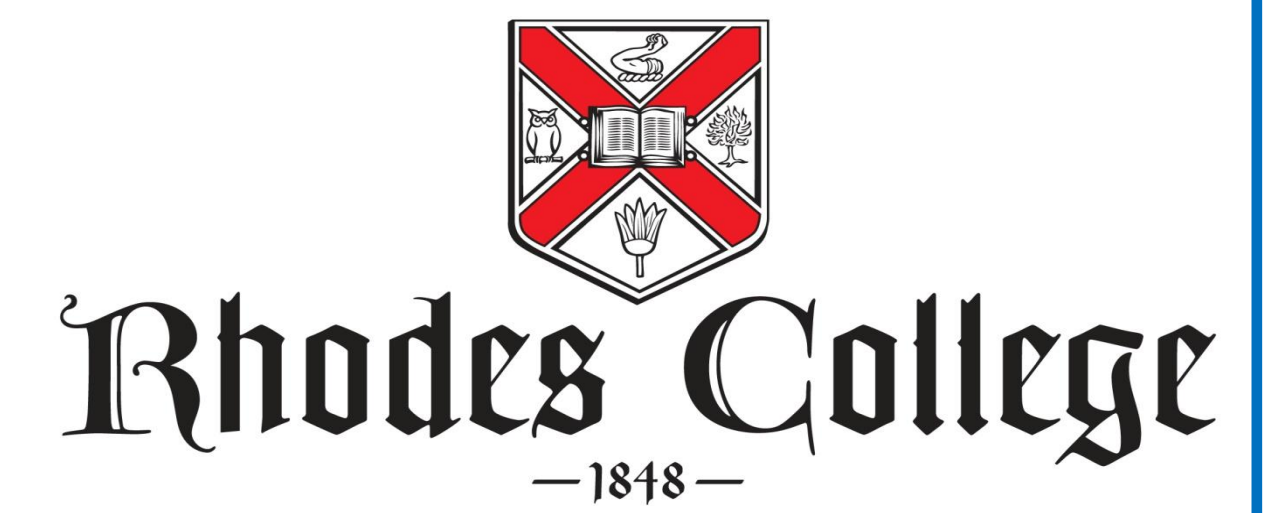


# Is the bufonid *Anaxyrus fowleri* resistant to chytrid fungus?

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

<sup>1</sup>Memphis Zoological Society, <sup>2</sup>Rhodes College, <sup>3</sup>Mississippi State University



## INTRODUCTION

Chytridiomycosis is an emerging skin disease of amphibians now widely believed to be a proximate driver of mass-mortalities and worldwide population die offs (Berger et al., 1998; Daszak et al., 1999). The disease is caused by the zoospore fungus *Batrachochytrium dendrobatidis* (*Bd*), which infects the superficial layers of the epidermis of its host and can cause a variety of symptoms and mortality (Longcore et al., 1999).

Although *Bd* is known to be lethal to many amphibian species, population declines to date show that species differ in their susceptibility, even in cases where the hosts are sympatric. Some species apparently have only moderate susceptibility where infections can cause severe declines in populations without the complete loss of all individuals, whereas other species test positive for the fungus and yet tolerate infection without any clinical signs of infection or population declines (Richmond et al., 2009).

Research shows that this apparent resistance can be attributed in some species to the production of large quantities of host defensive peptides or bacterial flora, inhibiting *Bd* growth (Richmond et al., 2009).

*Bd*'s rapid spread across all continents except for Antarctica has been exacerbated by the international trade of amphibians and has caused acute die-offs in many naïve populations (Picco and Collins, 2009; Schloegel et al., 2009). *Bd* can be introduced into new natural areas by movement of infected amphibians or in water or soil contaminated with *Bd* zoospores.

Here, we present preliminary results of a field study describing the presence and intensity of *Bd* infection in the Fowler's toad (*Anaxyrus fowleri*) in the Memphis, Tennessee metropolitan area.

## METHODS

**Data collection:** We collected a total of 159 adult Fowler's toads (Figure 1) from 11 locations and 60 recently metamorphosed toads from 5 of the same locations (recorded with GPS), roughly covering the entire study region.

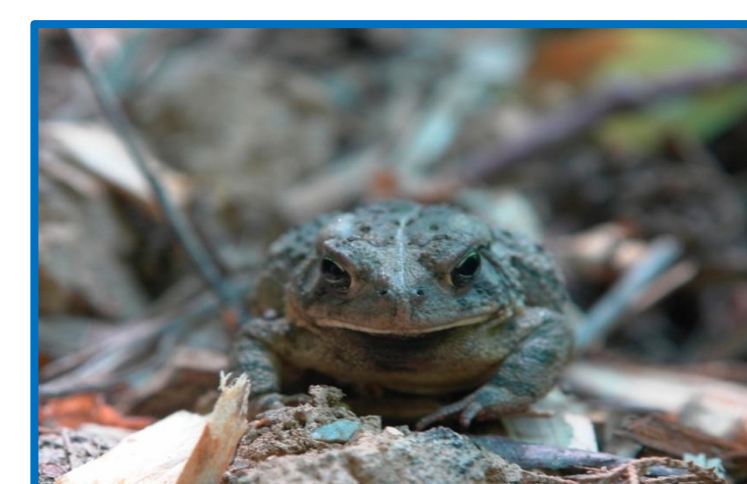


Figure 1. Adult Fowler's toad (*Anaxyrus fowleri*)

We collected 10 to 24 individuals (both male and female) from each location and swabbed the pelvic patch, hindlimbs, and the webbing between the toes where *Bd* infection is most concentrated (Figure 2).

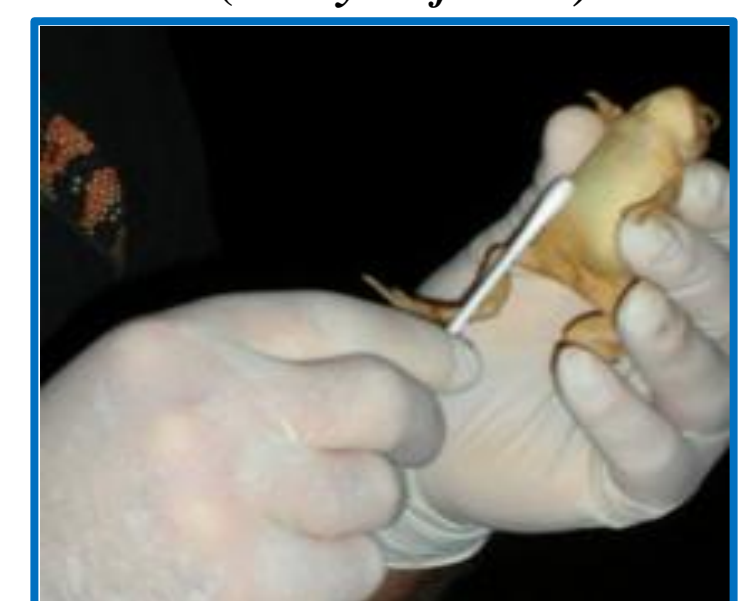


Figure 2. Swabbing *Anaxyrus fowleri* in the field

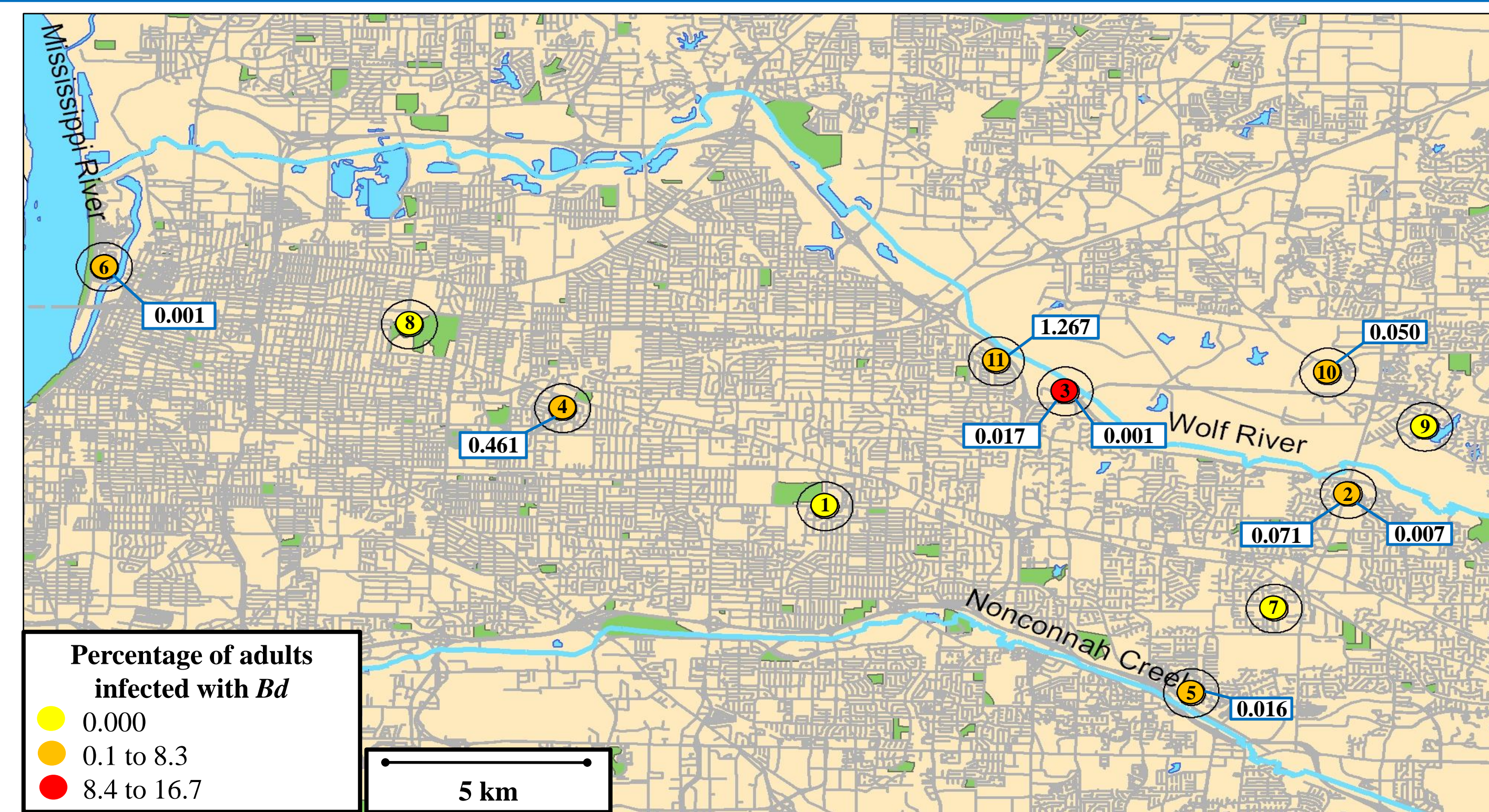
We analyzed swabs for the presence of *Bd* with quantitative (real time) Taqman PCR at the Amphibian Disease Laboratory of the San Diego Zoo's Institute for Conservation Research.

Swabs were tested in triplicates and the results shown in the text boxes in Figure 3 are mean quantity values of each triplicate.

**Data analysis:** We used the ERSI ArcMap program to map the locations of each study site. Points on the map have two buffers: the inner buffer has a 312m radius, which is the maximum reported movement distance of *A. fowleri* (Clarke, 1974), and the outer buffer has a radius of 624m to encompass possible greater movements (Figure 3).

We used linear regression and T-tests to determine whether the distance to the nearest river significantly affects *Bd* infection status.

## RESULTS

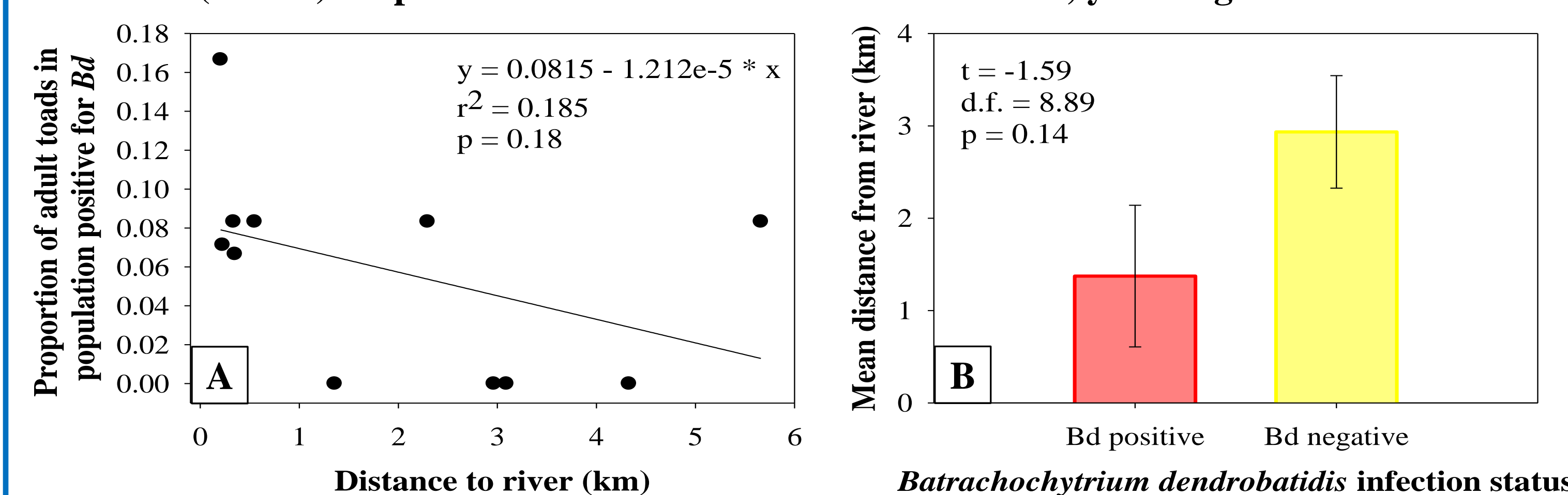


**Figure 3.** Spatial distribution of 11 study sites (denoted by numbers in circles) and the percentage of adult *Anaxyrus fowleri* sampled at each location that were positive for *Bd* (color-coded). Buffer zones represent 312 and 624 m from the center of each sample area to indicate accessibility to the nearest river. Text boxes provide mean qPCR quantity values of *Bd* detected for each positive toad at that site.

**Table 1.** Summary of *Batrachochytrium dendrobatidis* infection status of adult and metamorph *Anaxyrus fowleri* sampled in the Memphis, Tennessee metropolitan area.

Site ID	Site name	# adults sampled	# adults + for <i>Bd</i>	% of adults + for <i>Bd</i>	# metamorphs sampled	# metamorphs + for <i>Bd</i>	% of metamorphs + for <i>Bd</i>
1	Audubon Park	10	0	0.0			
2	Brookchase	24	2	8.3	12	0	0
3	Christian Brothers	12	2	16.7			
4	Chickasaw Gardens	12	1	8.3			
5	Madison Apts	15	1	6.7			
6	Mud Island	14	1	7.1	12	0	0
7	McVay Park	24	2	0.0			
8	Overton Park	12	0	0.0	12	0	0
9	Simpson Park	12	0	0.0			
10	Shelby Farms	12	1	8.3	12	0	0
11	Walnut Grove	12	1	8.3	12	0	0

- *Bd* was detected at 7 of 11 study sites (63.6%).
- Despite being widespread in Memphis, the percentage of toads infected and the quantity of *Bd* detected on positive individuals using qPCR were low.
- No metamorphs were confirmed positive for *Bd*.
- 5 of 7 (71.4%) *Bd* positive sites were within 624m of a river; yet 0 negative sites were.



**Figure 4.** (A) There is a non-significant negative correlation between the proportion of adult *Anaxyrus fowleri* infected with *Bd* and the distance (km) to the nearest river. (B) There is no significant difference in the distance to the nearest river for toads positive or negative for *Bd*.

## DISCUSSION

- *Bd* is relatively widespread in Fowler's toads in Memphis...BUT
  - The % of individuals infected at each site and the quantity of *Bd* detected on positive individuals is low (Table 1; Figure 3).
- The negative correlation between distance to river and proportion of individuals infected with *Bd* is non-significant (Figure 4B)...YET
  - Power analysis indicated that the low sample size (n=11) may prevent detection of a significant effect.
- No metamorphs were positive for *Bd* (Table 1)...WHY?
  - Infected tadpoles died prior to metamorphosis?
  - *Bd* only detectable in keratinized mouthparts of tadpoles and may not yet be detectable in skin of recent metamorphs?
  - Juvenile Fowler's toads are resistant to *Bd*?
- Anecdotal evidence from Memphis Zoo laboratory colony of Fowler's toads suggests resistance to *Bd*...SINCE
  - Fowler's toads infected with *Bd* can survive >5 months with *Bd*.
  - High-density housing of infected toads with non-infected toads does not result in infection of all individuals.

## FUTURE DIRECTIONS

- Examine *Bd* infection status of toads at additional sites and in rivers to improve statistical power and evaluate potential of rivers as transmission corridors.
- Collect samples from all life stages (tadpoles, metamorphs, and adults) at each site to confirm transmission dynamics within and among life stages.
- Collect samples from sympatric amphibian species to establish *Bd* infection status in amphibian communities in metropolitan Memphis, Tennessee.
- Examine Fowler's toads in captivity to assess virulence of *Bd* and resistance to *Bd* in this species.
  - Assess effects of housing density and temperature on *Bd* transmission and clearance rates.

## REFERENCES

- 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.
- Clarke, R. (1974) Activity and movement patterns in a population of Fowler's toad, *Bufo woodhousei fowleri*. *Am. Midl. Nat.* 92(2):257-274.
- 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
- Picco, A. and Collins, J. (2008) Amphibian commerce as a likely source of pathogen pollution. *Cons. Biol.* 2:1582-1589.
- Richmond, J., et al. (2009) Toward immunogenetic studies of amphibian chytridiomycosis: Linking innate and acquired immunity. *BioScience* 59(4):311-320.
- Schloegel, L., et al. (2009) Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biol. Cons.* 142:1420-1426.

## ACKNOWLEDGMENTS

This research was supported by a Memphis Zoo Conservation Action Network Grant to CKV and JRD and a SICB Charlotte Mangum Student Support Program award, the Rhodes student travel fund, and a Rhodes College Environmental Studies / Sciences Student Travel Support grant to DTE. We thank A. Graham, L. Lieb, S. Cassel, T. Boozalis, and C. Covic for field assistance and Dr. S. Boyle for GIS help. This research permitted by the Tennessee Wildlife Resources Agency and was approved by Rhodes College IACUC #099.

