Role of Emerging Infectious Diseases in Amphibian Population Declines: Chytrid Fungi and Ranavirus

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WFS 433/533: 11 April 2017
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Outline

I. Amphibian Declines and EIDs

II. Chytridiomycosis (Bd and Bsal)

III. Ranaviral Disease

Sixth Mass Extinction

Extinction rate is 100x higher than expected background rates

Kolbert (2014)
Worldwide Amphibian Population Declines

>100 Species Extinct since 1980

Amphibian Declines and Emerging Infectious Diseases

Science
306:1783-1786
EID 5:735-748

Nature
404:752-755
Biotropica
37:163-165

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

Ranaviruses

Frog Chytrid Fungus

Batrachochytrium dendrobatidis

>200 spp in decline
• Some Species: Highly Pathogenic
• Mostly Tropical at High Elevations
• Western United States

Mountain yellow-legged frog
(Rana muscosa)

Batrachochytrium dendrobatidis

Western United States

Boreal toad
Wyoming toad
CA red-legged frog
Chiricahua leopard frog?
The pathogen

- *Batrachochytrium dendrobatidis* (Bd):
  - Non-hyphal parasitic fungus (1 of 2 chytrid spp pathogenic to vertebrates)
- Infect keratinized tissue
  - Larvae: Mouthparts
  - Adults: Pelvic Region
- Life stages
  - Zoospore – aquatic, flagellated
  - Zoosporangium – zoospores discharged

Phylum: Chytridiomycota
Class: Chytridiomycetes
Order: Chytridiales

Histological Signs

Epidermis

- Zoosporangia
- Stratum Corneum
  - Normal Thickness: 2 – 5 µm
  - Infected: 60 µm

- Discharge Tube
- Proliferation of Epidermal Cells
  - Epidermal Hyperplasia
  - Sloughing

Cause of Mortality

- Osmoregulatory Inhibition (#1 cause; Voyles et al. 2009)
  - Decreased water uptake & ion exchange; altered electrolyte/solute levels (decrease Ca, actin & myosin cross-bridge cycle)
Batrachochytrium salamandrivorans: Determining the Risk to North America

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What do we know?

Salamandra salamandra

* 2010: 96% wild mortality in Netherlands
* 2013 & 2014: wild mortality in Belgium
* 2015: UK (trade) and Germany (captivity)
* 2016: Netherlands, Belgium, Germany (wild)
* Present in: (Vietnam, Thailand, Japan)
* Museum records in Asia >150 yrs

Unknown to occur in North America

Martel et al. 2013, PNAS;
Martel et al. 2014, Science;
Cunningham et al. 2015, Veterinary Record;
Sabino-Pinto et al. 2015, Amphibia-Reptilia

A lesion viewed under the microscope...

Dead cells (orange arrows)
Bthal thalli (black arrows)

Multifocal erosions and deep ulcerations of the skin throughout the body
Death generally occurs in under 2 weeks

Van Rooij et al. (2015)
*Why is Bsal a threat?*

- Infected no death
- Infected some death
- Infected 100%

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*Why is Bsal a threat?*

- 50% of salamander species occur in North America

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*Why is Bsal a threat?*

- Many SE States!
Risk Model: Yap et al. (2015)

Final Risk Assessment Model
- Relative Risk = SpRich * Log ClimSuit Bsal

Study Animals
Salamanders (12; 6) Frogs (4; 2)

Survival and Time to Death: EUWI

Of those that died, Median time to death = 16.7 days
Final Pathogen Prevalence: EUWI

Of those infected at the endpoint of the experiment, 50% died, 50% survived (dose-dep response)

Eurycea Diversity

Ecology and Pathology of Ranaviruses

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University of Tennessee
¹Center for Wildlife Health
²CVM Department of BDS
Ranavirus Characteristics

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

Family: Iridoviridae

Generae: Iridoviruses, Chloriridovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus

Species (6)
- Ambystoma tigrinum virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)
- Epizootic haematopoietic necrosis virus
- European catfish virus
- Saithe-Cooper Ranavirus

ICTV (2012)

Global Distribution of Ranavirus Cases: Amphibians

- All Latitudes, All Elevations
- 18 Families
- >100 Species
- 6 Continents: 1965, 1992/97

Case Example

Re-occuring Die-offs
Jamie Burichivich (USGS) and Megan Todd-Thompson (UT)

- Spotted & Marbled Salamander, Wood Frog
- Spring Peeper, Upland Chorus Frog

Green et al. (2002)
**Global Distribution of Ranavirus Cases: Reptiles**

- **4 Continents**: 1982, 1990s
- **Most FV3-like Ranaviruses: Captivity**
- **12 Families**: Agamidae, Anguidae, Boidae, Dactyloidae, Emyididae, Gekkonidae, Iguanidae, Lacertidae, Pythonidae, Testudinidae, Trionychidae, Varanidae
- **>30 Species**

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**Ranaviral Disease in Eastern Box Turtles**

- **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**

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**Ranaviral Disease in Eastern Box Turtles**

- **Gazette-Mail**
- **Steward Co., TN, Sept. 2015 John Hewlett**
- **Clendenin, WV July 2015**
Global Distribution of Ranavirus Cases: Fishes

- 22 Families:
  - Acipenseridae, Anguillidae, Centrarchidae, Channidae, Cyprinidae, Eleotridae, Esocidae, Gobiidae, Gobiostomatidae, Ictaluridae, Labridae, Latidae, Lepididae, Lutjanidae, Moronidae, Percidae, Poeciliidae, Salmonidae, Sciaenidae, Scophthalmidae, Serranidae, Siluridae
- >50 Species
- 4 Continents: 1991

Most non-FV3-like Ranaviruses

Duffus et al. (2015)

Ebola of Ectothermic Vertebrates

Hemorrhages

Friable Spleen:

“Apparent tropism for vascular endothelium”
R. Whittington

Organ Destruction

3 Primary Organs: Liver, Spleen, and Kidney

- Liver Necrosis
- Spleen Necrosis
- Kidney Degeneration

Mortality Can Be Rapid!
- Quickly as 3 days!

Hoverman et al. (2011a)

Miller et al. (2008, 2007)

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

Bollinger et al. (1999)
Potential Major Factor Contributing to Ranavirus Emergence

From 2000-2006, 25 million live amphibians per year imported to USA (Smith et al. 2009)

*Hong Kong = 56% infected

First Evidence of Amphibian Chytrid Fungus (Batrachochytrium dendrobatidis) and Ranavirus in Hong Kong Amphibian Trade

Captive Conditions

The perfect cauldron for virulence evolution!

- Abundant Hosts
- Multiple Strains
- Immunocompromised

Bullfrog Die-off: Alapaha, GA

Alapaha River

1998

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Bullfrog Die-off: Alapaha, GA

Possible Mechanism: Recombination
Claytor, Waltzek, et al. (in review): Chimeric FV3

Eight Significant Recombination Events involving 16 genes: ORF2L (envelope protein)
FV3-like ranavirus recombined with CMTV/CGSV-like ranavirus

Captive vs. Wild Isolates

Phylogeny, Life History, and Ecology Contribute to Differences in Amphibian Susceptibility to Ranaviruses
Hoverman et al. (2011a): 19 Species Tested
Opportunity for Spillover

Wastewater Rarely Decontaminated; Poor Animal Contaminant

Guaranteed Transmission

Site of Highly Virulent Ranavirus

Are Ranaviruses Capable of Causing Local Extirpations and Species Declines?

Collins & Crump (2009)

Muths et al. (2006)
Ranavirus die-offs with six species

Time to Extinction
Earl and Gray (2014)

Closed Population
- Hatching Stage
- Larval or Metamorph Stage

Exposure Interval (years)
- Every 50
- Every 25
- Every 10
- Every 5
- Every 2

300 years
25 years

Global Ranavirus Consortium, Inc.
http://www.ranavirus.org/

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