UNDERSTANDING AND MITIGATING EFFECTS OF CHYTRID FUNGUS TO AMPHIBIAN POPULATIONS IN ARIZONA

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EXECUTIVE SUMMARY

Chytrid fungus, *Batrachochytrium dendrobatidis*, was first implicated in declines of amphibian populations in South and Central America and Australia in the early 1990's. This pathogen had not been implicated in declines of North American amphibian populations until the late 1990's. Chytrids were first identified in Arizona in 1998; retrospective analyses indicate that they have been present since at least 1974. Volunteers from Tucson Herpetological Society assisted us in gaining insight into the epidemiology of this pathogen in wild frog populations. Volunteers searched sites for dead or dying anurans and recorded number and species encountered. Specimens were collected and submitted to the University of Arizona Veterinary Diagnostic Laboratory and Grand Canyon University for histology. We confirmed chytrids at over 12 sites and identified chytridiomycosis in *Ambystoma tigrinum, Bufo punctatus, Hyla wrightorum, Pseudacris triseriata, Rana catesbeiana, R. chiricahuensis, R. subaquavocalis,* and *R. yavapaiensis*. We found chytrids to infect frogs in river basins in central, eastern, and southeastern Arizona. Insights made during this study have affected changes in scientific collecting permit administration and design of conservation activities.

TABLE OF CONTENTS

Executive Summary i
Introduction 1
Methods 2
Results 2
Discussion 4
Literature Cited 8

FIGURES

Figure 1. Numbers of surveys conducted each month 2000-2001 3
Figure 2. Survey sites and outcome of Wintertime Die-Off Surveys 2000-2001 5

TABLES

Table 1. Chytrid results collected between 2000-2002 4
Table 2. Chytrid positive localities by major river drainage 4

APPENDIXES

Appendix A. Wintertime die-off survey form 9
Appendix B. Recommended amphibian release guidelines 10

Understanding and Mitigating Effects of Chytrid Fungus to Amphibian Populations in Arizona

Michael J. Sredl, Kimberleigh J. Field, and Anne M. Peterson

INTRODUCTION

Global decline of amphibian populations is one of the most perplexing and pressing environmental problems in our era. Because of their basic biology, amphibians have been called an important indicator species of global environmental health (Vitt and others 1990) and likened to a "canary in the coal mine." Reasons that have been used to support this notion include: 1) the biphasic life cycle of most amphibians (aquatic larvae that are usually herbivorous and terrestrial adults that are exclusively carnivorous) exposes them to both air- and water-borne toxicants that may be concentrated in either plant or animal foods; 2) they occur in habitats ranging from deserts and rain forests, to alpine and subpolar tundra, across the globe (only the marine environment lacks a significant amphibian fauna); 3) they can be an important constituent of the energy and nutrient cycles of many ecosystems and often comprise a significant fraction of total biomass (Burton and Likens 1975); 4) their moist, permeable skin leaves amphibians vulnerable to a variety of environmental insults from pollutants (both air and water-borne) to ultraviolet radiation.

Causal hypotheses leading to population declines in amphibians include the introduction of non-native predators and competitors, increased levels of ultraviolet radiation due to thinning of the ozone layer, acid precipitation, global climate change, environmental pollution, disease, or a combination of these factors (Blaustein and Wake 1990). While it has been evident for some time that many population declines are due to habitat destruction, others have not been associated with obvious environmental factors (National Research Council 1990; Crump and others 1992; Bradford and others 1994). Of particular concern are population declines in relatively undisturbed areas, where effects of agriculture, grazing, deforestation, or pollution are thought to be negligible. While it is possible that these fluctuations are normal (Pechmann and others 1991), recent mass mortality of amphibians in areas relatively free of human impact suggest that some local extinctions are not normal and may have been due to a newly identified disease, chytridiomycosis (Berger and others 1998).

A chytrid fungus (hereafter chytrid), *Batrachochytrium dendrobatidis*, the pathogen responsible for causing chytridiomycosis, has been detected in amphibian populations in Central America and Australia (Berger and others 1998). It had not been identified as a threat to Arizona amphibian populations until 1998 (Milius 1998). During the winters of 1998-1999 and 1999-2000, epidemics caused by chytrid fungus infection were studied by biologists in Arizona, including populations of Ramsey Canyon leopard frogs (*Rana subaquavocalis*). The demographic effects of chytrid epidemics can be dramatic (in 2 populations studied, over 100 dead lowland leopard frogs, *R. yavapaiensis,* were counted - Sredl unpublished data), but the geographic and seasonal scales at which they operate and species affected are poorly understood.

We used volunteers from Tucson Herpetological Society and the private sector to collect data that

provide insight into the epidemiology of this pathogen in wild populations of amphibians. Specifically, we characterize the geographical and temporal scales of chytrid fungus die-offs and their impacts to populations of Ramsey Canyon leopard frogs and other native amphibians. Finally, we outline preliminary protocols to prevent the spread of this pathogen within captive and wild populations.

METHODS

We collected field data on live, sick, and dead amphibians using visual encounter surveys. In our initial search, we used binoculars, where practical, to detect frogs from a distance. Following this search we proceeded to sample sites by walking the banks and using dip nets to sweep vegetation to detect additional frogs. We also searched for frogs in mud cracks and beneath debris and other cover sites. We sampled the entire perimeter of ponds and established linear segments of streams. Wintertime surveys were conducted 2 times a month until a die-off was detected, and non-winter surveys were conducted 3 times between the months of June-August.

In addition to visual encounter survey data, we recorded the site attributes (name of site, UTM coordinates, and elevation), date of survey, name of observers, survey effort (start and stop times and distance sampled), and air and water temperatures (Appendix A).

If sick or dead frogs were detected, specimens were preserved by placing them in 10% formalin or, if formalin was unavailable, chilling them on ice or placing them in a refrigerator. Specimens were delivered to the University of Arizona Veterinary Diagnostic Laboratory or Grand Canyon University for examination. Skin of specimens was examined using histology for the presence of chytrid fungus using the technique of Bradley and others (2002).

RESULTS

Between October 2000 and November 2002, 22 Tucson Herpetological Society volunteers surveyed 43 sites a total of 153 times. Over 750 volunteer hours were provided for these surveys (2000-2001: 317 hours; 2001-2002: 437 hours). Most surveys (74%) were conducted between October through March (Fig. 1).

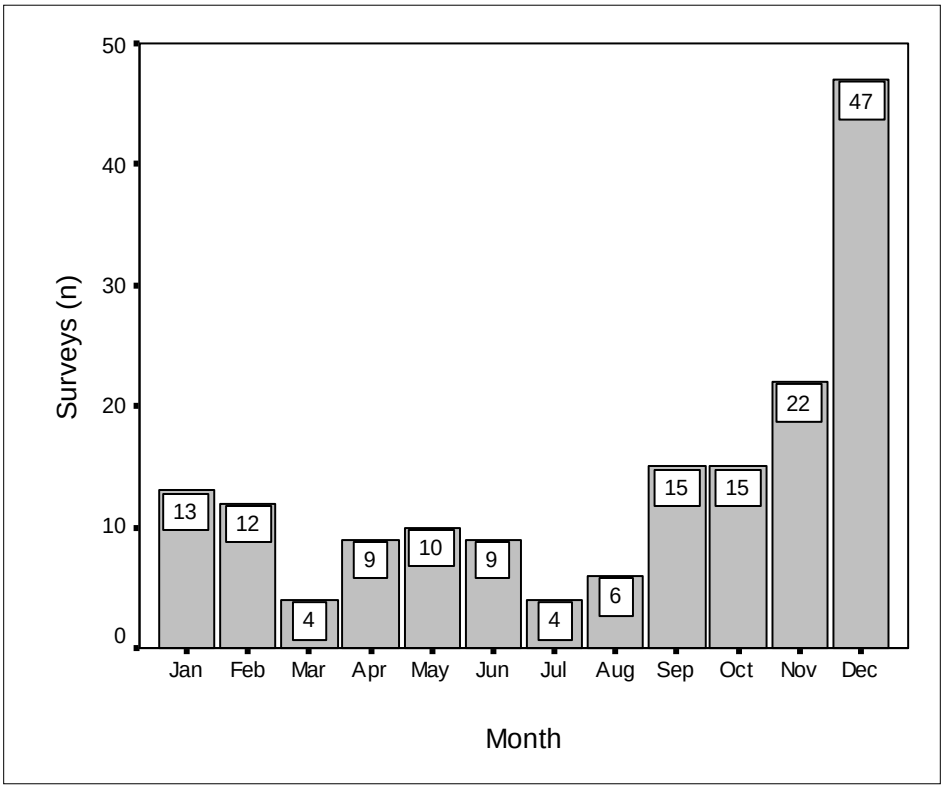


Figure 1. Numbers of surveys conducted each month 2000-2001.

These surveys detected 11 species: *Ambystoma tigrinum, Bufo punctatus, B. woodhousii, Pseudacris triseriata, Hyla arenicolor, H. wrightorum, Rana catesbeiana, R. chiricahuensis, R. pipiens, R. subaquavocalis*, and *R. yavapaiensis.* We confirmed chytrids in each of these species except *Hyla arenicolor.*

We detected chytrid positive frogs in May (n=3), January (n=2), April (n=2), November (n=2), December (n=2), February (n=1), July (n=1), August (n=1), and September (n=1).

Chytrid positive frogs were collected from 12 sites (Table 1). We surveyed sites in 7 river basins, and found chytrid die-offs in 6 of those: San Pedro, Santa Cruz, Yaqui, Verde, Gila, Little Colorado, and Concepcion (Table 2).

Table 1. Chytrid results collected between 2000-2002 expressed as number of specimens testing positive for the fungus over the total number of tested specimens. Numbers in parenthesis following the total number tested indicates the number of specimens that were undiagnosible.

Site	Chytrid Results	Site	Chytrid Results
Aliso Springs	0/4	Ramsey Canyon, Box	1/1
Bernstein Pond	0/1(1)	Ramsey Canyon, Meadow Pond	1/2
Buehman Canyon	9/10	SAGU 2	2/2
Butch Tank	0/1	Scotia Canyon	0/1
Carr Barn Pond	1/1(3)	Slaughter Ranch	1/1
Cinder Tank, Coconino	0/1	South East Yank Tank	0/3(3)
Montrose Canyon	2/2	Sycamore Canyon	2/2
Morgan City Wash	0/3	Sycamore Canyon/Yank Spring	1/1
Mosquito Lake	0/5	Thanksgiving Day Tank	1/3(1)
New Tank	1/1	Twenty seven Mile Lake	38/56*
North Tank	0/1	Upper Salt River	0/1
Pinetop Rearing Facility	0/3	Ramsey Canyon, Upper Trout	0/1(1)
Totals			**60/108(6)**

* results pending

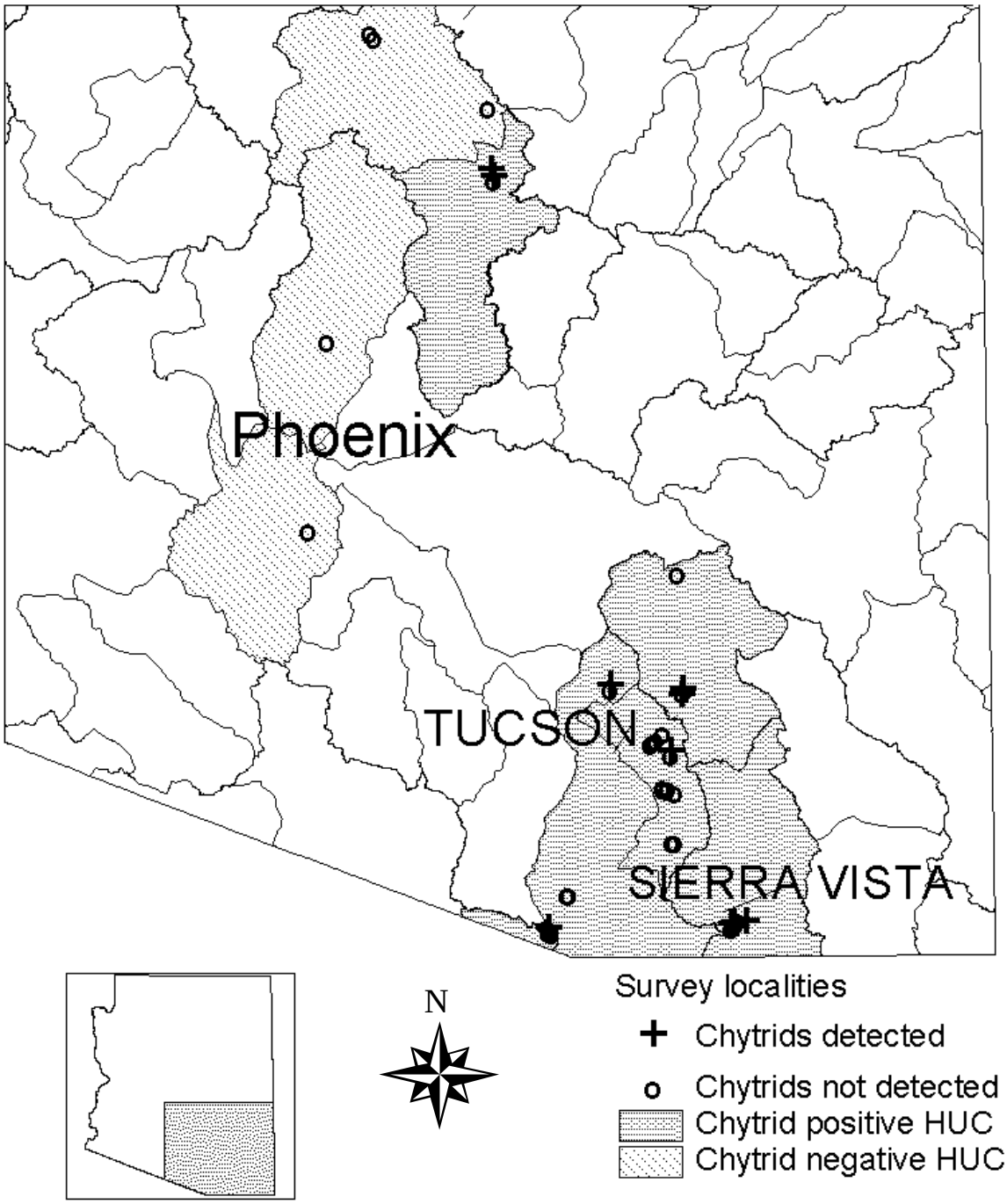
Table 2. Chytrid-positive localities by major river drainage. Positive localities/total localities surveyed. Number in parenthesis indicates number of localities where specimens were undiagnosable

Drainage	Chytrid Results
Santa Cruz	2/4
San Pedro	4/7(2)
Verde	2/5
Gila	1/3
Little Colorado	0/1
Yaqui	1/1
Conception	2/3(1)

DISCUSSION

While these surveys were opportunistic, the results contribute numerous insights to our understanding of the geographical, temporal, and taxonomic scope of this pathogen in Arizona. Preliminarily, chytrid fungus appears geographically widespread. Chytrids were found in frogs collected from every major river basin surveyed in Arizona, except the Little Colorado River.

Figure 2. Survey sites and outcome of Wintertime Die-Off Surveys 2000-2001. Sites where at least one chytrid positive individual was detected are denoted by a plus sign and sites where amphibians were not tested or chytrid negative individuals were found are denoted by an open circle. U.S. Geological Survey hydrological units (river basins) overlay survey sites.



(Retrospective studies are underway that will examine additional river basins, and provide additional insight into the geographical prevalence of this pathogen.)

Insights made during this study have affected changes in scientific collecting permit administration and conservation actions. As a result of the potential widespread distribution of this pathogen, the Arizona Game and Fish Department has placed stipulations that require individuals studying amphibian populations under a Scientific Collecting Permit to disinfect gear when moving between sites. Testing and prophylactic treatment of frogs for chytrid fungus prior to release has become a regular part of our conservation programs. The chytrid-negative frogs from the Little Colorado River were the result of this pre-release examination and spot testing of frogs from rearing facilities (Appendix B). These frogs were collected from the Department's Pinetop Rearing Facility, which was captively rearing *Rana chiricahuensis* for release to the wild.

That the largest number of chytrid-positive surveys in this study was found in May appears to contradict the cool-season pattern noted by Sredl (2000). However, the frogs collected during these May surveys were not part of a die-off (that is, no dead frogs were found at the time of collection). The presence of chytrid-positive frogs during warmer seasons has been noted by others (Sredl 2000; Bradley and others 2002), and chytrid-positive frogs collected during this time of the year likely relate more to disease history in the population and individual and seasonal variation in infection rates, than the likelihood of an epidemic.

Arizona has 29 species of amphibians in 8 families (Arizona Game and Fish Department 2002). This study has identified chytrids from 8 species in 4 families. Including data from other sources, chytrids have been confirmed in 12 Arizona species (*Ambystoma tigrinum, Bufo punctatus, Hyla wrightorum, H. arenicolor, Pseudacris triseriata, Rana berlandieri, R. blairi, R. catesbeiana, R. chiricahuensis, R. subaquavocalis, R. tarahumarae,* and *R. yavapaiensis*) in 4 families (Ambystomatidae, Bufonidae, Hylidae, and Ranidae).

While much information still needs to be collected, a few generalizations can be made. Chytrids have been confirmed from every species of the family Ranidae, except *Rana pipiens* and *R. onca,* the later species is likely extirpated from Arizona. Many members in this family of amphibians have experienced population declines since the mid-1970s (Clarkson and Rorabaugh 1989). Only one species of true toad (family Bufonidae) and no species of spadefoot toads (family Pelobatidae) have been known to become infected with chytrids. While no major declines of Arizona true toads and spadefoot toads have been noted, one true toad in western North America, *Bufo boreas,* has declined due at least in part to chytrids (D.E. Green unpublished data). Further investigation into these patterns is part of an ongoing study by the Department.

Through this study we have learned a great deal. However, there is still much to be done. Investigators have begun studies that should shed light on the following questions: where is chytrid fungus native?; has it become more of a problem now than it was historically?; if so, why; are chytrids everywhere, including high elevations?; how is the fungus maintained in the wild?; is it an obligate parasite?; what is the infection rate in wild populations?; does it vary seasonally?; and how

lethal is it? Answers to these and other questions will be needed before we understand the dramatic and perplexing decline of amphibian populations in Arizona and elsewhere and the role chytrid fungus played.

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Appendix A. Wintertime die-off survey form.

Amphibian Wintertime Die-Off Surveys

Please complete at least all * fields.

Site*						
Subsite*						
Easting						
Northing						
Elevation						
Date* (dd month yyyy)						
Observers*						
Start time*						
Stop time*						
Air temperature (°C)						
Water temperature						
Distance sampled*						
Species*						
# alive observed*						
# alive collected*						
specimen numbers (live)						
# dead observed*						
# dead collected*						
specimen numbers (dead)						
Date to VDL						
Notes on behavior of amphibians*						
Notes on condition of sick or dead amphibians*						
Other species observed*						
Photos? (y/n)*						
Notes/comments*						

Appendix B. Recommended amphibian release guidelines. Reviewed by Amphibian TAG received from Kevin Wright, the Phoenix Zoo May 8, 2001.

QUALIFICATIONS FOR A RELEASE PROGRAM:

1. No mortalities in release group in previous 30 days. No "cause of death unknown" or diagnosis of contagious disease as cause of death for 60 days prior to release. All mortalities should be examined by a pathologist skilled in diagnosing amphibian diseases. If sections of skin are submitted to the pathologist (instead of the whole animal), the sections should include at least 2 pieces of skin from the ventral pelvic region and/or ventral hind limb and/or feet or toes.
2. No unthriftiness or diagnosed illness in release group in previous 60 days. No obvious physical abnormalities – missing limbs, deformities of long bones, vertebral scoliosis or kyphosis, corneal lesions, skin lesions – detected. Diagnosis of certain diseases, such as mycobacteriosis, in a single individual may render the entire group unfit for release.
3. No medical treatments of the release group in previous 60 days.
4. All animals designated for release should be in permanent quarantine so that there is no overlap with care of exotic animals or native animals that have been exposed to exotic animals. Caretakers should "shower in" if they care for other herps either as part of their job or as pets. If a wild population has a known incidence of a given infectious agent (e.g., Lucke's herpesvirus), it may be safe to assume that released animals with that agent represent an acceptable risk.
5. All enclosures should be worked with separate tools and equipment to reduce cross-transmission. Disposable gloves should be worn and new ones used for each enclosure. Any enclosures with unthrifty animals should be worked last.
6. Water quality logs should be maintained. Adjustment to release site water conditions should occur 60 days prior to release.
7. Native prey items should be offered as the sole diet for 60 days prior to release.

PRE-RELEASE SCREENING PROTOCOL:

1. Depending on the size and life stage of the specimens to be released as well as the number of specimens destined to be released, a random sampling of animals may need to be assessed rather than an individual assessment of all animals within a group.
2. Obtain weight – reweigh if animal micturates or defecates; save cloacal water (urine) and feces separately for later analysis
3. Perform thorough physical exam; note body position, alertness, and respiratory rate prior to restraint; withdrawal reflex; blink reflex; righting reflex; ocular exam; oropharyngeal exam; Doppler assessment of cardiovascular system; heart rate; magnification-aided scrutinization of skin; transillumination of coelom; palpate abdomen; evaluate musculoskeletal system for obvious bony abnormalities
4. Obtain blood for culture, CBC, chemistries, and blood or serum banking or serology.

5. Obtain cloacal water or flush for parasites and banking. Urinalysis for glucose and other biologically important parameters.
6. Obtain fecal sample for parasites and banking.
7. Obtain C & S of skin, oropharynx and cloaca/rectum.
8. Obtain gill biopsy for parasites, DNA banking or frozen for future pathogen recovery attempts.
9. Obtain radiograph to r/o metabolic bone disease or other deformities
10. Obtain toe clip for identification; toe clip may be saved for chytrid histopathology, DNA banking or frozen for future pathogen recovery attempts. Consider PIT tags as permanent identification.
11. Ultrasonographic exam to reveal granulomatous disease
12. Skin scrape, gastric wash, tracheal (lung) wash for parasite or frozen for future pathogen recovery attempts
13. Random euthanasia of healthy specimens in the release population, if it is large enough, for thorough necropsy, histopathology and pathogen isolation attempts.

IMMEDIATE PRE-RELEASE ACTIVITIES:

1. 10 days and 2 days before release:

Soak in antifungal solutions (if this has never been used on this species before, try the treatment on a few individuals well ahead of time to determine tolerance).

Itraconazole: itraconazole diluted to 0.01% concentration in 0.6% saline (Sporanox, Janssen Pharmaceutical, Titusville, NJ) for up to 1 hr

Miconazole: miconazole diluted to 0.01% concentration in 0.6% saline as alternative (Conofite lotion, Schering-Plough Animal Health Corp., Union, NJ) for up to 1 hr. This solution is not tolerated as well as itraconazole since it contains alcohol.

2. At time of packing for transport:

Dip in benzalkonium chloride (2.0 mg/l) for at least 15 seconds. Rinse with fresh water before packing animal. If this has never been done before, try the treatment on a few individuals well ahead of time to determine tolerance.

Visual assessment of animals and approve or reject packing for transport

3. At release site:

Final visual assessment of animals and approve or reject release

Aquatic animals: Equilibrate water temperature and chemistries of transport container with release site water

Terrestrial animals: Equilibrate container temperature with release site temperature