# Ecology of Ranaviruses:
## A State of Understanding




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# Outline

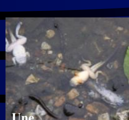
I. Ranavirus-Host Characteristics

II. Ecology: Species to Communities

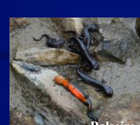
III. Effects of Stressors

IV. Commercial Trade & Pathogen Pollution

# Ranavirus Characteristics



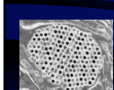

- •dsDNA, 150-280K bp
- •120-300 nm in diameter (3x smaller than bacteria)
- •Icosahedral Shape (20)




Family: Iridoviridae

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

Invertebrates | Ectothermic Vertebrates



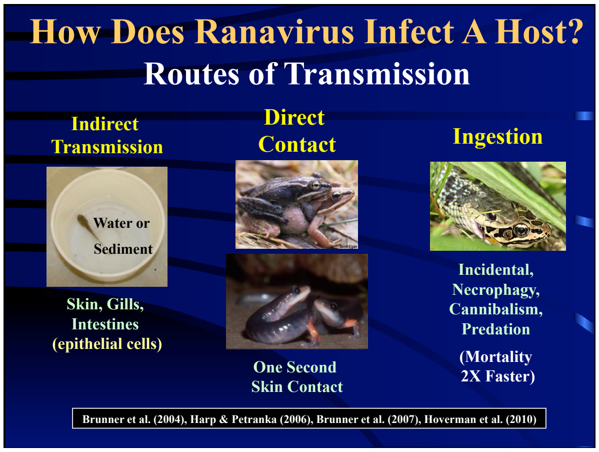
Paracrystalline Array

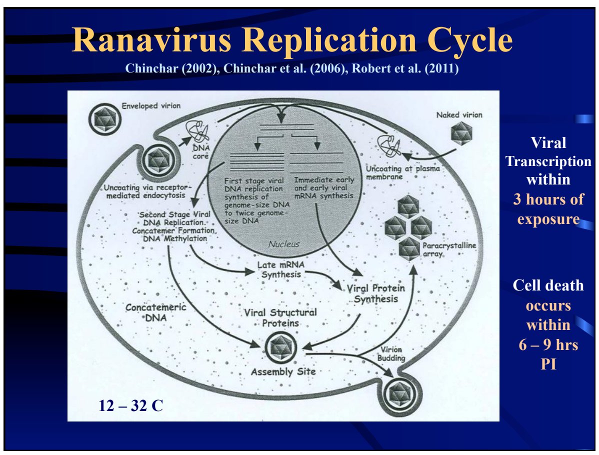
Species (6)

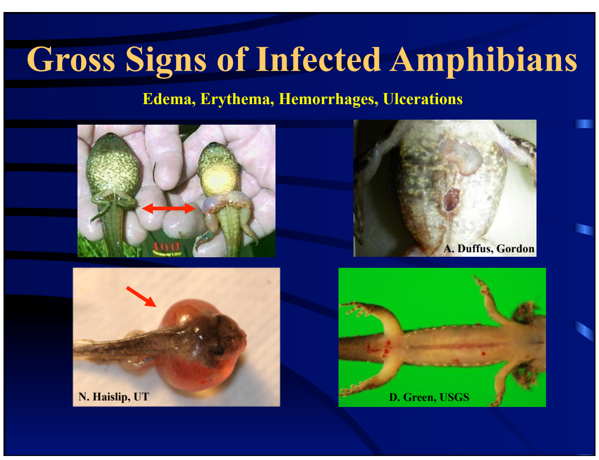
*Ambystoma tigrinum* virus (ATV)
Bohle iridovirus (BIV)
Frog virus 3 (FV3)
Epizootic haematopoietic necrosis virus
European catfish virus
Santee-Cooper Ranavirus

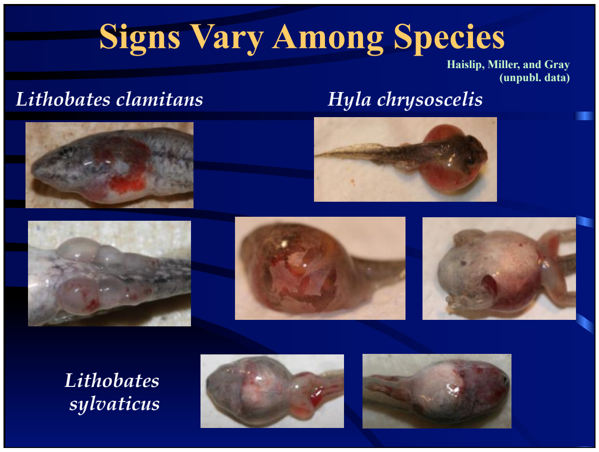

ICTV (2012)

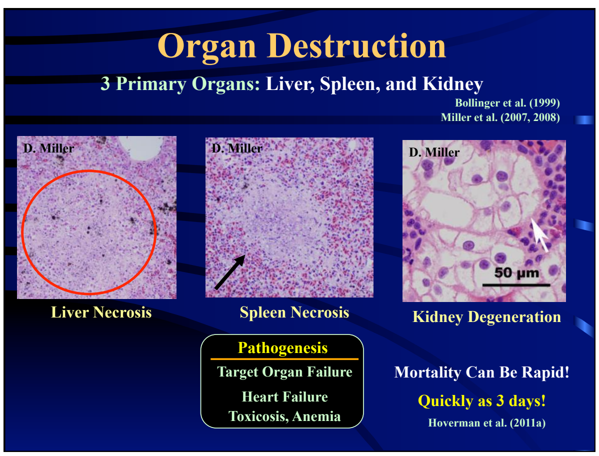
Chinchar et al. (2011)


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

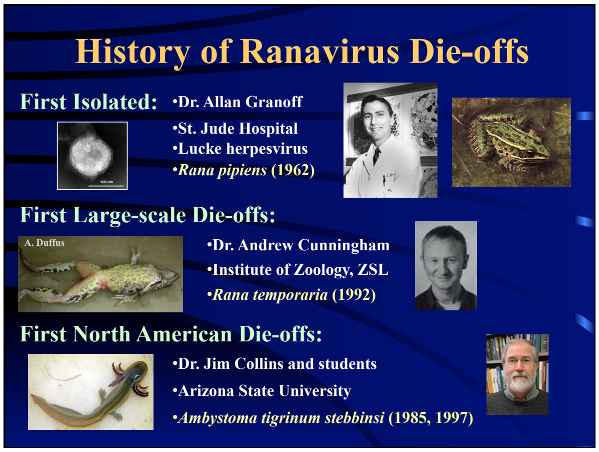

Ranavirus Replication Cycle
Chinchar (2002), Chinchar et al. (2006), Robert et al. (2011)
Enveloped virion
Naked virion
DNA core
Uncoating via receptor-mediated endocytosis
Uncoating at plasma membrane
First stage viral DNA replication synthesis of genome-size DNA to twice genome-size DNA
Immediate early and early viral mRNA synthesis
Nucleus
Second Stage Viral DNA Replication, Concatemer Formation, DNA Methylation
Late mRNA Synthesis
Viral Protein Synthesis
Paracrystalline array
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

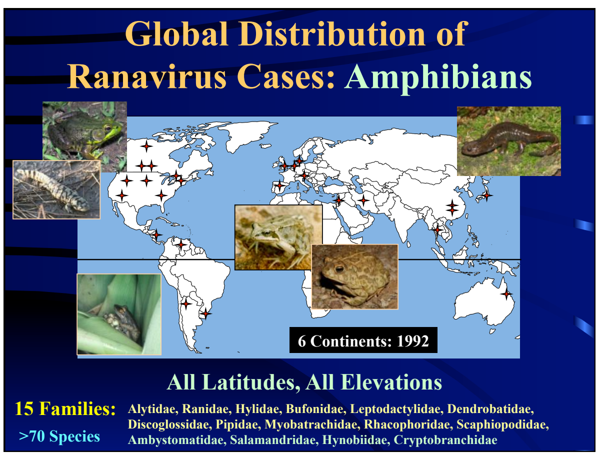

Gross Signs of Infected Amphibians
Edema, Erythema, Hemorrhages, Ulcerations
A. Duffus, Gordon
N. Haislip, UT
D. Green, USGS

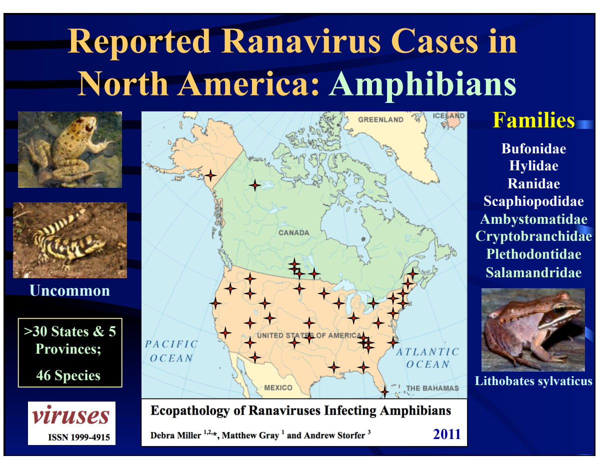

Signs Vary Among Species
Haislip, Miller, and Gray (unpubl. data)
Lithobates clamitans
Hyla chrysoscelis
Lithobates sylvaticus

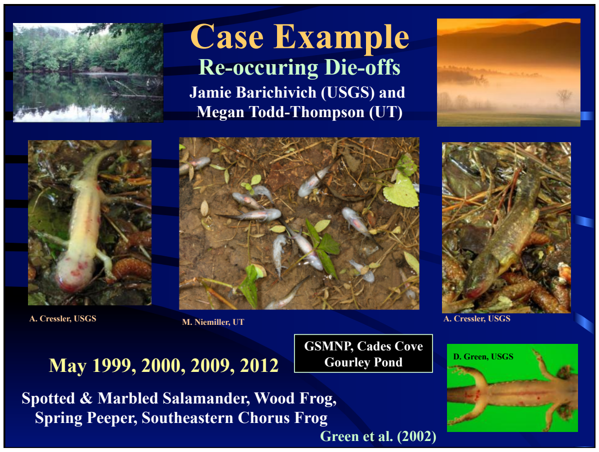

Organ Destruction
3 Primary Organs: Liver, Spleen, and Kidney
Bollinger et al. (1999)
Miller et al. (2007, 2008)
D. Miller
D. Miller
D. Miller
50 μm
Liver Necrosis
Spleen Necrosis
Kidney Degeneration
Pathogenesis
Target Organ Failure
Heart Failure
Toxicosis, Anemia
Mortality Can Be Rapid!
Quickly as 3 days!
Hoverman et al. (2011a)

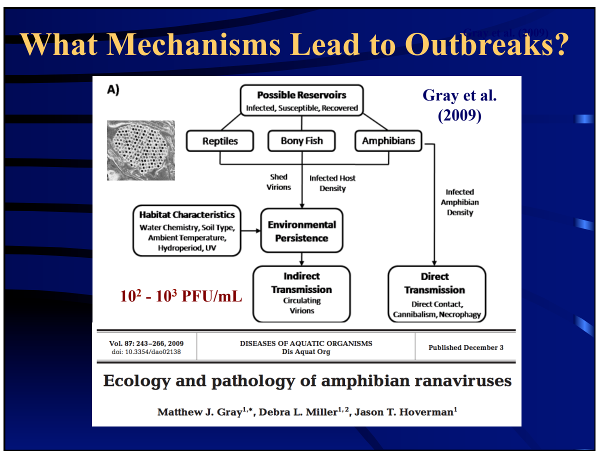

Maine 2013 Die-off
1000 carcasses/m2
≥200,000 dead
qPCR Confirmed
Wheelwright et al. (in review)
<24 hrs
6/14/13
6/15/13


History of Ranavirus Die-offs
First Isolated:
•Dr. Allan Granoff
•St. Jude Hospital
•Lucke herpesvirus
•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)

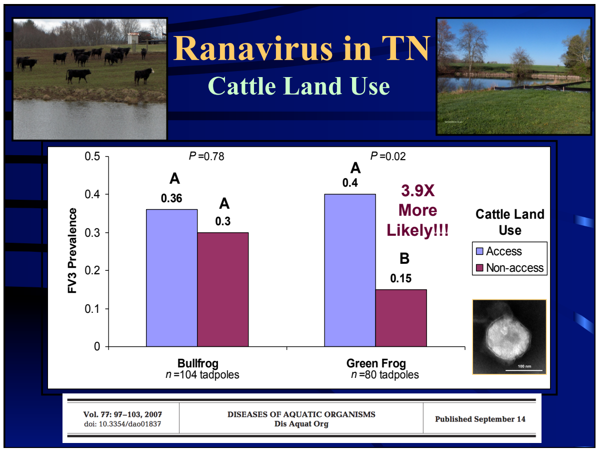

Global Distribution of Ranavirus Cases: Amphibians
6 Continents: 1992
All Latitudes, All Elevations
15 Families:
>70 Species
Alytidae, Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae, Discoglossidae, Pipidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae


Reported Ranavirus Cases in North America: Amphibians
Uncommon
>30 States & 5 Provinces;
46 Species
viruses
ISSN 1999-4915
GREENLAND
ICELAND
CANADA
UNITED STATES OF AMERICA
PACIFIC OCEAN
ATLANTIC OCEAN
MEXICO
THE BAHAMAS
Ecopathology of Ranaviruses Infecting Amphibians
Debra Miller 1,2,*, Matthew Gray 1 and Andrew Storfer 3
2011
Families
Bufonidae
Hylidae
Ranidae
Scaphiopodidae
Ambystomatidae
Cryptobranchidae
Plethodontidae
Salamandridae
Lithobates sylvaticus

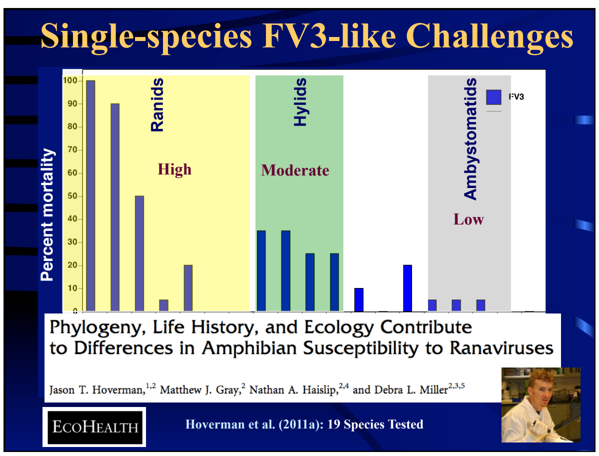

Case Example
Re-occuring Die-offs
Jamie Barichivich (USGS) and
Megan Todd-Thompson (UT)
A. Cressler, USGS
M. Niemiller, UT
A. Cressler, USGS
May 1999, 2000, 2009, 2012
GSMNP, Cades Cove
Gourley Pond
D. Green, USGS
Spotted & Marbled Salamander, Wood Frog,
Spring Peeper, Southeastern Chorus Frog
Green et al. (2002)


What Mechanisms Lead to Outbreaks?
A)
Possible Reservoirs
Infected, Susceptible, Recovered
Gray et al.
(2009)
Reptiles
Bony Fish
Amphibians
Shed
Virions
Infected Host
Density
Infected
Amphibian
Density
Habitat Characteristics
Water Chemistry, Soil Type,
Ambient Temperature,
Hydroperiod, UV
Environmental
Persistence
Indirect
Transmission
Circulating
Virions
Direct
Transmission
Direct Contact,
Cannibalism, Necrophagy
$10^2$ - $10^3$ PFU/mL
Vol. 87: 243–266, 2009
doi: 10.3354/dao02138
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published December 3
Ecology and pathology of amphibian ranaviruses
Matthew J. Gray[1,*], Debra L. Miller[1,2], Jason T. Hoverman[1]

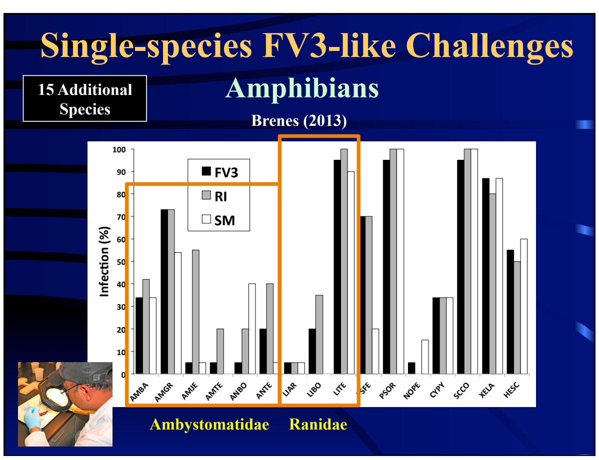

Species Challenges
FV3-like Ranaviruses
The
FUTURE
of Veterinary Medicine



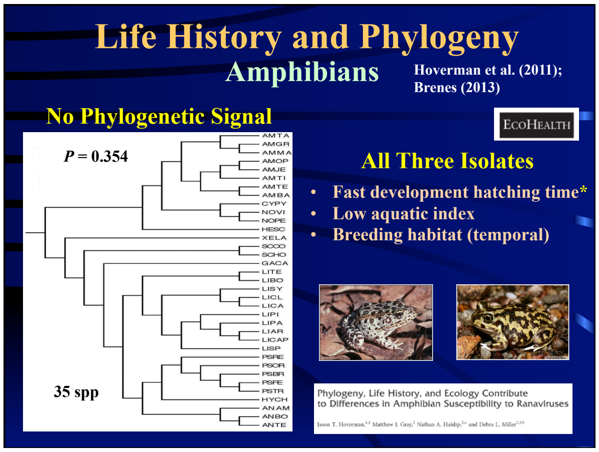
Ranavirus in TN
Cattle Land Use
FV3 Prevalence
P=0.78
P=0.02
A
0.36
A
0.3
A
0.4
3.9X More Likely!!!
B
0.15
Cattle Land Use
Access
Non-access
Bullfrog
n=104 tadpoles
Green Frog
n=80 tadpoles
Vol. 77: 97–103, 2007
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published September 14

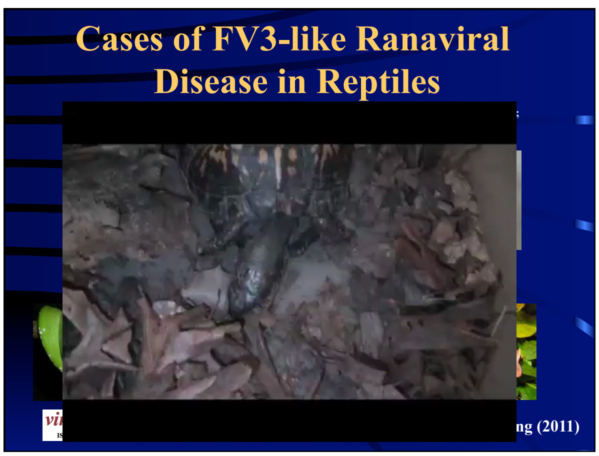



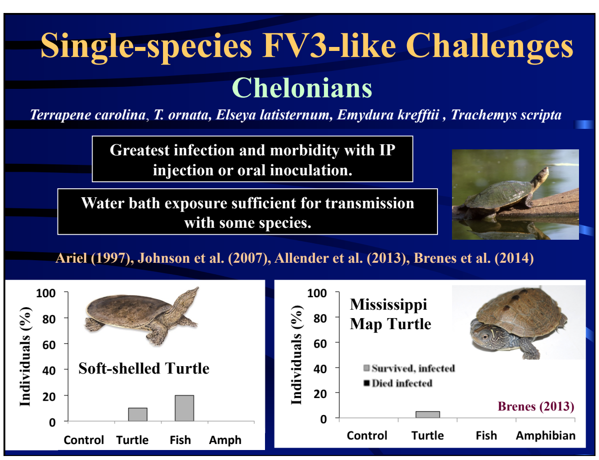
Single-species FV3-like Challenges
Percent mortality
Ranids
High
Hylids
Moderate
Ambystomatids
Low
FV3
Phylogeny, Life History, and Ecology Contribute to Differences in Amphibian Susceptibility to Ranaviruses
Jason T. Hoverman, Matthew J. Gray, Nathan A. Haislip, and Debra L. Miller
ECOHEALTH
Hoverman et al. (2011a): 19 Species Tested

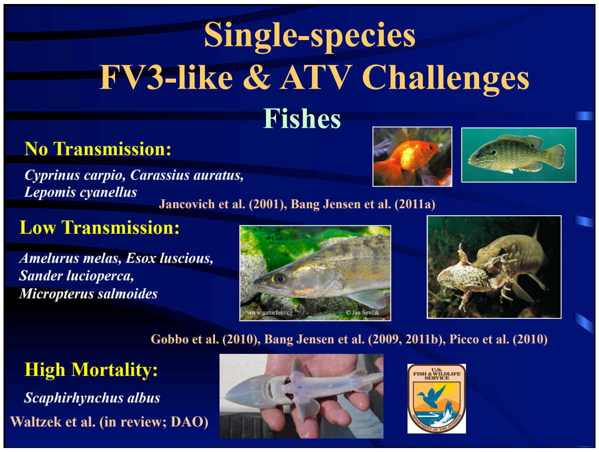



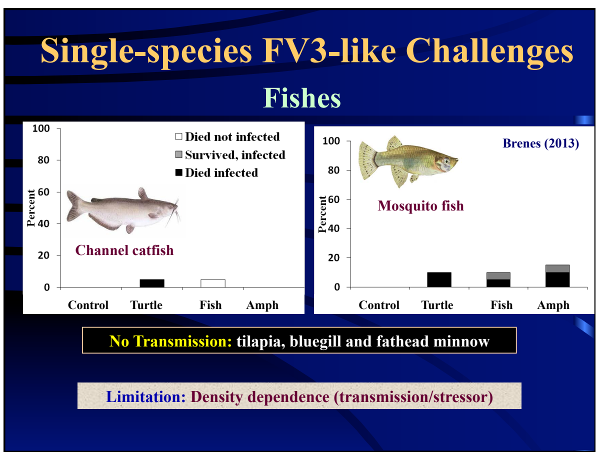
Single-species FV3-like Challenges
15 Additional Species
Amphibians
Brenes (2013)
Infection (%)
FV3
RI
SM
AMBA
AMGR
AMJE
AMTE
ANBO
ANTE
LIAR
LIBO
LITE
LISFE
PSOR
NOPE
CYPY
SCCO
XELA
HESC
Ambystomatidae
Ranidae

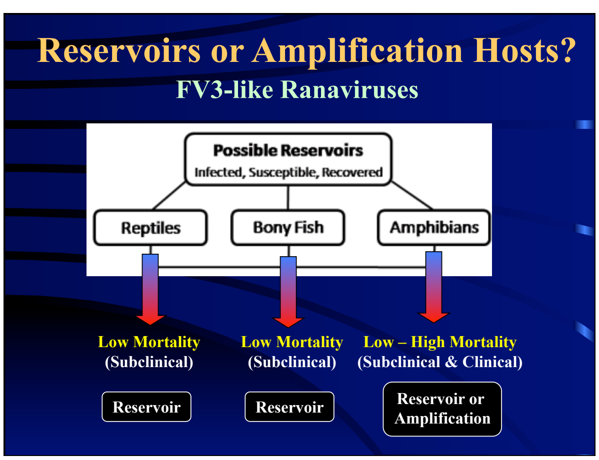

Life History and Phylogeny
Amphibians
Hoverman et al. (2011);
Brenes (2013)
No Phylogenetic Signal
EcoHealth
P = 0.354
AMTA
AMGR
AMMA
AMOP
AMJE
AMTI
AMTE
AMBA
CYPY
NOVI
NOPE
HESC
XELA
SCCO
SCHO
GACA
LITE
LIBO
LISY
LICL
LICA
LIPI
LIPA
LIAR
LICAP
LISP
PSRE
PSOR
PSBR
PSFE
PSTR
HYCH
ANAM
ANBO
ANTE
35 spp
All Three Isolates
• Fast development hatching time*
• Low aquatic index
• Breeding habitat (temporal)
Phylogeny, Life History, and Ecology Contribute
to Differences in Amphibian Susceptibility to Ranaviruses

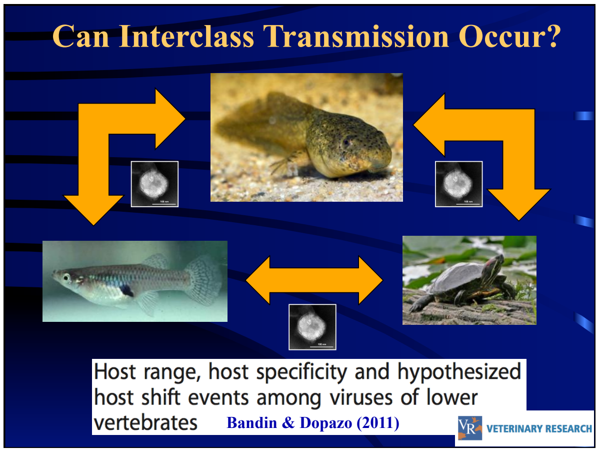

Cases of FV3-like Ranaviral
Disease in Reptiles


Single-species FV3-like Challenges
Chelonians
Terrapene carolina, T. ornata, Elseya latisternum, Emydura krefftii , Trachemys scripta
Greatest infection and morbidity with IP injection or oral inoculation.
Water bath exposure sufficient for transmission with some species.
Ariel (1997), Johnson et al. (2007), Allender et al. (2013), Brenes et al. (2014)
Individuals (%)
100
80
60
40
20
0
Soft-shelled Turtle
Control
Turtle
Fish
Amph
Individuals (%)
100
80
60
40
20
0
Mississippi
Map Turtle
Survived, infected
Died infected
Brenes (2013)
Control
Turtle
Fish
Amphibian

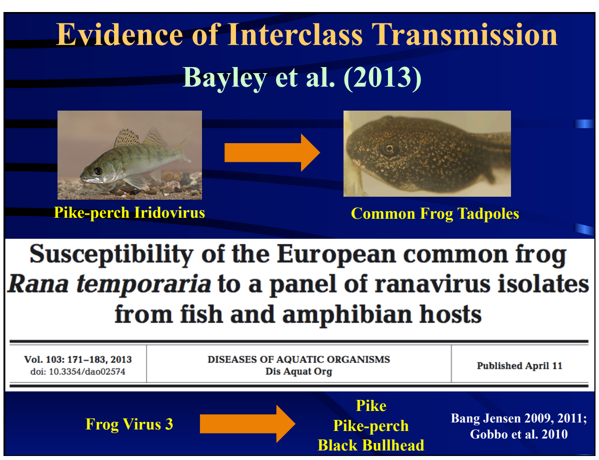

Single-species
FV3-like & ATV Challenges
Fishes
No Transmission:
Cyprinus carpio, Carassius auratus,
Lepomis cyanellus
Jancovich et al. (2001), Bang Jensen et al. (2011a)
Low Transmission:
Amelurus melas, Esox luscious,
Sander lucioperca,
Micropterus salmoides
Gobbo et al. (2010), Bang Jensen et al. (2009, 2011b), Picco et al. (2010)
High Mortality:
Scaphirhynchus albus
Waltzek et al. (in review; DAO)

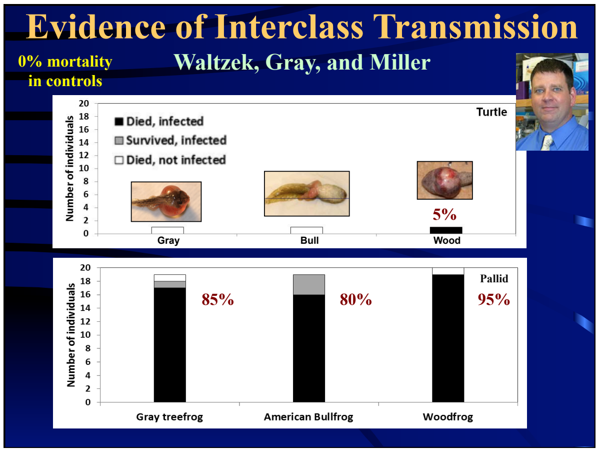

Single-species FV3-like Challenges
Fishes
100
80
60
40
20
0
Percent
Died not infected
Survived, infected
Died infected
Channel catfish
Control
Turtle
Fish
Amph
Brenes (2013)
Mosquito fish
No Transmission: tilapia, bluegill and fathead minnow
Limitation: Density dependence (transmission/stressor)

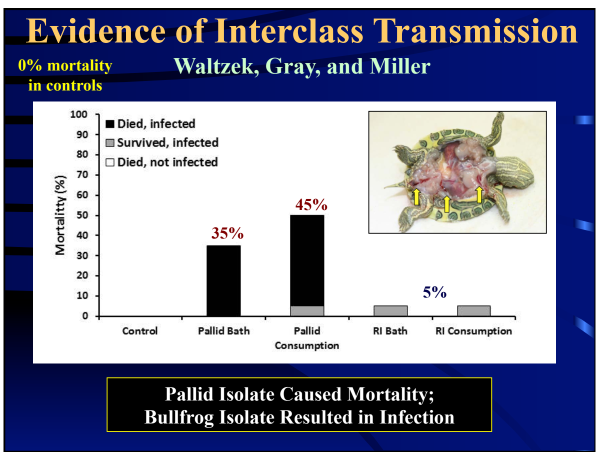

Reservoirs or Amplification Hosts?
FV3-like Ranaviruses
Possible Reservoirs
Infected, Susceptible, Recovered
Reptiles
Bony Fish
Amphibians
Low Mortality
(Subclinical)
Low Mortality
(Subclinical)
Low – High Mortality
(Subclinical & Clinical)
Reservoir
Reservoir
Reservoir or
Amplification

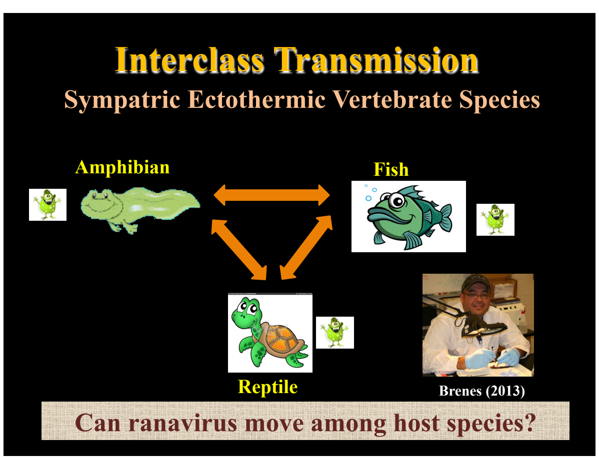

Can Interclass Transmission Occur?
Host range, host specificity and hypothesized host shift events among viruses of lower vertebrates
Bandin & Dopazo (2011)
VETERINARY RESEARCH


Evidence from the Wild
13 February 2012
The Washington Post
Make us your start page
POSTLOCAL
Deadly virus hits turtles, tadpoles in Montgomery County
North Branch Stream Valley State Park
26 of 31 Box Turtles Die from Ranaviral Disease
2008 – 2011
Larval anurans and salamanders dead too
Farnsworth and Seigel (2013)


Evidence of Interclass Transmission
Bayley et al. (2013)
Pike-perch Iridovirus
Common Frog Tadpoles
Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts
Vol. 103: 171–183, 2013
doi: 10.3354/dao02574
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published April 11
Frog Virus 3
Pike
Pike-perch
Black Bullhead
Bang Jensen 2009, 2011; Gobbo et al. 2010

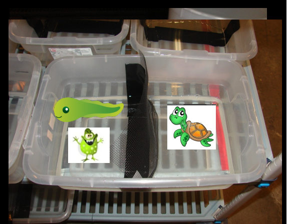

Evidence of Interclass Transmission
0% mortality in controls
Waltzek, Gray, and Miller
Died, infected
Survived, infected
Died, not infected
Turtle
Number of individuals
5%
Gray
Bull
Wood
Pallid
85%
80%
95%
Gray treefrog
American Bullfrog
Woodfrog


Evidence of Interclass Transmission
0% mortality in controls
Waltzek, Gray, and Miller
Died, infected
Survived, infected
Died, not infected
Mortalitty (%)
35%
45%
5%
Control
Pallid Bath
Pallid Consumption
RI Bath
RI Consumption
Pallid Isolate Caused Mortality;
Bullfrog Isolate Resulted in Infection


Interclass Transmission
Sympatric Ectothermic Vertebrate Species
Amphibian
Fish
Reptile
Brenes (2013)
Can ranavirus move among host species?

# Experiment

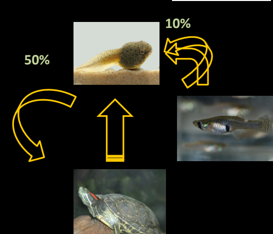
- Direct exposure
  - Exposed to $10^3$ PFU/mL
  - 3 days
- 12-L containers divided in half by a 2000 µm plastic mesh
- Different species in each side of the container



# Turtle and Fish Results

Brenes et al. (PLoS ONE, accepted)

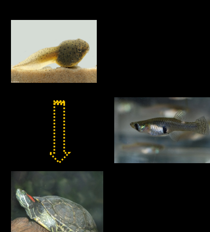
- All classes tested can transmit the virus
- Turtles infected tadpoles
  - 50% mortality
- Fish infected tadpoles
  - 10% mortality

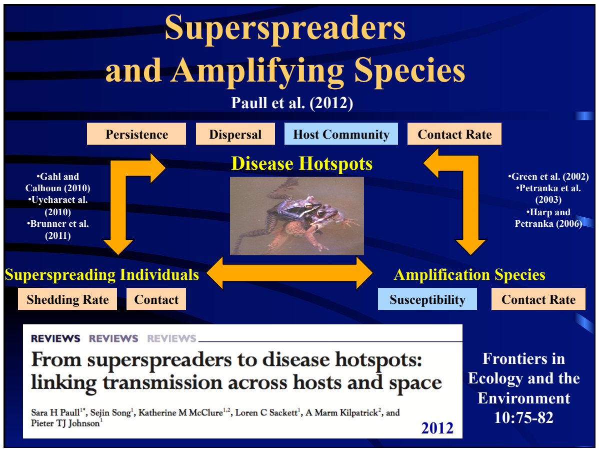


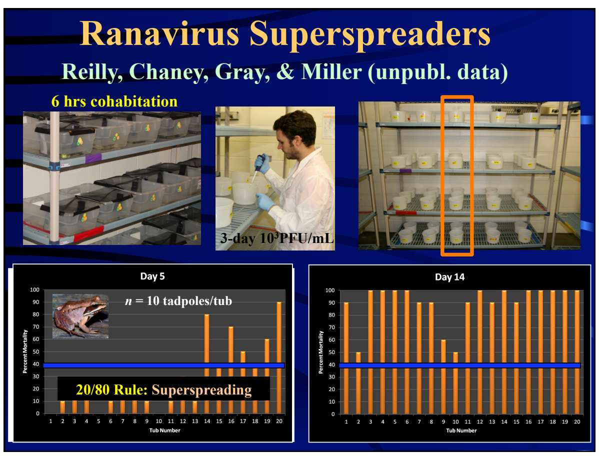

# Amphibian Results

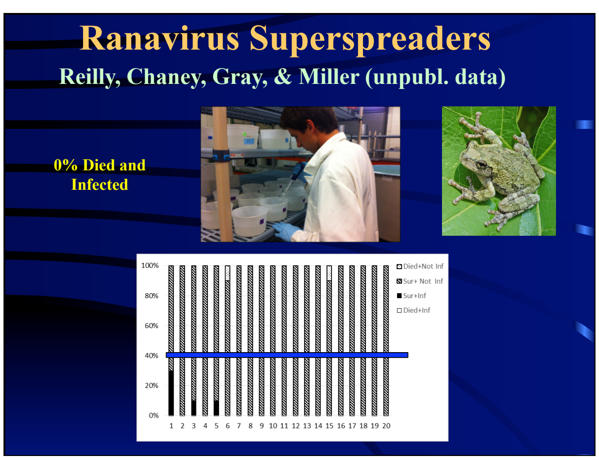
Brenes et al. (PLoS ONE, accepted)

- Amphibians transmitted to turtles but not fish
- No mortality of turtles or fish exposed to infected tadpoles
- Suggests that turtles and fish may be reservoirs of ranavirus
- Amphibians may be amplifying species




Superspreaders
and Amplifying Species
Paull et al. (2012)
Persistence
Dispersal
Host Community
Contact Rate
Disease Hotspots
•Gahl and Calhoun (2010)
•Uyeharaet al. (2010)
•Brunner et al. (2011)
•Green et al. (2002)
•Petranka et al. (2003)
•Harp and Petranka (2006)
Superspreading Individuals
Shedding Rate
Contact
Amplification Species
Susceptibility
Contact Rate
REVIEWS REVIEWS REVIEWS
From superspreaders to disease hotspots: linking transmission across hosts and space
Sara H Paull[1*], Sejin Song[1], Katherine M McClure[1,2], Loren C Sackett[1], A Marm Kilpatrick[2], and Pieter TJ Johnson[1]
2012
Frontiers in Ecology and the Environment 10:75-82


Ranavirus Superspreaders
Reilly, Chaney, Gray, & Miller (unpubl. data)
6 hrs cohabitation
3-day $10^3$ PFU/mL
Day 5
$n$ = 10 tadpoles/tub
20/80 Rule: Superspreading
Day 14


Ranavirus Superspreaders
Reilly, Chaney, Gray, & Miller (unpubl. data)
0% Died and Infected

## Community Level Transmission

Brenes, Gray, & Miller (unpubl. data)

Inoculated in Lab
$10^3$ PFU/mL FV3
Exposure Order

Appalachian: Wood frog, chorus frog, spotted salamander
Coastal Plains: Gopher frog, chorus, southern toad

**Does Exposure Order or Composition Matter?**

## Exposure Order Matters

Brenes (2013)

**Design**

*n* = 5 pools/trt
10 larvae/spp
60 days

**Exposure Treatments**

Only Wood Frogs
Only Chorus Frogs
Only Spotted Salamanders
Control

**Appalachian Community**

| | | | | |
|---|---|---|---|---|
| Wood Frogs | 100% (high) | 43% | Chorus Frogs | 52% |
| | | 12% | Spotted Salam | |
| Chorus Frogs | 44% (mod) | 72% | Wood Frogs | 40% |
| | | 3% | Spotted Salam | |
| Spotted Salam | 6% (low) | 24% | Chorus Frogs | 16% |
| | | 18% | Wood Frogs | |

## Community Composition Matters

Brenes (2013)

**Design**

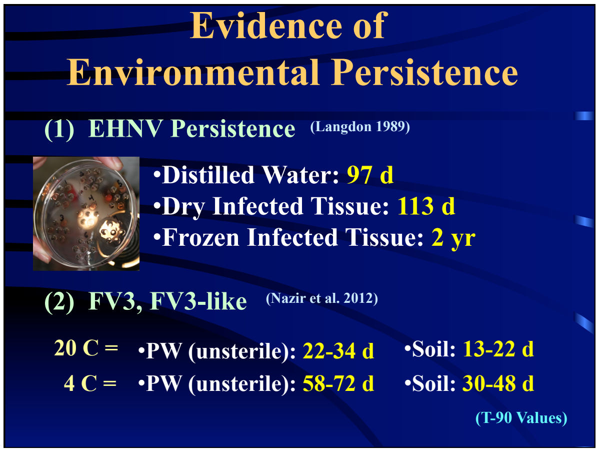
*n* = 5 pools/trt
10 larvae/spp
60 days

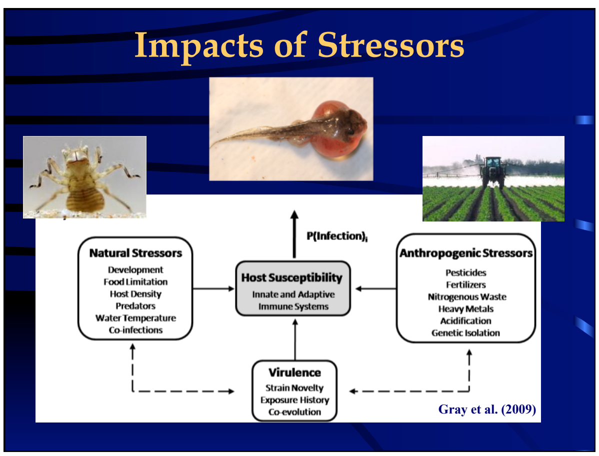
**Exposure Treatments**

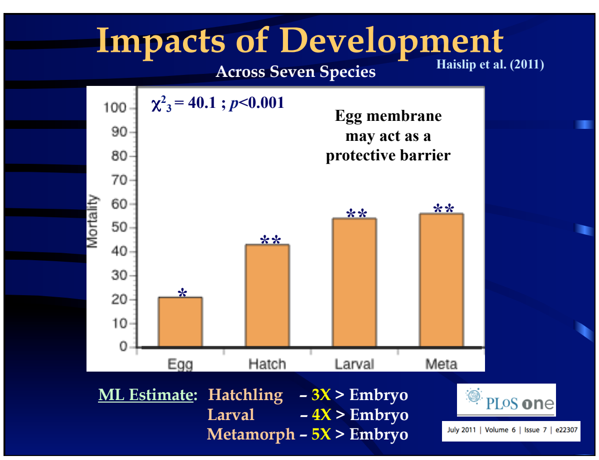
Only Gopher Frogs
Only Chorus Frogs
Only Southern Toad
Control

**Gulf Coastal Plain, USA**

| | | | | |
|---|---|---|---|---|
| Gopher Frogs | 100% (high) | 52% | Chorus Frogs | 62% |
| | | 34% | Southern Toad | |
| Chorus Frogs | 78% (high) | 70% | Gopher Frog | 68% |
| | | 58% | Southern Toad | |
| Southern Toad | 76% (high) | 32% | Chorus Frogs | 62% |
| | | 80% | Gopher Frog | |


Evidence of
Environmental Persistence
(1) EHNV Persistence (Langdon 1989)
•Distilled Water: 97 d
•Dry Infected Tissue: 113 d
•Frozen Infected Tissue: 2 yr
(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)

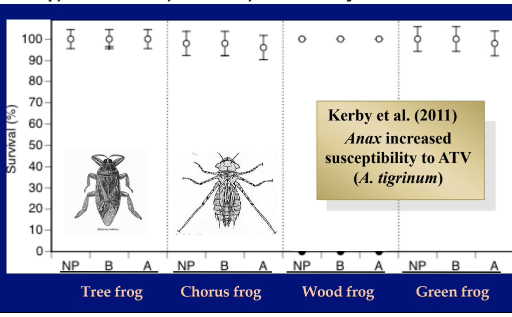

Impacts of Stressors
P(Infection)
Natural Stressors
Development
Food Limitation
Host Density
Predators
Water Temperature
Co-infections
Host Susceptibility
Innate and Adaptive Immune Systems
Anthropogenic Stressors
Pesticides
Fertilizers
Nitrogenous Waste
Heavy Metals
Acidification
Genetic Isolation
Virulence
Strain Novelty
Exposure History
Co-evolution
Gray et al. (2009)


Impacts of Development
Across Seven Species
Haislip et al. (2011)
$\chi^2_3 = 40.1$ ; $p<0.001$
Egg membrane may act as a protective barrier
Mortality
100
90
80
70
60
50
40
30
20
10
0
*
**
**
**
Egg
Hatch
Larval
Meta
ML Estimate: Hatchling – 3X > Embryo
Larval – 4X > Embryo
Metamorph – 5X > Embryo
PLoS one
July 2011 | Volume 6 | Issue 7 | e22307

## Natural stressors and disease risk: does the threat of predation increase amphibian susceptibility to ranavirus?

Can. J. Zool. 90: 893–902 (2012)

N.A. Haislip, J.T. Hoverman, D.L. Miller, and M.J. Gray




## Competing Temperature Hypotheses

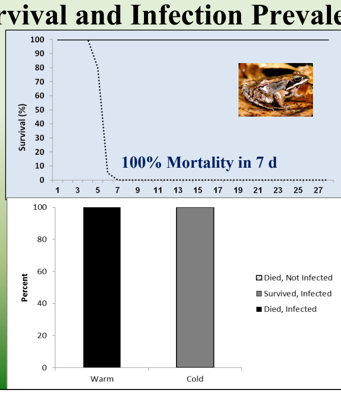
- **Virus Replication Hypothesis** Bayley et al. (2013)
  - Ranavirus replication increases with temperature up to 32 C **High Pathogenicity at Higher Temperatures**
- **Temperature Induced Stress Hypothesis**
  - Early Spring Breeding Species:
    - Stressed by Warm Temp
  - Summer Breeding Species:
    - Stressed by Cold Temp

**Pathogenicity is Species-specific and Related to Typical Water Temperature Experienced During Tadpole Development**

M. Brand

## Wood Frog
### Survival and Infection Prevalence

No Control Mortality




Warm
Cold

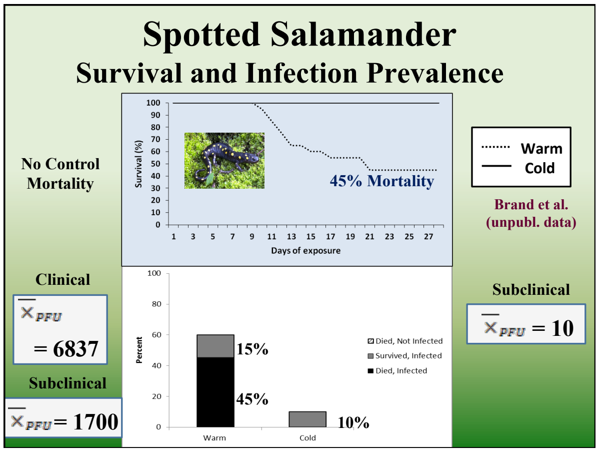
Brand et al. (unpubl. data)

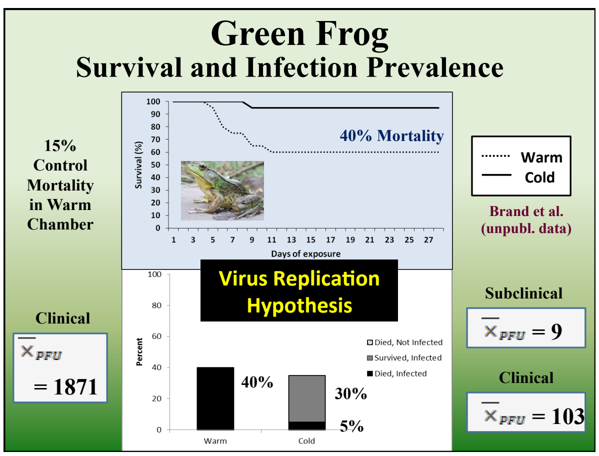
Clinical

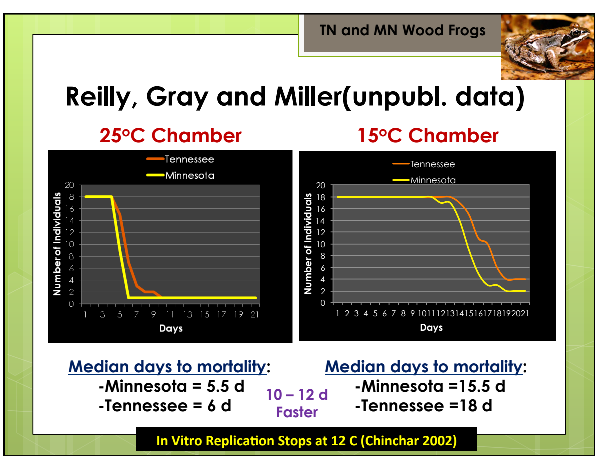
$\bar{X}_{PFU}$ = 152484

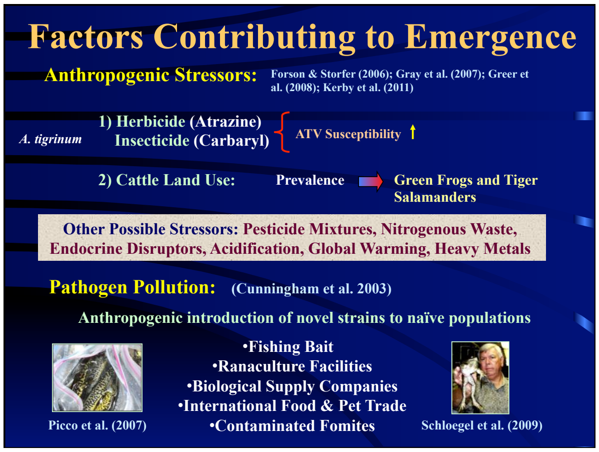
Subclinical

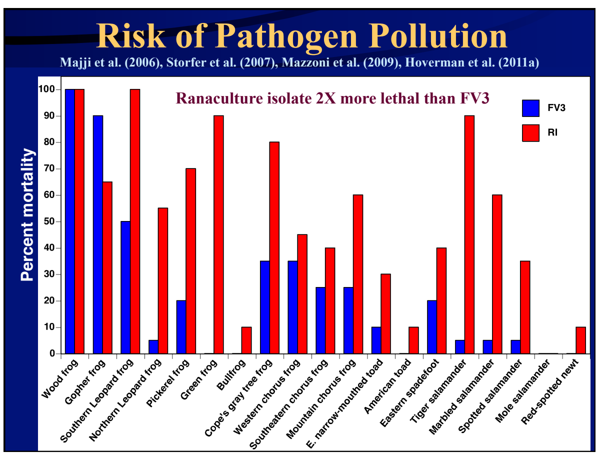
$\bar{X}_{PFU} = 84$

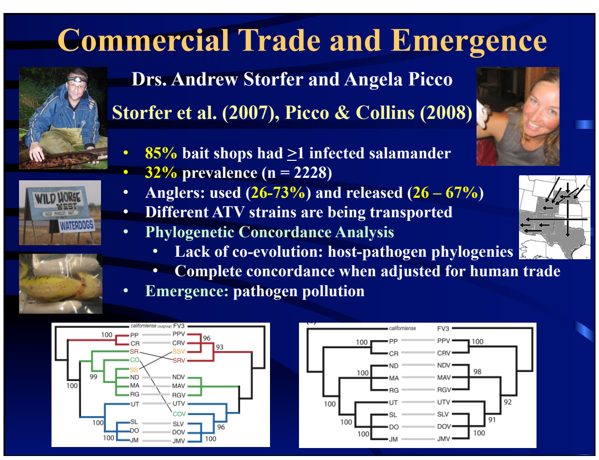

Spotted Salamander
Survival and Infection Prevalence
No Control Mortality
45% Mortality
Survival (%)
Days of exposure
Warm
Cold
Brand et al. (unpubl. data)
Clinical
$\bar{X}_{PFU}$ = 6837
Subclinical
$\bar{X}_{PFU}$ = 1700
Subclinical
$\bar{X}_{PFU}$ = 10
Percent
15%
45%
10%
Died, Not Infected
Survived, Infected
Died, Infected
Warm
Cold

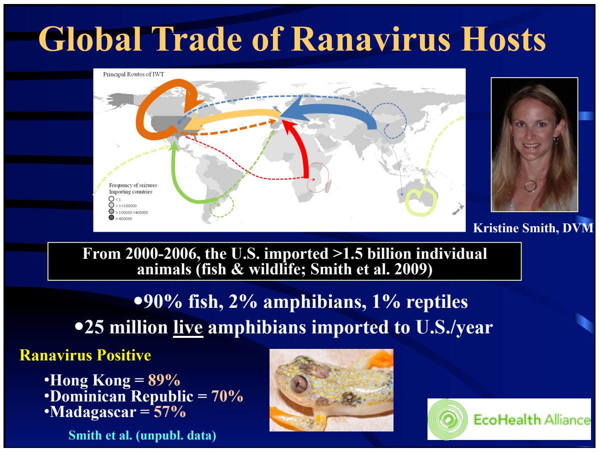

Green Frog
Survival and Infection Prevalence
15% Control Mortality in Warm Chamber
40% Mortality
Survival (%)
Days of exposure
Warm
Cold
Brand et al. (unpubl. data)
Virus Replication Hypothesis
Clinical
$\bar{X}_{PFU}$ = 1871
Subclinical
$\bar{X}_{PFU}$ = 9
Clinical
$\bar{X}_{PFU}$ = 103
Percent
40%
30%
5%
Died, Not Infected
Survived, Infected
Died, Infected
Warm
Cold


TN and MN Wood Frogs
Reilly, Gray and Miller(unpubl. data)
25°C Chamber
15°C Chamber
Tennessee
Minnesota
Number of Individuals
Days
Median days to mortality:
-Minnesota = 5.5 d
-Tennessee = 6 d
10 – 12 d Faster
Median days to mortality:
-Minnesota =15.5 d
-Tennessee =18 d
In Vitro Replication Stops at 12 C (Chinchar 2002)

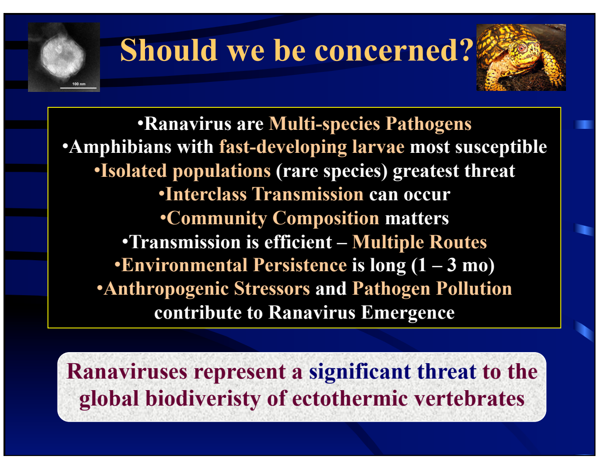

Factors Contributing to Emergence
Anthropogenic Stressors:
Forson & Storfer (2006); Gray et al. (2007); Greer et al. (2008); Kerby et al. (2011)
A. tigrinum
1) Herbicide (Atrazine)
Insecticide (Carbaryl)
ATV Susceptibility
2) Cattle Land Use:
Prevalence
Green Frogs and Tiger Salamanders
Other Possible 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
•Fishing Bait
•Ranaculture Facilities
•Biological Supply Companies
•International Food & Pet Trade
•Contaminated Fomites
Picco et al. (2007)
Schloegel et al. (2009)


Risk of Pathogen Pollution
Majji et al. (2006), Storfer et al. (2007), Mazzoni et al. (2009), Hoverman et al. (2011a)
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
Southeastern chorus frog
Mountain chorus frog
E. narrow-mouthed toad
American toad
Eastern spadefoot
Tiger salamander
Marbled salamander
Spotted salamander
Mole salamander
Red-spotted newt


Commercial Trade and Emergence
Drs. Andrew Storfer and Angela Picco
Storfer et al. (2007), Picco & Collins (2008)
• 85% bait shops had ≥1 infected salamander
• 32% prevalence (n = 2228)
• Anglers: used (26-73%) and released (26 – 67%)
• Different ATV strains are being transported
• Phylogenetic Concordance Analysis
• Lack of co-evolution: host-pathogen phylogenies
• Complete concordance when adjusted for human trade
• Emergence: pathogen pollution
WILD HORSE
WATERDOGS


Global Trade of Ranavirus Hosts
Kristine Smith, DVM
From 2000-2006, the U.S. imported >1.5 billion individual animals (fish & wildlife; Smith et al. 2009)
•90% fish, 2% amphibians, 1% reptiles
•25 million live amphibians imported to U.S./year
Ranavirus Positive
•Hong Kong = 89%
•Dominican Republic = 70%
•Madagascar = 57%
Smith et al. (unpubl. data)
EcoHealth Alliance

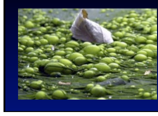

World Organization for Animal Health
OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008
OIE
Notifiable Diseases
Certification for Shipment
Schloegel et al. (2010)
International Transport of Animals
Disinfection: Johnson et al. (2003), Bryan et al. (2009); Gold et al (2014)
$75/ bottle
•Bleach ≥4%
•EtOH ≥70%
•Virkon ≥1%
•Nolvasan >0.75%


Should we be concerned?
•Ranavirus are Multi-species Pathogens
•Amphibians with fast-developing larvae most susceptible
•Isolated populations (rare species) greatest threat
•Interclass Transmission can occur
•Community Composition matters
•Transmission is efficient – Multiple Routes
•Environmental Persistence is long (1 – 3 mo)
•Anthropogenic Stressors and Pathogen Pollution contribute to Ranavirus Emergence
Ranaviruses represent a significant threat to the global biodiveristy of ectothermic vertebrates

Gray and Miller (2013)

## What can we do?

•Establish surveillance programs
(broad then focus on hotspots, >40% infection)
•Identify mechanisms of emergence
(natural, stressors, novel strains)
•Identify and implement intervention strategies
(break host-pathogen cycle, reduce stressors, biosecurity precautions)



## Global Ranavirus Consortium

http://fwf.ag.utk.edu/mgray/ranavirus/ranavirus.htm

Symposia
Discussion Groups
Website
Reporting System
Outreach Resources
Springer eBook

The goal of the GRC is to facilitate communication and collaboration among scientists and veterinarians conducting research on ranaviruses and diagnosing cases of ranaviral disease

GRC@LISTSERV.UTK.EDU