Effects of the Chytrid Fungus on the Tarahumara Frog (*Rana tarahumarae*) in Arizona and Sonora, Mexico

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Abstract—*We conducted histological analyses on museum specimens collected 1975-1999 from 10 sites in Arizona and Sonora to test for the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) in ranid frogs, focusing on the Tarahumara frog (*Rana tarahumarae*). During 1981-2000, frogs displaying disease signs were found in the field, and all of these we tested were infected with the chytrid pathogen. Population monitoring results at localities with the infection ranged from apparent disappearance, to decline and recovery, and, in some cases, apparent stability. One population was infected over a 17-year period without apparent population decline. Our results suggest that stress, notably cold, and possibly pollution, may influence disease severity. The presence of chytridiomycosis in Sonora was confirmed for* R. tarahumarae *(1982-1999)* R. yavapaiensis *(1999),* R. magnaocularis *(1985, 1999), and* R. pustulosa *(1985).*

Introduction

A chytrid fungus, *Batrachochytrium dendrobatidis*, has been linked to recent anuran population declines in Australia, tropical America, North America, Europe, and Africa. Berger and Speare (1998) discussed clinical signs of the disease it causes (chytridiomycosis), which include lethargy, ventral reddening, convulsions with hind limb extension, accumulation of sloughed skin on the body, and occasional ulcers, with death usually occurring a few days after the onset of disease signs. Chytridiomycosis is known in several anurans and one salamander species in Arizona (Collins et al. 2003; Bradley et al. 2000; Sredl and Caldwell 2000; Sredl 2000), and may have been a factor in the 1974-1983 extirpation of the Tarahumara frog in Arizona (Jones and Fernandez, unpublished). In this paper we review mortality episodes and declines of Tarahumara frog populations in Arizona and Sonora, and examine the relationships of these events to presence of chytrid fungus.

Materials and Methods

Both diurnal and nocturnal field searches were used to survey canyon streams for ranid frogs. Whenever possible, surveys were conducted during peak frog activity in the first few hours after dark, employing a stealthy search using headlamps. Whenever possible, diurnal surveys were also conducted. If Tarahumara frogs were not found, the deepest pools providing the most favorable habitat were often searched by hand for individuals hidden under rocks and ledges and among pool-bottom debris. Surveys usually consisted of two evening passes along the selected reach of stream followed by a diurnal re-survey of the same reach. In some cases, only a diurnal survey was feasible, and in these cases the thorough search by hand was generally employed.

Populations, even if limited to less than ten observed individuals, were considered to be "healthy" and reproductive if there were frogs of various sizes, as well as larvae, and few or no dead frogs were found. A population decline is assumed in this report for cases with significant numbers of dead or moribund frogs, when a previously "healthy" population yielded markedly fewer or no observed frogs than previously, and when the age distribution had collapsed to a single observed size/age category. A population was defined as "apparently extirpated" if $\geq$3 surveys yielded no observed individuals. Capture-recapture rates averaging 20% of the Santa Rita Mountains population, and the consistent observation of large numbers of frogs during multiple surveys in the 1970s suggest the reliability of survey results for this species. Tarahumara frogs live in a less concealing environment than some other ranid frogs, and therefore tend to yield more consistent survey results. Thus, we place considerable weight on our negative as well as positive survey results.

In 1999–2004 we selected specimens from the University of Arizona Herpetology Collection for histological examination at University's Veterinary Diagnostic Laboratory as described in Bradley et al. (2002). Our focus was on Sonoran frogs collected from 1981 to 1999 from populations where a decline had been observed or where frogs showed disease signs. Additional specimens from localities with no disease signs were randomly selected to test for the chytrid pathogen for comparison to the carefully chosen disease sample.

Population Trends and Chytrid Disease Observations in Arizona and Sonora

In this section, we describe the recent history of ranid frog declines and survey observations in Arizona and Sonora at Tarahumara frog localities. Observations are summarized in table 1 and fig. 1, and locality numbers are given in parentheses in text to assist the reader with time-lines and geography.

The first evidence of ranid frog decline in Arizona was on April 7, 1974, when C. J. May and D. Frost independently observed numerous dead and moribund Tarahumara and lowland leopard frogs (*R. yavapaiensis*) in Sycamore Canyon (2), Santa Cruz County. Leopard frogs and Tarahumara frogs tested positive for the chytrid pathogen (Jones and Fernandez, unpublished), and intensive searches demonstrate the species is extirpated. Chytridiomycosis is the likely cause of extirpation in this case. Lowland and Chiricahua (*R. chiricahuensis*) leopard frogs have persisted in the Pajarito Mountains, but have remained generally uncommon, with repeated mortality events and confirmed chytridiomycosis in the Chiricahua leopard frog in Sycamore Canyon (unpublished data).

Tarahumara frogs found in the Santa Rita Mountains (1), Santa Cruz County (Hale et al. 1977), were studied by mark-recapture beginning in 1975. On June 4, 1977, several frogs kept overnight in a bag died overnight, and others appeared lethargic. Although hands-off methods were adopted, all of the estimated 600-1,000 frogs had vanished from the core study area by 1978. Larvae remained, and metamorphosed through summer 1979; but no recruitment was observed; the last frog was found dead in May 1983; and none have been seen in the wild in Arizona since then (see Hale et al. 1995). Chiricahua leopard frogs also disappeared from Big Casa Blanca Canyon (1), although they persisted in small numbers in nearby Gardner Canyon.

Thus, in the space of 10 years, the Tarahumara frog, which had been present in at least two substantial populations in

Table 1—Summary of Chytrid analyses and history of Tarahumara frog status at selected localities in Arizona and Sonora. Annotations refer to Tarahumara frog unless specified. Code legend: (+ -) = positive or negative results of chytrid analysis for frog(s) collected for indicated year. Year = healthy population observed. Year = population decline observed. *Year* = Tarahumara frogs no longer observed. Species abbreviations: RATA = *Rana tarahumarae*; RAMA = *R. magnaocularis*; RAYA = *Rana yavapaiensis*.

Locality	Observation years	Annotations
1. Big Casa Blanca Cyn, Santa Rita Mts., Arizona	1975-6(-), **77,** 78-82, *83-present*	Abundant into 1977. Disease signs 1977. Decline to extirpation 1977-83.
2. Sycamore Canyon, Pajarito Mts., Arizona	1934-73, **74(+),** *75-present*	Abundant into 1974. Disease signs 1974. Disappeared abruptly.
3. A. El Chorro, Sierra La Madera, Sonora	2000(-)	Small apparently healthy population.
4. A. El Pulpito, Sierra San Luis, Sonora	2000(-)	One RAYA, one RATA tested negative.
5a. A. La Carabina (lower), Sierra El Tigre, Sonora	**1981, 82, 84, 86,** *98*	Disease signs 1981. Few frogs, some larvae 1982-86.
5b. A. La Carabina (middle), Sierra El Tigre, Sonora	**1982(+), 84, 86**	Decline apparent 1982. Disease signs 1982-86. Few frogs seen 1984, 1986.
5c. A. La Carabina (upper), Sierra El Tigre, Sonora	1982-83	Abundant with no evidence of disease.
6. A. El Tigre, Sierra El Tigre, Sonora	1981, 82-3(-), 86, 98, 99(+)	Abundant all visits. Possible disease signs 1986. Definite signs 1999.
7. A. La Sauceda, S. Oposura, Sonora	1981-82, 85-86, *98*	Possible extirpation from drought.
8. A. El Vaso, S. Huachinera, Sonora	1974, *85*	Habitat favorable. Ranids absent 1985.
9. A. La Colonia, Sierra El Rubi, Sonora	**1982(+),** *85,* 99(-)	Single dead RATA 1982. Healthy RATA, RAYA 1999.
10. A. Pinos Altos, Sierra El Rubi, Sonora	1975, *82, 85, 99*	Ranids absent 1980s. Only RAYA present 1999.
11a. A. Los Lavadaros, 11b. Cajon El Infierno, S. Aconchi, Sonora	1980, 82(-), 85, 98, 99	11a. Small numbers of frogs all visits. 11b. RATA absent 1998.
12. Canada El Zoquetal, Sierra Aconchi, Sonora	2000(-)	Apparently healthy population.
13. A. Las Uvalamitas, Sierra El Datil, Sonora	1983, 85, 89, *99*	Marginal habitat, possible extirpation from drought. RAMA present in 1999.
14a. A. El Aguaje, 14b.A. El Trigo, Rancho Trigo, Sonora	1968, 75(-), 82(+), 85, 99(+)	RATA, RAMA always abundant. Symptoms in captured frogs 1986, 99.
15. A. El Potrero, Sierra Milpillas, Sonora	1985, *99*	RATA, RAMA frogs 1985. Only RAMA present in 1999.
16. A. El Cobre, S. El Rincon, Sonora	1982-84, 85(+), 86, 93, *99*	Ranids abundant all visits. Symptoms in captured RATA, RAPU, RAMA 1985. RATA absent 1999.

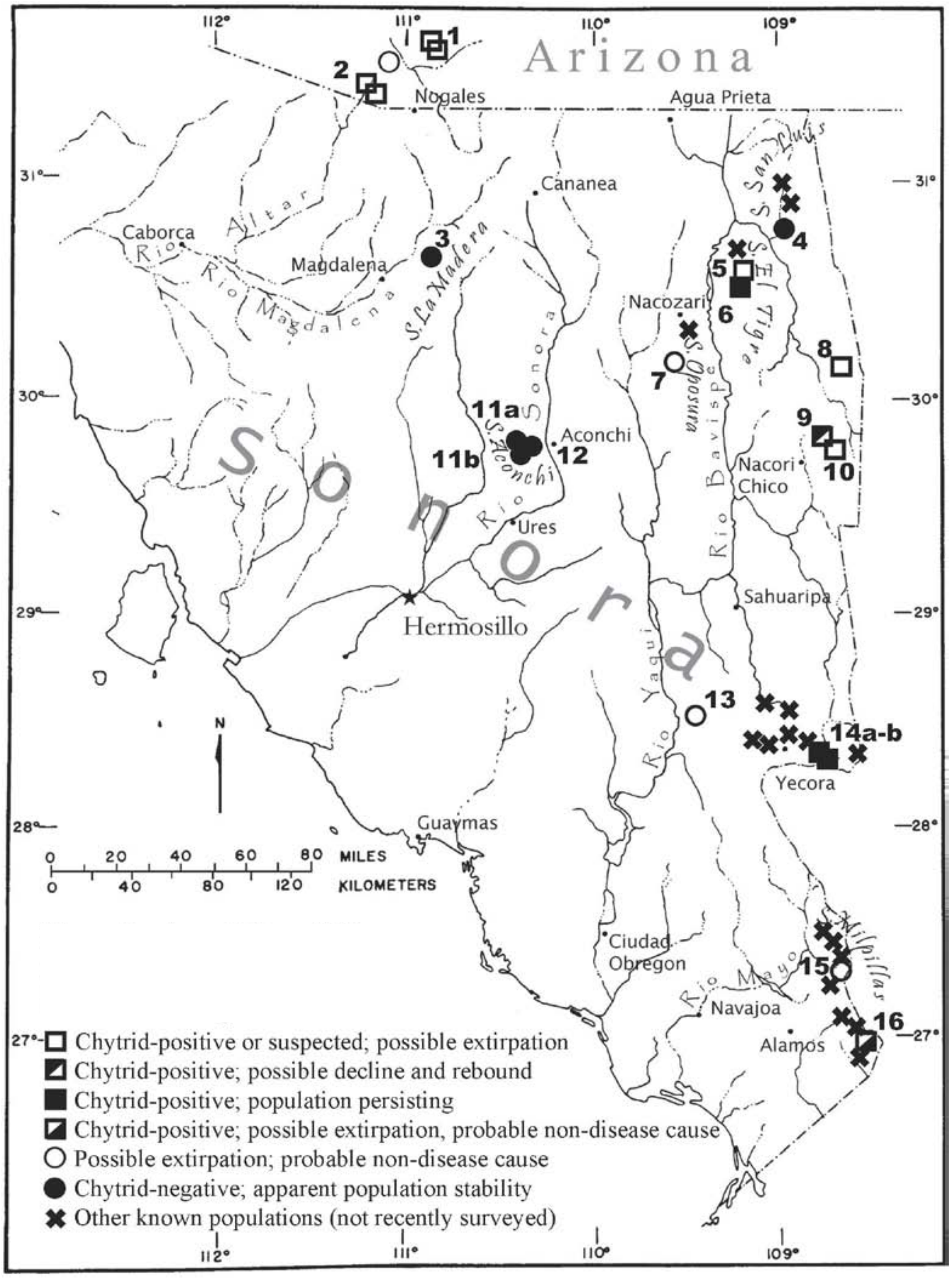

Figure1—Association of disease evidence with observed status of the Tarahumara frog in Arizona and Sonora.

Arizona, was completely extirpated during massive, rapid population-level mortality episodes. In the closely observed Santa Rita population, the primary event apparently occurred over a single winter, and within 6 years the population was extirpated. The symptoms observed indicate that disease, probably chytridiomycosis, was associated with this decline, although specimens were not tested.

In fall 1981, we observed a catastrophic die-off at Arroyo La Carabina, Sierra El Tigre (5a), in northern Sonora. From late October to mid-November, moribund frogs increased from 42% to 94% in observed samples totaling (approximately) 50 and 60 frogs, respectively. Symptoms often exacerbated by capture and handling, included ataxia (incoordination), fixed mydriasis (enlarged fixed pupils unresponsive to light), loss of the righting response, complete loss of responsiveness, extension of the limbs into a splayed-out position, and death, consistent with chytridiomycosis (Berger et al. 1998; Berger and Speare 1998; Pessier et al. 1999). An initial hypothesis was that this mortality stemmed from heavy metal poisoning (Hale et al. 1995), although it now appears more likely that chytridiomycosis was the cause. As seen in the Santa Rita Mountains decline, occasional frogs were observed in 1982 and 1984, and larvae through 1986. Frogs 5 km higher in the drainage (5b) exhibited disease symptoms, tested chytrid-positive, and declined; but they were not extirpated when last visited in 1986. Five km farther upstream in the upper reaches of the canyon (5c), frogs were abundant and displayed no symptoms when last visited in 1982-1983. Since 1986, only the lower reach of the canyon has been surveyed (in 1998) and no frogs were found.

In marked contrast, Tarahumara frogs surveyed in Arroyo El Tigre (6), also in Sierra El Tigre, in 1981-1983, 1986, spring 1998 and fall 1999, remained consistently abundant, although chytridiomycosis has probably been present since at least 1986. No frogs have been found dead in the stream, but in spring 1986 a Tarahumara frog was found with fixed mydriasis, and a red-spotted toad (*Bufo punctatus*) was underweight, lethargic, and displayed fixed mydriasis and locomotor impairment. Three Tarahumara frogs collected in 1999 for captive propagation quickly displayed disease symptoms, and they, plus a lowland leopard frog from the same sample, tested chytrid-positive. In this and the following case, populations persisted without apparent decline despite evidence of disease.

Near Yecora, in east-central Sonora, a Tarahumara frog collected at Rancho El Trigo (14a and 14b) in fall 1982 showed a mild chytrid infection. In 1985, several captive individuals exhibited disease symptoms, and some died. In fall 1999, captive frogs displayed disease symptoms or died overnight in a bag; of these, 3 of 4 Tarahumara and 3 of 5 leopard frogs (cf. *R. magnaocularis*) showed mild to moderate chytrid infection. Despite this evidence of a chytrid infection from 1982-1999, surveys have always found healthy populations of both species.

At another site in northeastern Sonora, Arroyo La Colonia (9), a single dead Tarahumara frog collected in fall 1982 was severely infected with the chytrid fungus. No ranids were observed there in summer 1985, but in fall 1999, reproductive populations of both Tarahumara and lowland leopard frogs were found, and a tested Tarahumara frog was chytrid negative. We believe this population declined or was extirpated, but rebounded or was recolonized, over a period of 17 years.

At two nearby localities, Tarahumara frogs may have declined due to disease, although we can only infer this as a plausible explanation. Tarahumara frogs were found at Arroyo El Vaso (8) in 1974, but not in 1985. Similarly, at Arroyo Pinos Altos (10), Tarahumara frogs were collected in 1969, but were not found in 1982 or 1985, and only a single lowland leopard frog was seen in 1999. Both these localities have apparently suitable habitat, and are close to Arroyo La Colonia (discussed above), where frogs were similarly absent in the 1985 survey, but where chytridiomycosis was confirmed for 1982. In the absence of other plausible explanations, we attribute apparent declines at localities (8) and (10) to disease as a reasonable hypothesis.

In southern Sonora, we infer non-chytrid-related causes for apparent population declines. At Arroyo El Cobre, Rancho Choquincahui (16), we studied the Tarahumara frog and sympatric ranids—Rana de Cascada (*R. pustulosa*), and big-eyed leopard frog (*R. magnaocularis*)—from 1982-1986. In fall 1985, 7 of 21 frogs held overnight showed symptoms ranging from lethargy to death. Histology confirmed chytrid infections in 3 of 5 specimens, one of each species. Tarahumara frogs remained abundant in fall 1993, but were not found in 1999 or 2002, while Rana de Cascada and big-eyed leopard frogs remained abundant through 2002 (J. Rorabaugh and M. Sredl, personal communication). During 11 visits to Arroyo El Cobre and multiple surveys between 1982 and 1999, dead frogs were occasionally found, but no large declines were observed. We suspect that anthropogenic habitat changes (forest replacement by exotic buffel grass [*Pennisetum ciliare*], with associated stream sedimentation) allowed Rana de Cascada to replace Tarahumara frogs (Hale, unpublished). Ranid frogs have persisted despite a long record of chytrid infection, and we have no reason to assume that disease was involved in the disappearance of the Tarahumara frog.

There are other instances for which we suspect that declines were unrelated to disease. Arroyo La Sauceda (7) and A. Las Uvalamitas (13) supported Tarahumara frog populations during 1981-1989, but in both cases only leopard frogs were found in 1998-1999. These streams had marginal habitat and Tarahumara frogs probably declined due to drought conditions (unpublished observations) in the 1990s. In another instance, Tarahumara frogs were present in Arroyo El Potrero (15) in 1985, but only big-eyed leopard frogs were found in 1999. Apparent increase of a native predatory fish (*Gila* sp.) was coincident with this change, and additional field observations (Hale, unpublished) suggest that this leopard frog may co-exist with such fish more readily than the Tarahumara frog does.

Four localities yielded chytrid-negative tests and showed no indications of population decline. Tarahumara frogs were seen in all except one survey at Arroyo Los Lavaderos (11a and b), and a 1982 specimen tested chytrid-negative. Similarly, Tarahumara frogs collected in 2000 from newly discovered populations at Arroyo El Chorro (3), Arroyo El Pulpito (4), and Cañada El Zoquetal (12)—all with no evidence of decline or age structure imbalance—tested chytrid-negative.

Discussion and Conclusions

Tarahumara frog populations in Sonora responded in a variety of ways to chytrid infections (table 1), ranging from no detectable response to apparent extirpation. In Arizona, the Sierra El Tigre, and other areas in northeastern Sonora, the disease was associated with at least three (1, 2, and 5a), and perhaps two other (8 and 10) apparent extirpations, as well as one case where surveys indicated a disease-associated decline followed by a rebound. Further south, we have not confirmed an association between chytridiomycosis and population decline, although the disease was confirmed present. There appear to have been a number of extirpations related to causes other than disease.

In many instances, stress of capture and handling produced disease signs and mortality in frogs that were not noticeably sick, and other stressors including acid rain and heavy metals from copper smelters (Hale et al. 1995) could have been involved. Stress could reduce immune response (Carey et al. 1999; but see Carey et al. 2003), general health, or overall anti-pathogen competency (see Rollins-Smith et al. 2002). The association of mortality episodes with cooler seasons, high latitudes and altitudes, and areas without warm springs in leopard frogs (Sredl and Caldwell 2000; Sredl 2000; Randy Jennings, personal communications; Rosen, unpublished) and in Tarahumara frogs all point toward cold as a stress factor.

In Arizona, we recorded a February 20 water temperature of 8.6 ºC in plunge-pool habitat at Big Casa Blanca Canyon (1) and lower minima there are a certainty. In contrast, at Arroyo El Cobre (16) in southern Sonora, where ranids remain abundant despite chytridiomycosis, minima are higher (e.g., observed minimum at 13 ºC on December 29). At Arroyo El Tigre (6) and Rancho Trigo (14), frogs persist despite the disease, but they do so near springs where water emerges at a near-constant 20-22 ºC. Thus, while the virulence with which the disease affected some populations suggests this disease is novel, and its arrival has coincided with mass mortality and previously unexplained population losses in southwestern ranid frogs, mortality and population decline are not a universal result of chytrid infection. Other factors appear to modulate the impact of the pathogen, and the most apparent of these is temperature. Such modulation may play a stronger role in disease impacts than is currently demonstrable.

While our findings are cause for concern, chytridiomycosis-related declines were not confirmed in the southern portion of the Tarahumara frog's range, in the heart of the Sierra Madre Occidental. This extensive mountainous region, dissected by numerous barrancas and innumerable lesser drainages, is well suited to the Tarahumara frog. Sightings by other researchers (Tom Van Devender, George Ferguson, Phillip Jenkins, personal communication) in this region within the last decade suggest the species is still abundant and probably widespread there. In contrast, in Arizona and northern Sonora, population losses occurred in isolated pockets of favorable habitat where extirpations may not be recoverable via natural re-colonization. Thus, while we do not suggest that the Tarahumara frog is immanently threatened with extinction; many populations in the northern third to half of its range have been infected, and several have disappeared. The longer term impact of this disease, which seems to be the best explanation for the systematic decline of this unique Madrean species, remains to be seen. Disease effects may be synergistically entwined with habitat modification, pollution, and the further spread of non-native species, factors that may have contributed to these events and are likely still suppressing native ranid populations.

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