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Amber Teacher, University of Helsinki
Megan Todd-Thompson, University of Tennessee
Yumi Une, Azabu University
I. Emerging Infectious Diseases

II. Are Ranaviruses a Threat?

III. Ranavirus Pathology and Ecology

IV. Future Research Directions
Amphibian Declines and Emerging Infectious Diseases

Chytrid Fungus

Larvae: 80-100%

Adults: >95% (Europe)

Ranaviruses

Science 306:1783-1786

Nature 404:752-755

EID 5:735-748

Biotropica 37:163-165

34% in Risk of Extinction

43% in Decline
Commonly Asserted

- Ranavirus Die-offs are not Widespread
- Ranavirus only affect Common Species
- No Evidence that Ranaviruses are Capable of Causing Declines

Does the current knowledge of the ranavirus host-pathogen system support these claims?
History of Ranavirus Die-offs

First Isolated:
• Dr. Allan Granoff
• St. Jude Hospital
• *Rana pipsiens* (1962)

First Large-scale Die-offs:
• Dr. Andrew Cunningham
• Institute of Zoology, ZSL
• *Rana temporaria* (1992)

First North American Die-offs:
• Dr. Jim Collins and students
• Arizona State University
• *Ambystoma tigrinum stebbinsi* (1985, 1997)
Global Distribution of Ranavirus Die-offs

All Latitudes, All Elevations

11 Families: Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae, Discoglossidae, Rhacophoridae, Myobatrachidae, Ambystomatidae, Salamandridae, Hynobiidae
Reported Amphibian Die-offs in North America: *Ranavirus*

Families
- Ranidae
- Hylidae
- Bufonidae
- Ambystomatidae
- Salamandridae

Uncommon
- Lithobates sylvaticus

>30 States & 20 Spp; 5 Provinces
Are Ranavirus Die-offs Widespread?

**YES**, Ranavirus Die-offs are Widespread!

ARMI 2006
(110; 34 states)
43% = *Ranavirus*
16% = fungi
10% = protozoan

D. Green, unpubl. data
Common Species Only?
Case Examples
Japan: Azabu University
Dr. Yumi Une

Introduced American Bullfrogs:

Asian Salamanders: Hynobiidae

Experimental Challenges
11 Species (7: 100%)
1. Hynobius nebulosus
2. Hynobius lichenatus
3. Hynobius nigrescens
4. Hynobius tokyoensis
5. Cynops ensicauda
6. Cynops pyrrhogaster
7. Rana (Pelophylax) porosa porosa

Sept 2008 & 2009
Case Examples
North America
Jamie Barichivich (USGS) and Megan Todd-Thompson (UT)

May 2009
Spotted & Marbled Salamander, Wood Frog, Spring Peeper, Southeastern Chorus Frog

GSMNP, Cades Cove
Gourley Pond

A. Cressler, USGS
M. Niemiller, UT
A. Cressler, USGS
D. Green, USGS
Southern Appalachia: What about Plethodontids?

2007-2009

12 Species & 4 Genera:

- Desmognathus
- Eurycea
- Plethodon
- Gyrinophilus

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

>97% MCP similarity with the ranavirus FV3

**Prevalence Chart**

- **DECO** n=33: 61%
- **DEMO** n=15: 53%
- **DEQU** n=29: 45%
- **DEIM** n=34: 38%
- **EUWI** n=42: 33%
- **DEOC** n=10: 30%
- **DESA** n=46: 26%
- **PLIO** n=29: 3%

**P < 0.001**

Spp: n ≥ 10
Another Family of Concern

2009

Hiwassee River

Little River

17 of 40 individuals  43%

First Report in Cryptobranchidae
Do Ranaviruses Only Affect Common Species

NO, Ranaviruses Cause Die-offs in Uncommon Species Also!

Cryptobranchidae?  Plethodontidae?

Who Cares?
### Species of Concern: Uncommon

#### Tennessee

<table>
<thead>
<tr>
<th>Species</th>
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</thead>
<tbody>
<tr>
<td>Desmognathus aeneus</td>
</tr>
<tr>
<td>Desmognathus welteri</td>
</tr>
<tr>
<td>Desmognathus wrighti</td>
</tr>
<tr>
<td>Eurycea junaluska</td>
</tr>
<tr>
<td>Gyrinophilus palleucus</td>
</tr>
<tr>
<td>Gyrinophilus gulolineatus</td>
</tr>
<tr>
<td>Pseudotriton montanus</td>
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<tr>
<td>Plethodon dorsalis</td>
</tr>
<tr>
<td>Plethodon richmondi</td>
</tr>
<tr>
<td>Plethodon wehrlei</td>
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<tr>
<td>Plethodon welleri</td>
</tr>
<tr>
<td>Plethodon yonahlossee</td>
</tr>
<tr>
<td>Plethodon aureolus</td>
</tr>
<tr>
<td>Plethodon jordani</td>
</tr>
<tr>
<td>Cryptobranchus alleganiensis</td>
</tr>
<tr>
<td>Siren intermedia</td>
</tr>
<tr>
<td>Aneides aeneus</td>
</tr>
<tr>
<td>Ambystoma barbouri</td>
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<tr>
<td>Ambystoma talpoideum</td>
</tr>
<tr>
<td>Hemidactylium scutatum</td>
</tr>
<tr>
<td>Hyla gratiosa</td>
</tr>
<tr>
<td>Hyla versicolor</td>
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<tr>
<td>Acris gryllus</td>
</tr>
<tr>
<td>Pseudacris brachyphona</td>
</tr>
<tr>
<td>Rana areolata</td>
</tr>
<tr>
<td>Rana capito</td>
</tr>
<tr>
<td>Scaphiopus holbrookii</td>
</tr>
</tbody>
</table>
**Species of Concern: Uncommon**

Southeastern United States

**Federally Listed:**
- *Rana capito sevosa*, *Ambystoma cingulatum*,
- *Phaeognathus hubrichti*, *Ambystoma bishopi*

**Species of Concern:**

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Alabama</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>2) Arkansas</td>
<td>25</td>
<td>12</td>
</tr>
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<td>3) Florida</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>4) Georgia</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>5) Kentucky</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>6) Louisiana</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>7) Mississippi</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>8) North Carolina</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>9) South Carolina</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>10) Tennessee</td>
<td>26</td>
<td>14</td>
</tr>
</tbody>
</table>

113 Species and 25 Genera Total 50% U.S.
Capable of Causing Local Exterminations?
Evidence of Local Extinction

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

Recruitment at most wetlands failed due to ranavirus

Persistence Possible from Source Populations

Rescue Effect
Is there evidence that ranaviruses can cause amphibian population declines?

**YES**, Recurring die-offs → Local Population Extinction

Greatest Threat:
- Rare species
- Isolated Populations (no rescue effect)

Common Species:
- Keep common species common!
Pathology and Ecology of Amphibian Ranaviruses

A. Cressler, USGS
**Ranavirus Characteristics**

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

**Family: Iridoviridae**

**Genera:** *Iridovirus, Chloriridovirus, Ranavirus, Megalocytiviruses, and Lymphocystiviruses*

- Invertebrates
- Ectothermic Vertebrates

**Species (6):**
- *Ambystoma tigrinum* virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)

**Virion**

*Amphibian Declines*

Chinchar et al. (2006)

Docherty et al. (2003)

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

Jancovich et al. (1997)
Ranavirus Replication Cycle

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

Protein synthesis within hours of infection

Cell death occurs within 6 – 9 hrs PI

12 – 32 C
Ranavirus: Gross Signs

Edema, Erythema, Hemorrhages, Ulcerations

N. Haislip, UT

A. Duffus, ZSL

D. Green, USGS
Ranavirus: Internal Signs

Kidney Hemorrhages  Pale and Swollen Liver

Disease Can Progress Fast: 1-3 d signs, 3-7 d mortality; 2 wks die-off
Imagine if Ranaviruses could Infect Humans

Monday

Fever

Wednesday

Hands, Feet, Legs Swollen

Friday

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

Sunday

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

Humans
Ebola, Anthrax, Elephantiasis

There is no Cure!
Ranavirus: Histopathological Signs

3 Primary Organs: Kidney, Liver and Spleen


Pathogenesis
- Target Organ Failure
- Heart Failure
- Toxicosis, Anemia

D. Miller

Kidney Degeneration

Spleen Necrosis

Viral Inclusions
Routes of Transmission

**Horizontal vs. Vertical:**

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

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**Ingestion**

- Oral inoculation
- Water Bath Contaminated Sediment
  
  - Time to signs: 1 – 2 weeks
  - Time to mortality: 1 – 3 weeks

**Necrophagy**

- 3 – 7 days

**Cannibalism**

- Brunner et al. (2004), Pearman et al. (2004), Harp & Petranka (2006), Hoverman et al. (2010)

**Environmental Persistence**

- (2-4 weeks)
Ectothermic Reservoirs
Reptiles and Fish

Are Amphibian Ranaviruses Lethal to other Ectothermic Vertebrates?

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

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

Blind Pony Hatchery
Ranavirus Ecology

N. Haislip, University of Tennessee
Variation in Susceptibility to *Ranavirus* Among Species and Tadpole Developmental Stages

Nathan A. Haislip
M.S. Candidate
Stage Comparison
Across All Species

$\chi^2_3 = 40.1 ; p<0.001$

Egg membrane may act as a protective barrier

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

**χ²₃ = 143.4 ; P<0.001**

- **Species Comparison**
  - *H. chrysoscelis* - 10X > *P. feriarum*
  - *L. sylvaticus* - 7X > *L. pipiens*
  - *S. holbrookii* - 65X > *P. feriarum*
  - *L. Pipiens* - 13X > *P. feriarum*

**ML Estimate:**
- *A. americanus* - 4X > *P. feriarum*
- *H. chrysoscelis* - 10X > *P. feriarum*
- *L. pipiens* - 13X > *P. feriarum*
- *L. clamitans* - 1.3X > *L. pipiens*, 16X > *P. feriarum*

**Logistic Analyses**
- **Species*Stage Interactions**
- **Rapid Developers**
**Ranids**

**Lithobates clamitans**

**Lithobates pipiens**

**Across Ranids, Metamorph Stage - Most Susceptible**

**Lithobates sylvaticus**

Immune Function Drops
Hylids

Pseudacris feriarum

For HYCH, Hatchling and Larval Stages - Most Susceptible
Toads

Anaxyrus americanus

Similar to Ranids

Considerable Variation in Susceptibility among Developmental Stages

- Immune Function
- Co-Evolutionary or Ecological Factors
Ranavirus Ecology

Red = Well Studied

Blue = More Studies Needed

No Color = Very Little Information
Pathogenicity of a Ranaculture
*Ranavirus* Isolate

Jason T. Hoverman
Post-doctoral Research Associate
Pearson’s chi-square test

RI = ranaculture isolate
Southern Leopard Frog
Pickerel Frog
Green Frog

Percent survival

- Control
- FV3
- RI

Day

0  3  6  9  12  15  18  21
Cope’s Gray Treefrog
American Toad

![Graph showing percent survival over days for American Toads in different conditions: Control, FV3, RI. The graph indicates a decrease in survival rate over time for the RI condition.]
Eastern Narrow-mouthed Toad
Eastern Spadefoot

Captive Facilities
Sources of Highly Virulent Ranaviruses
Factors Contributing to Emergence

**Pathogen Pollution:** (Cunningham et al. 2003)

Anthropogenic introduction of novel strains to naïve populations

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

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

1) Herbicide (Atrazine)  
2) Cattle Land Use: FV3 Prevalence  
   
A. tigrinum  
Fertilizer (sodium nitrate)  

Leukocytes ↓  
ATV Susceptibility ↑  
Inconclusive  

Green Frogs: 4X in access  

**Other Possible Stressors:** Pesticide Mixtures, Nitrogenous Waste, Endocrine Disruptors, Acidification, Global Warming, Heavy Metals
Why Should We Care?

Aren’t Ranaviruses A Natural Host-Pathogen System?

YES, but to the best of our knowledge the frequency of die-offs is increasing

Are Humans a Cause?
World Organization for Animal Health

OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008

International Transport of Animals
Notifiable Diseases
Certification for Shipment
Schloegel et al. (2010)

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

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

$50/bottle
Important Research Directions
Pressing Research Directions

Southeast Species

1) Surveillance & Monitoring

2) Experimental Challenges

Tennessee

*Hyla gratiosa*  *Acris gryllus*

*Hyla versicolor*  *Rana areolata*

*Siren intermedia*  

*Ambystoma barbouri*

*Ambystoma talpoideum*
Pressing Research Directions
Mechanisms Driving Outbreaks?

1) Cattle Use: Nitrogenous Waste

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

3) Strain Virulence

Do Ranaviruses from One Region Represent Novel Pathogens in Another Region?
Pressing Research Directions
A Mechanism Driving Future Outbreaks
Temperature-induced Stress

Ectothermic Vertebrates

Ranavirus Replication Increases with Temperature
Pressing Research Directions
Reservoirs and Persistence

1) Fish and Reptiles

2) Persistence
Pathogen Ecology
Spatially Structured Breeding Sites

\[
\begin{align*}
I(t)_{ijkl} & \quad S(t|I)_{ijkl} \\
I(t|k)_{ijkl} & \quad S(t|I)_{ijkl}
\end{align*}
\]

\[
EM(t)_{ijkl}
\]

\[
P(N_t)_{il} > 0
\]

\[i = \text{species} \quad j = \text{age class} \quad k = \text{pathogen} \quad l = \text{wetland}\]
Collaborators

University of Georgia

Dr. Debra Miller
Dr. Sandy Baldwin
Dr. Jason Hoverman

University of Tennessee

Nathan Haislip
Kevin Hamed

Funding:

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

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