A Questionable Role for Amphibian Chytrid Fungus in the Decline of the Relict Leopard Frog

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Bradford et al. 2004

Relict leopard frog declines – Original perspective:

- Habitat
- Disturbance regimes
- Introduced nonnative predators and competitors

Disease

Emergent Disease – Chytridiomycosis

Caused by *Batrachochytrium dendrobatidis (Bd)*

Disease was not originally considered a threat

Batrachochytrium dendrobatidis

Thermal tolerance

- Growth of *Bd* in culture occurs at temperatures between 4–25° C
- Growth slows at higher temperatures, with an upper thermal limit ≥ 28° C

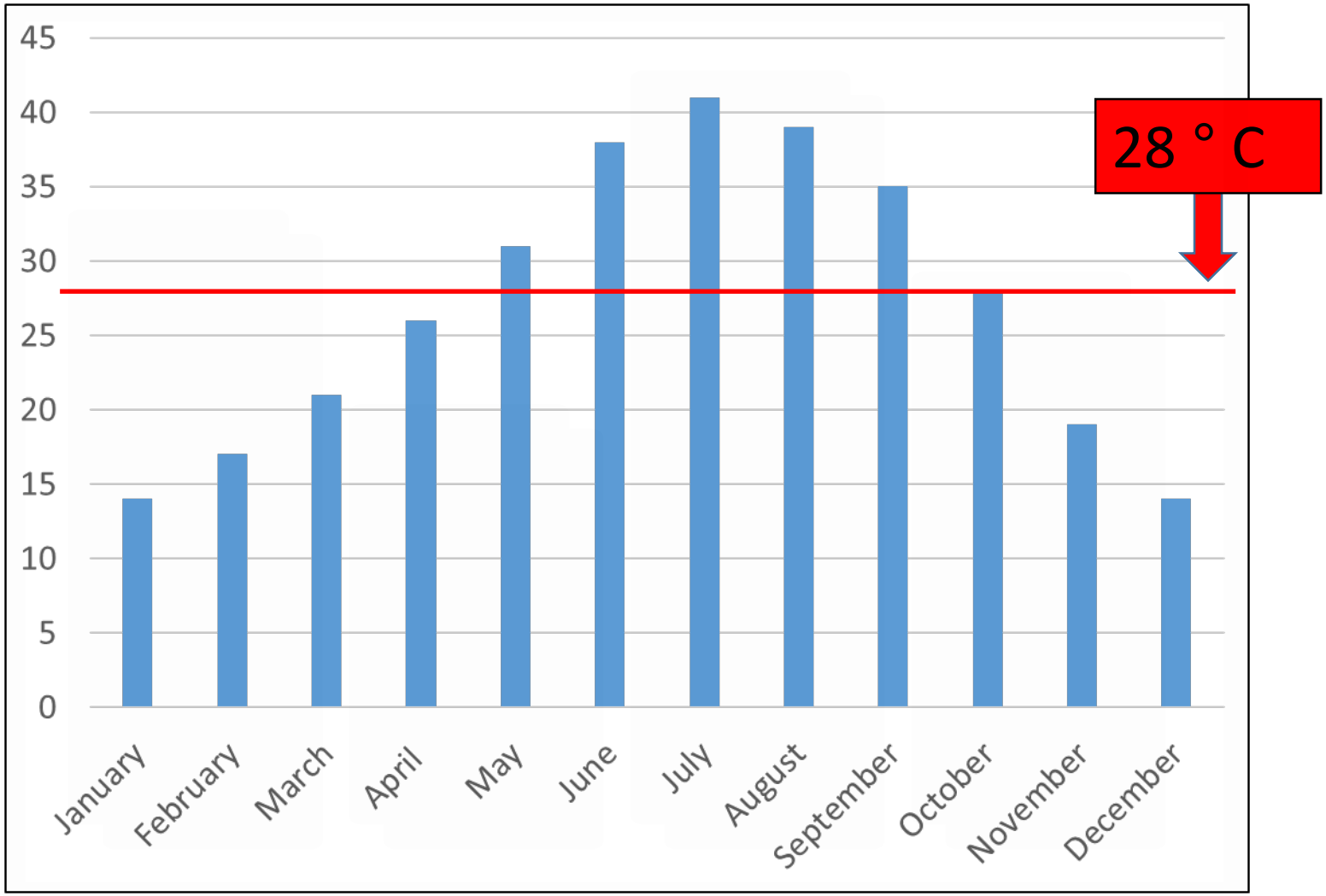
(Piotrowski et al. 2004)

Batrachochytrium dendrobatidis

Thermal tolerance

- Anticipated that hot ambient temperatures common in the Mojave Desert would greatly limit *Bd* infection prevalence and intensities

Average Monthly High Temperatures in Mojave Desert



Batrachochytrium dendrobatidis

Thermal tolerance

- In habitats and regions where temperatures commonly occur above thermal optimum for *Bd* growth, the pathogen should be absent or limited

  Piotrowski et al. 2004; Puschendorf et al. 2009

- Considerable support for this hypothesis

  Occurrence and impact of *Bd* tends to be limited in regions and during seasons that are characterized by high temperatures

  Berger et al. 2004; Drew et al. 2006; Kriger et al. 2007; Savage et al. 2011; Whitfield et al. 2012

Batrachochytrium dendrobatidis

High temperature

- Shown to limit pathogenicity or clear *Bd* infection in some amphibians

  (e.g.Woodhams et al. 2003)

- Amphibian immune system function appears more robust at higher temperatures

  (Rollins-smith et al. 2011)

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Nothing a Hot Bath Won't Cure: Infection Rates of Amphibian Chytrid Fungus Correlate Negatively with Water Temperature under Natural Field Settings

Matthew J. Forrest[1*], Martin A. Schlaepfer[2,3]

- Working on closely related lowland leopard frogs, showed..
- "Geothermal springs appear to provide protective habitat where amphibians can persist despite the presence of *Bd*"

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Nothing a Hot Bath Won't Cure: Infection Rates of Amphibian Chytrid Fungus Correlate Negatively with Water Temperature under Natural Field Settings

Matthew J. Forrest[1*], Martin A. Schlaepfer[2,3]

Made the observation that,

"All naturally occurring *R. onca* populations are now associated with perennial geothermal springs with source temperatures exceeding 30°C"

Speculated,

> Elevated water temperature appears to provide frogs refuge from *Bd* in the wild
>
> "…Relict leopard frog…survival may be contingent on geothermal watersheds"

New perspective

- If all remaining naturally occurring populations are associated with geothermal watersheds…..
- Disease may limit current distribution
- Disease may have been a facilitator of the historical decline of the relict leopard frog, specifically chytridiomycosis

Research Aims

- To assess *Bd* presence/prevalence in amphibians at historical sites for relict leopard frogs

  We conducted field sampling along a large portion of the historical sites for relict leopard frogs

- To assess susceptibility of relict leopard frogs to *Bd*

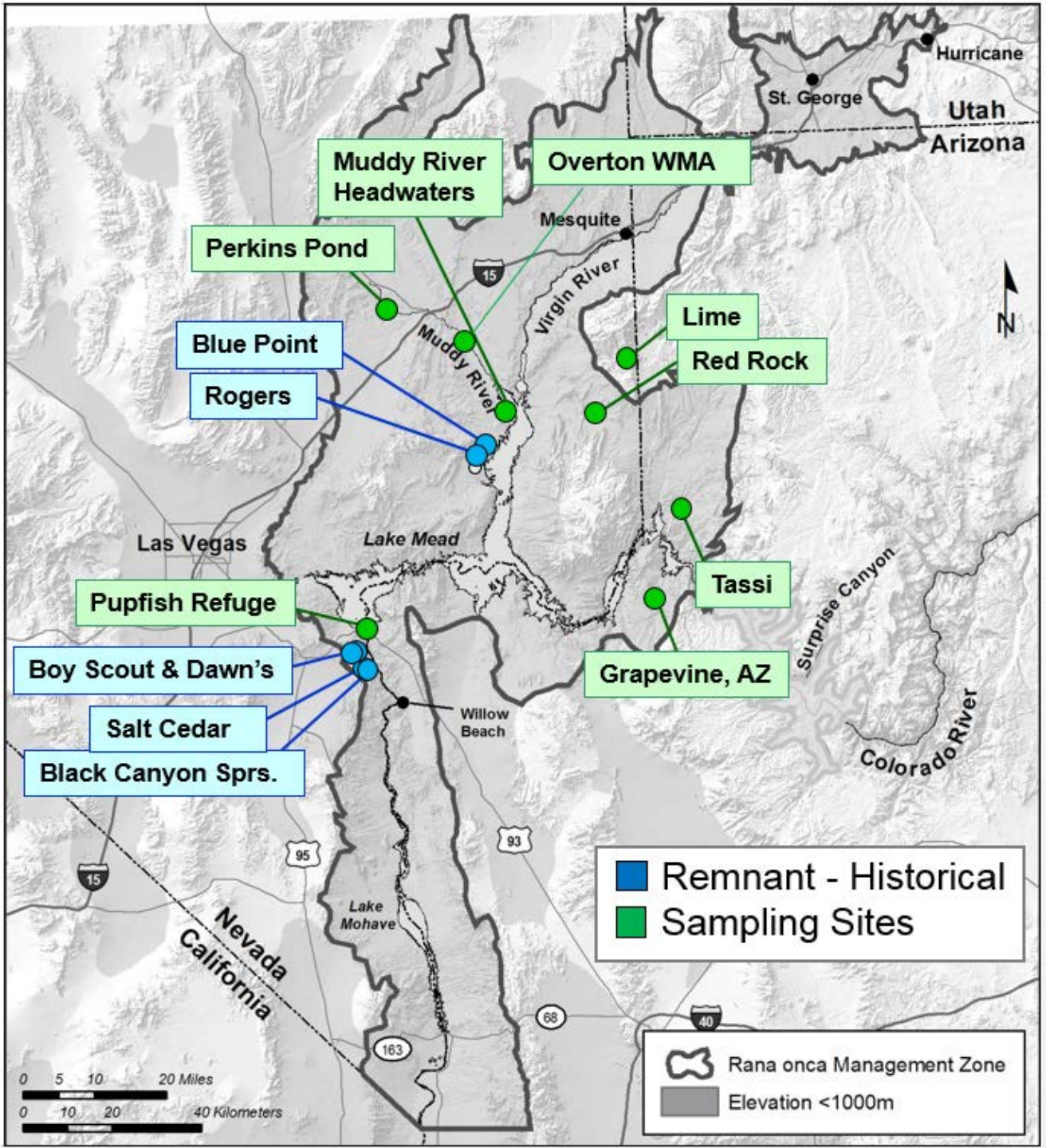
  We conducted a laboratory exposure study

Conservation Implications

- Management strategy for the species has emphasized establishing new populations
- If RLF are highly susceptible, translocations may be limited to geothermal springs or to isolated sites where *Bd* was absent.

Field Sampling

Sampling sites – targeting a large portion of the historical range of relict leopard frogs



Field Sampling

Methods:

- Sampled predominately in early Spring
- Swabbed every anuran captured
- Determined infection via qRTPCR

Field Sampling

Methods:

Sampled 5 different species

- ✓ American bullfrog
- ✓ Baja California treefrog
- ✓ Red-spotted toad
- ✓ Relict leopard frog
- ✓ Woodhouse's toad

Field Sampling

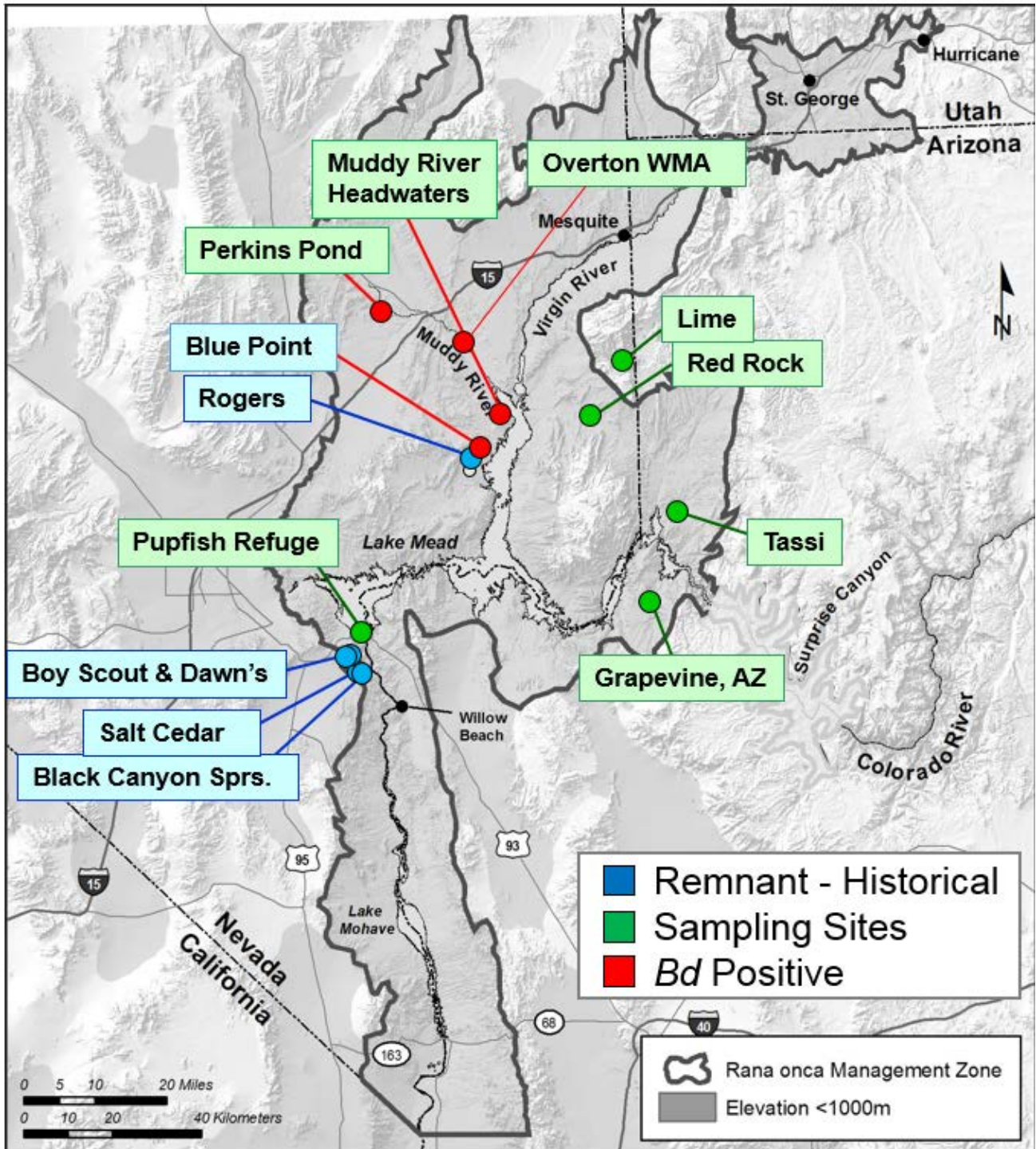
Results:

Sampled 376 anurans

Bd detected at

4 water sites, on

- ✓ American bullfrog
- ✓ Baja California treefrog
- ✓ Red-spotted toad
- ✓ Relict leopard frog
- ✓ Woodhouse's toad



Field Sampling – pit tagged individuals

- Pit-tagged relict leopard frogs at Northshore allowed us to observe *Bd* infections temporally

- Three frogs were sampled multiple times
  - One appeared to have cleared infection
  - Another maintained infection across 13 months
  - An additional frog was seen healthy 8 months after testing positive, although not retested.

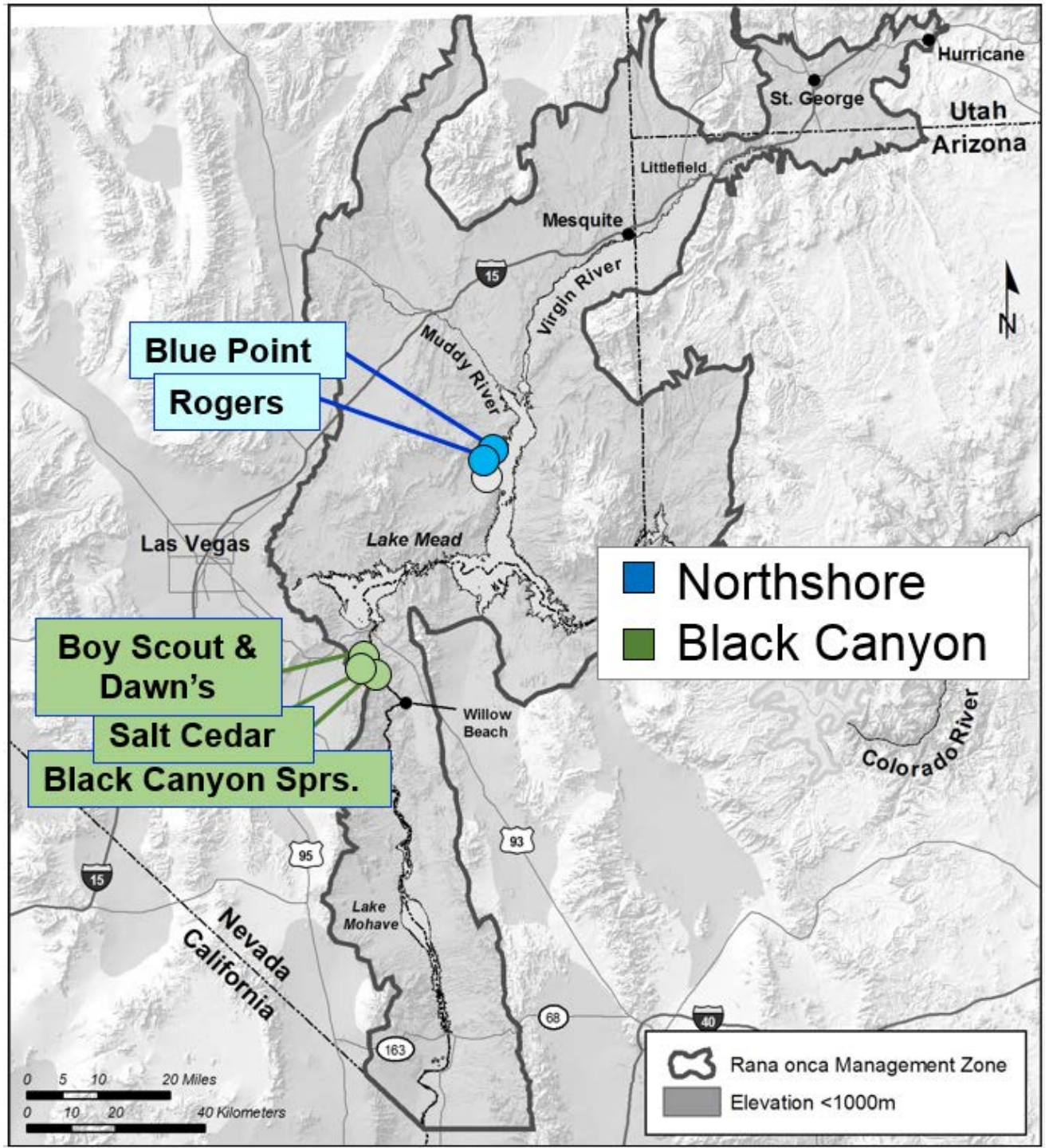
Laboratory Studies

We **predicted:**

- Relict leopard frogs would be highly susceptible to *Bd*
- There would be a difference in susceptibility between a potentially *Bd* naïve population (**Black Canyon**) and a population that has individuals currently infected with *Bd* (**Northshore**)

Source Populations

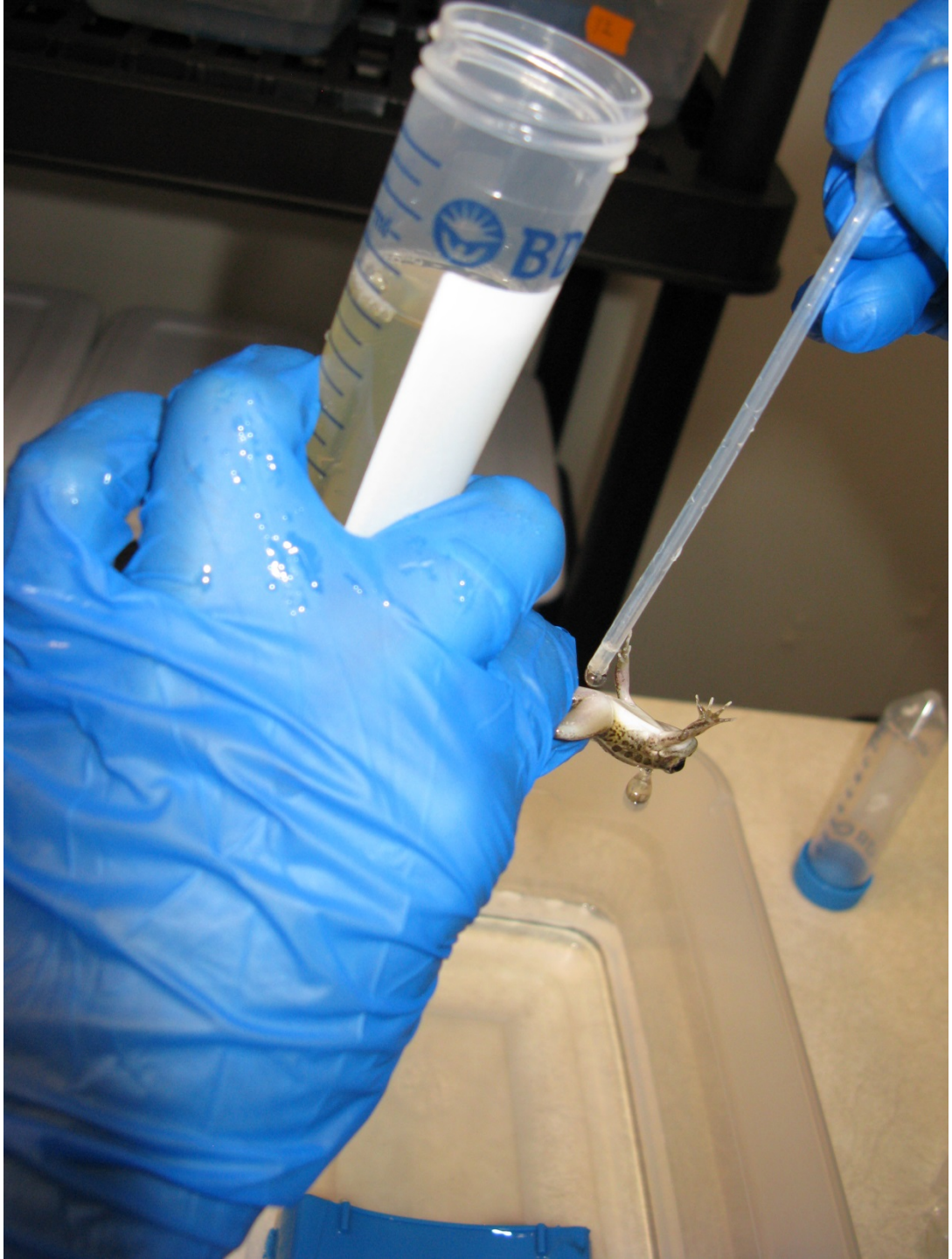
General area where egg masses were collected for headstarting



Laboratory Studies – Methods

- Initial experiment using 60 total juvenile frogs from Northshore and Black Canyon populations
- Used *Bd* strain CJB7

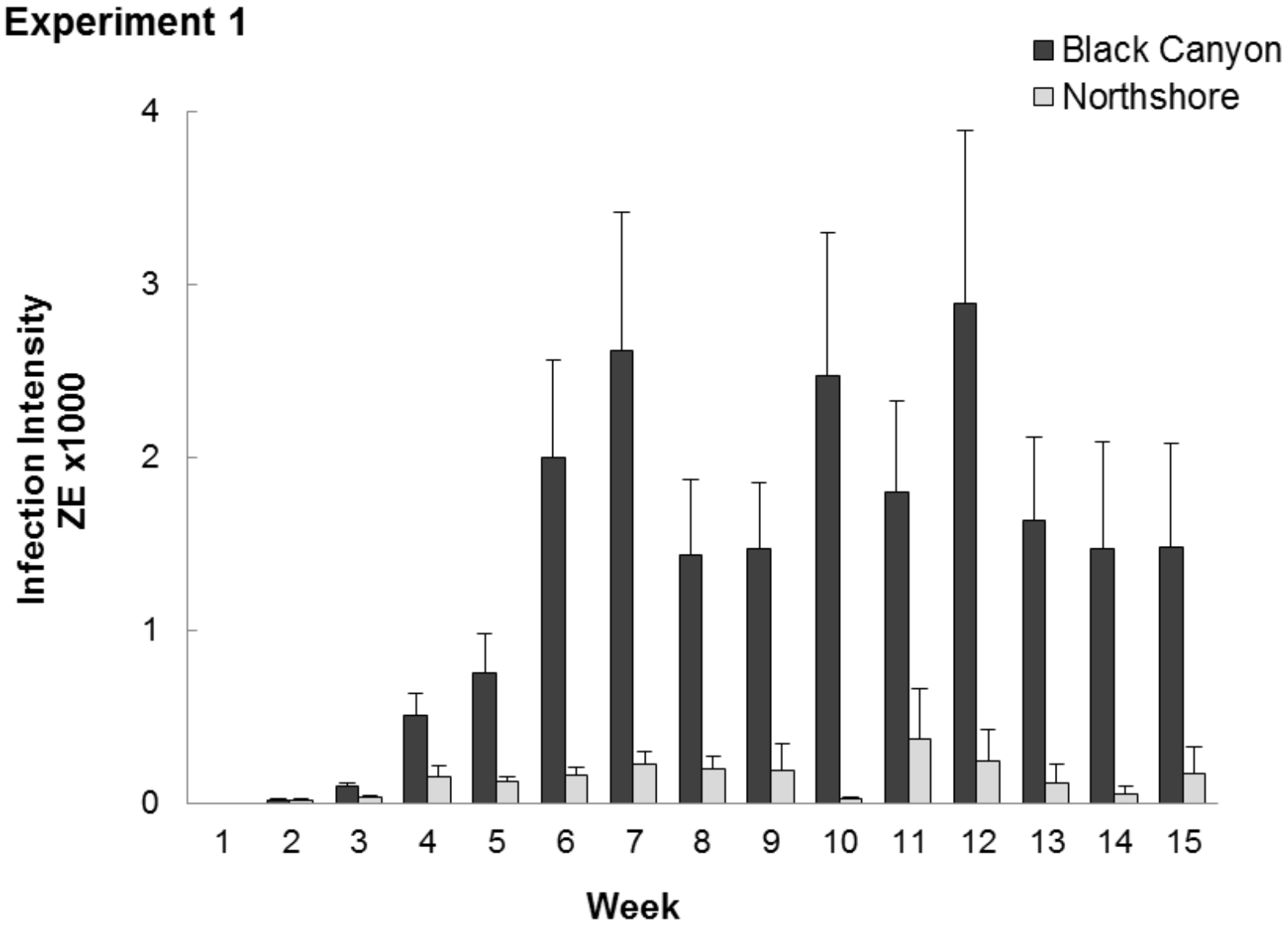
  Collected in 2009 From Mountain Yellow-legged Frog (*Rana muscosa*) at Sixty Lakes Basin in southern Sierra Nevada, California
- Separated frogs into three groups
  - Black Canyon
  - Northshore
  - Control


BD

Results

- Infectability
  - All exposed frogs became infected
  - No control animals became infected
- Survivorship
  - No difference in survivorship between infected and control animals
- Infection Clearance
  - Northshore 90%, Black Canyon 20%
- Infection trend comparisons

Results – Infection Trends



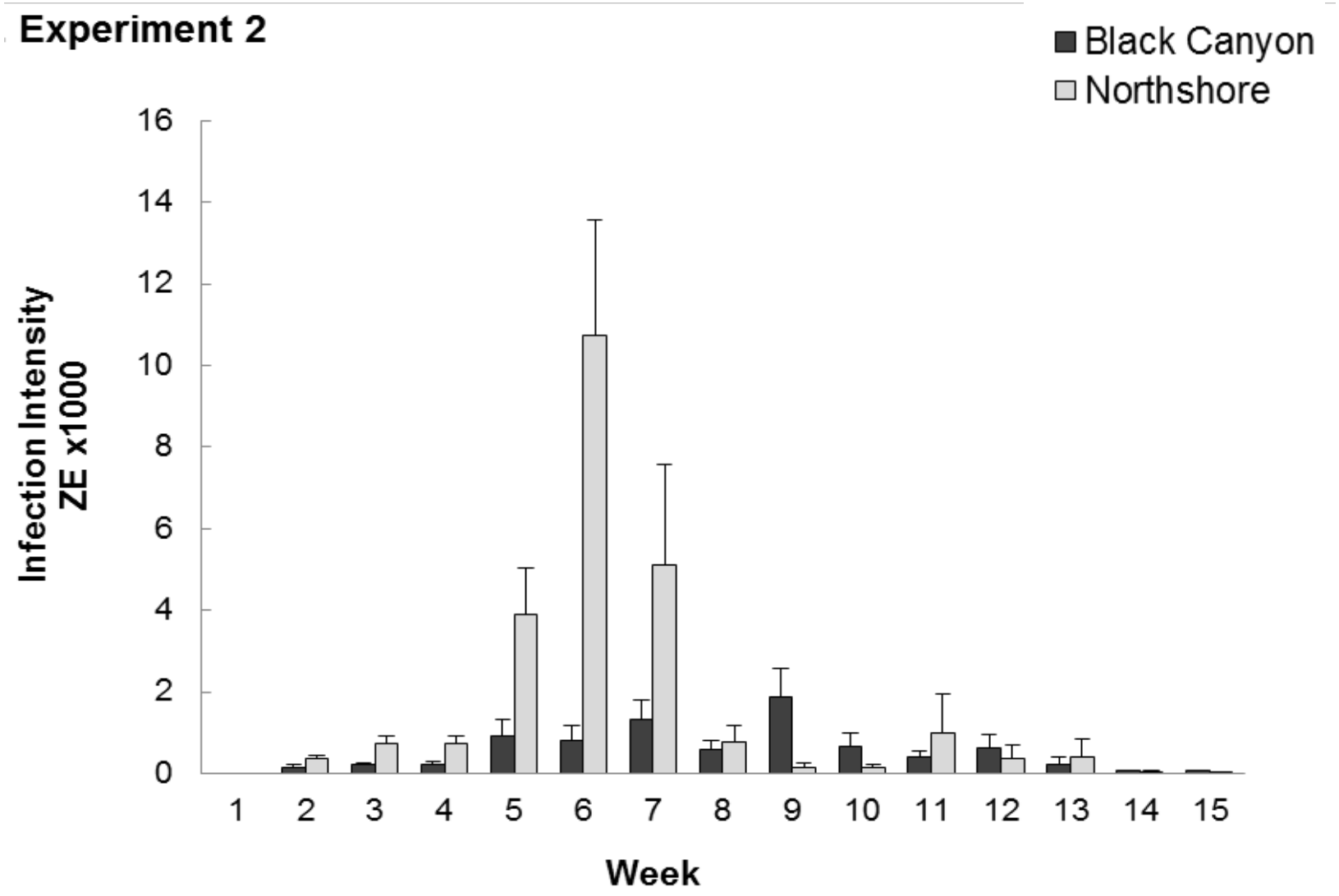
Second Laboratory Experiment – Methods

- Evaluate an additional strain to address possible difference in variance between strains
- Repeated experiment using *Bd* strain Section Line Lake
  - Collected in 2011 from Cascades Frog (*Rana cascadae*) at Section Line Lake in Klamath Mountains, California
- Divided 54 frogs from each locality into 3 groups:
  - Black Canyon
  - Northshore
  - Control

Results

- Infectability
  - All except two frogs in SLL experiment became infected
  - No control animals became infected
- Survivorship
  - No difference in survivorship between infected and control animals
- Infection Clearance
  - Northshore 88%, Black Canyon 61%
- Infection trend comparisons

Results – Infection Trends



Conclusions

- Relict leopard frogs are susceptible to *Bd* infection
- Survivorship does not appear to be significantly effected by at least two strains in the laboratory
- Frogs appear to be able to clear infection in the lab at high proportions (64% overall)
- There is some evidence that frogs in the wild can persist despite infection
  - Clearance of infection in field and laboratory
  - > one year survival in the wild

Discussion – However…

- Laboratory environment
- Relict leopard frogs have inherent resistance or may have evolved resistance
  - Speculation questioning how this might occur in thermal systems
- Attenuation of *Bd* strains
- Conservation – Results bode well for the future conservation of the species, not limiting potentially suitable sites

Future directions

- Isolate the local strain
- Perform an additional exposure study

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