Ranaviruses:
Cold Blooded Killers!




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University of Tennessee, Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries

12:20 p.m.
17 February 2010
160 Plant Biotechnology Building

Outline

I. Emerging Infectious Diseases

II. Die-offs and TN Surveillance

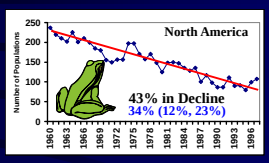
III.Ranavirus Pathology and Ecology

IV.Future Research Directions

Amphibian Declines
and Emerging Infectious Diseases

Science
306:1783-1786

EID 5:735-748

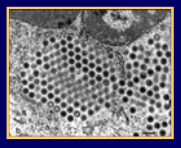


Nature
404:752-755

Biotropica
37:163-165


Chytrid Fungus


Adults: >95% (Europe)
Larvae: 80-100%


Ranaviruses

History of Ranavirus Die-offs

First Isolated:
- Dr. Allan Granoff
- St. Jude Hospital
- *Rana pipiens* (1962)



First Large-scale Die-offs:

- Dr. Andrew Cunningham
- Institute of Zoology, ZSL
- *Rana temporaria* (1992)

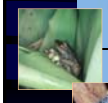
First North American Die-offs:

- Dr. Jim Collins and students
- Arizona State University
- *Ambystoma tigrinum stebbinsi* (1985, 1997)

Global Distribution of Ranavirus Die-offs








Wide Host Range

5 Continents, All Latitudes, All Elevations

11 Families: Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae, Discoglossidae, Rhacophoridae, Myobatrachidae, Ambystomatidae, Salamandridae, Hynobiidae

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



Uncommon

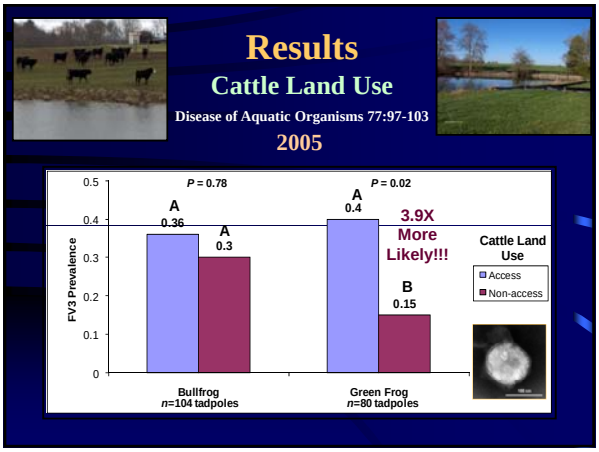
>30 States & 20 Spp; 4 Provinces

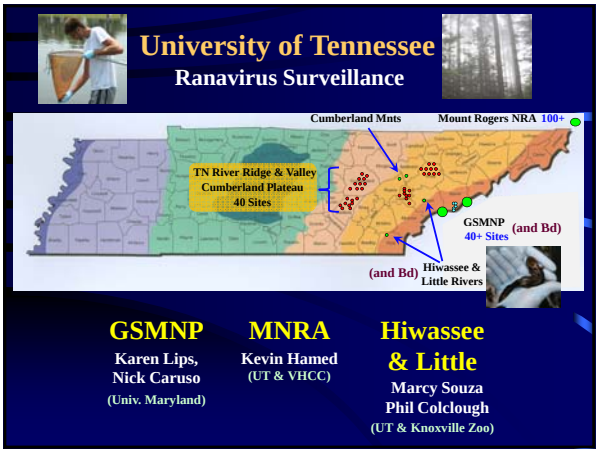
Families

Ranidae
Hylidae
Bufonidae
Ambystomatidae
Salamandridae

Lithobates sylvaticus


Smoky Mountains
Cades Cove: Gourley Pond
Jamie Barichivich (USGS) and
Megan Todd-Thompson (UT)
A. Cressler, USGS
M. Niemiller, UT
A. Cressler, USGS
May 2009
Spotted & Marbled Salamander, Wood Frog,
Spring Peeper, Southeastern Chorus Frog
D. Green, USGS


Results
Cattle Land Use
Disease of Aquatic Organisms 77:97-103
2005
P = 0.78
P = 0.02
A
0.36
A
0.3
A
0.4
B
0.15
3.9X
More
Likely!!!
Cattle Land
Use
Access
Non-access
FV3 Prevalence
0.5
0.4
0.3
0.2
0.1
0
Bullfrog
n=104 tadpoles
Green Frog
n=80 tadpoles

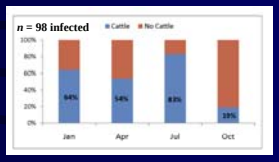

University of Tennessee
Ranavirus Surveillance
Cumberland Mnts
Mount Rogers NRA 100+
TN River Ridge & Valley
Cumberland Plateau
40 Sites
GSMNP
40+ Sites
(and Bd)
(and Bd)
Hiwassee &
Little Rivers
GSMNP
Karen Lips,
Nick Caruso
(Univ. Maryland)
MNRA
Kevin Hamed
(UT & VHCC)
Hiwassee
& Little
Marcy Souza
Phil Colclough
(UT & Knoxville Zoo)

Pond Surveillance

2008 Sampling

Seasons and Land use:

Green Frog, Bullfrog, Pickerel Frog, Newt, Tiger and Spotted Salamanders



Ranavirus Hotspots:

Knox Country

- Jan: 1 Pond = 57%
- Apr: 4 Ponds >30% (43%)
- July: 1 Pond = 33%
- Oct: 5 Ponds > 50% (90%, 100%)

Oct Die-off:

Green frog, Bullfrog, Newts

Southern Appalachia:

What about Plethodontids?

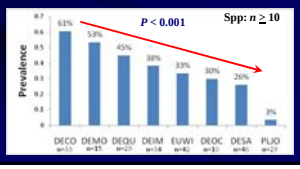
2007-2009

12 Species & 4 Genera:

>97% MCP similarity with the ranavirus FV3

Desmognathus
Eurycea
Plethodon
Gyrinophilus

Black-bellied Salamander	Pygmy Salamander
Spotted Dusky Salamander	Santeetlah Dusky Salamander
Imitator Salamander	Spring Salamander
Seal Salamander	Jordan's Salamander
Ocoee Salamander	Blue Ridge 2-lined Salamander
Shovel-nosed Salamander	Three-lined Salamander



Aquatic vs. Terrestrial

Another Family of Concern

2009

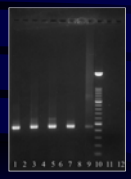
Hiwassee River

Little River

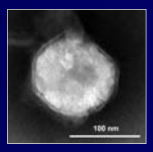
17 of 40 individuals → 43%

Hellbenders are Suitable Hosts

Ecology and Pathology of Amphibian Ranaviruses



A. Cressler, USGS



Ranavirus Characteristics

Docherty et al. (2003)

Granoff et al. (1965); Rafferty (1965)

- dsDNA, 150-280K bp
- 120-300 nm in diameter (3x smaller than bacteria)
- Icosahedral Shape (20)

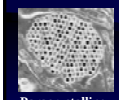
Chinchar et al. (2006)

Jancovich et al. (1997)

Family: Iridoviridae

Genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Megalocytivirus*, and *Lymphocystivirus*

Invertebrates — Ectothermic Vertebrates



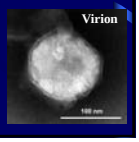
Paracrystalline Array

Amphibian Declines

Species (6)

- *Ambystoma tigrinum* virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)

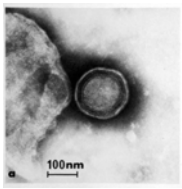
Virion



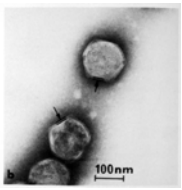
Candidate Species: *R. catesbeiana* virus Z (RCV-Z)

Ranavirus Replication Cycle

Chinchar (2002), Chinchar et al. (2006)



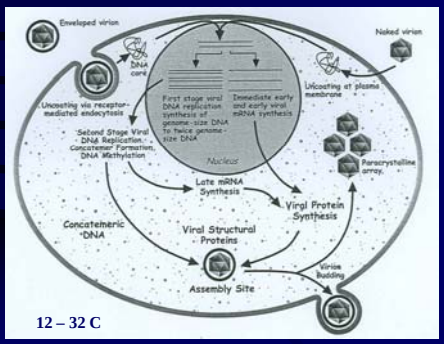
Enveloped Virion



Non-enveloped

Ranavirus Replication Cycle

Chinchar (2002), Chinchar et al. (2006)



12 – 32 C

Protein synthesis within hours of infection

Cell death occurs within 6 – 9 hrs PI

Ranavirus: Gross Signs

Edema, Erythema, Hemorrhages, Ulcerations

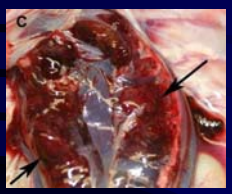
A. Duffus, ZSL

N. Haislip, UT

D. Green, USGS

Ranavirus: Internal Signs

Kidney Hemorrhages



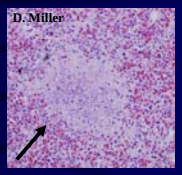
Pale and Swollen Liver

Ranavirus: Histopathological Signs

3 Primary Organs: Kidney, Liver and Spleen

Chinchar (2002), Chinchar et al. (2003)

Kidney Degeneration



Spleen Necrosis

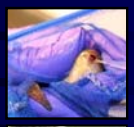
Viral Inclusions

Pathogenesis
Target Organ Failure
Heart Failure
Toxicosis, Anemia

Disease Can Progress Fast: 1-3 d signs, 3-7 d mortality; 2 wks die-off

Routes of Transmission

Oral inoculation



Ingestion

5 – 7 days

Necrophagy
Cannibalism

Water Bath Contaminated Sediment

Time to signs: 1 – 2 weeks
Time to mortality: 2 – 4 weeks

Gruia-Gray & Desser (1992)

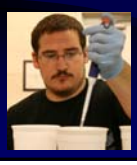
Invertebrates
(needs to be tested!)

Brunner et al. (2004), Pearman et al. (2004), Harp & Petranka (2006)

Horizontal vs. Vertical:

- Only Horizontal Transmission Demonstrated
- Duffus et al. (2008): Vertical Transmission Suspected

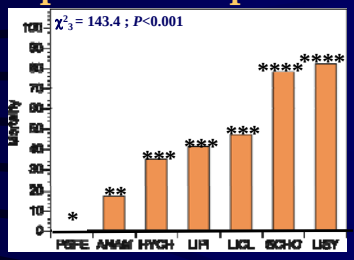
Variation in Susceptibility to *Ranavirus* Among Species and Tadpole Developmental Stages



Nathan A. Haislip
M.S. Candidate

Species Comparison

Across All Development Stages

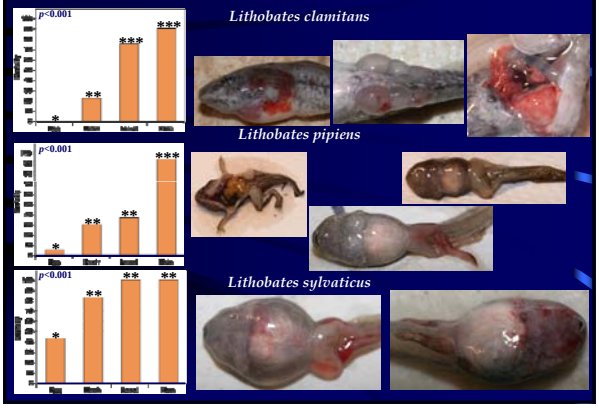


Logistic Analyses Species*Stage Interactions

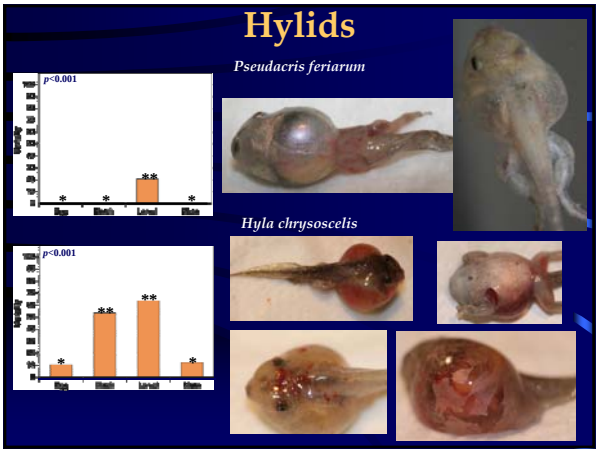
ML Estimate:

A. americanus – 4X > *P. feriarum*
H. chrysoscelis – 10X > *P. feriarum*
L. pipiens – 13X > *P. feriarum*
L. clamitans – 1.3X > *L. pipiens*, 16X > *P. feriarum*
S. holbrookii – 65X > *P. feriarum*
L. sylvaticus – 7X > *L. pipiens*
– 70X > *P. feriarum*

Ranids

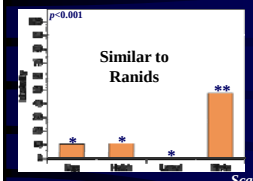


Hylids

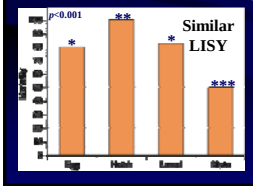


Toads

Anaxyrus americanus



Scaphiopus holbrookii



Fast Developers

Potential Natural Stressors

1) Development (N. Haislip)
- Immune Function: Egg, Hatchling, Larval, Metamorph, Adult

2) Water Temperature
- Positive Relationship: Virus Replication; Immune Function

3) Population Density
- Competition (L. Rucker)
- Contact Probability

4) Genetic Diversity Pearman and Garner (2005)
- Genetically Isolated Populations More Susceptible

5) Predation (N. Haislip)
- Exposure to Predators: Corticosterone Synthesis
- Elevated Corticosterone: Increased Parasite Infection

Belden and Kiesecker (2005)

6) Other Pathogens
- Secondary Infection: Ranaviruses, Bd, *Aeromonas hydrophila*, *Saprolegnia*

Factors Contributing to Emergence

Anthropogenic Stressors: Forson & Storfer (2006); Gray et al. (2007)

A. tigrinum

1) Herbicide (Atrazine)
Fertilizer (sodium nitrate)
→ Leukocytes ↓ → ATV Susceptibility ↑
Inconclusive

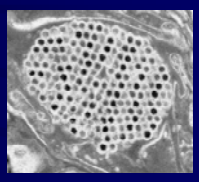
2) Cattle Land Use: FV3 Prevalence ➡ Green Frogs: 4X in access

Novel Strain Introduction: "Pathogen Pollution"

1) Salamander Bait Trade
- Introduction of Novel Strains
  Jancovich et al. (2005), Picco et al. (2007), Storfer et al. (2007)

2) Ranaculture Facilities
- More Virulent Strains
  Majji et al. (2006), Hoverman et al., unpubl. data

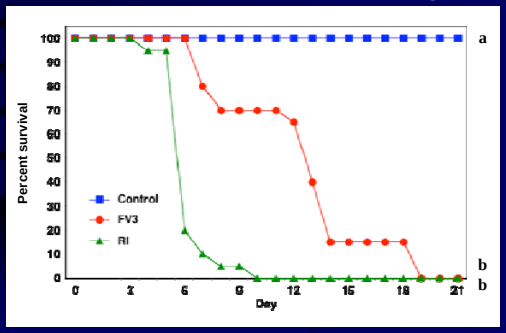
Pathogenicity of a Ranaculture *Ranavirus* Isolate



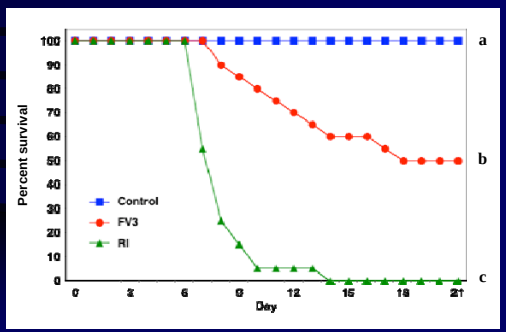
Jason T. Hoverman
Post-doctoral
Research Associate

Wood Frog

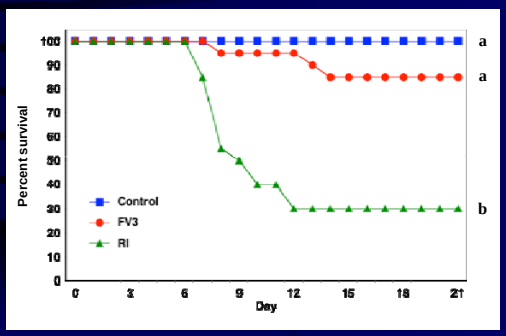
Pearson's chi-square test



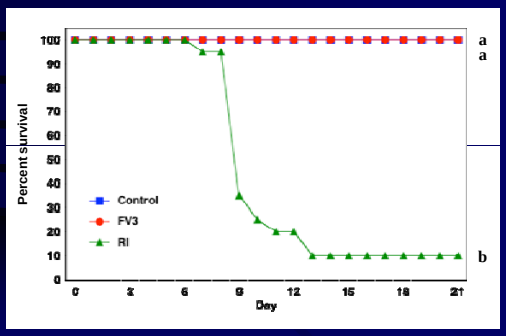
Southern Leopard Frog



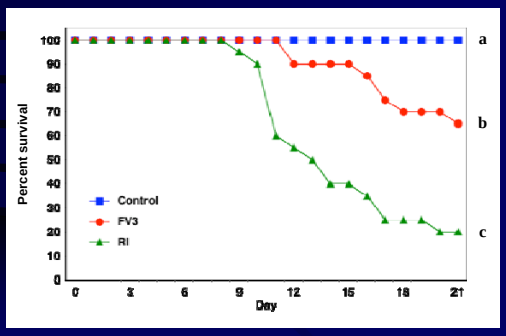
Pickerel Frog



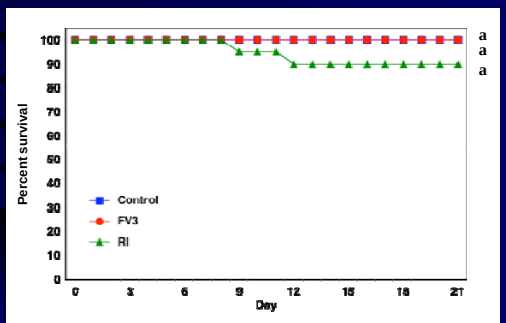
Green Frog



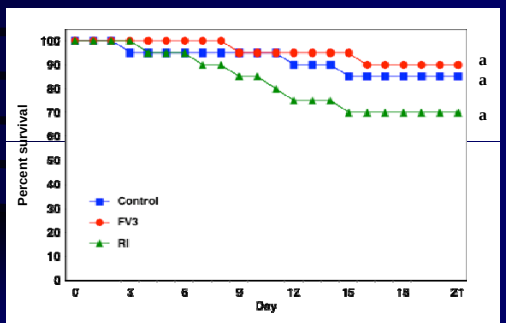
Cope's Gray Treefrog



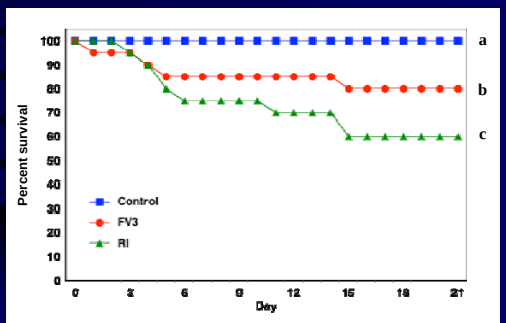
American Toad



Eastern Narrow-mouthed Toad



Eastern Spadefoot



World Organization for Animal Health

OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008

OIE
Notifiable Diseases
Certification for Shipment
Schloegel et al. (2009)

International Transport of Animals

Disinfection: Johnson et al. (2003), Bryan et al. (2009)

$50/ bottle

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

Other Ectothermic Hosts
Reptiles and Fish

Are Amphibian Ranaviruses Lethal to other Ecothermic Vertebrates?

Reptiles: *Gopherus polyphemus*, *Testudo hermanni*, *Terrapene carolina carolina*, *Trionyx sinensis*, *Uroplatus fimbriatus*, and *Chondropython viridis*
(Marschang et al. 1999, 2005; Hyatt et al. 2002; Allender et al. 2006)

Fish:
- BIV & barramundi: Experimental Challenge (Moody & Owens 1994)
- FV3 & pallid sturgeon: 2009 Die-off J. Briggler, MO Dept of Conservation

Blind Pony Hatchery

Important Research Directions

Pressing Research Directions

Southeast Species

1) Surveillance & Monitoring







2) Experimental Challenges


Tennessee

Hyla gratiosa *Acris gryllus*
Hyla versicolor *Rana areolata*
Siren intermedia
Ambystoma barbouri
Ambystoma talpoideum

Pressing Research Directions

Mechanisms Driving Outbreaks?

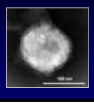
1) Cattle Use: Nitrogenous Waste





2) Pesticides: Atrazine, Carbaryl Malathion, Endosulfan, Glyphosate Mixtures?

3) Strain Virulence



Do Ranaviruses from One Region Represent Novel Pathogens in Another Region?

Hoverman et al.: Captive Facilities

Pressing Research Directions

A Mechanism Driving Future Outbreaks

Temperature-induced Stress

Ectothermic Vertebrates

Ranavirus Replication Increases with Temperature

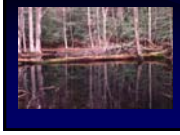

Pressing Research Directions

Reservoirs and Persistence

1) Fish and Reptiles



2) Persistence






Pathogen Ecology

i = species
j = age class

k = pathogen
l = wetland

Spatially Structured Breeding Sites


$EM(t)_{ijkl}$

Host-Pathogen Community

$I(t)_{ijkl}$

$S(t)_{ijkl}$


$IM(t)_{ijkl}$

$P(N_t)_{il} > 0$

Collaborators

University of Georgia


Dr. Debra Miller

Dr. Sandy Baldwin

University of Tennessee



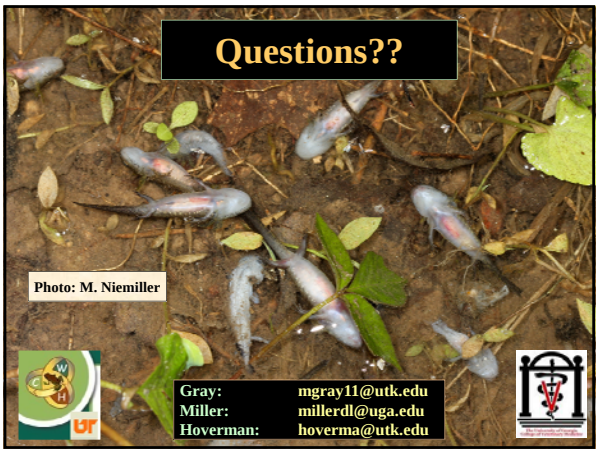
Dr. Jason Hoverman

Nathan Haislip

Kevin Hamed

Funding:

- UGA Veterinary Diagnostic & Investigational Laboratory (Tifton)
- UT Institute of Agriculture
- Tennessee Wildlife Resources Agency
- Assoc. Reptile & Amphibian Veterinarians


Questions??
Photo: M. Niemiller
Gray: mgray11@utk.edu
Miller: millerdl@uga.edu
Hoverman: hoverma@utk.edu