Ecology and Pathology of Ranaviruses




M. Niemiller

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Outline

I. Ranavirus Die-offs and Host Effects

II. UT Research: Host-Pathogen Interactions

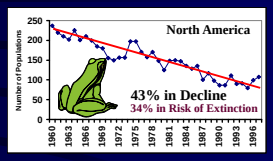
III. Can Ranaviruses Contribute to Declines?

IV. Anthropogenic Effects and Disinfectants

Amphibian Declines and Emerging Infectious Diseases

Science
306:1783-1786

EID 5:735-748

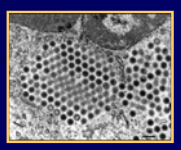


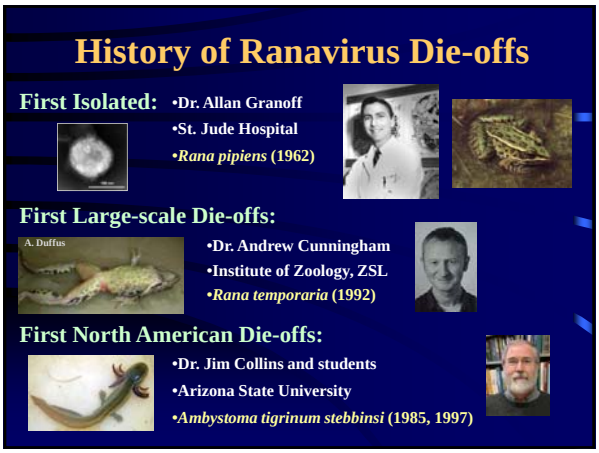
Nature
404:752-755

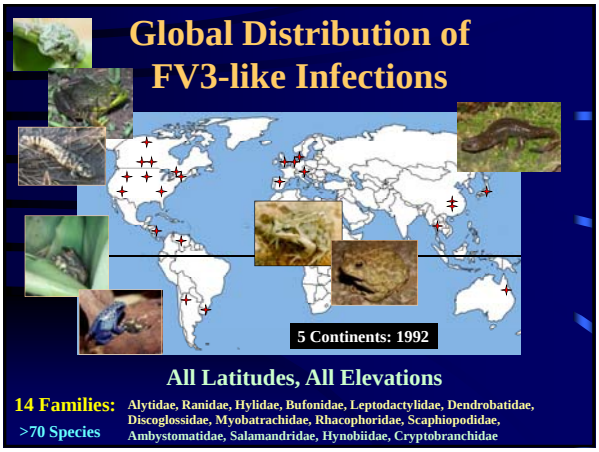
Biotropica
37:163-165


Chytrid Fungus


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

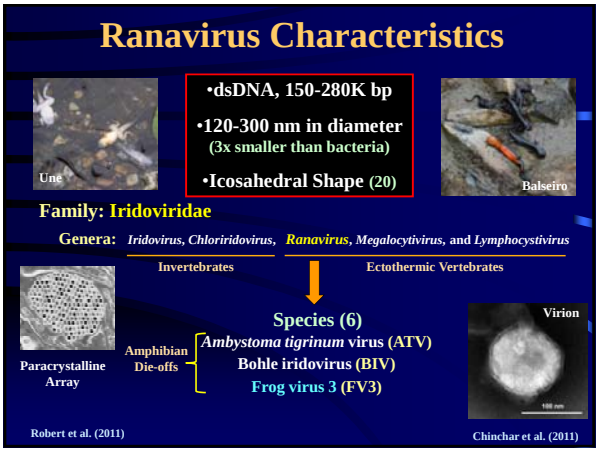

Ranaviruses

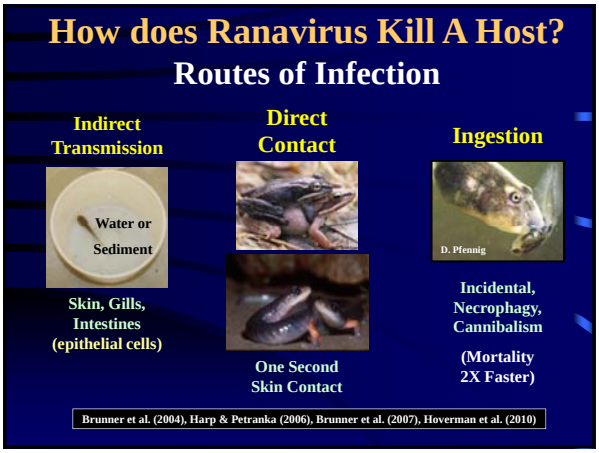

History of Ranavirus Die-offs
First Isolated:
•Dr. Allan Granoff
•St. Jude Hospital
•Rana pipiens (1962)
First Large-scale Die-offs:
A. Duffus
•Dr. Andrew Cunningham
•Institute of Zoology, ZSL
•Rana temporaria (1992)
First North American Die-offs:
•Dr. Jim Collins and students
•Arizona State University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


Global Distribution of FV3-like Infections
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


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

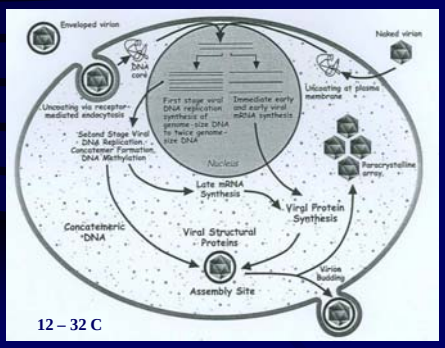

Case Example
North America
Jamie Barichivich (USGS) and
Megan Todd-Thompson (UT)
A. Cressler, USGS
M. Niemiller, UT
A. Cressler, USGS
GSMNP, Cades Cove
Gourley Pond
May 2009
D. Green, USGS
Spotted & Marbled Salamander, Wood Frog,
Spring Peeper, Southeastern Chorus Frog


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)
Ambystoma tigrinum virus (ATV)
Bohle iridovirus (BIV)
Frog virus 3 (FV3)
Amphibian
Die-offs
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)



12 – 32 C

Protein synthesis within hours of infection

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

Signs Vary Among Species

Haislip et al. (unpubl. data)

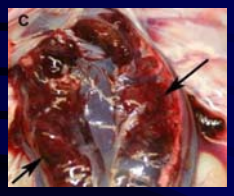
Lithobates clamitans

Hyla chrysoscelis

Lithobates sylvaticus

Internal Signs of Ranaviral Disease

Kidney Hemorrhages

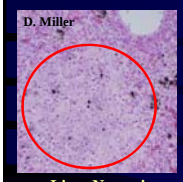


Pale and Swollen Liver

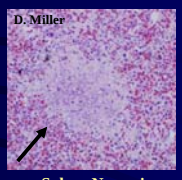
Organ Destruction

3 Primary Organs: Liver, Spleen, and Kidney

Bollinger et al. (1999)
Miller et al. (2007, 2008)



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

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

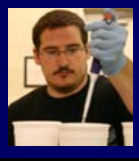
Begging Dr. Death (Jack Kevorkian) for a quick end!

University of Tennessee
Ranavirus Research

Gray, Miller, Hoverman, Haislip,
Bryan, Brenes, Hilzinger and others

- Species Susceptibility
- Isolate Virulence
- Developmental Stage
- Risk of Predation
- Disinfectants

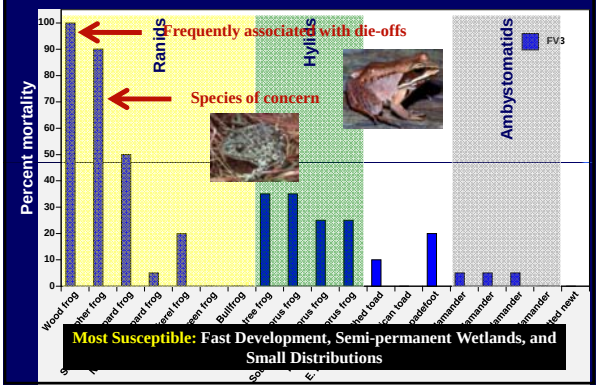
- Community Level Effects
- Interclass Transmission
- Environmental Persistence



Broad Host Range

Hoverman et al. (2011): 19 Species Tested

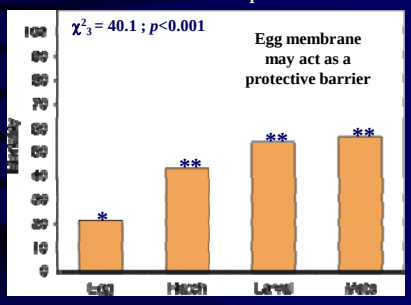
Expanding to 40 spp (2012)



Impacts of Development

Across Seven Species

Haislip et al. (2011)

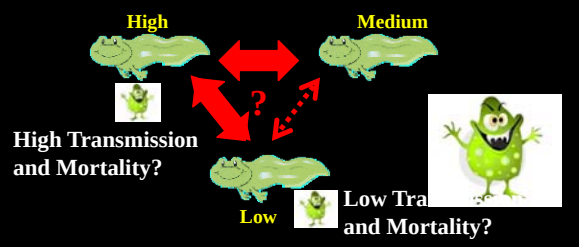


ML Estimate: Hatchling – 3X > Embryo
Larval – 4X > Embryo
Metamorph – 5X > Embryo

Community Level Transmission

Does it Matter Who is Infected?

Species with Different Susceptibilities

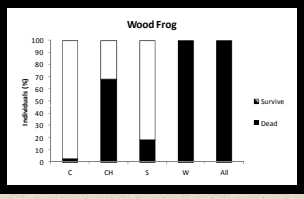


Can Certain Species Drive Outbreaks?

Results: Wood Frog

Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al.
(unpubl. data)



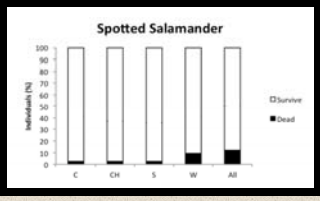
Wood frogs experienced greatest mortality in all treatments

100% Mortality: All exposed or they were exposed.
65% Mortality: Chorus frogs exposed.
15% Mortality: Spotted salamanders exposed.

Results: Spotted Salamander

Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al.
(unpubl. data)



Spotted Salamanders experienced lowest mortality among treatments

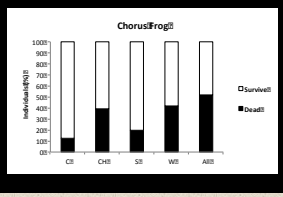
0 – 15% Mortality

Slightly higher mortality if wood frogs or all species exposed.

Results: Upland Chorus Frog

Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al.
(unpubl. data)



Chorus frogs experienced moderate mortality among treatments

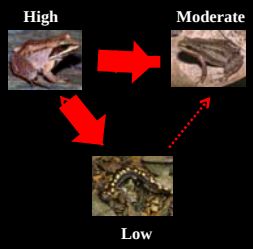
20 – 50% Mortality

Similar mortality if wood frogs, all species, or only chorus frogs were exposed

Pathogen Transmission

Community Predictions Confirmed

- **Wood frog tadpoles appear to function as superspreaders**
- **Exposure of upland chorus frog tadpoles caused an outbreak.**
- **Exposure of spotted salamander larvae was insufficient to cause an outbreak.**



What about other Ectothermic Vertebrates?

13 February 2012

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, 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

Blind Pony Fish Hatchery, Missouri

Wild Case

Identical with *R. aurora* ranavirus

Mao et al. (1999)

Experimental Challenges

Anurans

- 2-L Tubs
- Pallid & Turtle
- 3 Day WB
- 21 Days

10^3 PFU/mL

Turtles

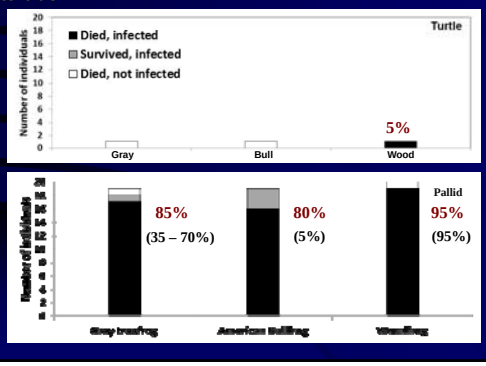
- 12-L Tubs
- Pallid & Bullfrog
- 3 Day WB
- Infected WF (3 d)
- 28 Days

n = 20 / trt + 20 controls
Water Change: 3d
Morbidity & Mortality
qPCR for Infection

Transmission to Anurans

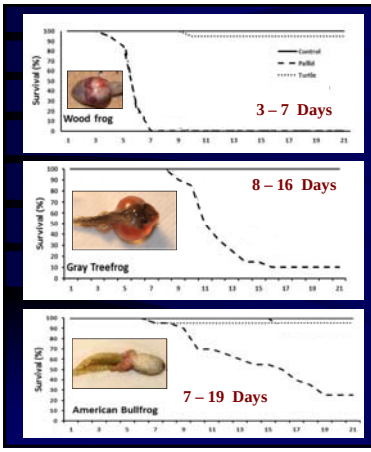
0% mortality in controls

Final Mortality

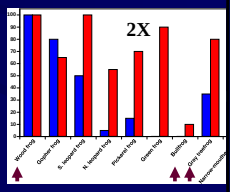


Survival

Pallid Isolate



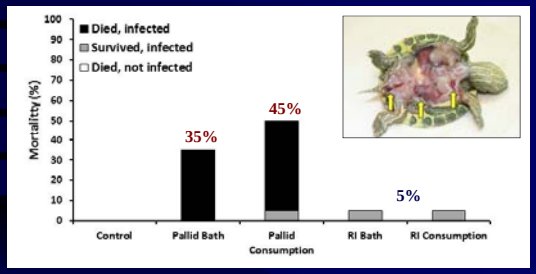
Resulted in faster mortality than any other previously tested isolate



Hoverman et al. (2011)

Transmission to Turtles

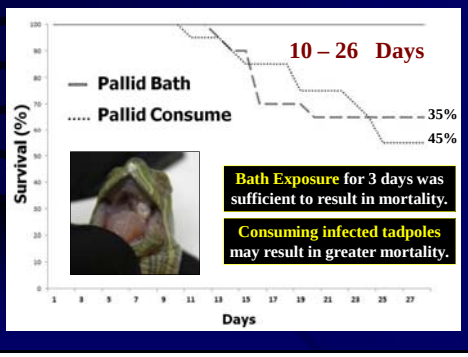
Final Mortality



Pallid Isolate Caused Mortality;
Bullfrog Isolate Resulted in Infection

Transmission to Turtles

Survival Curves



Bath Exposure for 3 days was sufficient to result in mortality.

Consuming infected tadpoles may result in greater mortality.

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

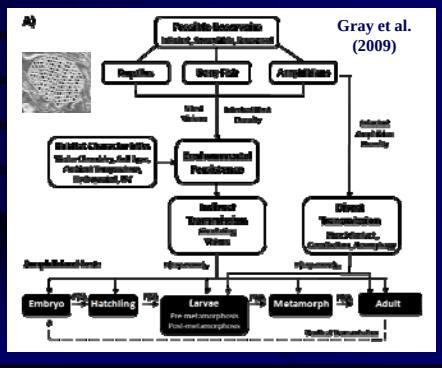
Mutual Reservoirs

Isolate Dependent

Community Level Epizootic Cases

MD, NC

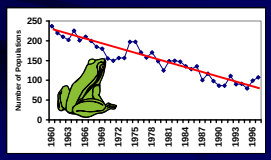
Ranavirus Ecology

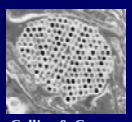


Gray et al. (2009)

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

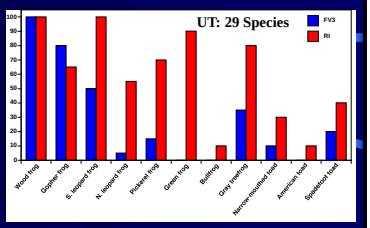
(3) Clustering of Individuals

- Sexually transmitted disease

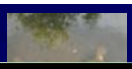
Evidence of Alternate Hosts

(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

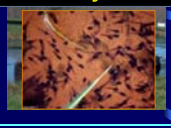
(1) Breeding (Brunner et al. 2004)

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?

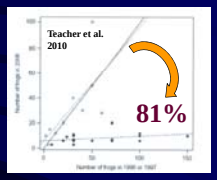
YES, all 3 characteristics met in the Ranavirus-Host System

Caveat: Community and Site Dependent!

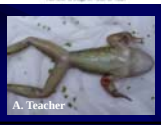
Evidence of Declines

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

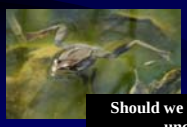
Biological Conservation 138:371-380

Wetlands 23:278-290

Recruitment at most wetlands failed due to ranavirus

Persistence Possible from Source Populations

Rescue Effect



Uncommon Species?

Should we be concerned with a few uncommon species?

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)

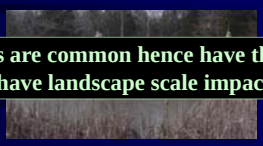
If uncommon species are highly susceptible, ranaviruses could have a significant impact on amphibian communities.

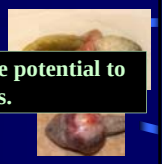
10) Tennessee = 26 species (14 genera)

Ranavirus Landscape Prevalence

Tennessee Ponds

Hoverman et al. (2012)





Ranaviruses are common hence have the potential to have landscape scale impacts.

Ranavirus Distribution: 83% of Ponds Sampled

Hotspots: ≥40% in 15 out of 40 Ponds Sampled

- 85% Infection
- 100% Infection

(morbid and dead)

Take Home Messages

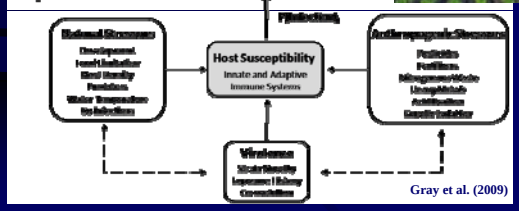
Should we be Concerned?

- •Ranavirus Die-offs have Global Distribution
- •Ranavirus Prevalence can be High
- •Ranaviruses Infect Multiple Amphibian Species with Different Susceptibilities
- •Community Composition may Matter
- •Interclass Transmission is Possible – Abundant Reservoirs
- •Ranavirus Persistence may be Long
- •High Transmission: Breeding and for Schooling Spp.

Epidemiological Theory Supports the Premise that Ranaviruses Could Cause Local Population Extirpations and Contribute to Species Declines

Uncommon Species with High Susceptibility are at Greatest Risk!

Impacts of Stressors



Gray et al. (2009)

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

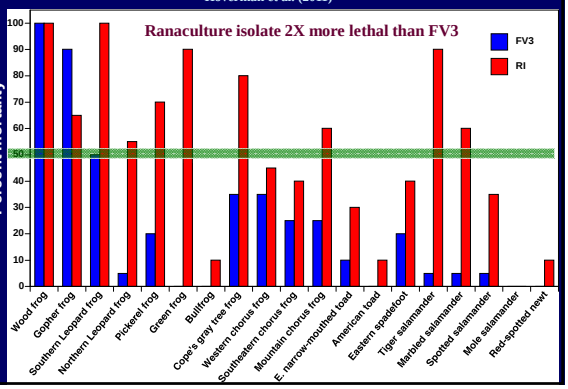
Picco et al. (2007)

- •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)



World Organization for Animal Health

OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008

Notifiable Diseases
Certification for Shipment
Schloegel et al. (2010)

International Transport of Animals

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

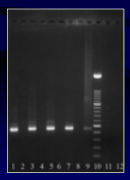
$75/ bottle

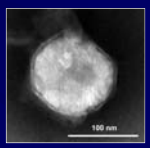
- Bleach ≥4%
- EtOH ≥70%
- Virkon ≥1%
- Nolvasan >0.75%

Second International Conference of Ranaviruses

2013 International Conference of the Wildlife Disease Association

27 – 29 July 2013: Knoxville, TN






Questions??
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