Ecology and Pathology of Ranaviruses

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Outline

I. Ranavirus Die-offs and Host Effects

II. UT Research: Host-Pathogen Interactions

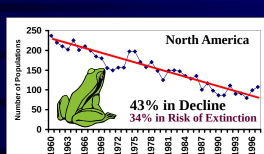
III. Anthropogenic Effects and Disinfectants

IV. Can Ranaviruses Contribute to Declines?

Amphibian Declines and Emerging Infectious Diseases

Science 306:1783-1786

EID 5:735-748



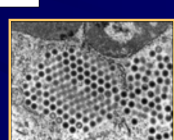
Nature 404:752-755

Biotropica 37:163-165

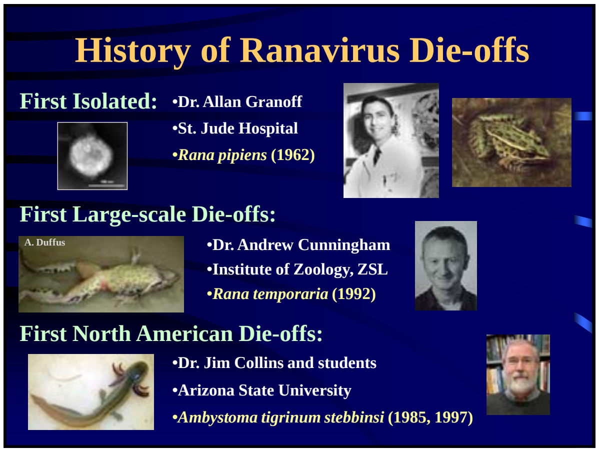
Chytrid Fungus

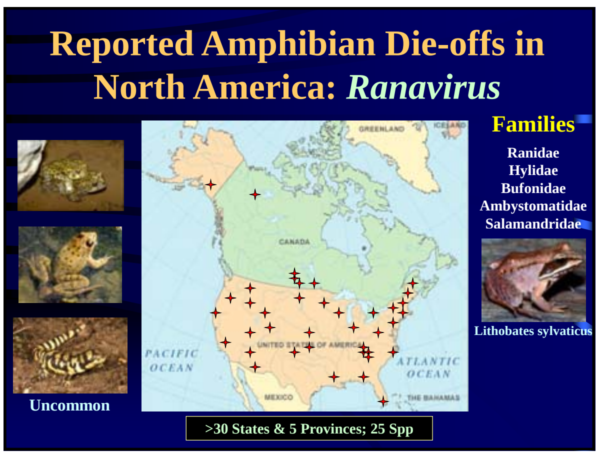
Adults: >95% (Europe)

Larvae: 80-100%

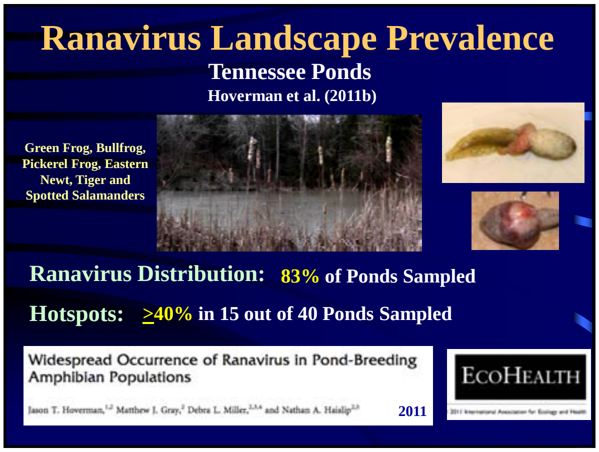


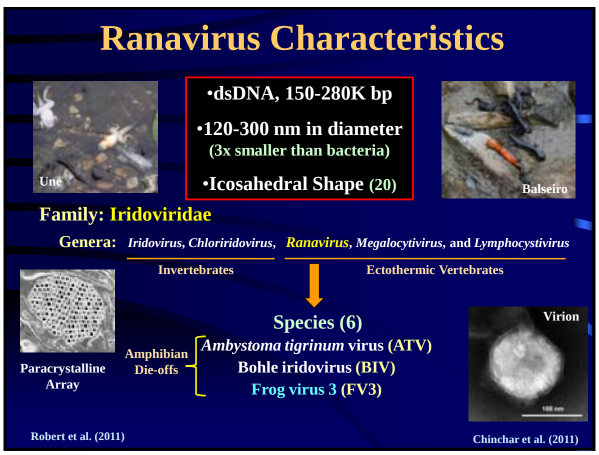
Ranaviruses

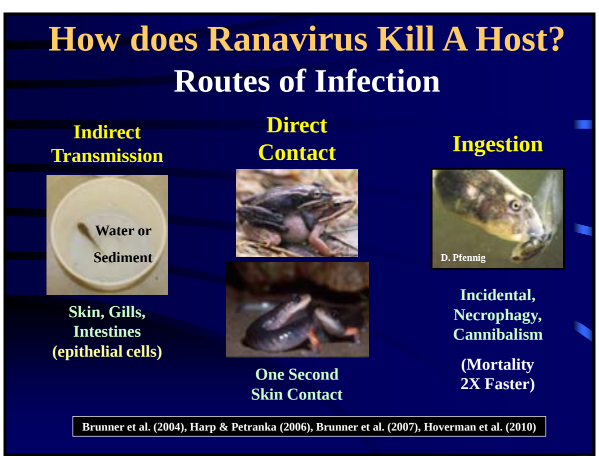

History of Ranavirus Die-offs
First Isolated:
•Dr. Allan Granoff
•St. Jude Hospital
•Rana pipiens (1962)
First Large-scale Die-offs:
A. Duffus
•Dr. Andrew Cunningham
•Institute of Zoology, ZSL
•Rana temporaria (1992)
First North American Die-offs:
•Dr. Jim Collins and students
•Arizona State University
•Ambystoma tigrinum stebbinsi (1985, 1997)

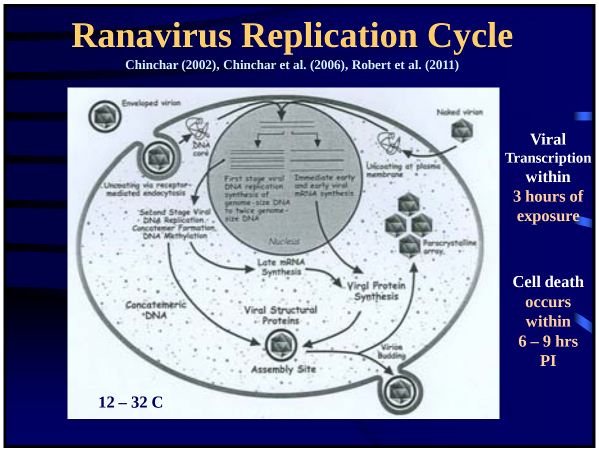

Reported Amphibian Die-offs in North America: Ranavirus
Families
Ranidae
Hylidae
Bufonidae
Ambystomatidae
Salamandridae
Lithobates sylvaticus
Uncommon
GREENLAND
ICELAND
CANADA
UNITED STATES OF AMERICA
PACIFIC OCEAN
ATLANTIC OCEAN
MEXICO
THE BAHAMAS
>30 States & 5 Provinces; 25 Spp

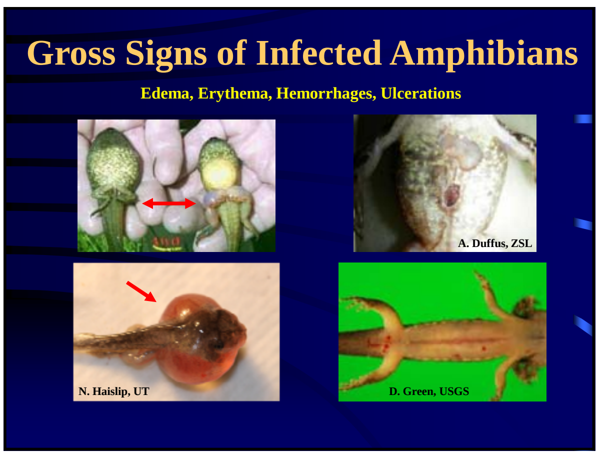

Case Example
North America
Jamie Barichivich (USGS) and
Megan Todd-Thompson (UT)
A. Cressler, USGS
M. Niemiller, UT
A. Cressler, USGS
GSMNP, Cades Cove
Gourley Pond
D. Green, USGS
May 2009, 2012
Spotted & Marbled Salamander, Wood Frog,
Spring Peeper, Southeastern Chorus Frog

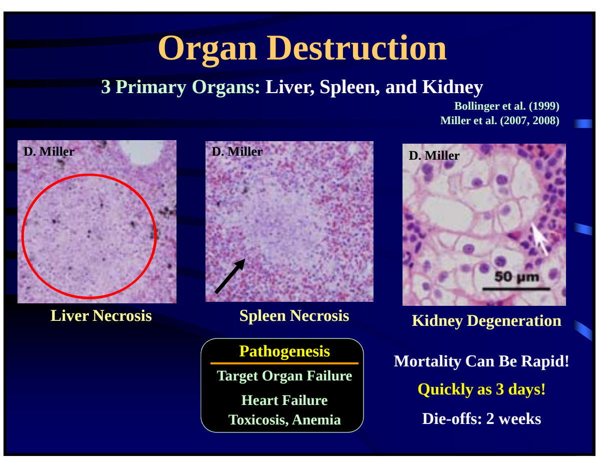

Ranavirus Landscape Prevalence
Tennessee Ponds
Hoverman et al. (2011b)
Green Frog, Bullfrog, Pickerel Frog, Eastern Newt, Tiger and Spotted Salamanders
Ranavirus Distribution: 83% of Ponds Sampled
Hotspots: ≥40% in 15 out of 40 Ponds Sampled
Widespread Occurrence of Ranavirus in Pond-Breeding Amphibian Populations
Jason T. Hoverman, Matthew J. Gray, Debra L. Miller, and Nathan A. Haislip
2011
EcoHealth

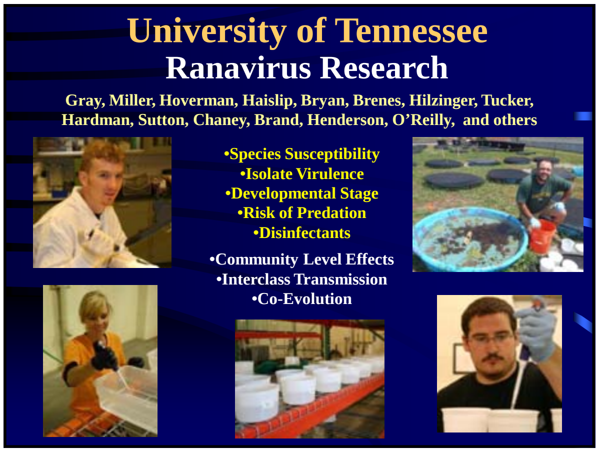

Ranavirus Characteristics
•dsDNA, 150-280K bp
•120-300 nm in diameter
(3x smaller than bacteria)
•Icosahedral Shape (20)
Une
Balseiro
Family: Iridoviridae
Genera: Iridovirus, Chloriridovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus
Invertebrates
Ectothermic Vertebrates
Species (6)
Amphibian Die-offs
Ambystoma tigrinum virus (ATV)
Bohle iridovirus (BIV)
Frog virus 3 (FV3)
Paracrystalline Array
Virion
Robert et al. (2011)
Chinchar et al. (2011)

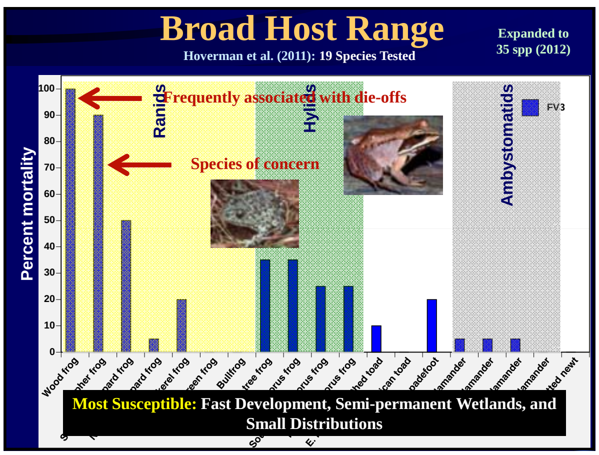

How does Ranavirus Kill A Host?
Routes of Infection
Indirect Transmission
Direct Contact
Ingestion
Water or Sediment
D. Pfennig
Skin, Gills, Intestines (epithelial cells)
One Second Skin Contact
Incidental, Necrophagy, Cannibalism
(Mortality 2X Faster)
Brunner et al. (2004), Harp & Petranka (2006), Brunner et al. (2007), Hoverman et al. (2010)

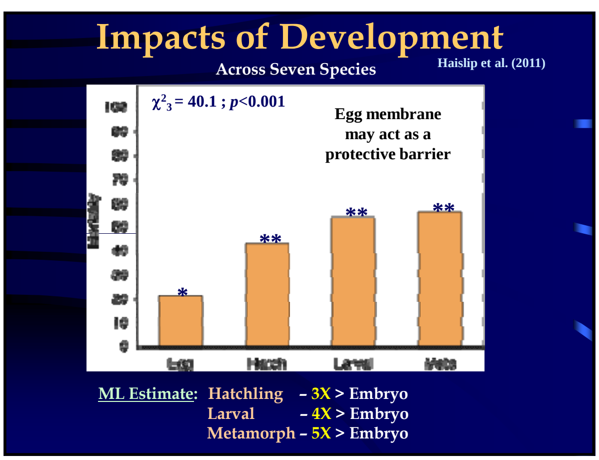

Ranavirus Replication Cycle
Chinchar (2002), Chinchar et al. (2006), Robert et al. (2011)
Enveloped virion
Naked virion
DNA core
Uncoating via receptor-mediated endocytosis
Uncoating at plasma membrane
First stage viral DNA replication synthesis of genome-size DNA to twice genome-size DNA
Immediate early and early viral mRNA synthesis
Second Stage Viral DNA Replication, Concatemer Formation, DNA Methylation
Nucleus
Paracrystalline array
Late mRNA Synthesis
Viral Protein Synthesis
Concatemeric DNA
Viral Structural Proteins
Assembly Site
Virion Budding
12 – 32 C
Viral Transcription within 3 hours of exposure
Cell death occurs within 6 – 9 hrs PI


Gross Signs of Infected Amphibians
Edema, Erythema, Hemorrhages, Ulcerations
A. Duffus, ZSL
N. Haislip, UT
D. Green, USGS


Organ Destruction
3 Primary Organs: Liver, Spleen, and Kidney
Bollinger et al. (1999)
Miller et al. (2007, 2008)
D. Miller
D. Miller
D. Miller
50 µm
Liver Necrosis
Spleen Necrosis
Kidney Degeneration
Pathogenesis
Target Organ Failure
Heart Failure
Toxicosis, Anemia
Mortality Can Be Rapid!
Quickly as 3 days!
Die-offs: 2 weeks


University of Tennessee
Ranavirus Research
Gray, Miller, Hoverman, Haislip, Bryan, Brenes, Hilzinger, Tucker, Hardman, Sutton, Chaney, Brand, Henderson, O'Reilly, and others
•Species Susceptibility
•Isolate Virulence
•Developmental Stage
•Risk of Predation
•Disinfectants
•Community Level Effects
•Interclass Transmission
•Co-Evolution


Broad Host Range
Hoverman et al. (2011): 19 Species Tested
Expanded to 35 spp (2012)
Percent mortality
Ranids
Hylids
Ambystomatids
FV3
Frequently associated with die-offs
Species of concern
Wood frog
Bullfrog
Most Susceptible: Fast Development, Semi-permanent Wetlands, and Small Distributions


Impacts of Development
Across Seven Species
Haislip et al. (2011)
χ^2_3 = 40.1 ; p<0.001
Egg membrane may act as a protective barrier
ML Estimate: Hatchling – 3X > Embryo
Larval – 4X > Embryo
Metamorph – 5X > Embryo

Community Composition Matters:
host identity affects outcome of ranavirus outbreaks in larval amphibian communities

Roberto Brenes,
Matthew Gray, and Debra Miller
University of Tennessee
Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries

Objectives

1. Effects of species susceptibility on transmission and the likelihood of a community-level outbreak
2. Determine if highly susceptible species can function as an amplification species

Treatments

- Completely Randomized Design (5 treatments)
  - Species A Exposed Only; Others Not
  - Species B Exposed Only
  - Species C Exposed Only
  - All Exposed
  - None Exposed

•Controlled Exposure

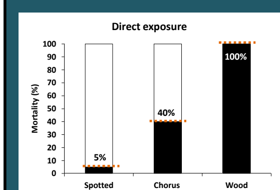
- •2-L containers
- •10^3 PFU/mL
- •Ranavirus Isolate = Frog Virus 3
- •Control = MEM Eagle media
- •Exposure = 3 days then distributed to mesocosms

Hoverman et al. (2011)

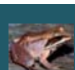
Aquatic Mesocosms

- Mesocosm Site
- Aged to emulate natural conditions
- *n* = 25 pools, 5 pools per treatment
- Each pool = 10 random larvae/species
- Duration = 60 days

Appalachian Community: Direct Exposure Mortality

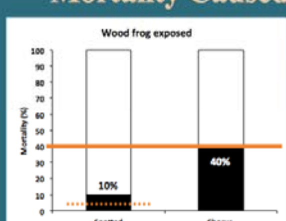






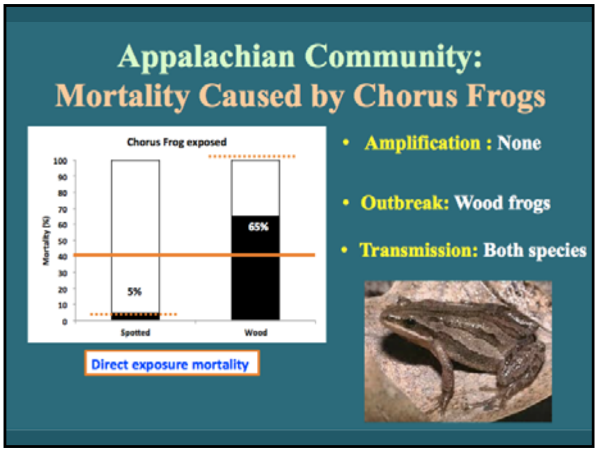
- Amplification: mortality 2X greater than direct exposure (Paull et al., 2012)
  - Spotted Salamander:
    - $\geq$10% mortality
  - Chorus Salamander:
    - $\geq$80% mortality
  - Wood Frog:
    - Not possible

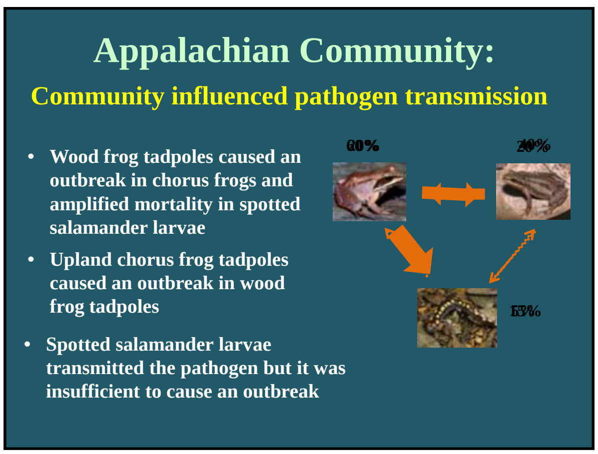
Appalachian Community: Mortality Caused by Wood Frogs



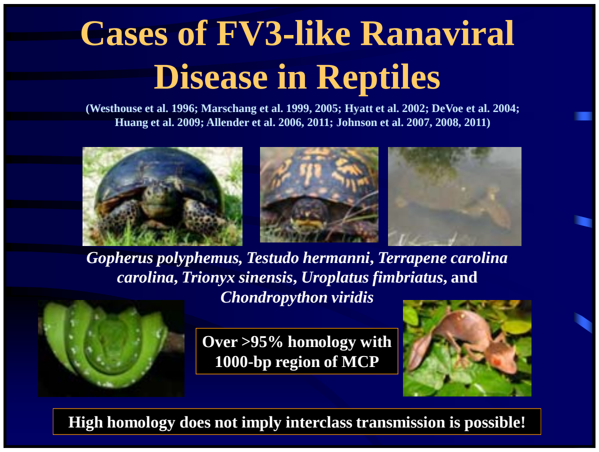
Direct exposure mortality

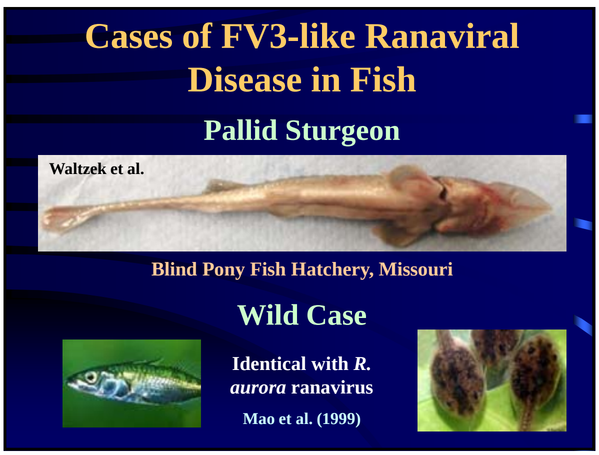
- Amplification : Spotted salamander (twice as high)
- Outbreak : Chorus frogs
- Transmission: Both species

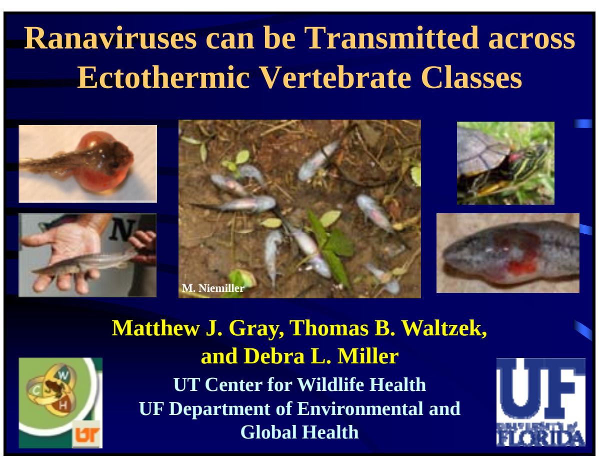

Appalachian Community:
Mortality Caused by Chorus Frogs
Chorus Frog exposed
Mortality (%)
5%
65%
Spotted
Wood
Direct exposure mortality
• Amplification : None
• Outbreak: Wood frogs
• Transmission: Both species

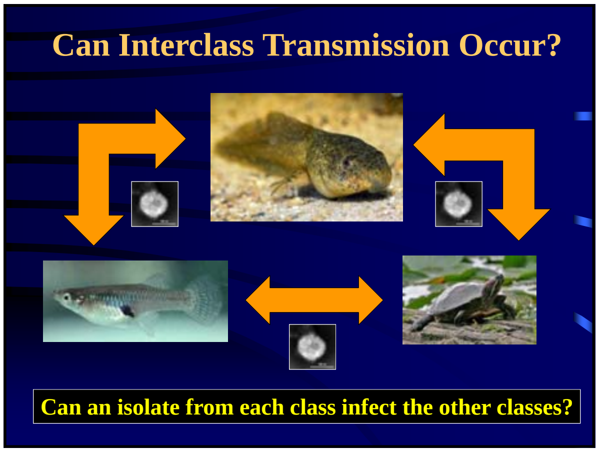

Appalachian Community:
Community influenced pathogen transmission
• Wood frog tadpoles caused an outbreak in chorus frogs and amplified mortality in spotted salamander larvae
• Upland chorus frog tadpoles caused an outbreak in wood frog tadpoles
• Spotted salamander larvae transmitted the pathogen but it was insufficient to cause an outbreak

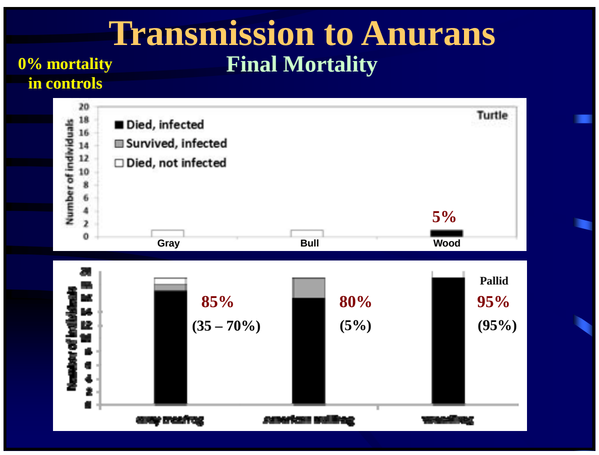

What about other Ectothermic Vertebrates?
13 February 2012
The Washington Post
Make us your start page
POSTLOCAL
North Branch Stream Valley State Park
Deadly virus hits turtles, tadpoles in Montgomery County
26 of 31 Box Turtles Die from Ranaviral Disease
2008 – 2011
Larval anurans and salamanders dead too
Farnsworth and Seigel, Towson U.

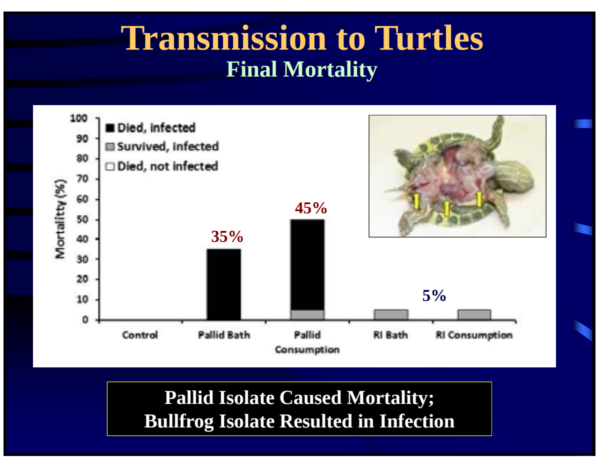

Cases of FV3-like Ranaviral Disease in Reptiles
(Westhouse et al. 1996; Marschang et al. 1999, 2005; Hyatt et al. 2002; DeVoe et al. 2004; Huang et al. 2009; Allender et al. 2006, 2011; Johnson et al. 2007, 2008, 2011)
Gopherus polyphemus, Testudo hermanni, Terrapene carolina carolina, Trionyx sinensis, Uroplatus fimbriatus, and Chondropython viridis
Over >95% homology with 1000-bp region of MCP
High homology does not imply interclass transmission is possible!


Cases of FV3-like Ranaviral Disease in Fish
Pallid Sturgeon
Waltzek et al.
Blind Pony Fish Hatchery, Missouri
Wild Case
Identical with R. aurora ranavirus
Mao et al. (1999)


Ranaviruses can be Transmitted across Ectothermic Vertebrate Classes
M. Niemiller
Matthew J. Gray, Thomas B. Waltzek, and Debra L. Miller
UT Center for Wildlife Health
UF Department of Environmental and Global Health


Can Interclass Transmission Occur?
Can an isolate from each class infect the other classes?


Transmission to Anurans
Final Mortality
0% mortality
in controls
Turtle
Died, infected
Survived, infected
Died, not infected
Number of individuals
5%
Gray
Bull
Wood
Pallid
85%
(35 – 70%)
80%
(5%)
95%
(95%)


Transmission to Turtles
Final Mortality
Died, infected
Survived, infected
Died, not infected
Mortality (%)
35%
45%
5%
Control
Pallid Bath
Pallid Consumption
RI Bath
RI Consumption
Pallid Isolate Caused Mortality;
Bullfrog Isolate Resulted in Infection

Conclusions

- Interclass Transmission is possible
- Pallid isolate was more virulent than box turtle or bullfrog
    - 15 – 65%
    - 1 – 10 days
    (One Isolate)
- Turtle and bullfrog isolates resulted in infection in wood frogs and red-eared sliders, respectively


Transmission of ranavirus between ectothermic vertebrate hosts

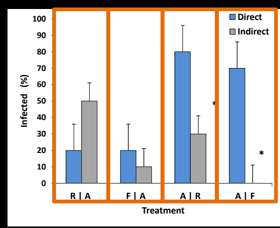
Roberto Brenes[1]*, Matthew Gray[1], Debra Miller[1,2], Rebecca. P. Wilkes[2], and Thomas. B. Waltzek[3]
[1]Center for Wildlife Health and [2]College of Veterinary Medicine, University of Tennessee
[3]College of Veterinary Medicine, University of Florida

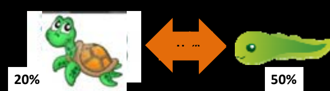
Experiment

- Direct exposure
    - Exposed to 10^3 PFU/mL
    - 3 days
- 15.5 L containers divided by 2000-μm plastic mesh
- Different species in each side of the container
    - One side exposed

Results

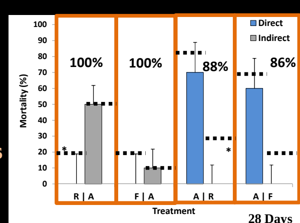
- Turtles infected amphibians
- Amphibians infected turtles
- Fish infected amphibians
- Amphibians did not infect fish





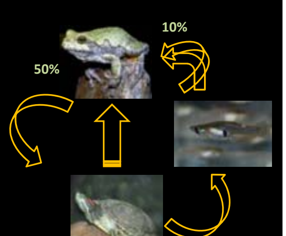
Results

- Not all species caused mortality
- Turtles caused mortality in amphibians
- Amphibians did not cause mortality in turtles
- Fish caused mortality in amphibians
- Amphibians did not cause mortality in fish

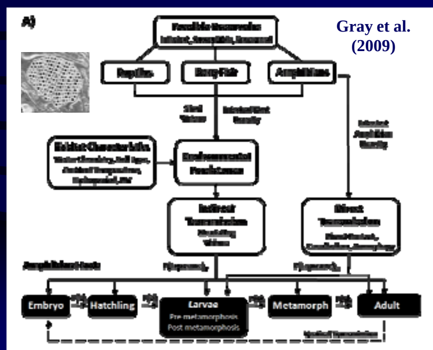


Turtle and Fish Results

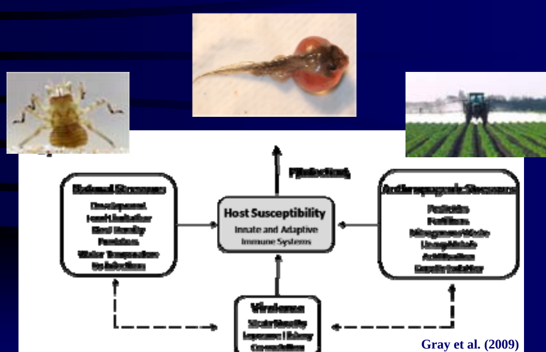
- All classes tested can transmit the virus
- Turtles infected tadpoles
  - 50% mortality
- Fish infected tadpoles
  - 10% mortality



Ranavirus Ecology



Impacts of Stressors



Factors Contributing to Emergence

Anthropogenic Stressors: Forson & Storfer (2006); Gray et al. (2007); Greer et al. (2008); Kerby et al. (2011)

A. tigrinum
1) Herbicide (Atrazine)
Insecticide (Carbaryl)
ATV Susceptibility ↑

2) Cattle Land Use: Prevalence → Green Frogs and Tiger Salamanders

Other Possible Stressors: Pesticide Mixtures, Nitrogenous Waste, Endocrine Disruptors, Acidification, Global Warming, Heavy Metals

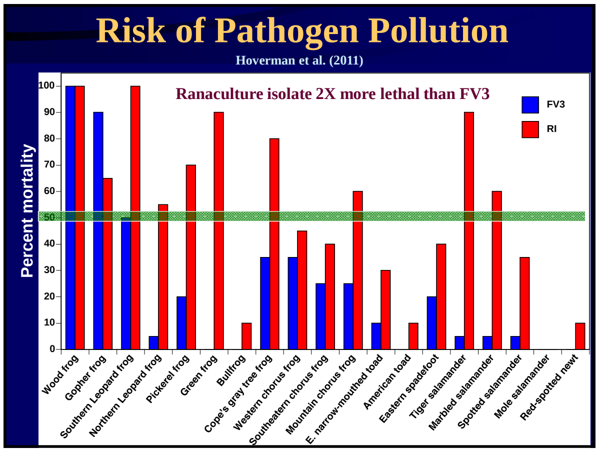
Pathogen Pollution: (Cunningham et al. 2003)

Anthropogenic introduction of novel strains to naïve populations

Picco et al. (2007)

- Fishing Bait
- Ranaculture Facilities
- Biological Supply Companies
- International Food & Pet Trade
- Contaminated Fomites

Schloegel et al. (2009)

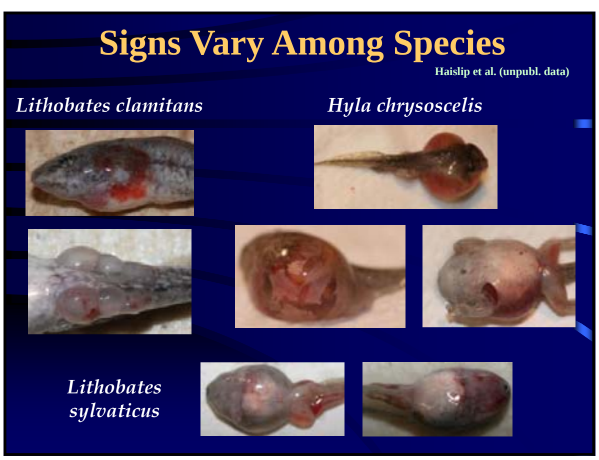

Risk of Pathogen Pollution
Hoverman et al. (2011)
Ranaculture isolate 2X more lethal than FV3
FV3
RI
Percent mortality
0
10
20
30
40
50
60
70
80
90
100
Wood frog
Gopher frog
Southern Leopard frog
Northern Leopard frog
Pickerel frog
Green frog
Bullfrog
Cope's gray tree frog
Western chorus frog
Southeatern chorus frog
Mountain chorus frog
E. narrow-mouthed toad
American toad
Eastern spadefoot
Tiger salamander
Marbled salamander
Spotted salamander
Mole salamander
Red-spotted newt


World Organization for Animal Health
OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008
OiE
Notifiable Diseases
Certification for Shipment
Schloegel et al. (2010)
International Transport of Animals
Disinfection: Johnson et al. (2003), Bryan et al. (2009)
$75/ bottle
•Bleach ≥4%
•EtOH ≥70%
•Virkon ≥1%
•Nolvasan >0.75%


Are Ranaviruses Capable of Contributing to Species Declines?
M. Niemiller
Matthew J. Gray
University of Tennessee
Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries

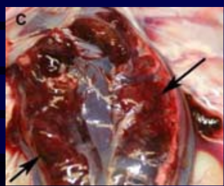

Global Distribution of
Ranaviral Disease
5 Continents: 1992
All Latitudes, All Elevations
14 Families:
>70 Species
Alytidae, Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae,
Discoglossidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae,
Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae


Death and Destruction


Signs Vary Among Species
Haislip et al. (unpubl. data)
Lithobates clamitans
Hyla chrysoscelis
Lithobates sylvaticus

Internal Signs of Ranaviral Disease

Kidney Hemorrhages



Pale and Swollen Liver

It attacks quickly killing hosts as quickly as 3 days!

Imagine if Ranaviruses could Infect Humans

Monday

Fever

Wednesday

Hands, Feet, Legs Swollen

No Amphibian Pathogen: Diversity of Gross Signs or Kills as Quickly

There is no Cure!

Friday

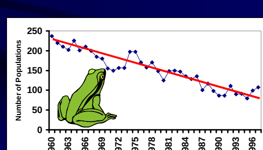
Bedridden, Body Enlarged 2X, Lesions, Hemorrhaging Internally and from Orifices

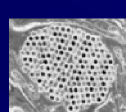
Sunday

Begging Dr. Death (Jack Kevorkian) for a quick end!

Are Ranaviruses Capable of Causing Local Extirpations and Species Declines?

Muths et al. (2006)





Collins & Crump (2009)

Traditional Theory
(Anderson and May 1979)

Extirpation is possible if:

Frequency Dependent

(1)Multiple Host Pathogens Where Susceptibility Differs

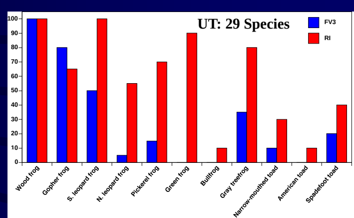
Is at least one of these conditions satisfied in the ranavirus-host system?

- Survive Outside Host

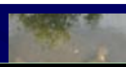
(3) Clustering of Individuals
- Sexually transmitted disease

Evidence of Alternate Hosts
(Moody and Owens 1994, Marschang et al. 1999, 2005; Hyatt et al. 2002; Allender et al. 2006; Duffus et al. 2008, Picco et al. 2010; J. Briggler, J. Hoverman, D. Miller, B. Rothermel, unpubl. data)

(1) Multiple-host Pathogen:



(2) Other Ectothermic Vertebrates



Ranaviruses can infect multiple host species & some serve as asymptomatic carriers – #1 Met

Evidence of Environmental Persistence

(1) EHNV Persistence (Langdon 1989)

Ranaviruses can be remain viable outside the host for considerable duration (permanent wetlands at colder temperatures). – #2 Met

(2) FV3, FV3-like (Nazir et al. 2012)

20 C =	•PW (unsterile): 22-34 d	•Soil: 13-22 d
4 C =	•PW (unsterile): 58-72 d	•Soil: 30-48 d

(T-90 Values)

Evidence of Individual Clustering and Transmission

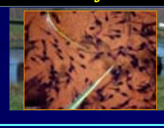
(1) Breeding (Brunner et al. 2004)

Frequency dependent transmission is possible in larval and adult age classes – #3 Likely.

(2) Larval Clustering

- Increase Infection Rates
- Vegetation Reduction

(Greer et al. 2008)



Local Extirpations and Declines?

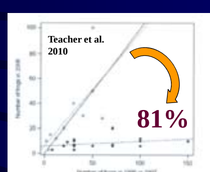
YES, all 3 characteristics met in the Ranavirus-Host System

Caveat: Community and Site Dependent!

Evidence of Declines

Dr. Amber Teacher
Southeastern England
1996/97 and 2008

Animal Conservation 13:514-522



Ranavirus (+) populations
81% Median Reduction

Evidence of Re-occuring Die-offs

Dr. Jim Petranka
Tulula Wetland Complex, NC
1998-2006

Biological Conservation 138:371-380

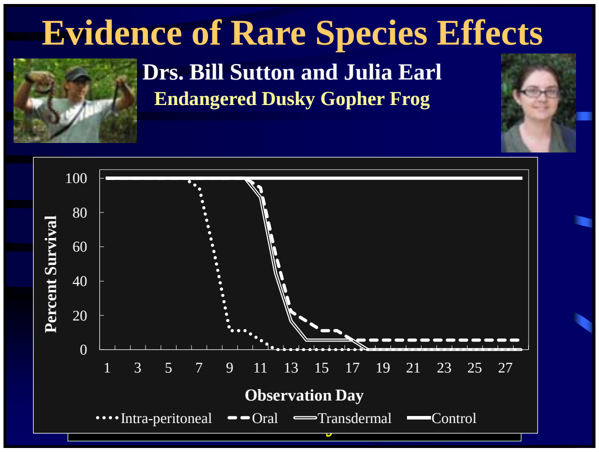
Wetlands 23:278-290

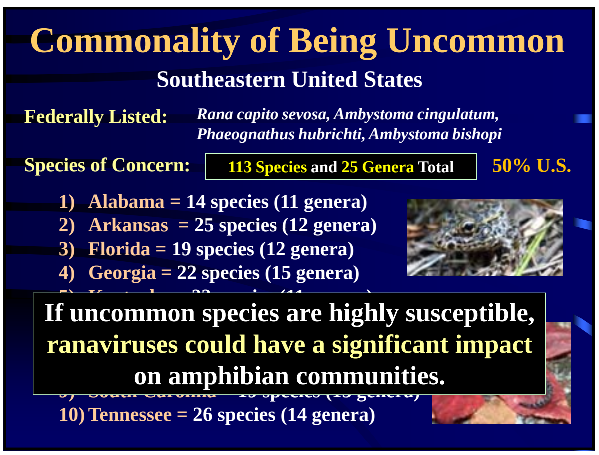
Recruitment at most wetlands failed due to ranavirus

Persistence Possible from Source Populations

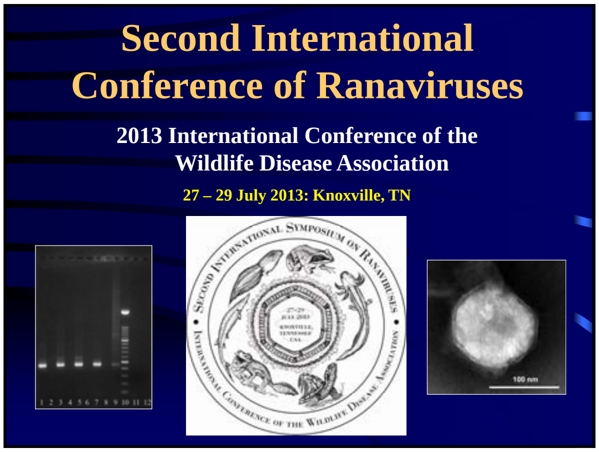
Rescue Effect

Uncommon Species?


Evidence of Rare Species Effects
Drs. Bill Sutton and Julia Earl
Endangered Dusky Gopher Frog
Percent Survival
100
80
60
40
20
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Observation Day
Intra-peritoneal
Oral
Transdermal
Control


Commonality of Being Uncommon
Southeastern United States
Federally Listed:
Rana capito sevosa, Ambystoma cingulatum, Phaeognathus hubrichti, Ambystoma bishopi
Species of Concern:
113 Species and 25 Genera Total
50% U.S.
1) Alabama = 14 species (11 genera)
2) Arkansas = 25 species (12 genera)
3) Florida = 19 species (12 genera)
4) Georgia = 22 species (15 genera)
If uncommon species are highly susceptible, ranaviruses could have a significant impact on amphibian communities.
10) Tennessee = 26 species (14 genera)


Take Home Messages
Should we be Concerned?
•Ranavirus Die-offs have Global Distribution
•Ranavirus Prevalence can be High
•Ranaviruses Infect Multiple Amphibian Species with Different Susceptibilities
•Community Composition Matters
•Interclass Transmission is Possible – Abundant Reservoirs
•Ranavirus Persistence is Long
•High Transmission: Breeding and for Schooling Spp.
•Anthropogenic Stressors and Pathogen Pollution contribute to Ranavirus Emergence
Epidemiological Theory Supports the Premise that Ranaviruses Could Cause Local Population Extirpations and Contribute to Species Declines


Second International Conference of Ranaviruses
2013 International Conference of the Wildlife Disease Association
27 – 29 July 2013: Knoxville, TN
Second International Symposium on Ranaviruses
International Conference of the Wildlife Disease Association


Questions??
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Miller: dmille42@utk.edu