

Daniel T. Eastlack<sup>1,2</sup>, Jon R. Davis<sup>1,2</sup>,  
Andrew J. Kouba<sup>1</sup> and Carrie K. Vance<sup>1,3</sup>

<sup>1</sup>Memphis Zoological Society, Memphis Tennessee. <sup>2</sup>Rhodes College, Department of Biology Memphis Tennessee. <sup>3</sup> Department of Biochemistry and Molecular Cell Sciences, Mississippi State University, Starkville MS.

# The amphibian extinction crisis and Chytridiomycosis

- Chytridiomycosis is now widely believed to be a proximate driver of mass-mortalities and worldwide population die offs (Berger et al., 1998; Daszak et al., 1999)

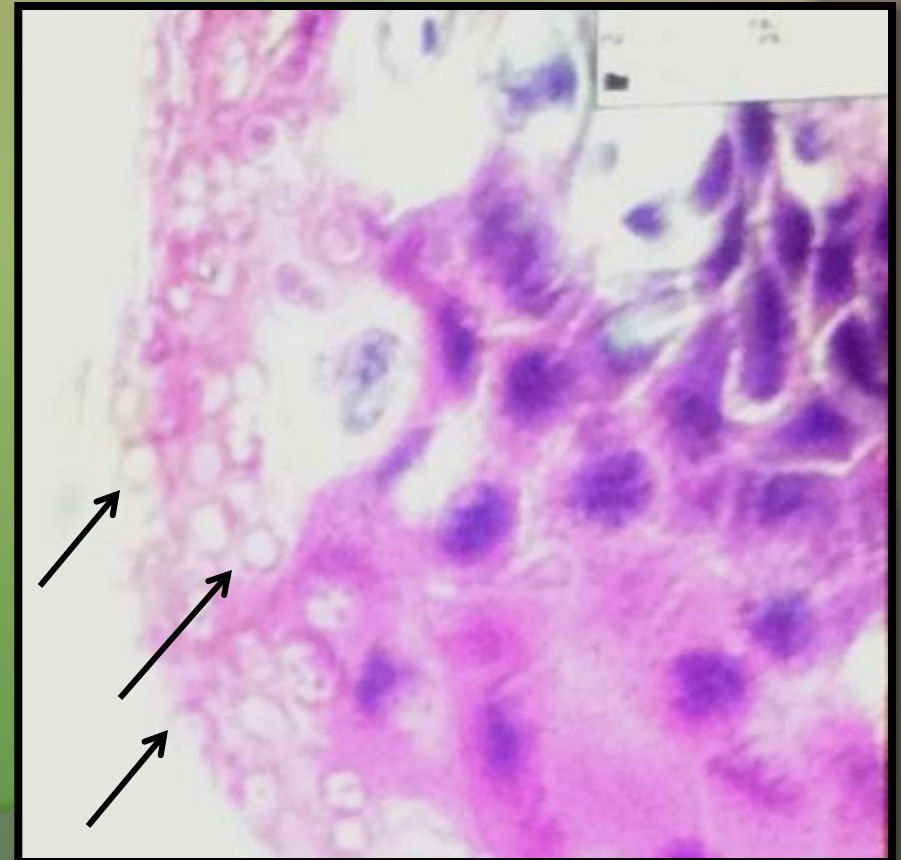


- Caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*)
- Disruption to cutaneous function may be the mechanism by which *Bd* produces mortality (Voyles, 2009).

# Detection of *Bd* zoospores in amphibian skin

*Bd* infection is determined either by:

- histology (Berger et al., 1999)
- quantitative polymerase-chain reaction (qPCR) (Annis et al., 2004).



# New method for chytrid detection: Near Infrared Reflectance Spectroscopy (NIRS)

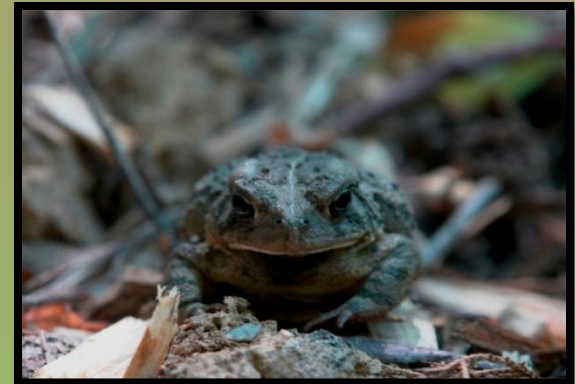
- *Bd* affects the stratum corneum in post-metamorphic amphibians
  - Alterations in the hydration of the keratin layers are signatures of *Bd* infection
- NIRS has been shown to distinguish *organized* bound water in the lipid phase, the primary and secondary hydration layers of keratin, and water in *bulk* in the stratum corneum layer of skin (Martin, 1994)

**Hypothesis: these factors differ in infected and non-infected individuals, and thus could create distinguishably different NIRS spectra of amphibian skin.**

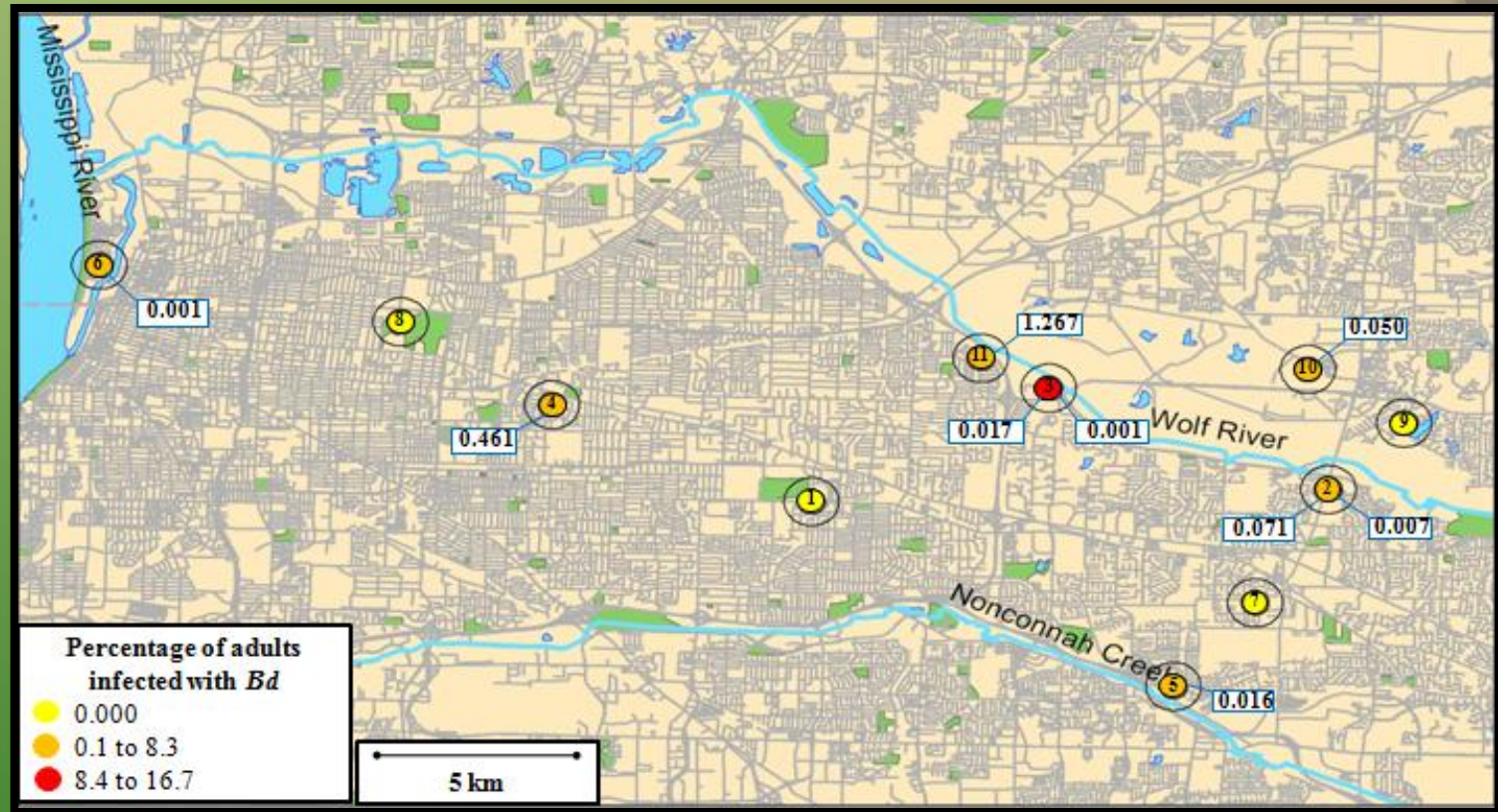
# Project Objective

*Use Fowler's toads as a model species to develop a novel and rapid method of Bd screening based on Near Infrared Reflectance Spectroscopy (NIRS) both in-situ and ex-situ.*

- Why Fowler's toads (*Anaxyrus fowleri*) ?
  - Common local species
  - Model for endangered Wyoming toad and Boreal toad
- Collected 159 adult Fowler's toads from 11 locations in Shelby County
- qPCR analysis for *Bd*
  - Swabs were analyzed using Taqman qPCR by the San Diego Amphibian Disease laboratory



# *Bd* distribution in Fowler's toads



- *Bd* was detected at 7 of 11 study sites However,
  - the percentage of toads infected per site was very low, and
  - the quantity of *Bd* detected on positive individuals using qPCR was low—thresh hold of detection.
- Only two animals exhibited strong *Bd* load for spectral profiling

# NIRS spectra collection

- Adults were NIRS scanned with two contact probes using our ASD FieldSpec 3 NIRS system.



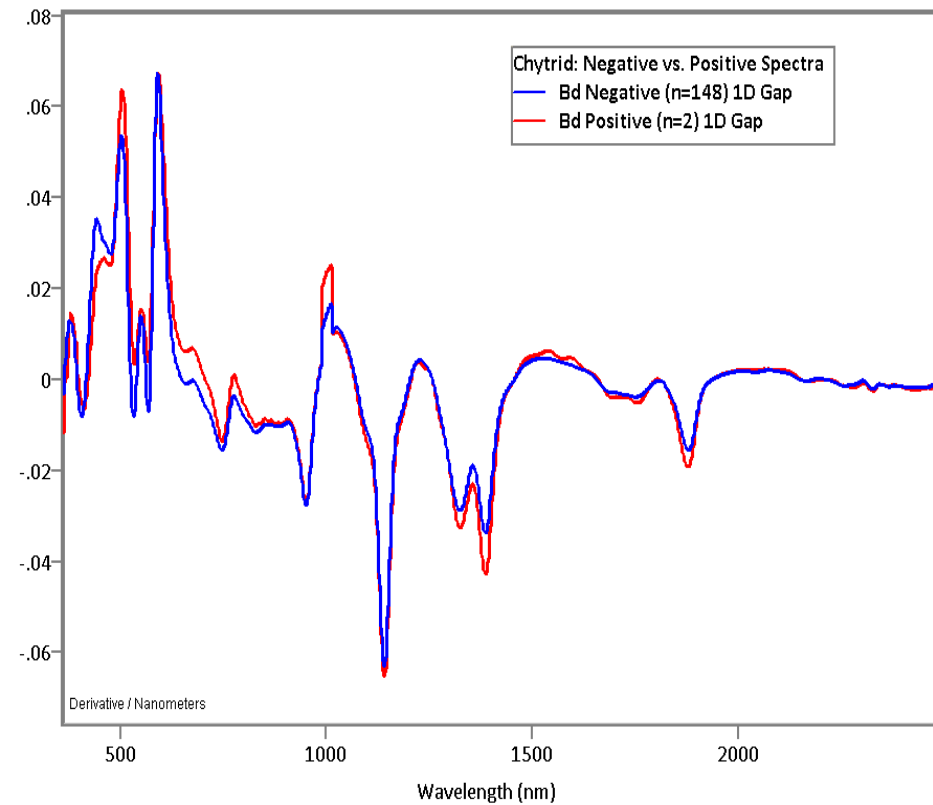
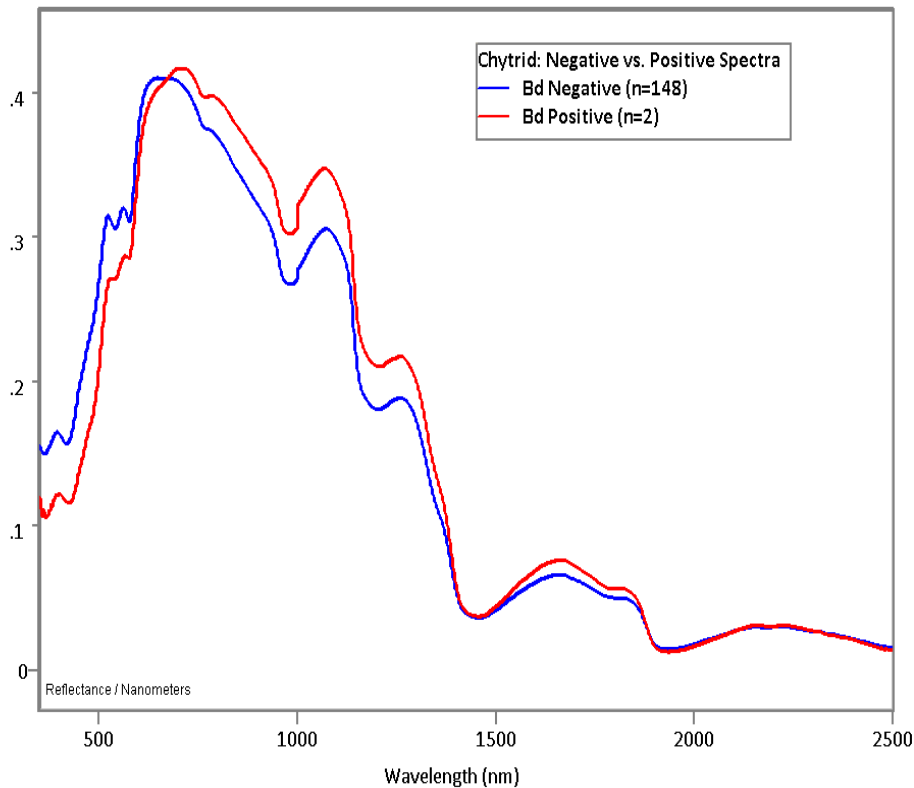
NIRS Screening of Fowler's toad  
(*Anaxyrus fowleri*)



NIRS ASD Field Spec 3®

- Couple the qPCR results with the NIRS spectra for each individual

# Fowler's Toad Spectra



## Reflectance Spectra

## 1<sup>st</sup> Derivative GAP processing

- But how can we determine whether these characteristics of interest are significant and the result of the presence of chytrid infection?

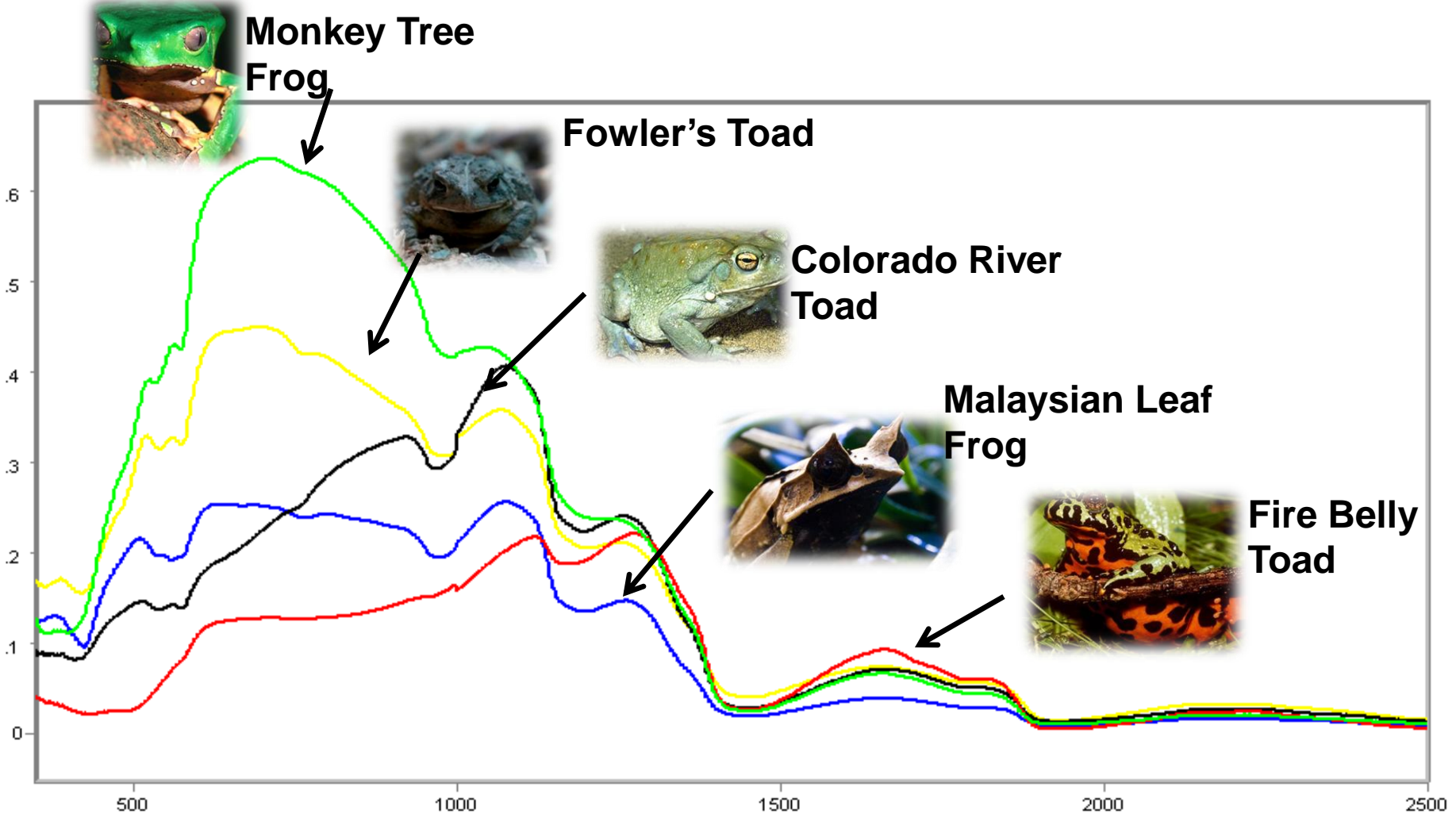
# Chemometrics:

## The statistics of chemical spectra

- Computer program Grams/AI®
- Building a calibration model
  - A group of samples which that display the maximum variability of the character of interest
  - Principal component regression (PCR)
- Validation
  - “leave one out” cross validation
- Prediction of future unknowns

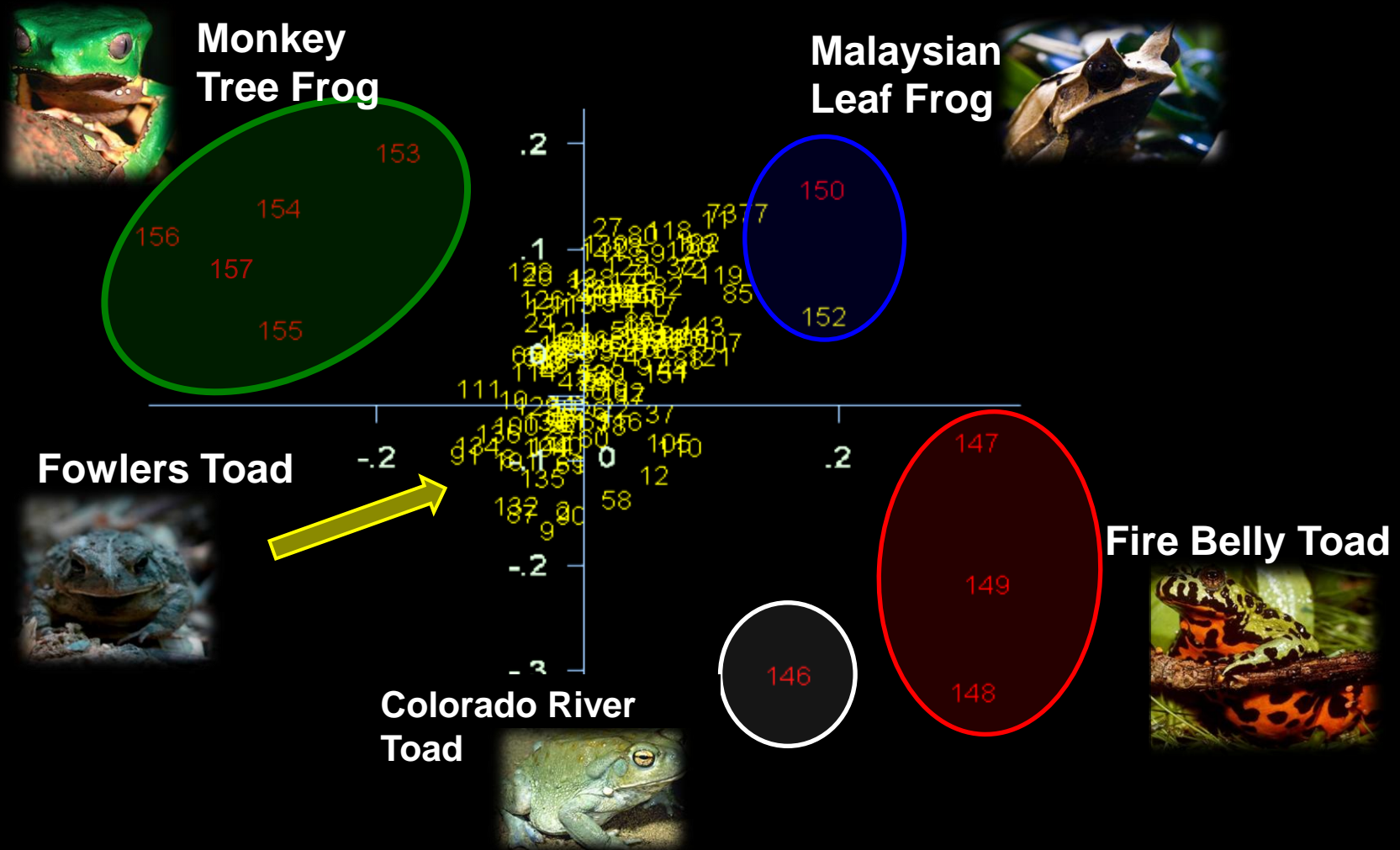
*To test validity of this process, we first compared different species to Fowler's toads*

# Averaged spectra from multiple species



These spectra *look* different from the fowler's toad spectra, but are there significant statistical differences?

# Calibration model prediction results



Chemical differences in skin properties go beyond water and color.  
Processed from spectral data between 800nm-2300nm

# Male vs. Female Fowler's Toads

## Prediction results

Prediction Results	Female Library	Male Library
Female Unknowns (n=47)	94%	6%
Male Unknowns (n=96)	12%	88%

- Discrimination could be due to:
  - Differences in chemical make-ups of stratum corneum, i.e., fat ratio or protein composition
  - Differences in internal structures, i.e., ovarian tissue

# Discussion

- Only 2 Fowler's toads had significantly strong *Bd*-positive qPCR results
  - Prediction results from non-infected library could not exclude these
  - More positives are needed to test against it
- More positives to build a calibration library for *Bd*-infected individuals
- Why were infection levels so low?
  - Do Fowler's toads have resistance to *Bd*?

# Discussion

- Why will this technique be useful if *Bd* infection can already be detected?

# Future Studies

- Acquiring more *Bd*-positive NIRS spectra from wild Fowler's toads
- Creating spectral libraries for other species such as the Leopard frog, and the endangered Boreal toad and Gopher frog
- Testing further the applicability *in situ* of our field NIRS system

# Acknowledgements

- Memphis Zoo Conservation Action Network Grant to CKV and JRD
- Memphis Zoo Conservation and Research Department
- Memphis Zoo Herpetarium staff
- Mississippi State University for spectroscopy equipment
- Allan Pessier from the San Diego Zoo for histology images and qPCR
- Field help from A. Graham, L. Lieb, S. Cassel, T. Boozalis, and C. Covic



MISSISSIPPI STATE  
UNIVERSITY™

# Works Cited

Annis, S., F. Dastoor, H. Ziel, P. Daszak, and J. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *Journal of Wildlife Diseases* 40(3):420-428

Berger, L., et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proc. Nat. Acad. Sci.* 95:9031– 9036.

Daszak, P., et al. (1999) Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Dis.* 5:735–748.

Longcore , J., et al. (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227.

Martin, K. (1994) Interpreting the near-infrared reflectance of skin: Water content and scattering. *In: Near Infrared Spectroscopy: The Future Waves. Proceedings of the 18<sup>th</sup> IFSCC Congress: Depth-profiling of skin by near-infrared spectroscopy.*