Poor Biosecurity Could Lead to Disease Outbreaks in Amphibian Populations

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GRC Ranavirus Course
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Outline

- Background
  - Amphibian Declines
  - Ranavirus
  - Common Field Techniques
- Methods
- Results
- Discussion
- Questions

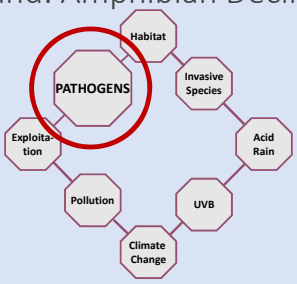
Background: Amphibian Declines

9.3% Extinct, Extinct in the wild, or Critically Endangered

24.3% Endangered or Vulnerable

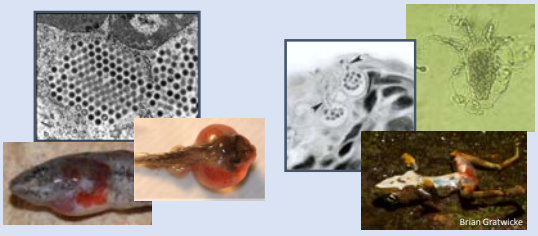
Holt et al
Science 2012

Background: Amphibian Declines



BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

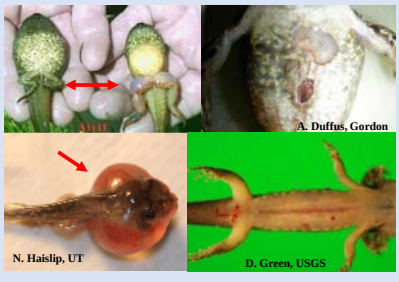
Background: Pathogens



Ranaviruses Chytrid

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Background: Ranavirus



"Ebola" of Amphibians

- Edema
- Erythema
- Hemorrhages
- Ulcerations

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

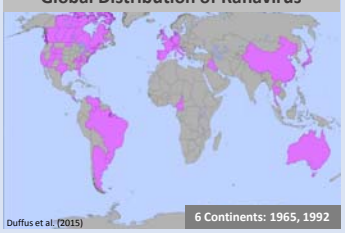
Background: Ranavirus

18 Families:

Alytidae, Ranidae, Hylidae, Bufonidae, Centrolenidae, Craugastoridae, Dendrobatidae, Discoglossidae, Leptodactylidae, Pipidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae

>100 Species

Global Distribution of Ranavirus



Duffus et al. (2015)

All Latitudes, All Elevations

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Background: Ranavirus Transmission

Indirect

Skin, Gills, Intestines

Direct Contact

One Second Skin Contact

Ingestion

Incidental, Necrophagy, Cannibalism, Predation

Brunner et al. (2004), Harp & Petranka (2006), Brunner et al. (2007), Hoverman et al. (2010), Robert et al. (2011)

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Cases of Pathogen Pollution

- White-nose syndrome
- Ranavirus through bait salamanders (Jancovich et al 2005)
- Bsal into Europe (Martel et al 2014)
- Bd release through captive breeding program (Walker et al 2008)

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Sampling Process

- Catch individuals
- Hold individuals in container
- Process individuals
- Release individuals

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Research Objectives

1. Determine if cohousing animals in buckets affects ranavirus transmission
2. Determine if changing gloves between animals reduces ranavirus transmission.

BACKGROUND METHODS RESULTS DISCUSSION QUESTIONS

Methods: Study Animal

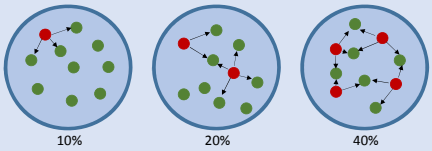
Wood Frog, *Lithobates sylvaticus*

- Highly susceptible to Ranavirus
  - > 90% mortality when exposed in water

BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Methods: Cohousing

- Different lengths of time (15 min, 30 min, 60 min)
- Densities of infected individuals (10%, 20%, 40%)



3 time periods * 3 densities * 10 individuals → 90 individuals/replicate
90 individuals/replicate * 5 replicates → 450 individuals
10 controls

Methods: Cohousing

Our Experiment | Field Example

Clip tails of infected individuals (for identification and pathogen verification)

Methods: Cohousing

Our Experiment | Field Example

Cohouse in buckets (for 15, 30, or 60 minutes)

Methods: Cohousing

Our Experiment | Field Example

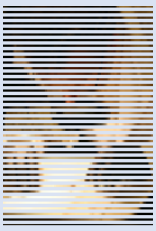
Retrieve from bucket

Methods: Cohousing

Our Experiment | Field Example

Place in individual tubs and monitor for 14 days

Methods: Cohousing



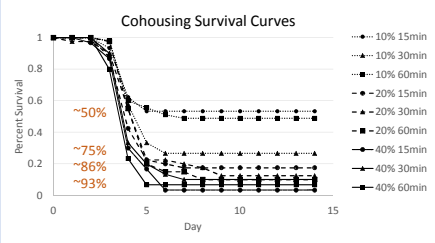
Necropsy all that die (Euthanize all at end)

Methods: Cohousing

Analyze with qPCR

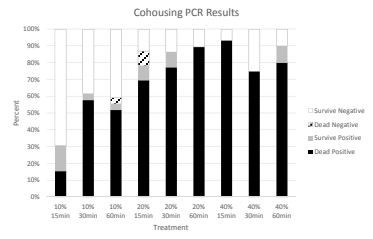
BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Results: Cohousing



BACKGROUND METHODS **RESULTS** DISCUSSION QUESTIONS

Results: Cohousing



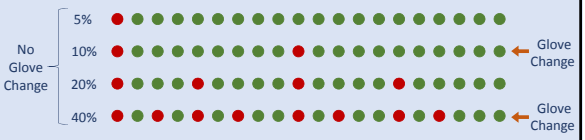
- 91% of infected died
- 8% of infected survived
- 1.6% mortality with no detected infection

3 of 5 replicates

BACKGROUND METHODS **RESULTS** DISCUSSION QUESTIONS

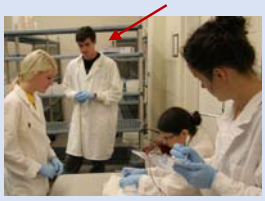
Methods: Glove Change

- Change gloves or don't change gloves
- Vary density of infected individuals



((4 densities no change) + (2 densities change)) * 20 individuals = 120 individuals
120 individuals per replicate x 5 replicates → 600 individuals
10 controls

Methods: Glove Change



1 person handles ONLY infected individuals

1 person handles ONLY clean individuals

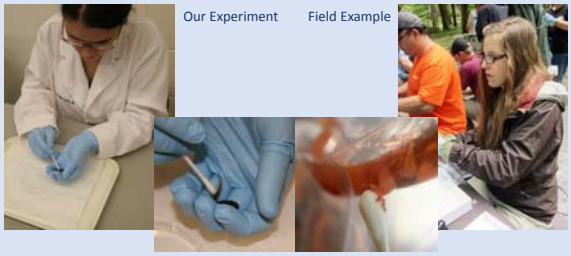
Methods: Glove Change

Place individual on tray

Methods: Glove Change

Our Experiment

Field Example



Swab individual

BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Methods: Glove Change

Keep swabs from infected individuals

BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Methods: Glove Change

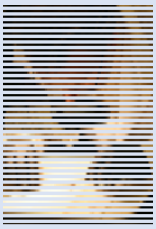
Our Experiment

Field Example

Monitor individuals for 14 days

BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Methods: Glove Change



Necropsy all that die (Euthanize all at end)

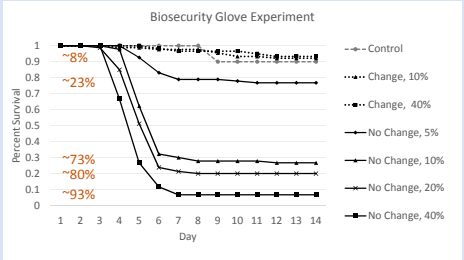
BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Methods: Glove Change

Analyze with qPCR

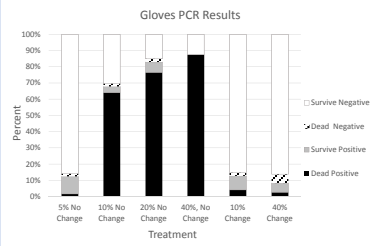
BACKGROUND **METHODS** RESULTS DISCUSSION QUESTIONS

Results: Glove Change



BACKGROUND METHODS **RESULTS** DISCUSSION QUESTIONS

Results: Glove Change



- 85% of infected died
- 15% of infected survived
- 2% mortality with no detected infection

3 of 5 replicates

BACKGROUND METHODS **RESULTS** DISCUSSION QUESTIONS

Discussion

- Cohousing tadpoles resulted in pathogen transmission
  - Even short time periods matter
  - Lower infection prevalence → Lower mortality
    - 10% prevalence → 50% mortality
    - 20% prevalence → 90% mortality
- Gloves can transfer virus
  - Even at low infection prevalence (10% → 70% mortality)

BACKGROUND METHODS RESULTS **DISCUSSION** QUESTIONS

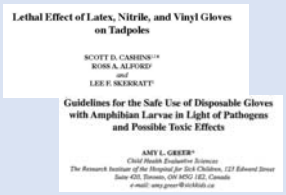
Biosecurity Practices

- House animals individually (i.e. Ziploc bags or tupperware)

BACKGROUND METHODS RESULTS **DISCUSSION** QUESTIONS

Biosecurity Practices

- House animals individually (i.e. Ziploc bags or tupperware)
- Use new gloves for every individual


Lethal Effect of Latex, Nitrile, and Vinyl Gloves on Tadpoles

SCOTT D. CASHINS[1,3]
ROSS A. ALFORD[2]
and
LEE F. SKERRATT[1]

Guidelines for the Safe Use of Disposable Gloves with Amphibian Larvae in Light of Pathogens and Possible Toxic Effects

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"We wish to emphasize that discontinuing glove use may unnecessarily lead to increased spread of harmful pathogens."

BACKGROUND METHODS RESULTS **DISCUSSION** QUESTIONS

Biosecurity Practices

- Clean up!

•Bleach ≥4%
•EtOH ≥70%
•Virkon ≥1%
•Nolvasan >0.75%

BACKGROUND METHODS RESULTS **DISCUSSION** QUESTIONS

Conclusion

- Cohousing and handling both affect ranavirus transmission
- Researchers have mistakenly furthered disease spread before
- Only YOU can limit disease outbreaks!

BACKGROUND METHODS RESULTS **DISCUSSION** QUESTIONS

Swabbing Example: https://www.youtube.com/watch?v=a5CtPrGOK8c

Breakout Session

- Group 1 –Terrestrial salamanders
- Group 2 – Tadpoles
- Group 3 – Adult frogs
- Group 4 – Reptiles

Contributors and Funding:

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Morgan Gaynor
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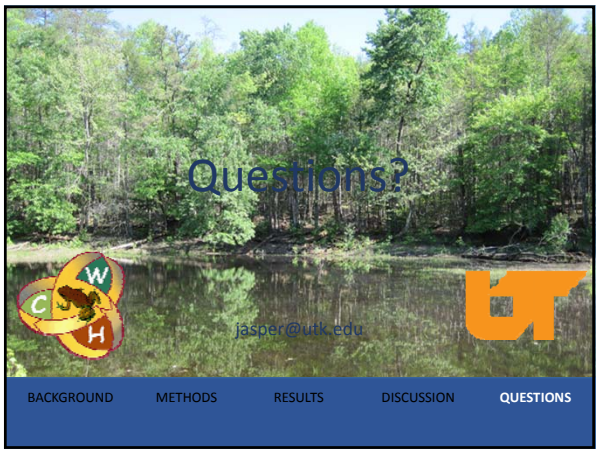
Questions?
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BACKGROUND
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