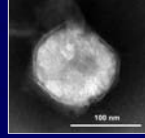
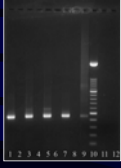


Impacts of Stressors on *Ranavirus* Prevalence in American Bullfrog and Green Frog Tadpoles



Matthew J. Gray, Debra Miller, and Charles Baldwin



UT Center for Wildlife Health
UGA College of Veterinary Medicine



Amphibian Declines and Emerging Infectious Diseases

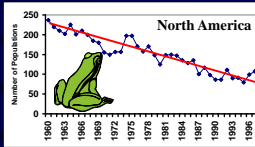
Science
306:1783-1786

EID 5:735-748

Batrachochytrium dendrobatidis



Chytrid Fungus



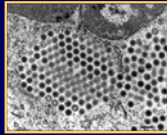
Nature
404:752-755

Biotropica
37:163-165



All latitudes and elevations

Larvae: 80-100% (Adults: UK)

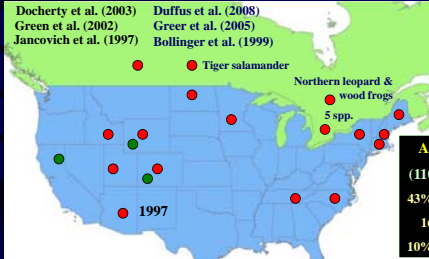


Ranavirus

Reported Amphibian Die-offs in North America: *Ranavirus*

Docherty et al. (2003)
Green et al. (2002)
Jancovich et al. (1997)

Duffus et al. (2008)
Greer et al. (2005)
Bollinger et al. (1999)











ARMI 2006
(110; 34 states)
43% = *Ranavirus*
16% = fungi
10% = protozoan

12 States & 12 Spp = *Ranavirus*
3 States & 3 Spp = Chytrid

Ranaviruses Represent The Greatest Pathogen Threat to Loss of Amphibian Biodiversity in North America.




Ranavirus Characteristics

- Iridoviridae
 - Large dsDNA
 - Virion: icosahedral
- Transmission
 - Contaminated water or soil
 - Casual or direct contact
 - Cannibalism or necrophagy
- Reservoirs
 - Amphibians
 - Reptiles
 - Fish
- Environmental Persistence
 - Aquatic Environment: 1 – 2 months
- Pathology
 - Gross Signs
 - Edema, Erythema
 - Organ Failure (necrosis or apoptosis)


Emergence of Ranavirus

Anthropogenic Stressor Hypothesis


Decrease Water Quality Decreases in White Blood Cells

Forson and Storfer 2006
Gray & Miller unpubl. data




Ranavirus

Immunocompromised




Mass Mortality Event




Impacts of Cattle on Amphibians


Access



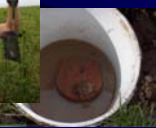
Non-access




Postmetamorphic



Health of Amphibian Larvae?



Larval



Freshwater Biology 53: in press
Journal of Wildlife Management 72: in press

Research Objectives

Anthropogenic Stressor

→ Cattle Decrease Water Quality

- 1) **Cattle access** in wetlands on *Ranavirus* prevalence in tadpoles of two common amphibian species

Natural Stressors

→ Water Temperature & Development

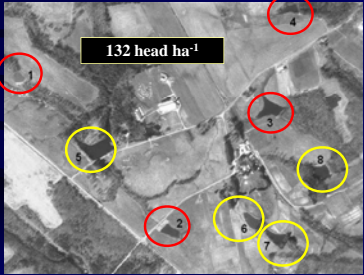
- 2) **Seasonal** variation in *Ranavirus* prevalence
- 3) Trends in *Ranavirus* prevalence among tadpole **developmental** stages



Study Area and Tadpole Sampling

Cumberland Plateau University of Tennessee Crossville, TN
 Plateau Research and Education Center

4 Access >10 years 132 head ha⁻¹ 4 Non-access Never



n = 104 *R. catesbeiana* *n* = 80 *R. clamitans*

Winter (15 Feb), Summer (15 June), Autumn (15 Oct) 2005

Methods

Necropsy

- Transported to University of Tennessee
- Benzocaine hydrochloride
- Development stage
Gosner 1960
- Tissues collected & preserved
- UGA Veterinary Diagnostic & Investigational Lab





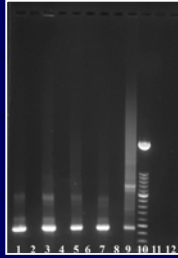
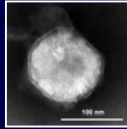
Methods

Ranavirus Testing and Identification

Organ Homogenate: liver, spleen, kidney, heart, gills and lungs

Techniques:

- Histology
- Electron microscopy
- PCR
- Sequencing



100% homology with the MCP gene for the Ranavirus FV3

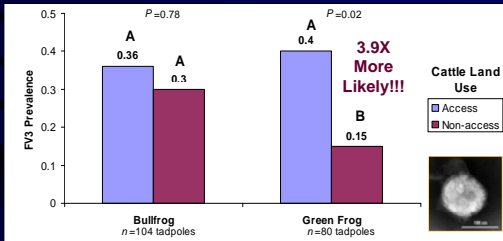
Positive Samples Totalled → Prevalence

Electrophoresis of Ranavirus



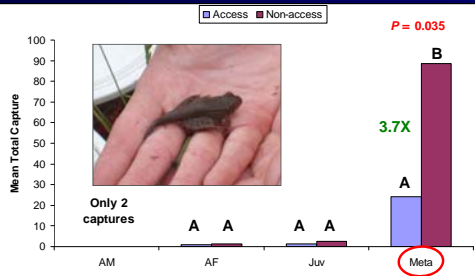
Results

Cattle Land Use



Statistical Tests: Logistic Regression and Maximum Likelihood Estimation

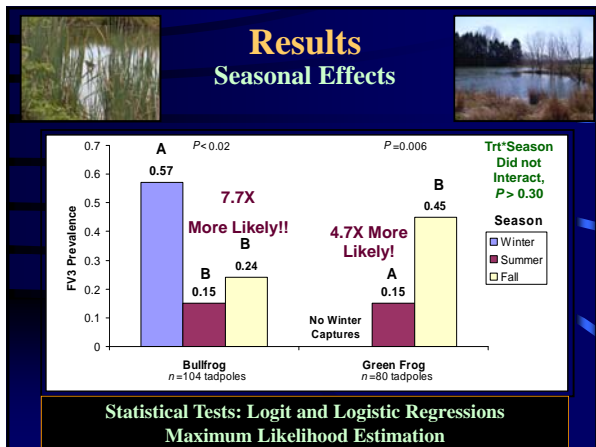
Does Higher FV3 Prevalence Imply Negative Consequences to Population?

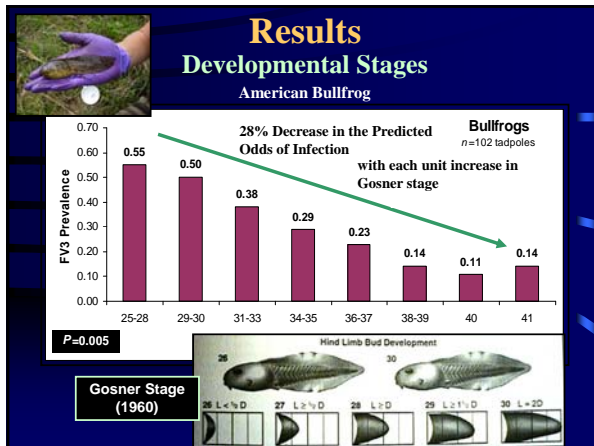


Postmetamorphic Green Frogs









Possible Mechanisms Driving Trends

- Water Temperature:**
 T lymphocyte proliferation and serum complement activity less at low temperature in *R. pipiens*.
Maniero and Carey (1997)
- Raffel et al. (2006): + WBCs and temperature
Rojas et al. (2005): + Survival and temperature: *Ranavirus* ATV
- Developmental Stages:**
 Tadpole immunity increases through development in *Xenopus laevis*.
Rollins-Smith (1998)

Conservation Implications

- Cattle access is an anthropogenic stressor
- Cattle exclusion may reduce *Ranavirus* emergence

USDA Conservation Programs

- Environmental Quality Incentives Program
- Conservation Reserve Program (CP-21)

Provide 75-90% Cost Share

Acknowledgments

University of Georgia



Dr. Debra Miller

Dr. Sandy Baldwin

University of Tennessee



Chandler Schmutzer

Liz Burton

Funding: •UGA Veterinary Diagnostic & Investigational Laboratory (Tifton)



•UT Institute of Agriculture

•Tennessee Wildlife Resources Agency

•Assoc. Reptile & Amphibian Veterinarians



Questions??



Diseases of Aquatic Organisms 77:97-103
