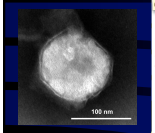
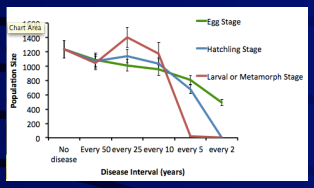
# Ranaviruses:
## Cry Wolf or Real Threat?



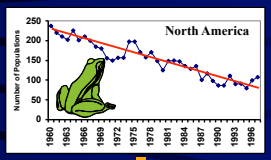




**Matthew J. Gray**
University of Tennessee
Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries

3 February 2014, 4:00 PM, Lecture Hall B
UF Aquatic Animal Health Program

# Presentation Road Map


Life History




Die-offs

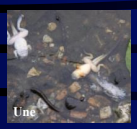
Theory

Monitoring

Limited

Simulations

# Ranavirus Characteristics

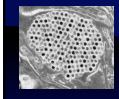



- dsDNA, 150-280K bp
- 120-300 nm in diameter (3x smaller than bacteria)
- Icosahedral Shape (20)


Family: Iridoviridae

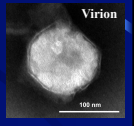
Genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Megalocytivirus*, and *Lymphocystivirus*

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**


Skin, Gills, Intestines (epithelial cells)

(3 hrs viral transcription)

**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), Robert et al. (2011)

## Gross Signs of Infected Amphibians

Edema, Erythema, Hemorrhages, Ulcerations

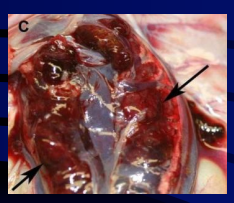
A. Duffus, Gordon

N. Haislip, UT

D. Green, USGS

## Internal Signs of Ranaviral Disease

Kidney Hemorrhages




Pale and Swollen Liver

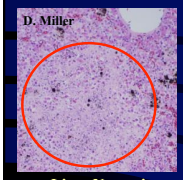

It attacks quickly killing hosts as fast as 3 days!

Hoverman et al. (2011a)

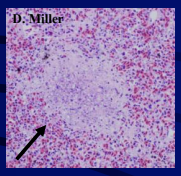
## Organ Destruction

3 Primary Organs: Liver, Spleen, and Kidney

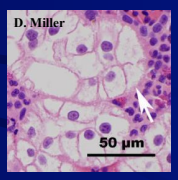
Bollinger et al. (1999)
Miller et al. (2007, 2008, 2011)




Liver Necrosis




Spleen Necrosis




Kidney Degeneration

**Pathogenesis**

Target Organ Failure

Heart Failure
Toxicosis, Anemia

?

## What if Ranaviruses could Infect Humans?

12 – 32 C

Chinchar (2002)

**Monday**

Fever

**Wednesday**

Hands, Feet, Legs Swollen

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

There is no Cure!

**Friday**

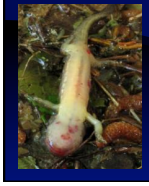
Bedridden, Body Enlarged 2X, Lesions, Hemorrhaging Internally and from Orifices

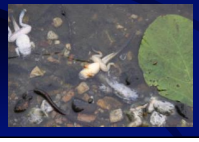
**Sunday**

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

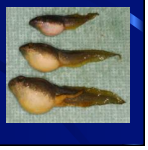
## Mass Mortality Events

Larvae





Adults



## History of Ranavirus Die-offs

**First Isolated:** •Dr. Allan Granoff

•St. Jude Hospital
•Lucke herpesvirus
•*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 Cases: Amphibians














**All Latitudes, All Elevations**

**15 Families:** Alytidae, Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae, Discoglossidae, Pipidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae

**>70 Species**

## Reported Ranavirus Cases in North America: Amphibians

**Uncommon**

**>30 States & 5 Provinces; 46 Species**


**Families**

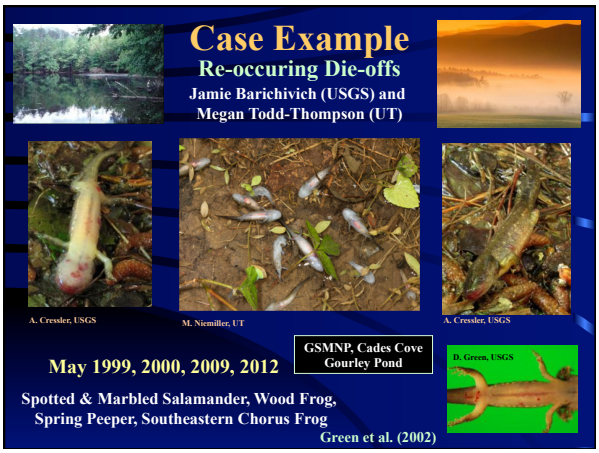
Bufonidae
Hylidae
Ranidae
Scaphiopodidae
Ambystomatidae
Cryptobranchidae
Plethodontidae
Salamandridae

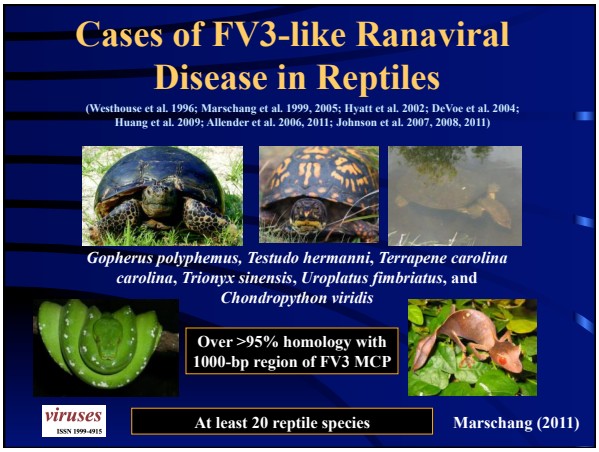
**Lithobates sylvaticus**

*viruses*
ISSN 1999-4915

**Ecopathology of Ranaviruses Infecting Amphibians**

Debra Miller [1,2,*], Matthew Gray [1] and Andrew Storfer [3] **2011**


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


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 FV3 MCP
viruses
ISSN 1999-4915
At least 20 reptile species
Marschang (2011)


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
View Photo Gallery — Biologists say an alarming number of turtles rescued from the path of the
Intercounty Connector's construction have died of a virus they fear could devastate Maryland's
ecosystem.
26 of 31 Box
Turtles Die
from
Ranaviral
Disease
2008 – 2011
Larval
anurans and
salamanders
dead too
Farnsworth
and Seigel
(2013)

## Cases of Ranaviral Disease in Fishes



*Ictalurus melas, I. nebuosa, Silurus glanis, Psetta maxima, Sander lucioperca, Perca fluviatilis, P. flavescens, Oncorhynchus mykiss, Pomoxis nigromaculatus, Gambusia affinis, Epinephelus tauvina*

EHNV, ECV | At least 30 fish species | LMBV, SGIV

**Iridovirus infections in finfish – critical review with emphasis on ranaviruses**

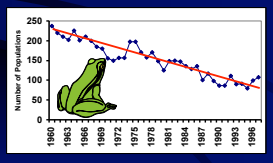
Journal of Fish Diseases 33:95-122

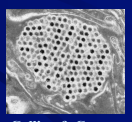
R J Whittington, J A Becker and M M Dennis

World Organisation for Animal Health (OIE) Reference Laboratory for EHN Virus, Faculty of Veterinary Science, The University of Sydney, Sydney, Australia

## 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 Species Where Susceptibility Differs**

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

- Survive Outside Host

**(3) Clustering of Individuals**

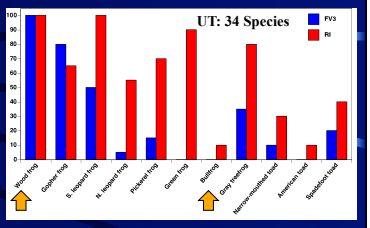
- Sexually transmitted disease

# 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; Hoverman et al. 2011a; Brenes 2013; Waltzek et al. in review)

(1) Multiple-species Pathogen:



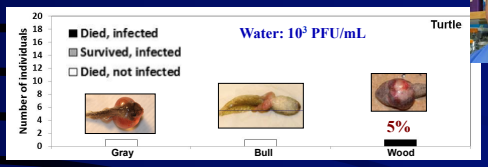


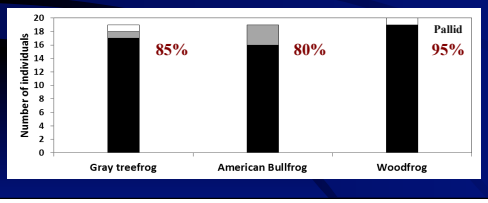

(2) Other Ectothermic Vertebrates

# Evidence of Interclass Transmission

0% mortality in controls

Waltzek, Gray, and Miller




# Evidence of Interclass Transmission

0% mortality in controls

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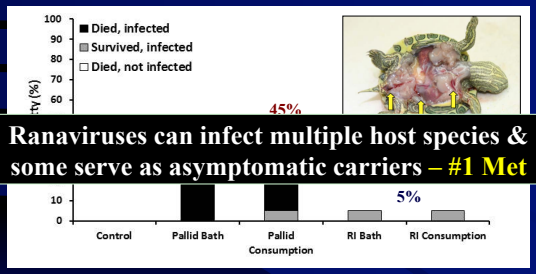





**Ranaviruses can infect multiple host species & some serve as asymptomatic carriers – #1 Met**

**Pallid Isolate Caused Mortality; Bullfrog Isolate Resulted in Infection**

## 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 Frequency Dependent Transmission

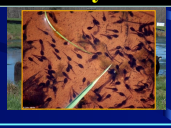
(1) Breeding (Brunner et al. 2004)

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

(2) Larval Clustering

- Increase Contact Rates
- Vegetation Reduction

(Greer et al. 2008)



Local Extirpations and Declines?

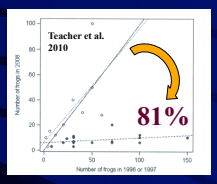
YES, all 3 characteristics met in the Ranavirus-Host System

## Evidence of Local Extinction

Dr. Amber Teacher
Southeastern England

Animal Conservation 13:514-522

1996/97 and 2008





Ranavirus (+) populations 81% Median Reduction


Larger Populations Greatest Proportional Declines

## Evidence of Local Extinction

Dr. Jim Petranka

Tulula Wetland Complex, NC

1998-2006

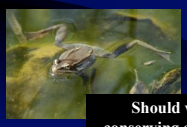
Biological Conservation 138:371-380

Wetlands 23:278-290

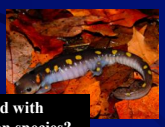
Recruitment at most wetlands failed due to ranavirus

Persistence Possible from Source Populations

Rescue Effect



Uncommon Species?



Should we be concerned with conserving a few uncommon species?

## Commonality of Being Uncommon

Southeastern United States

Federally Listed: *Rana 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)
5) Kentucky = 22 species (11 genera)
6) Louisiana = 15 species (10 genera)

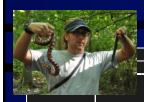
RISK?
Testing Susceptibility for Uncommon Species

## Evidence of Rare Species Effects

Sutton et al. (in review)

Endangered Dusky Gopher Frog

Animal Conservation





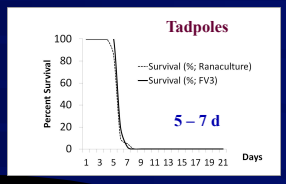


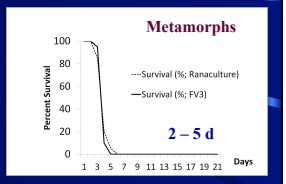

*n* = 18 /trt

## Evidence of Rare Species Effects

**Chaney, Gray, Miller & Kouba**

**Threatened Boreal Toad**



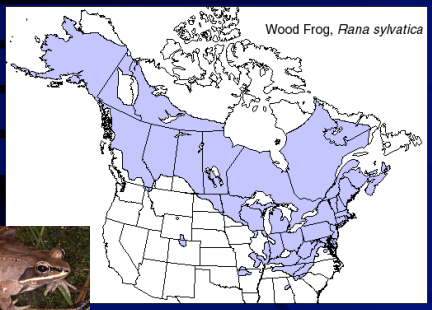




**Genetically isolated populations are more susceptible to FV3-like ranaviruses than mixed populations (Pearman and Garner 2005)**

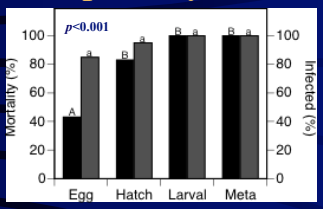
## Any Concern for Common Species?

**Wood Frog Example**

**Most Widely Distributed Species in North America**




## Stage Susceptibility of Wood Frogs




$10^3$ PFU/mL

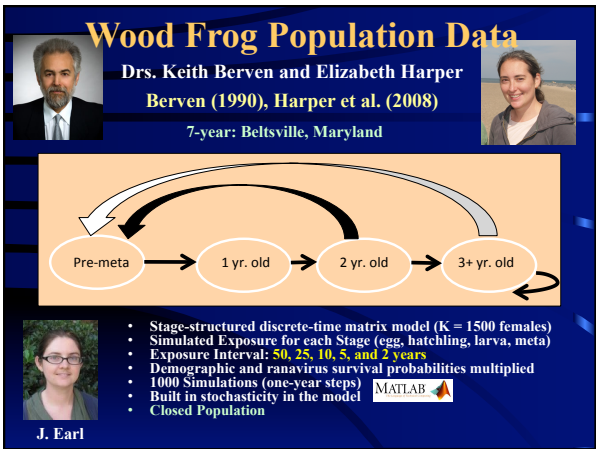
**Haislip et al. (2011)**

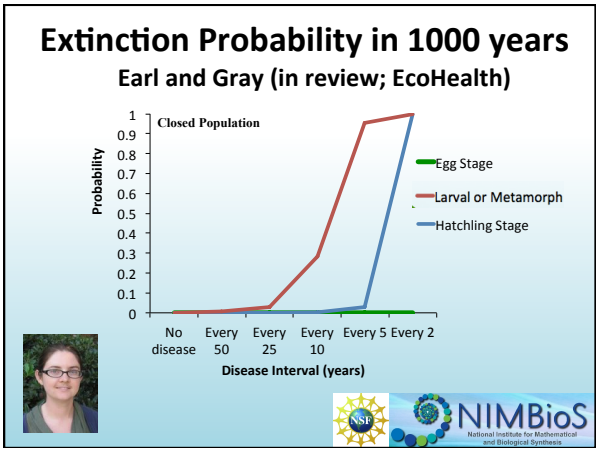
PLoS one

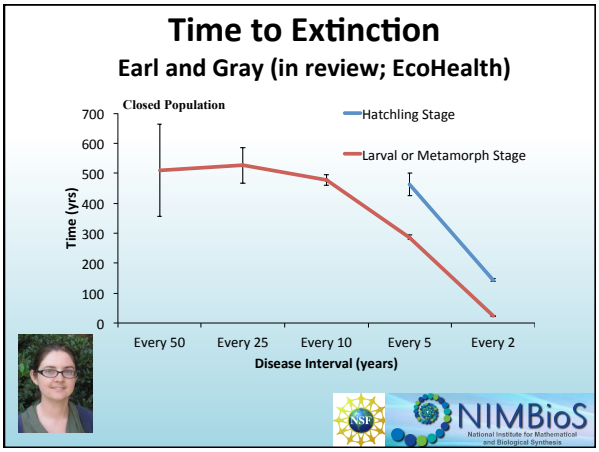
July 2011 | Volume 6 | Issue 7 | e22307

**Egg = 60% Survival**
**Hatchling = 20% Survival**

**Larvae = 0% Survival**
**Metamorph = 0% Survival**

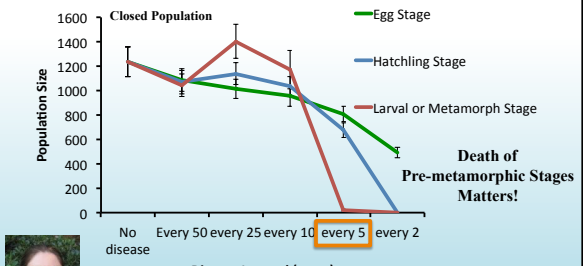

Wood Frog Population Data
Drs. Keith Berven and Elizabeth Harper
Berven (1990), Harper et al. (2008)
7-year: Beltsville, Maryland
Pre-meta
1 yr. old
2 yr. old
3+ yr. old
Stage-structured discrete-time matrix model (K = 1500 females)
Simulated Exposure for each Stage (egg, hatchling, larva, meta)
Exposure Interval: 50, 25, 10, 5, and 2 years
Demographic and ranavirus survival probabilities multiplied
1000 Simulations (one-year steps)
Built in stochasticity in the model
Closed Population
MATLAB
J. Earl


Extinction Probability in 1000 years
Earl and Gray (in review; EcoHealth)
Closed Population
Probability
Egg Stage
Larval or Metamorph
Hatchling Stage
No disease
Every 50
Every 25
Every 10
Every 5
Every 2
Disease Interval (years)
NIMBioS


Time to Extinction
Earl and Gray (in review; EcoHealth)
Closed Population
Hatchling Stage
Larval or Metamorph Stage
Time (yrs)
Every 50
Every 25
Every 10
Every 5
Every 2
Disease Interval (years)
NIMBioS

## Female Population Size

**Earl and Gray (in review; EcoHealth)**

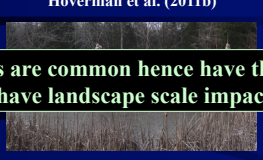


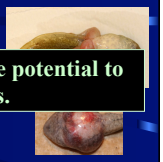

NSF NIMBioS National Institute for Mathematical and Biological Synthesis

## Ranavirus Landscape Prevalence

*n* = 40 ponds
2 years, 4 seasons

**Tennessee Ponds**
Hoverman et al. (2011b)





**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

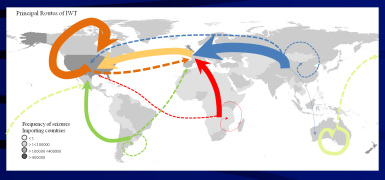
- 85% Infection
- 100% Infection

(morbid and dead)

EcoHealth

EcoHealth 9:36-48

## 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

# Cry Wolf or Valid Risk?

## Should we be Concerned?

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

Epidemiological Theory AND Initial Simulations Support the Premise that Ranaviruses Could Cause Local Population Extirpations and Contribute to Species Declines

More Research: Species Susceptibility, Community Composition, More Complex Simulations (dispersal, transmission), Long-Term Monitoring

# 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

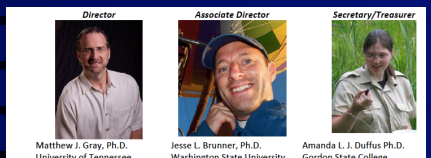
Bylaws Approved

GRC@LISTSERV.UTK.EDU

Membership in 2014

# Global Ranavirus Consortium

## Executive Committee and Board





Questions??
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FUTURE