

**Detection of (*Batrachochytrium dendrobatidis*), the Chytrid Fungus
Associated with Global Amphibian Declines, in Montana Amphibians**

Bryce A. Maxell

Montana Natural Heritage Program, Helena, Montana (406) 444-3655 bmaxell@mt.gov

Grant Hokit

Biology Professor, Carroll College, Helena, Montana

Jeff Miller

Biology Professor, American University, Cairo, Egypt

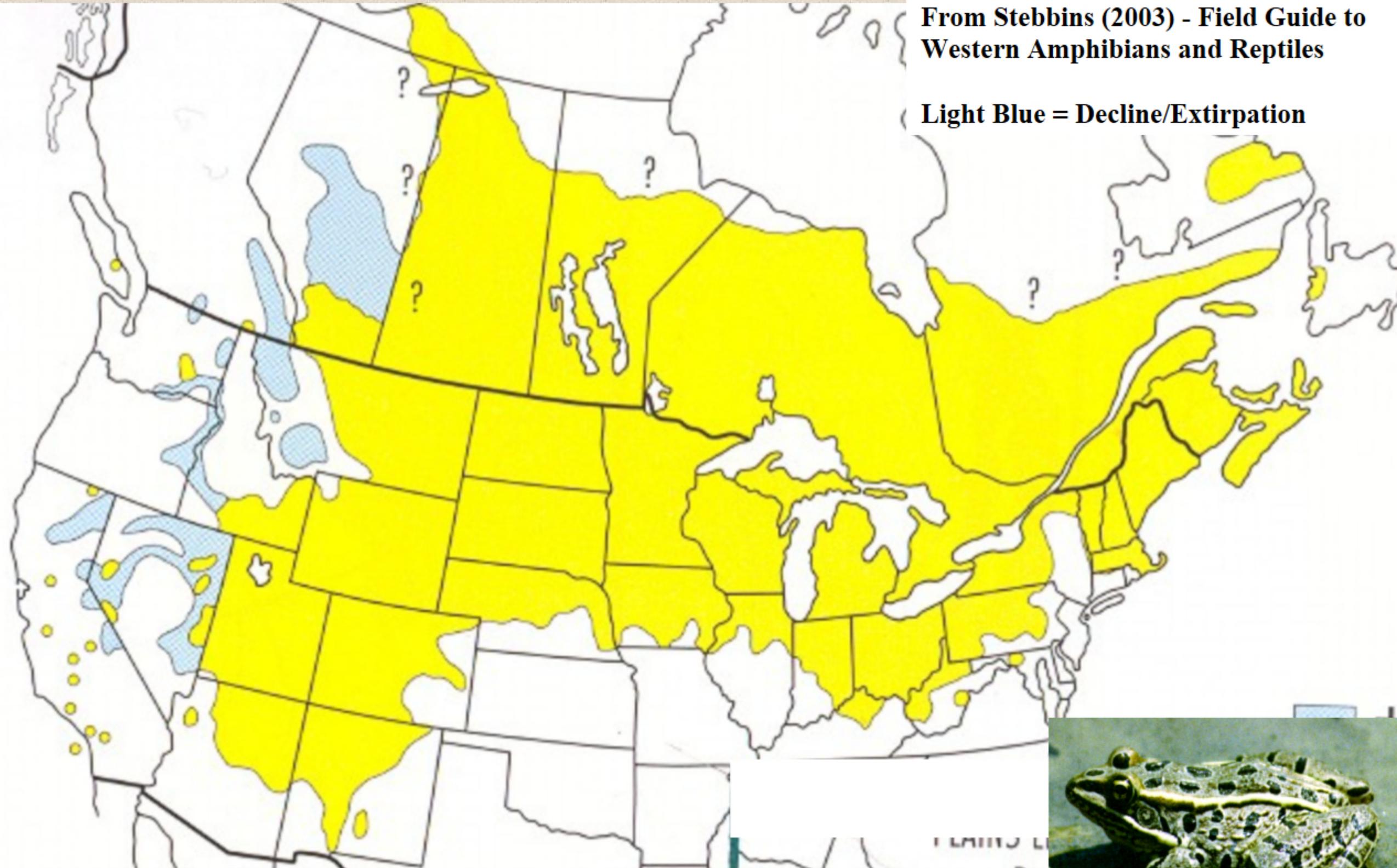
Kirwin Werner

Biology Professor, Salish Kootenai College, Pablo, Montana

Northern Leopard Frog (*Rana pipiens*)

From Stebbins (2003) - Field Guide to Western Amphibians and Reptiles

Light Blue = Decline/Extirpation



Pre and Post-1990 Distributions



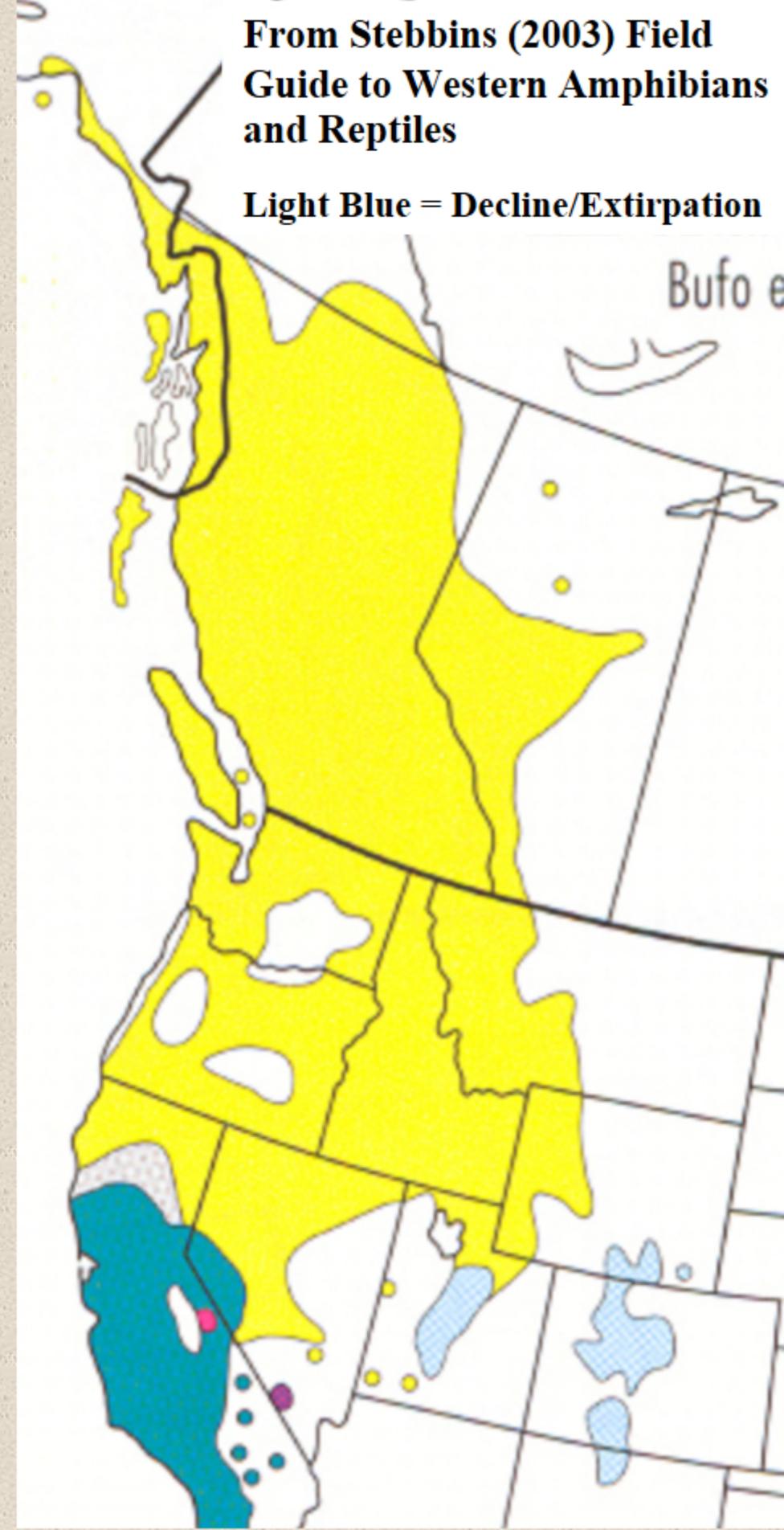
Western Toad (*Bufo boreas*)

Cause for Concern in Western Montana

- * No quantitative historic data to assess change in status
- * Historic qualitative accounts list them as “abundant” “common”, or “the most abundant amphibian in western Montana
- * Recent extirpations in southern part of range
- * Still widespread in Montana and appear to be relatively more common in northwest Montana
- * Now only found breeding in 2-5% of lentic sites
- * Majority of breeding sites have limited reproduction
- * Only a handful of breeding clusters with what appears to be viable populations

From Stebbins (2003) Field
Guide to Western Amphibians
and Reptiles

Light Blue = Decline/Extirpation



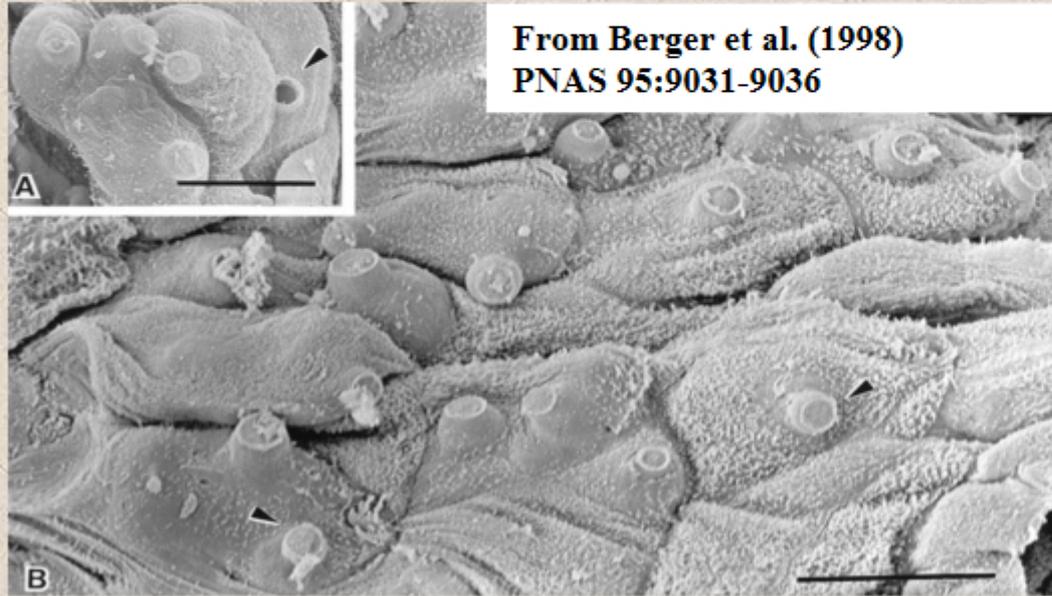
Project Goals

1. **Test amphibians across Montana to determine if *B. dendrobatidis* is present.**
2. **If *B. dendrobatidis* is detected identify prevalence and determine if there are any correlations between, elevation, habitat type, human activity in the area, and species present or breeding at a site.**
3. **Test tissue samples from museum voucher specimens gathered prior to declines in regions where declines have been well documented to see if *B. dendrobatidis* was present prior to declines.**

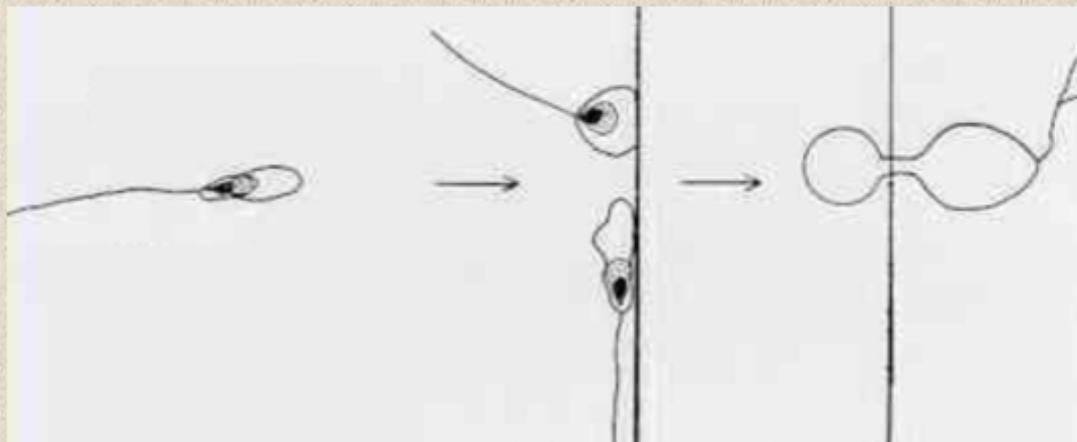
Batrachochytrium dendrobatidis

A Fungal Pathogen Linked to Amphibian Declines

Life History



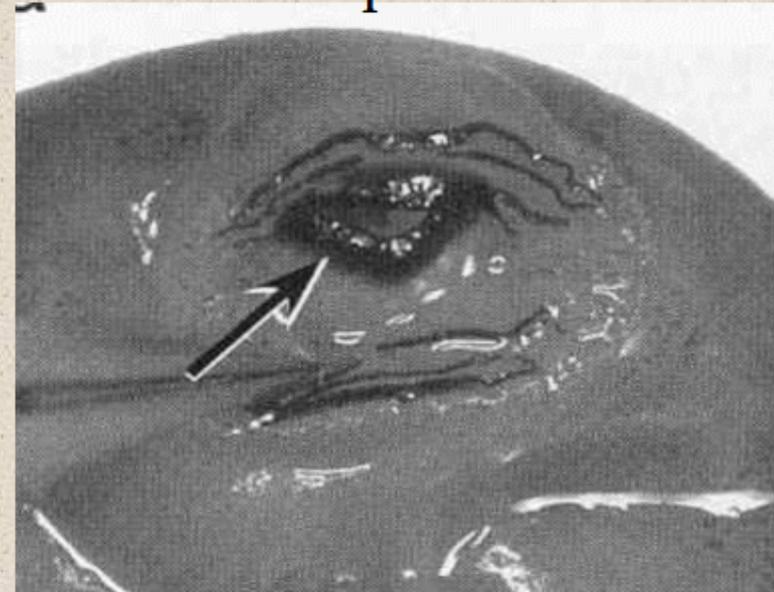
Chytrid thallus digests keratin in epidermal skin cells
Sporangia release clonal zoospores via discharge tubes



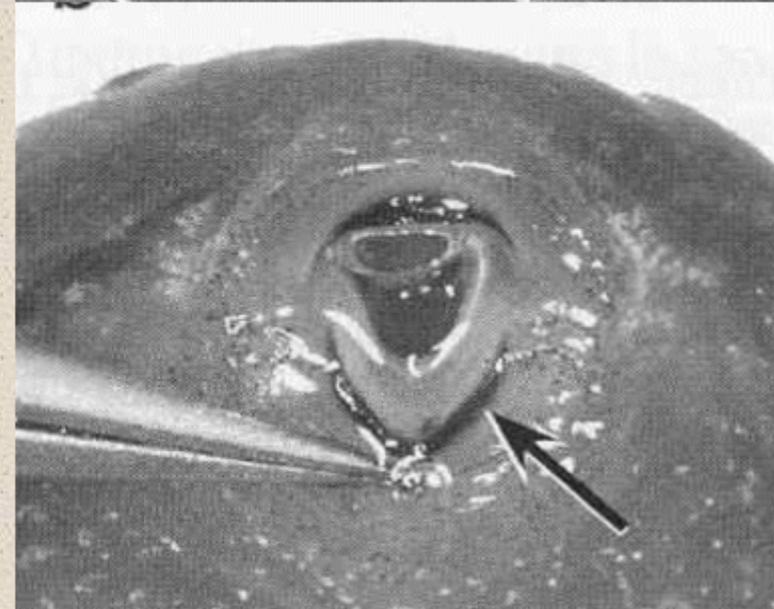
Free-swimming flagellated zoospores penetrate host cells

Field Identification

- Lethargy & skin sloughing in adults
- Keratinized mouthparts reduced or absent in tadpoles



Normal



Infected

From Vredenburg (2001)
Herp Review 32(3):151-152

Batrachochytrium dendrobatidis

A Fungal Pathogen Linked to Amphibian Declines

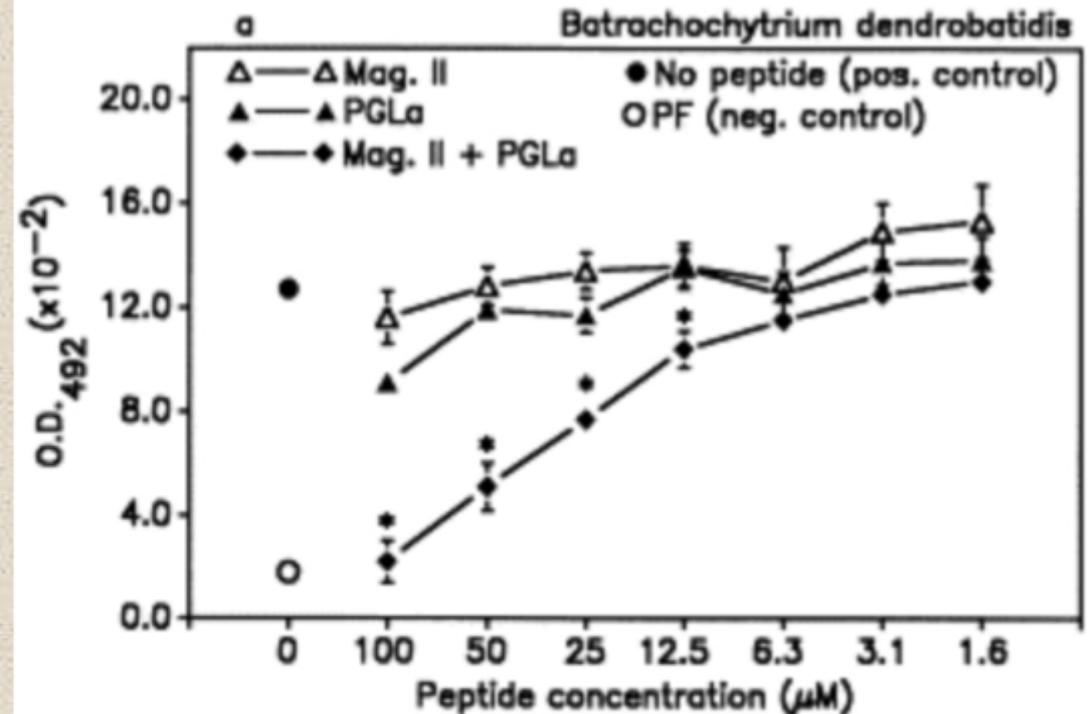
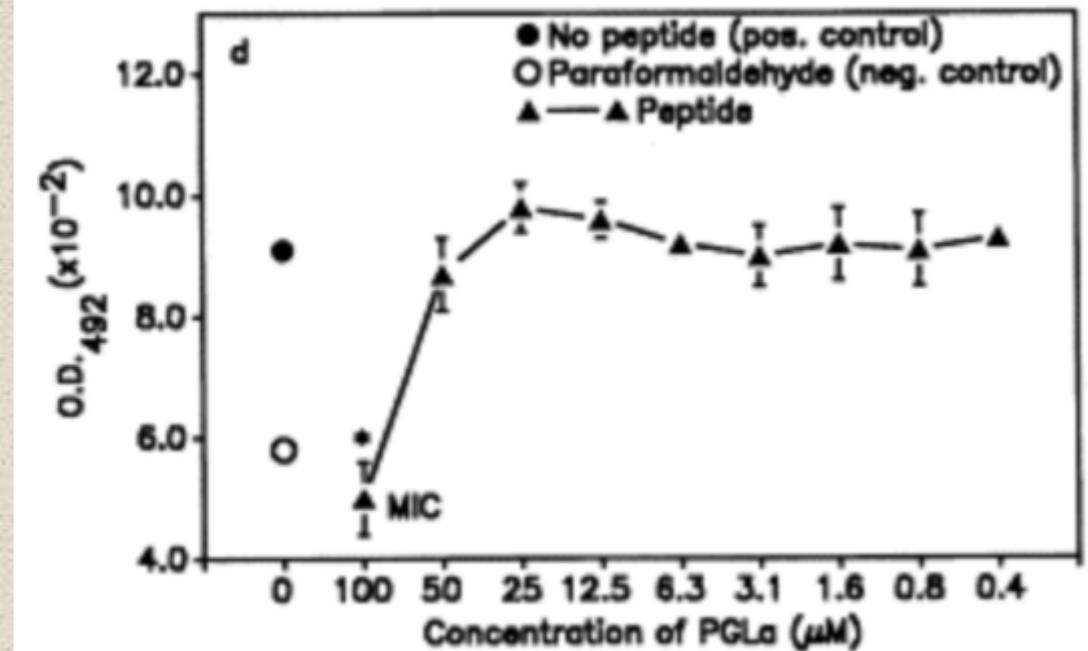
- Linked to declines/dieoffs on 6 continents
- Earliest record from northern leopard frog specimen in Colorado from 1974.
- Same species with little genetic divergence (EID)
- Detected and suspected of causing declines in 94+ species in 14 families
- Has been found in 20+ species in N.A.
- Grows at temperatures of 6 to 28 °C, but dies at extended exposure above ~32°C
- Zoospores do not withstand dessication
- Not virulent to all amphibian species
- May be cured by elevating body temperatures or application of fungicides

Natural Resistance

- Amphibian Skin Peptides Inhibit Growth of *B. dendrobatidis*
- Zoospores are 2-4 times more sensitive than sporangia to peptides
- Peptide production and effectiveness varies with temperature

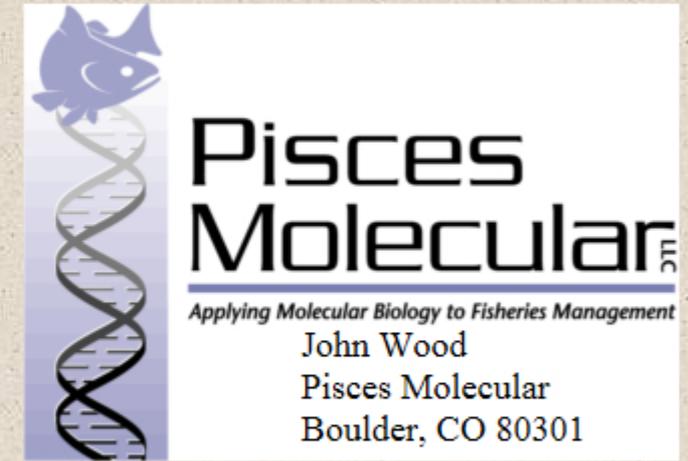
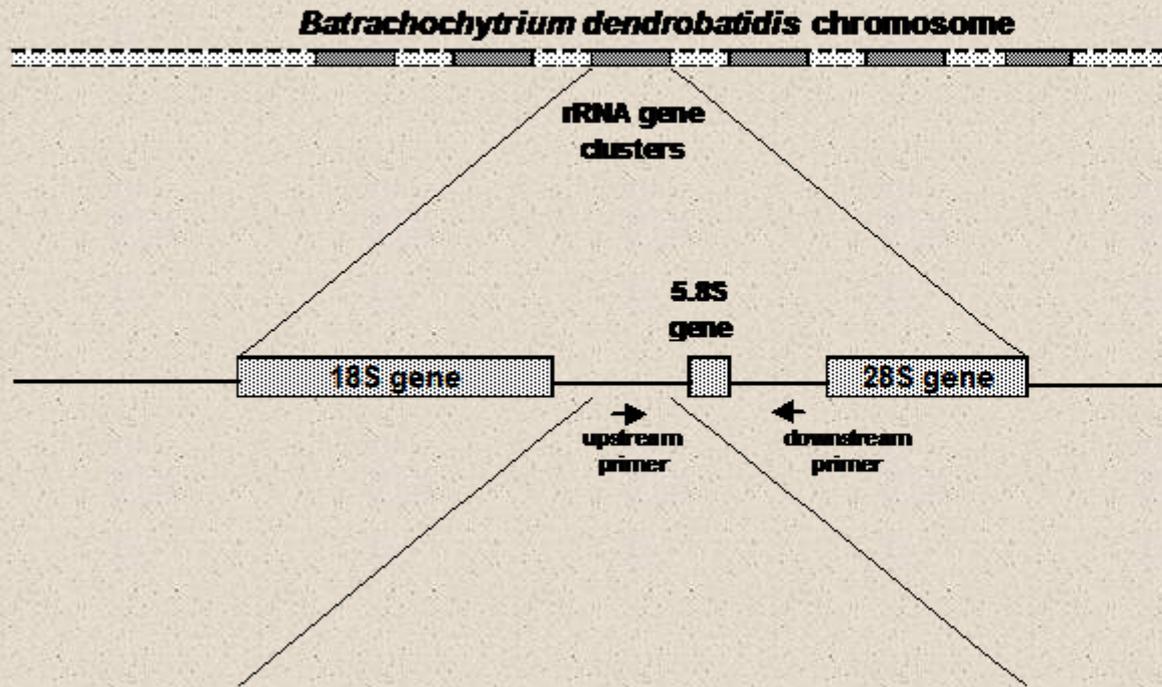
Other Stressors May Inhibit Peptide Synthesis and Release

- Pesticides
- Herbicides
- Other pollutants
- Other pathogens
- Cold temperatures



From Rollins-Smith et al. (2002)
Dev & Comp Immunology 26:63-72.

Detecting *B. dendrobatidis* with PCR primers for ITS region of rDNA

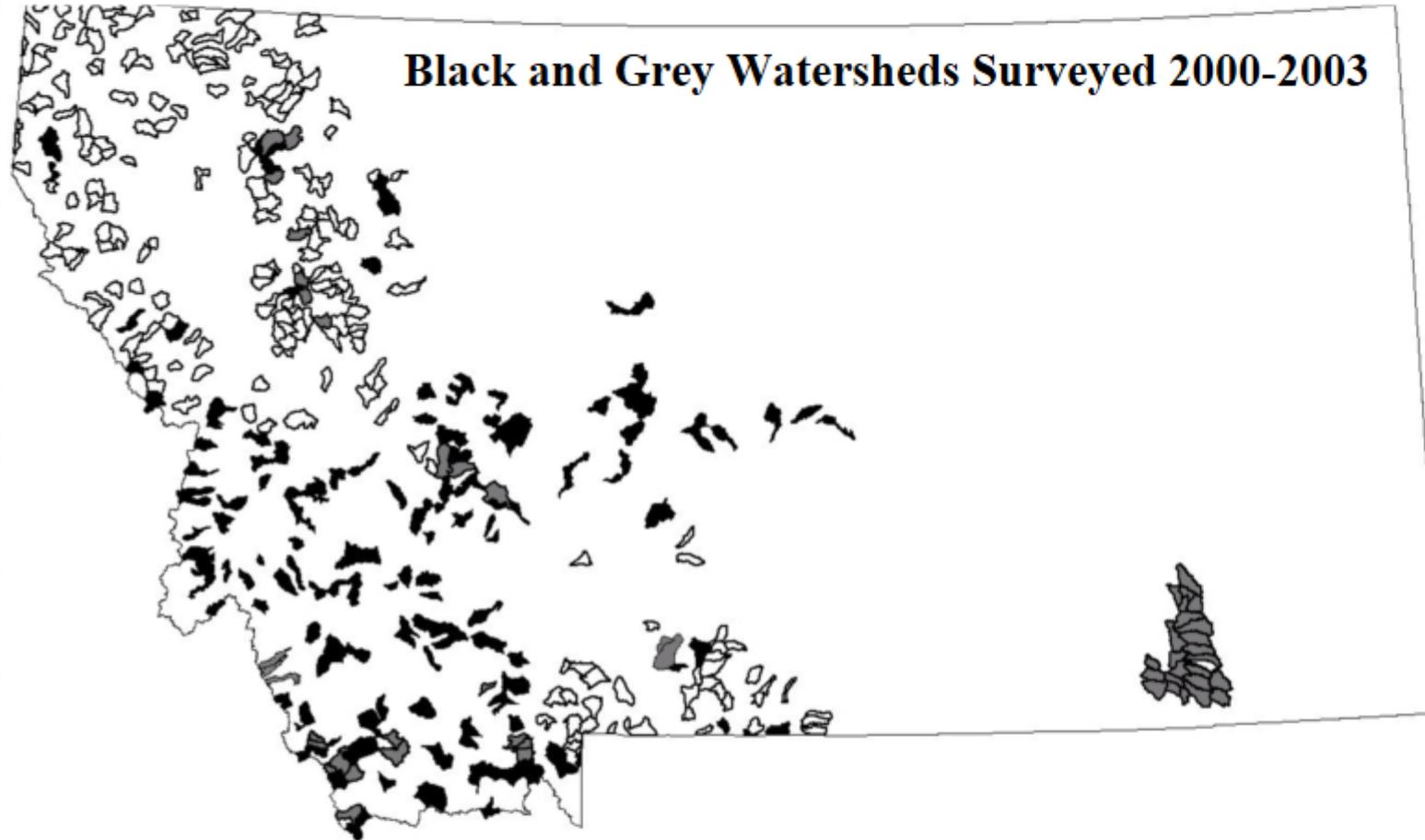


Sample Source	Results
Skin Scrapings	good
Toes	good
Pelvic Patch Tissues	good
Animal washes	less sensitive
Tadpole mouthparts	poor
Sentinel tadpoles	poor
Pond water filtrates	In progress
Insects	In progress

Number of Zoospores in Sample	PCR Detection Signal
1,000,000	+++
100,000	+++
10,000	+++
1,000	++ or +++
100	++ or +++
10	- or +
1	-
0	-

Sources for Tissues Tested for *B. dendrobatidis*

1. Amphibian Inventory Project



2. USGS BRD at Lost Trail NWR & Glacier Park

3. Kirwin Werner - Eastern Montana

4. Jeff Miller - Flathead Valley (M.S. Thesis at UM in 1975)

Tissue Types

- Toes placed directly in ethanol
- Dried toes from roadkill
- Toes and skin from formalin fixed and ethanol preserved vouchers
- Toes from formalin fixed and formalin preserved vouchers

Tissues Tested for *B. dendrobatidis*

Species (Total N)	No. 1970s Negatives (no positive samples)	No. Recent Negatives	No. Recent Positives	Recent Prevalence (Recent N)
Long-toed Salamander (1)	0	1	0	0% (n = 1)
Tiger Salamander (1)	0	1	0	0% (n = 1)
Western Toad (46)	11	21	14	40% (n = 35)
Great Plains Toad (2)	0	2	0	0% (n = 2)
Woodhouse's Toad (2)	0	2	0	0% (n = 2)
Boreal Chorus Frog (4)	0	2	2	50% (n = 4)
Columbia Spotted Frog (24)	9	8	7	47% (n = 15)
Northern Leopard Frog (18)	10	5	3	38% (n = 8)

***No signs of morbidity in positive animals**

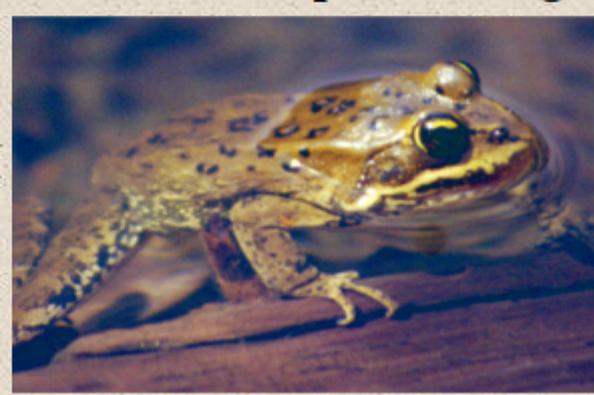
Overall Recent Prevalence = 38% (n = 68)

Western Toad

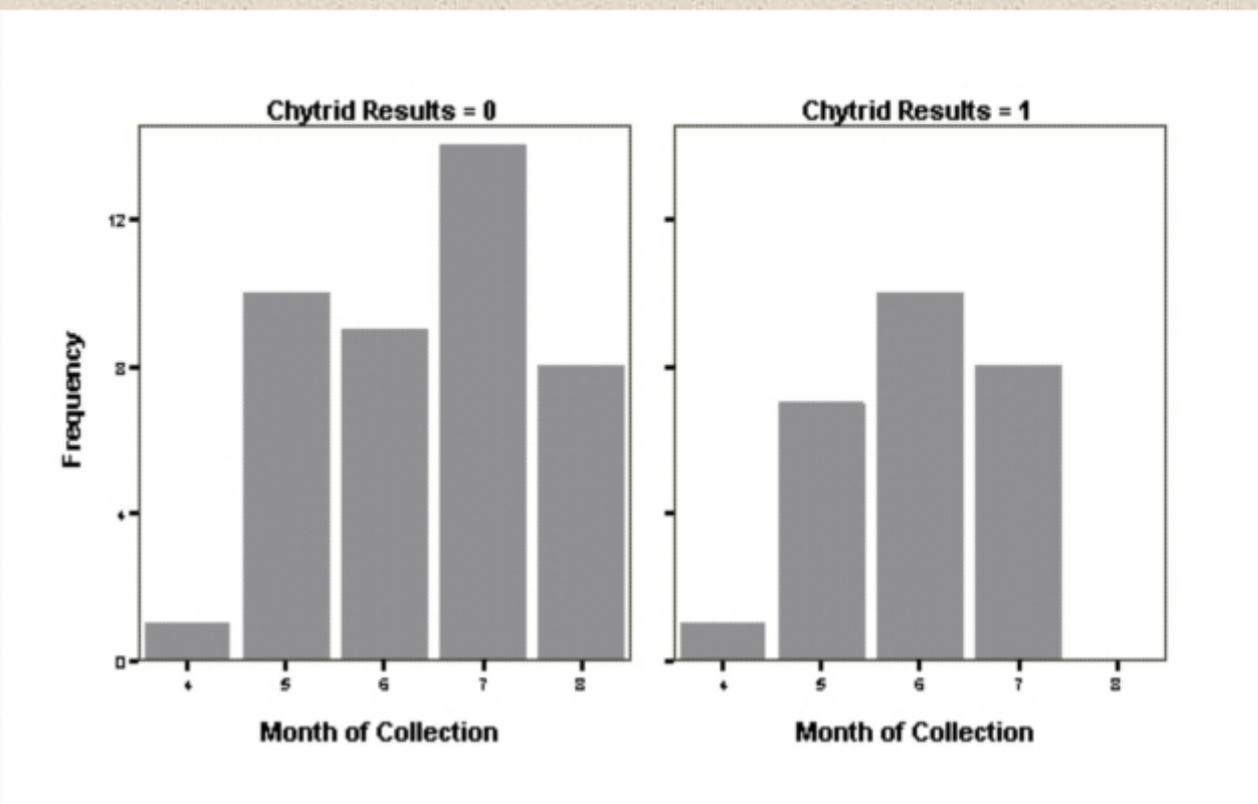
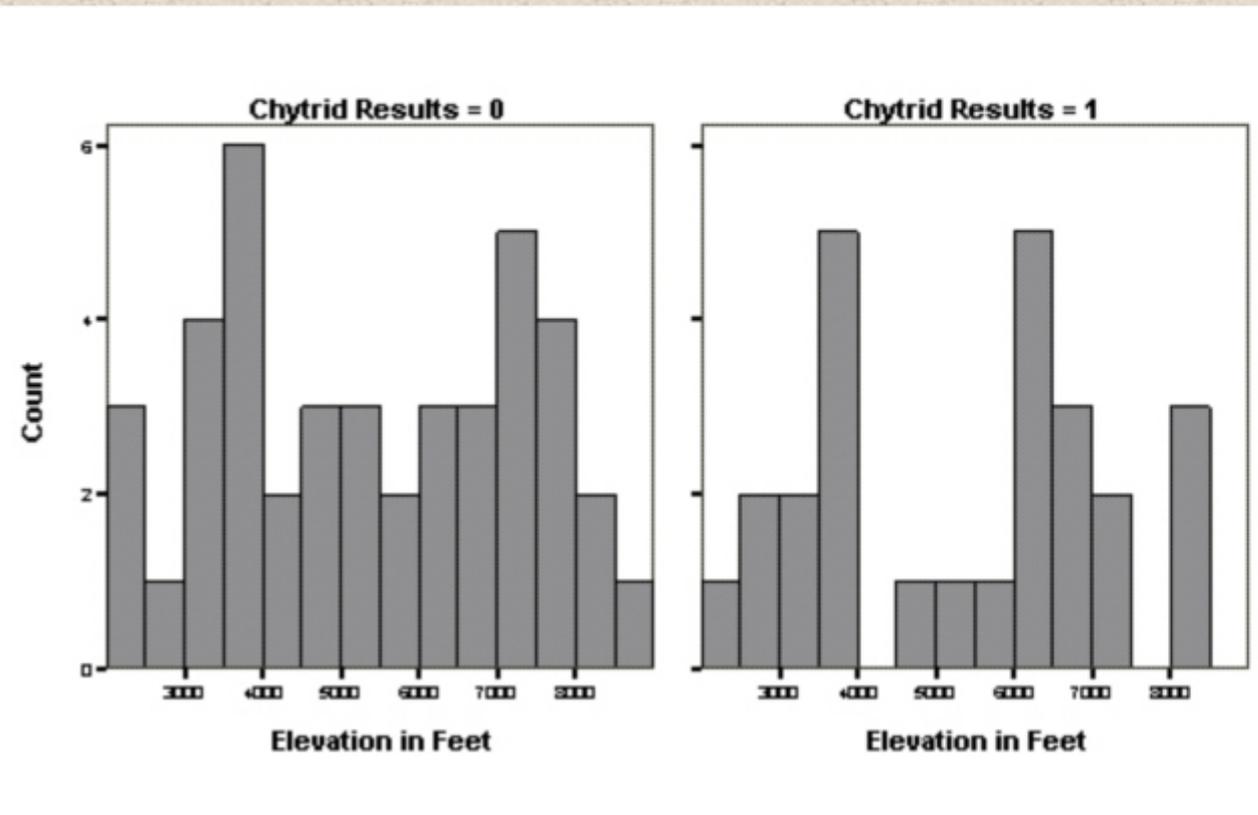
Boreal Chorus Frog

Columbia Spotted Frog

Northern Leopard Frog



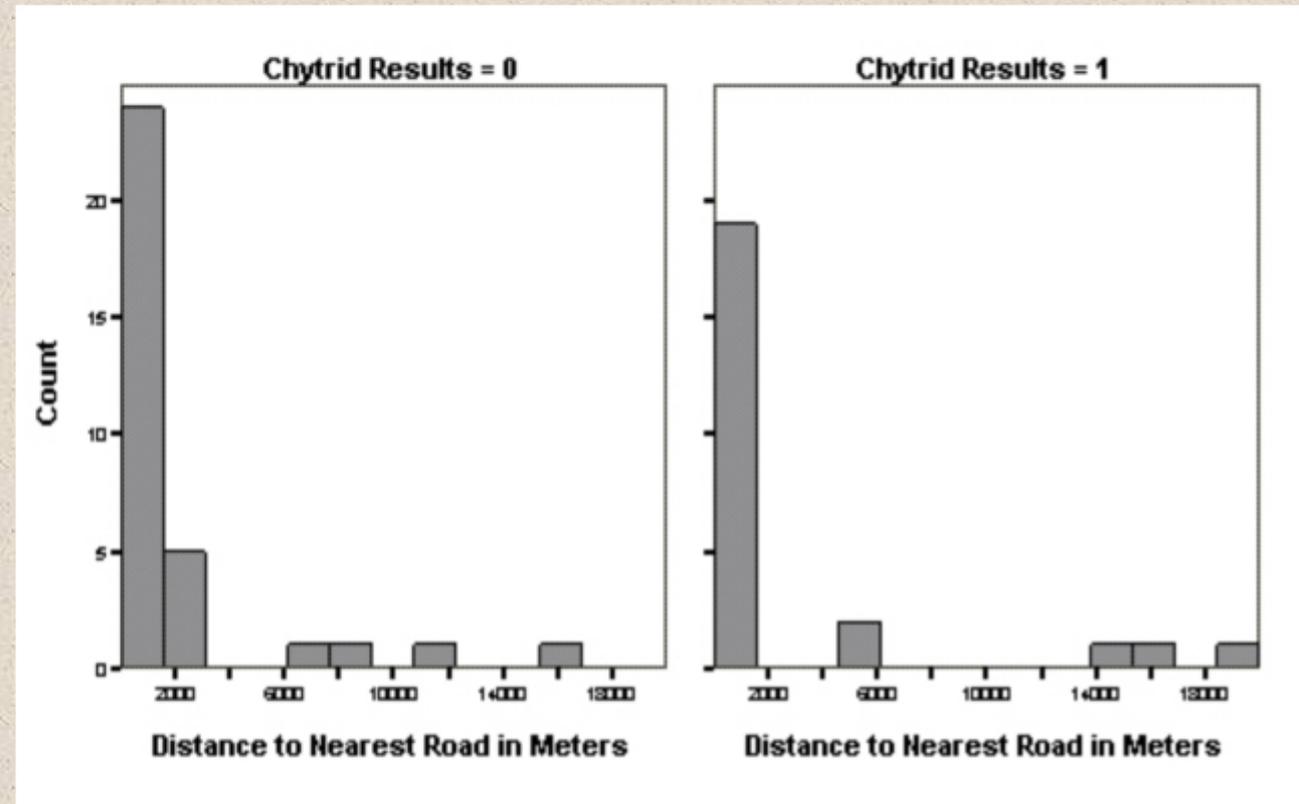
Detection of *B. dendrobatidis* Relative to Elevation, Season, and Habitat Type



Habitat Type	Positive Samples	Negative Samples
Permanent Water Body	6	26
Ephemeral Water Body	5	7
Away from Water	3	8

Detection of *B. dendrobatidis* Relative to Human Activity, Distance to Roads and Method of Preservation

Human Activity	Positive Sites	Negative Sites
Low	7	9
Moderate	7	25
High	5	4



Method of Preservation	Positive Samples	Negative Samples
Dry	2	5
Ethanol	22	17
Formalin Fixed EtOH Stored	2	9
Formalin Fixed & Stored	0	11

Amphibian Presence and Breeding at Lentic Sites Positive and Negative for Detection of *B. dendrobatidis*

Species (N for sites in species range)	Number (%) Negative Sites Detected	Number (%) Negative Sites Breeding	Number (%) Positive Sites Detected	Number (%) Positive Sites Breeding
Long-toed Salamander (- = 10, + = 9)	6 (60%)	6 (60%)	4 (44%)	4 (44%)
Tiger Salamander (- = 10, + = 7)	3 (30%)	2 (20%)	0 (0%)	0 (0%)
Western Toad (- = 15, + = 14)	12 (80%)	9 (60%)	10 (71%)	5 (36%)
Great Plains Toad (- = 8, + = 5)	2 (25%)	1 (13%)	0 (0%)	0 (0%)
Woodhouse's Toad (- = 7, + = 5)	5 (71%)	2 (29%)	4 (80%)	2 (40%)
Boreal Chorus Frog (- = 10, + = 10)	5 (50%)	3 (30%)	5 (50%)	3 (30%)
Pacific Treefrog (- = 0, + = 1)	-	-	1 (100%)	1 (100%)
Columbia Spotted Frog (- = 15, + = 14)	13 (87%)	7 (47%)	14 (100%)	5 (36%)
Northern Leopard Frog (- = 22, + = 19)	4 (18%)	2 (9%)	3 (16%)	2 (11%)

Future Areas of Research

- **Can PCR based methods be used for formalin fixed voucher specimens?**
- **Earliest date for evidence of *B. dendrobatidis* in Montana?**
- **Is it widespread in eastern Montana?**
- **How many species are infected?**
- **What is prevalence within and among populations?**
- **What are vectors and reservoirs?**
- **What are effects of pathogen load on survival and fecundity for different species?**
- **What factors depress immune or peptide responses to *B. dendrobatidis*?**
- **Modeling population and metapopulation impacts.**



Summary

- **No evidence that *B. dendrobatidis* was present in NW Montana prior to declines**
- **No evidence that *B. dendrobatidis* caused declines, but the timeline for declines fits with other declines thought to have been caused by *B. dendrobatidis*.**
- ***B. dendrobatidis* is currently widespread in Montana across a variety of elevations, climate regimes, and levels of human impact**
- ***B. dendrobatidis* is currently present in at least 4 Montana amphibians and has a relatively high prevalence and apparently low virulence in the infected populations examined**
- **We have a lot to learn about *B. dendrobatidis* and its possible impacts**
- **Managers should avoid additional stressors to species of concern**
- **Any ill or dead animals detected should be immediately frozen or preserved in ethanol to allow for future pathology**
- **Personnel working in aquatic habitats should follow protocols to prevent spread of this and other waterborne pathogens**

ACKNOWLEDGMENTS

Amphibian Inventory Funding Sources

- **U.S. Forest Service Region 1**
- **Montana Department of Fish, Wildlife, and Parks**
- **USGS BRD's Amphibian Research and Monitoring Initiative**
- **Bureau of Land Management**
- **Montana Department of Environmental Quality Wetlands Grant Program**

PCR Based Identification of *B. dendrobatidis*

- **IUCN's Declining Amphibians Population Task Force - Funding**
- **USGS BRD's Amphibian Research and Monitoring Initiative - Funding**
- **Pisces Molecular LLC - testing**
- **Seanna Annis, Joyce Longcore, John Wood, and Janet Epp – development of PCR based based identification of *B. dendrobatidis***

Others

- **David Earl Green at National Wildlife Health Center**
- **Lisa Eby's Lab Group at University of Montana**