians; at night we used headlamps. Healthy animals were captured by hand; identified to species, sex, and age where possible; measured (snout-to-vent length); and weighed (mass) with a spring-scale before release. I noted time and location (meter along transect, right or left bank, height over water or ground, distance from water), as well as any notable behavior or appearance. The species, age, sex, and location of dead or dying animals (December 1996–January 1997) were noted, and the bodies were frozen or preserved in either 95% alcohol or 10% buffered formalin. After 1–14 days of storage, all carcasses were transported to the United States and shipped to the Animal Health Diagnostic Center in Maryland, where diagnostic analyses were performed.

Tadpoles were dipnetted out of streams, bagged, and returned to the lab, where they were identified to species when possible (Lips & Savage 1996), staged (Gosner 1960), measured, and released.

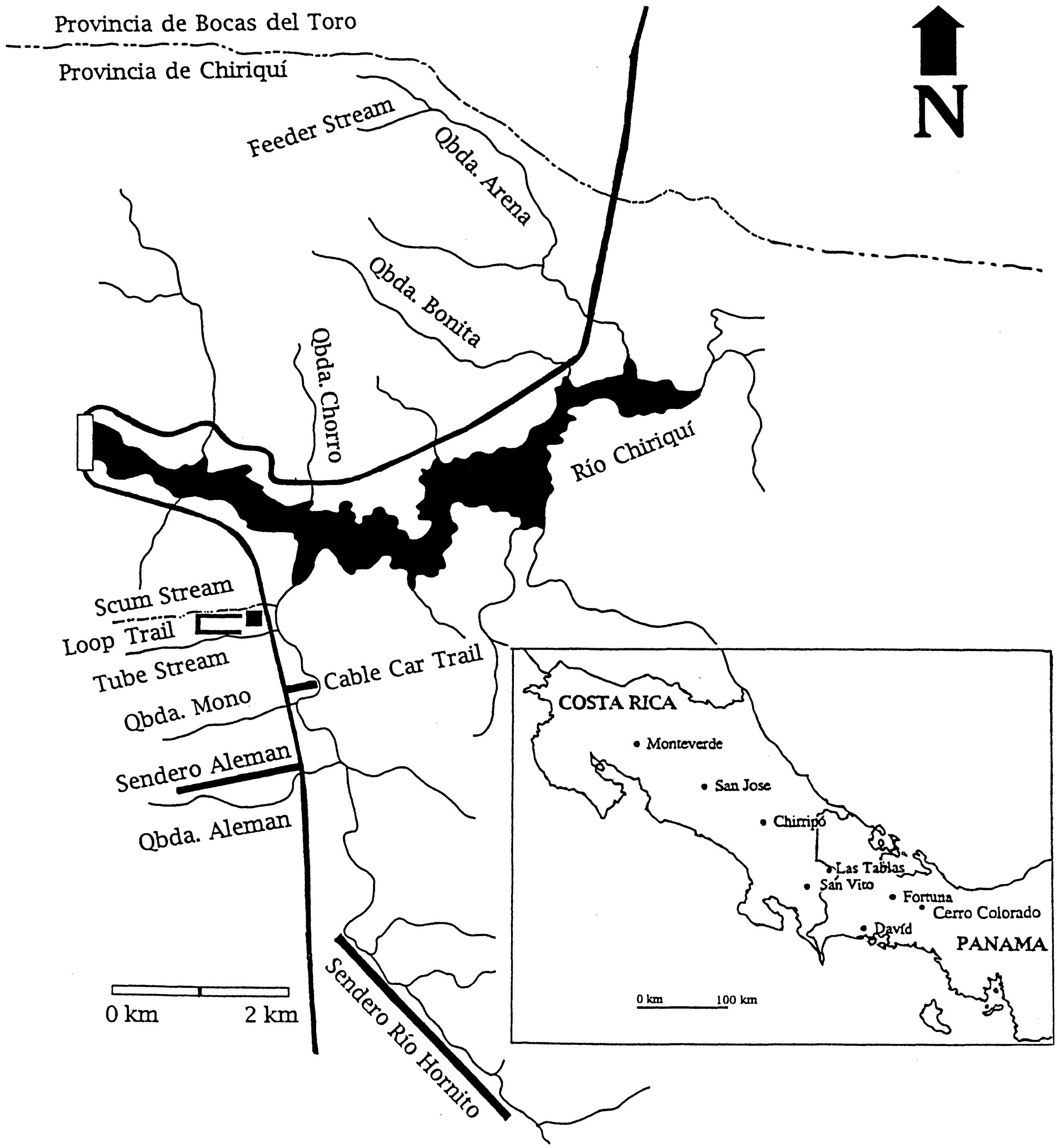

Figure 1. Map of Costa Rica and western Panama (inset) and the watershed of the Río Chiriquí and most of the Reserva Forestal Fortuna. Square indicates approximate location of station, short black lines represent terrestrial transects, riparian transects are labeled by stream name, black shading represents the lake, and the highway is represented by the long black strip.

Results

All streams were similar in acidity in December 1996–January 1997. The average pH of the 26 streams was 7.34 ± 0.22. Stream acidity over 9 days in 1996–1997 was about the same as that in 1976, when the average acidity of five streams and two rivers was 7.56 ± 0.25 (Addames 1977). Rain water (4.84 ± 0.28, $n = 7$), however, was significantly ($p < 0.001$) more acidic than stream water.

Adult Riparian Surveys

In 1993 I surveyed no trails but monitored Tube Stream 13 times and Scum Stream 16 times and found a total of 194 adult amphibians of nine species. During a brief visit in May–June 1994, I surveyed no trails but monitored Quebrada Aleman and Tube Stream twice each and captured a total of 19 individuals belonging to eight species. In December 1994–January 1995, I captured four species along four stream transects for a total of 77 adult captures, and 16 adults of seven species along three terrestrial transects. Between 28 December 1996 and 18 January 1997, I completed 48 surveys (44 riparian and 4 terrestrial) and captured 351 animals of 29 species along 33 km of transects. This included 307 captures of 24 species from seven stream transects and 44 captures of 10 species from four terrestrial transects. In July–August 1997, I surveyed nine streams 36 times and found only six individuals of five species (*Bolitoglossa lignicolor*, *Bufo coniferus*, *Eleutherodactylus cruentus*, *E. fitzingeri*, *Hyla uranochroa*). Between December 1997 and January 1998, I surveyed five streams 13 times and found one species (*Cochranella prosoblepon*). Both the number of captures per minute and the number of captures per meter have declined for all riparian transects since 1995 (Fig. 2). Correlations between year of survey and both the number of captures per minute and the number of captures per meter showed a strong, negative relationship for the six streams with 3 or more years of capture data (Table 1).

During December 1996–January 1997, I found 54 dead or dying frogs (15% of total captures) belonging to 10 species from four streams. During repeated morning visits to streams, I found most dead and dying animals "frozen" in their normal calling positions, so it appeared as if they came to the stream the previous night and died in place. Dying individuals were often lethargic and had no righting response. Some experienced convulsions and trembling of the limbs and head or were thin, but none had obvious wounds or lesions. Histological examinations of formalin-fixed amphibians revealed that 18 of 18 adult anurans were infected with a fungus (Berger et al. 1998). Dead frogs had the greatest infection, dying animals had a high infection, but the apparently healthy animals collected as controls had low levels of fungal infection (D. E. Green, personal communication). Histological sections of skin from seven anurans collected 12 months previously showed no evidence of this fungus (D. E. Green, personal communication).

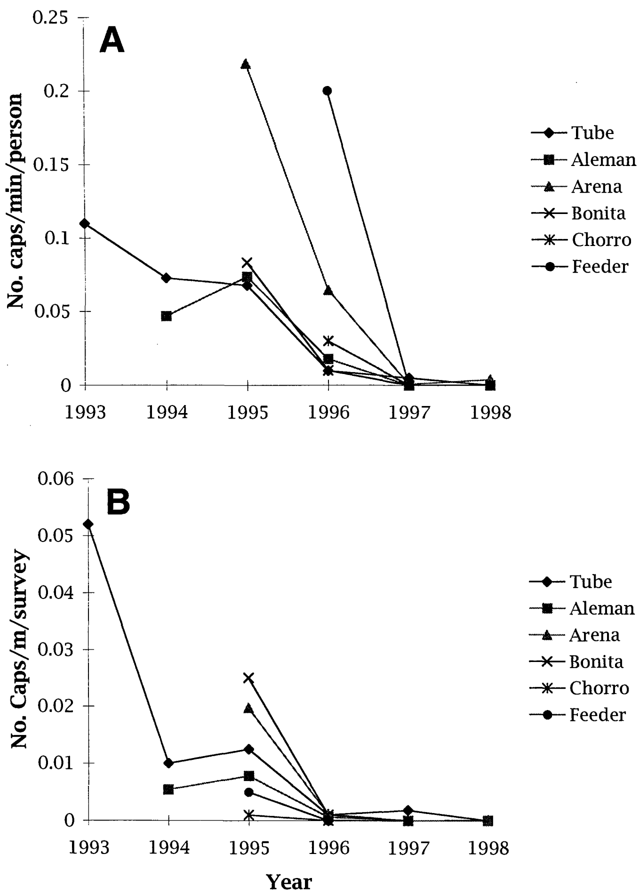

Figure 2. Average annual amphibian captures for six streams (Tube, Aleman, Arena, Bonita, Chorro, Feeder) per minute per person (a) and per meter per survey (b). All riparian transects show a large reduction in the amphibian capture rate following the event of adult mortality in December 1996.

It is not yet possible to say whether any amphibian species have disappeared from Fortuna, although some species have declined and were not seen in July–August 1997, including *Hyla colymba*, *H. debilis*, *Phyllomedusa lemur*, *Atelopus varius*, *Eleutherodactylus fleishmanni*, *E. punctariolus*, *E. rugulosus*, and *E. emcelae*.

Adult Terrestrial Surveys

Terrestrial transects were initiated in December 1994–January 1995, when three trails were surveyed four times, resulting in 16 captures of seven species (*Eleutherodactylus cruentus*, *E. gollmeri*, *E. caryophyllaceus*, *E.* sp., *Bufo haematiticus*, *Colostethus* spp., and *Rana*

Table 1. Pearson product-moment correlations of amphibian captures per minute per person and captures per meter per survey by year (1993–1997) for stream and terrestrial transects in Fortuna, Panama.

Transect	*Captures/ minute/person*	*Captures/ meter/survey*
Stream		
Tube Stream	−0.9516[a]	−0.7976
Quebrada Aleman	−0.8239	−0.8135
Quebrada Arena	−0.8955	−0.7998
Quebrada Bonita	−0.9157	−0.8831
Quebrada Chorro	−0.8660	−0.8660
Feeder Stream	−0.8660	−0.8660
Terrestrial		
Hornito	−0.8772	−0.9999[b]
Loop	−0.2352	−0.1663
Cable Car	−0.9926	−0.9999[b]

[a]$p < 0.01$.
[b]$p < 0.05$.

warzewitschii). In December 1996–January 1997, four trails were surveyed four times, resulting in 25 captures of seven *Eleutherodactylus* species (*E. cruentus*, *E. caryophyllaceus*, *E. pardalis*, *E. gollmeri*, *E. diastema*, *E. bransfordii*, and *E.* sp.). In July–August 1997, I surveyed five trails 13 times and found seven species (*Bolitoglossa colonnea*, *Oedipina complex* sp. A, *Eleutherodactylus diastema*, *E. caryophyllaceus*, *E. pardalis*, *E. podiciferus*, and *E. gollmeri*). Between December 1997 and January 1998 I surveyed two trails five times and found two species (*E. diastema* and *E. cruentus*). I never found dead frogs along the terrestrial transects, the number of species encountered remained the same, and no species had disappeared, although both the number of captures per minute and the number of captures per meter declined for four of five surveyed trails since 1994 (Fig. 3). Correlations between the year of survey and both captures per minute and captures per meter are strongly negative for the Sendero Río Hornito and Cable Car Trail, but not for Loop Trail (Table 1).

In addition to visual encounter surveys along streams and trails, I cleared three leaf litter plots (Jaeger & Inger 1994) between 13 and 30 July 1997. Each plot was intentionally placed in a relatively flat area within 5, 20, or 50 m from streams, so the chance of capturing anurans (especially *Colostethus* spp.) was high. After 145 minutes of searching an area that totaled 114 m^2 (two plots 5×5 m^2; 1 plot 8×8 m^2), I had neither seen nor captured a single animal.

Tadpoles

In 1993 I collected 434 tadpoles belonging to 11 species from Scum Stream, and 18 tadpoles of 4 species from Tube Stream. In December 1994–January 1995, 37 tadpoles of 3 species from Scum Stream and 35 tadpoles of 6 species were collected from Tube Stream. All 524 tadpoles of these 12 species had the full, normal complement of keratinized tooth rows and jaw sheaths. In December 1996–January 1997, I collected 106 tadpoles from Quebradas Aleman, Chorro, Mono, Scum, and Tube Streams. Thirteen individuals of 4 species (centrolenid sp., *Hyla uranochroa*, *H. colymba*, and *H. debilis*) showed either partial or complete loss of the keratinized mouthparts, and all five streams produced some individuals with these deformities.

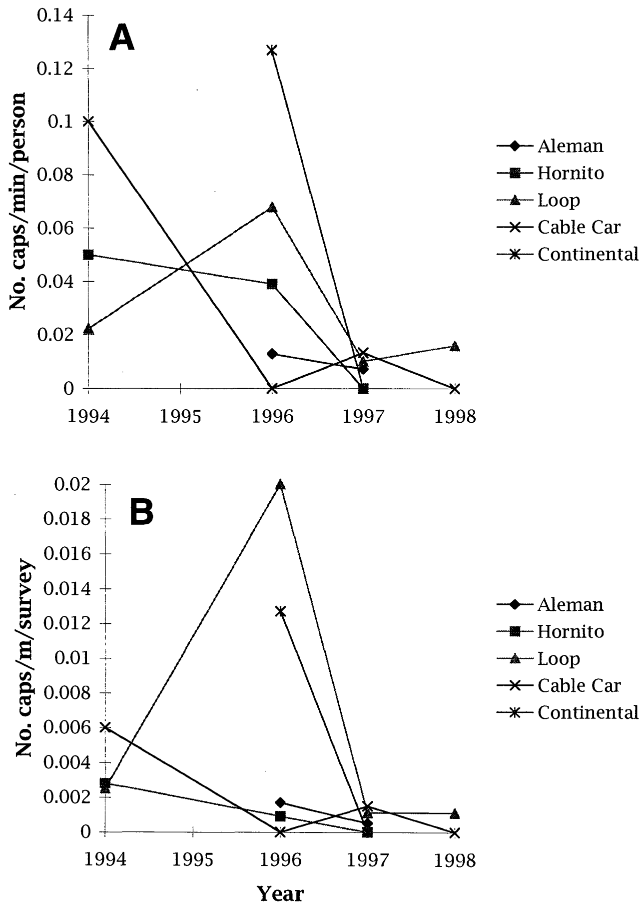

Figure 3. Average annual amphibian captures for five terrestrial transects (Aleman, Hornito, Loop, Cable Car, and Continental) per minute per person (a) and per meter per survey (b). All show reduced amphibian captures following the event of adult mortality in December 1996.

In July–August 1997, 133 tadpoles of only 6 species were found in the seven streams surveyed, and it was noticeably difficult to find large quantities of tadpoles. Haphazard netting over 3 days in Quebrada Aleman produced no tadpoles, and all streams had fewer species; for example, only *H. lancasteri* was found in Scum Stream (11 species in 1993, 3 species in 1994, and 4 species in December 1996–January 1997), only *H. urano-*

chroa was found in Tube Stream (4 species in 1993, 6 species in December 1994–January 1995, 9 species in December 1996–January 1997), and only *Colostethus* spp. was found in Quebrada Chorro (5 species in December 1996–January 1997). Despite intensive searches, no tadpoles of *H. colymba*, *H. debilis*, *H. graceae*, or *Phyllomedusa lemur* were found during July–August 1997. Of the 133 tadpoles captured from six streams, a roadside ditch, and the garbage pit, 15 (11%) partially or completely lacked keratinized mouthparts, but not all species were affected. None of the 15 *Smilisca phaeota* (garbage pit), the 25 *Bufo coniferus*, or the 39 *Rana warzewitschii* (roadside ditch) had any obvious loss of keratinized mouthparts. Four of 23 (17%) *H. lancasteri*, 5 of 6 (83%) centrolenids, and 5 of 5 (100%) *H. uranochroa* showed loss or reduction of keratinized mouthparts.

Discussion

Reserva Forestal Fortuna

Fortuna has experienced a sudden and massive decline in the abundance and diversity of anurans, especially riparian species. Following an event of adult mortality, the diversity and abundance of amphibians began to decline and tadpoles were first seen without keratinized mouthparts. The fact that I found dead frogs at all is extremely unusual. Roberto Ibañez (personal communication) and his assistants counted 24,050 frogs during diurnal and nocturnal censuses in central Panama and never encountered a dead anuran, yet 15% of my 361 captures were dead. Widespread mortality across five families and 10 species argues for a highly virulent or novel disease or for selective poisoning, or indicates that the anuran fauna has become susceptible to previously nonlethal organisms or conditions.

The combination of adult mortality along streams, tadpole mouthpart abnormalities, and reduced abundance of all riparian species suggests that the cause of death at Fortuna might be found in the water, and it supports the idea of the fungus as the cause of decline. During December 1996–January 1997, some species of the families Bufonidae (*Bufo haematiticus*, *Atelopus varius*) and Leptodactylidae (*Eleutherodactylus emcelae*, *E. rugulosus*) were found dead in large numbers and are now difficult to find throughout the reserve. All these species either spend much of their adult lives near streams or congregate there to look for mates. Other taxa, including some Leptodactylidae (*E. caryophyllaceus*), Plethodontidae (*Oedipina*, *Bolitoglossa*), and Hylidae (*H. zeteki*), were not found dead and are still present at Fortuna. These animals either lack an aquatic stage (all salamanders, all *Eleutherodactylus*) or, in the case of *Hyla zeteki*, spend their entire life in water-filled plants in the canopy. The major difference between survivors and declining species is that most riparian species have been affected, whereas those that are forest-dwellers have been less affected. The genus *Eleutherodactylus* supports this point: stream-side species such as *E. emcelae* and *E. rugulosus* have disappeared, whereas forest species such as *E. caryophyllaceus* and *E. gollmeri* are still present.

The clinical signs of the dead frogs from Fortuna were similar to those from dead and dying frogs from Las Tablas (Lips 1998). All of the dead, post-metamorphic anurans from Panama were infected with a chytridiomycete fungus (Berger et al. 1998), and some of the dead frogs from Las Tablas had a possible skin infection (Lips 1998). Chytrids are common, ubiquitous fungi found in aquatic habitats and soils, or as parasites of algae, plants, or insects. These fungi typically break down chitin, cellulose, and keratin, but none have been reported as a vertebrate parasite (Berger et al. 1998). I suggest three possible hypotheses to explain their presence in these frogs. First, an introduced species of fungus might cause widespread mortality because resident frogs would not have evolved resistance to a foreign pathogen (Leighton 1995). Stocking of exotic fishes or human or animal visitors to the reserve could have introduced an exotic fungus (e.g., Van Riper et al. 1986). Second, these fungi may have been normal residents of these tropical streams, but recent changes in the environment or their biological interactions might have caused a host shift or increased virulence. Third, the immune system of these frogs might have been compromised by changes in the environment (Carey 1993; Pounds & Crump 1994). Because only upland or montane frogs have experienced unexplained declines in tropical regions, I hypothesize that conditions or synergistic effects associated with higher elevations are involved.

Possible environmental cofactors that have been shown to affect amphibian survival and that might contribute to these declines include climate change, acid rain, and environmental contaminants. Amphibians are dependent on moisture for survival and reproduction, so changes in rainfall are a likely stress factor. For example, multiple El Niño events in recent years (Weylan et al. 1996) and deforestation have changed patterns of precipitation in many tropical areas (Pounds & Crump 1994; Laurance et al. 1996) and could stress amphibians. Rainfall was acidic at Fortuna during this episode of mortality, but it is unlikely that this was the cause of death in these frogs; there was no rain at all for the first 2 weeks of the 1996–1997 survey, yet frogs were dying in large numbers throughout the 3-week period. Also, *Hyla zeteki* spends its entire life cycle in bromeliads filled with rainwater; if the cause of death were in the rain, this species should have been affected. Environmental contaminants (e.g., agrochemicals, industrial pollution, heavy metals) could directly affect taxa of many classes

Table 2. Examples of taxonomic similarities in patterns of anuran survival and decline at the three Central American sites that have experienced amphibian declines.*

Monteverde	*Las Tablas*	*Fortuna*
Surviving		
Bufonidae		
Bufo coniferus		*Bufo coniferus*
Hylidae		
Hyla pseudopuma	*Hyla calypsa*	*Hyla lancasteri*
	H. picadoi	*H. zeteki*
H. uranochroa		*H. uranochroa*
Smilisca phaeota		*Smilisca phaeota*
Leptodactylidae		
Eleutherodactylus cruentus	*Eleutherodactylus cruentus*	
E. hylaeformis	*E. hylaeformis*	
E. diastema		*Eleutherodactylus diastema*
		E. gollmeri
		E. caryophyllaceus
Declining		
Bufonidae		
Atelopus varius	*Atelopus chiriquiensis*	*Atelopus varius*
Bufo haematiticus		*Bufo haematiticus*
B. periglenes	*Bufo fastidiosus*	
Hylidae		
Phyllomedusa lemur		*Phyllomedusa lemur*
Hyla tica	*Hyla tica*	
H. rivularis	*H. rivularis*	
		Hyla debilis
		H. colymba
		H. graceae
Leptodactylidae		
Eleutherodactylus rugulosus	*Eleutherodactylus rugulosus*	*Eleutherodactylus rugulosus*
	E. punctariolus	*E. emcelae*

**Not all species are found at all sites, but species tend to show the same response (survive or decline) at those sites where they occur. Monteverde data from Pounds et al. (1997), Las Tablas data from Lips (1998), and Fortuna data from this study.*

and phyla, consistent with observed anuran mortality at Fortuna. Accumulation of environmental contaminants could directly kill frogs or might act indirectly, weakening adults so that they became more susceptible to disease. Many of these chemicals vaporize readily, can be transported long distances via the atmosphere (Rapaport et al. 1985), and are in widespread use throughout the tropics (Colborn et al. 1993).

Lower Central America

At least three upland protected sites in lower Central America have suffered amphibian population declines in the past 10 years in a sequential and directional pattern. Based on the similarities of declines, habitats, and species affected, I hypothesize that declines at Las Tablas and Fortuna were caused by the same agent that reduced the montane amphibian fauna of Monteverde (Pounds et al. 1997). Monteverde is about 250 km to the northwest of Las Tablas and experienced a sudden unexplained decline in its anurans in 1987-1988. Because declines occurred so rapidly, no surveys or collections were made, but about 40% of anurans have declined or disappeared from Monteverde and have not been seen since (Pounds et al. 1997). Understanding the patterns of mortality and population decline among the anuran species at these three sites might explain what kind of agent is involved or its location in the environment; several species of anurans have declined or disappeared, but a few species have survived. There is remarkable similarity among both the affected taxa and the surviving species at each of these sites (Table 2), and the same species or a close relative shows the same response at two or three sites.

I hypothesize that these three examples of sudden and sequential amphibian decline resulted from the same causative agent(s), and I suggest that an aquatic, microbial pathogen is the most likely candidate. If this is true, then the timing and sequence of declines outlines directional movement among sites from the northwest to southeast, a pattern typical of disease epidemics (Laurance et al. 1997). New evidence provided by E. Lindquist (personal communication) supports this hypothesis. Between February and June 1994, Lindquist found one dead *Atelopus chiriquiensis* and one dead *Rana warzewitschii* from a few streams on the northern and eastern slopes of Cerro Pando, a site geographically intermediate to Las Tablas and Fortuna and at a time temporally intermediate to declines at those two sites. Diagnostic examination of five *Atelopus* revealed that four of five individuals were infected with the chytrid parasite (D. Green, personal communication). In December 1997, Lindquist found no amphibians of any kind along upland streams at either Cerro Pando or Las Nubes, another site located midway between Las Tablas and Fortuna.

Conclusion

I suggest that declines or disappearances in these Neotropical amphibian populations are not a series of coincidental, unrelated events but are indications of the spread of a virulent, infectious, and highly contagious disease. Necropsy data suggest that this skin parasite is the proximate cause of mortality, but the ecology of closely related chytrids suggests that additional, synergistic factors might be involved. The similarity of declines in lower Central America and Australia (Lips 1998) and the presence of two morphologically similar microbes in the skin of anurans (Berger et al. 1998) should provide impetus for biologists to look for common causes of amphibian declines at sites around the globe.

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