Biotic factors contributing to the emergence of ranavirus in North American amphibian communities

Roberto Brenes
University of Tennessee
Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries

Wednesday, 2 November 2011, 12:20 pm, 160 PBB

Amphibians in Distress

- **IUCN Red List** (IUCN 2011)
  - 12% birds
  - 23% mammals
  - 32% amphibians
- **Amphibians**
  - 43% in decline
  - 1 in 3 species in threat of extinction
    (Stuart et al. 2004)

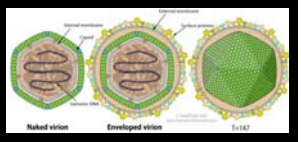
Southern Yellow-legged Frogs
Sierra Nevada, CA

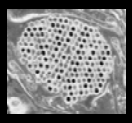
What is killing the frogs?

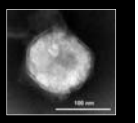
- **Emerging infectious diseases** (Daszak et al. 2003, Hayes et al. 2010)
- ***Batrachochytrium dendrobatidis*** **(Bd): Tropics**
  (Lips et al. 2005, Lips et al. 2006, Kilpatrick et al. 2010)
- **Ranaviruses: North America and Europe**
  (Duffus et al. 2008, Gray et al. 2009, Teacher et al. 2010)

Iridoviridae

- Double stranded DNA
- Invertebrates
  - *Chloridirivirus* and *Iridovirus*
- Lower Vertebrates
  - *Lymphocystivirus*
  - *Megalocytivirus*
  - *Ranavirus*







(Mao et al. 1997, Mao et al. 1999, Bandin and Dopazo 2011)

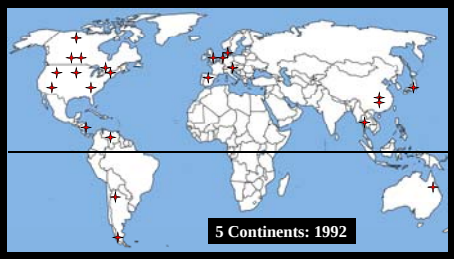
Ranavirus Hosts

Poikilothermic Hosts

- **Fish** (Chinchar et al. 2001, Gobbo et al. 2010, Jensen et al. 2011)
- **Turtles** (Johnson et al. 2008, Allender et al. 2011)
- **Snakes** (Hyatt et al. 2002)
- **Salamanders** (Jancovich et al. 2003, Geng et al. 2010)
- **Frogs** (Green et al. 2002, Miller et al. 2007).

Most Declines

Global Distribution of Ranavirus



All Latitudes, All Elevations

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

(Miller et al. 2011)

Cases of Ranavirus Infection and Disease in North America

Mortality

Ranidae
Hylidae
Bufonidae
Ambystomatidae
Salamandridae

Infection

Cryptobranchidae
Plethodontidae
Scaphiopodidae

Are all species equally susceptible?

Hoverman et al. (in press)

- Susceptibility among 19 amphibian species.
- Mortality: 0 to 95%.

Wood Frog (95%): High susceptibility

Gray Treefrog (70%): Moderate susceptibility

Eastern Newt (10%): Low susceptibility

Eastern Narrowmouth Toad: NO Mortality

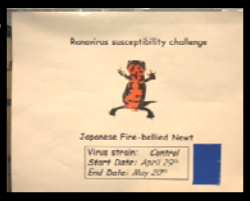
Hoverman et al. (in press)

Phylogenetic Ancestral Character Reconstruction

- Ambystomatidae, Hylidae, and Ranidae
  - Low, intermediate and high susceptibility, respectively
- Host Traits
  - Semi-permanent habitats
  - Narrow range distributions
  - Rapid development

Chapter I: Objectives

1. Test differences in ranavirus susceptibility among 40 amphibian species
   - ➢19 to 40 species
2. Relate susceptibility to evolutionary and life history characteristics
   - ➢Do previous correlations hold?

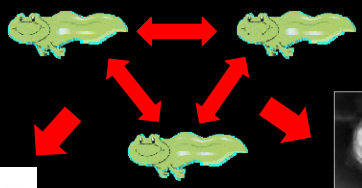


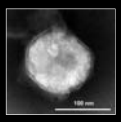
Identify High Risk Species
Guide Conservation Strategies

Community Interactions

Larval Amphibians

Different Species Interact





Pathogen Transmission

Competition for Resources

- ➢Shedding
- ➢Contact
- ➢Necrophagy & Cannibalism

Community Level Transmission

Does it Matter Who is Infected?

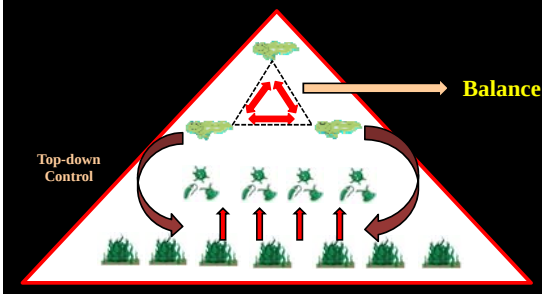
Species with Different Pathogenicities

High Transmission and Mortality?

Low Tra and Mortality?

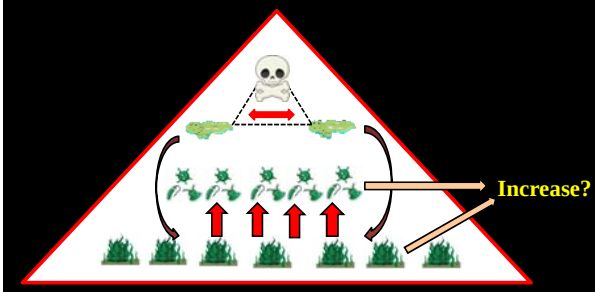
Can Certain Species Drive Outbreaks?

Ecosystem Interactions

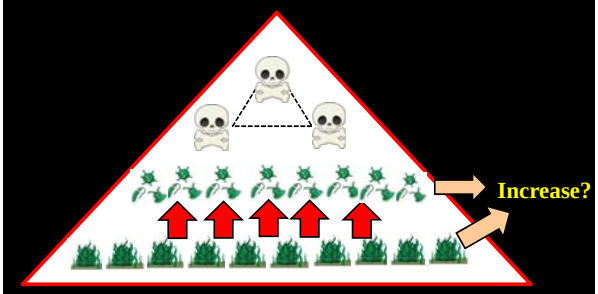


Stabilizing Effect among Trophic Levels

Can Disease Drive Ecosystem Processes?



Disease Induced Trophic Cascades



Disease and Trophic Cascades

Batrachochytrium dendrobatidis

Central Panama Streams: Massive *Bd* Epizootic

- 269% increase in chlorophyll levels
- 220% increase in AFDM
- 140% increase inorganic sediments (Connelly et al. 2008a)
- Algal community shift from small diatoms to large upright algal species (Connelly et al. 2008b)
- Macroinvertebrates shift from detritivores to grazers (Whiles et al. 2006a, Colon-Gaud et al. 2009, 2010a, and 2010b)

Only Known Case of Disease-induced Trophic Cascades in Amphibians

Can Ranavirus Outbreaks Affect Ecosystem Processes?

Chapter 2: Objectives

1. Effects of Species Susceptibility on Ranavirus Transmission and Likelihood of a Community-level Outbreak
2. Effects of Ranavirus Associated Mortality on Trophic Level Interactions

Chapter 1 Methods

Species Susceptibility using Controlled Experimental Challenges

Increase Number of Tested Species
19 to 40 Species
3 to 8 Families

Target Species:
≥4 species per family

Family	Hoverman et al. (in press)	2011	2012 (projection)
Ranidae	Wood frog	Crawfish frog	Plains Leopard frog
	Gopher frog	Foothill Yellow-legged Frog	Cuban treefrog
	Southern leopard frog		Rana sp.
	Northern leopard frog		
	Pickerel frog		
	Green frog		
	American bullfrog		
Hylidae	Cope's gray tree frog	Pacific treefrog	
	Southeastern chorus frog		
	Western chorus frog		
	Mountain chorus frog		
			Cricket Frog
			Barking treefrog
			Bird voice treefrog
Bufonidae	American toad	Southern Toad	Fowler's Toad
		Western Toad	Bufo sp.
Microhylidae	Eastern narrow-mouthed toad		
Scaphiopodidae	Eastern spadefoot		Plains spadefoot
			New Mexico spadefoot
			Couch's Spadefoot
Ambystomatidae	Tiger salamander	Streamside Salamander	
	Marbled salamander		
	Spotted salamander		
	Mole salamander		
Salamandridae	Red-spotted newt	Striped Newt	California Newt
		Japanese Fire Newt	
Plethodontidae		Four toed Salamander	
Total	19	9	12

Fill in Family Gaps

Egg Mass Collection

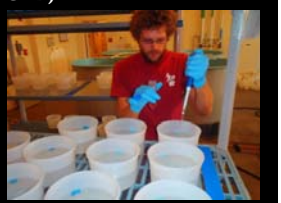
- Wild populations
- Vertical Transmission Unknown to Occur
- Joe Johnson Animal Research and Teaching Unit (JARTU)
- Cultured in mesocosms until ready to test (Gosner 30)

Scientific Collection Permit #1990

All Methods Follow:
Hoverman et al. (in press)

Experimental Challenges

- JARTU C111 Lab
- 2-L tubs (1 L of water)
- Four treatments (*n* = 20/trt, CRD)
  - FV3
  - Great Smoky Mountains
  - Ranaculture Isolate
  - Control
- 10^3 PFU/mL
- Exposed for 3 days
- Duration = 21 days



All Methods Follow:
Hoverman et al. (in press)

Animal Care
IACUC Protocol #2009-0511

- Check 2X per day
- Fed every 3 days
  - **Tadpoles:** TetraMin® (12% of body mass)
  - **Salamanders:** 3 mL of concentrated zooplankton
- 100% water change: 3 days
- Morbid or dead individuals removed
- Survivors humanely euthanized at 21 days

All Methods Follow:
Hoverman et al. (in press)

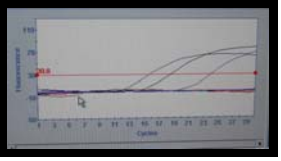
Ranavirus Testing

Necropsy:

- Section of Liver and Kidney Extracted
- Frozen at -80C
- Animal stored in 10% buffered formalin

Extraction and PCR:

- gDNA Extraction
  - QIAamp DNA Mini Kit
- DNA Quantification
  - Qubit™ fluorometer
- Viral Infection & Load Quantification
  - TaqMan qPCR assay

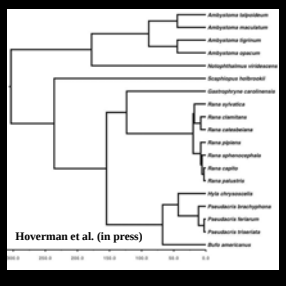


Analysis

- **Phylogenetic Comparative Methods** (Ord and Martins 2006, Stephens and Wiens 2008)

•Ancestral Character Reconstruction

- Breeding Habitat
- Breeding time
- Time of metamorphosis
- Size at metamorphosis
- Clutch size
- Hatching time
- Adult body size
- Time of Maturity
- Range size



Hoverman et al. (in press)

Life History Characteristics

Scientific name	Breeding habitat	Breeding time	Time to metamorphosis (days)	Size at metamorphosis (mm)	Clutch size	Hatching time (d)	Adult body size (mm)	Time to maturity (yr)	Range size (sq km)
Rana sylvatica	1.5	2	65.0	17.0	3000	12.0	52.5	2.0	8,884,571
Rana cuplla	2.5	2	161.5	32.5	1450	8.5	77.0	1.8	148,967
Rana sphenocephala	3	3	82.5	26.5	1550	14.5	70.5	2.0	1,649,839
Rana pipiens	3.5	2	100.0	34.0	3800	14.5	70.5	2.0	6,852,031
Rana palustris	3	2	75.0	23.0	3000	14.5	59.5	2.0	3,348,573
Rana clamitans	3	4	242.5	29.5	3000	8.0	73.5	1.0	3,541,109
Rana catesbeiana	4	3	547.5	60.0	11000	8.0	121.0	2.0	4,432,906
Hyla chrysoscelis	3	4	55.0	16.5	90	5.0	41.5	2.0	2,484,281
Pseudacris triseriata	2.5	2	55.0	10.0	1000	8.5	29.0	1.0	698,650
Pseudacris feriarum	2.5	2	55.0	10.0	1000	8.5	27.0	1.0	868,550
Pseudacris brachyphona	1.5	2	47.0	12.0	158	8.5	28.5	1.0	255,456
Bufo americanus	2	3	57.5	9.5	11000	7.5	70.5	3.0	4,928,210
Scaphiopus holbrooki	1	4	37.0	10.3	4200	3.0	50.5	1.5	1,001,976
Gastrophryne carolinensis	1.5	4	45.0	10.3	775	1.3	27.0	1.5	1,516,976
Ambystoma tigrinum	2	3	100.0	62.0	3700	34.5	195.0	2.0	1,857,255
Ambystoma opacum	2	1	150.0	57.5	119	100.0	98.5	2.0	1,697,972
Ambystoma maculatum	2.5	2	203.5	51.0	625	34.0	154.5	2.5	3,557,209
Ambystoma talpoideum	2	1	105.0	49.0	445	33.0	87.5	2.0	546,868
Notophthalmus viridescens	3.5	4	90.0	20.0	350	27.5	89.5	1.4	2,373,532

For New Species: Conant and Collins 1998, Petranka 1998, Lannoo 2005, Elliot et al. 2009 (amphibian life history references)

Phylogenetic Analysis

Ranavirus Susceptibility Response Variable = Infection Prevalence per Isolate

- Phylogenetic generalized least squares (Martins and Hansen 1997)
- Branch Lengths Estimated using TimeTree
- Phylogenetic Signal Tested using "Picante" in R Software
- Correlation with Life History Traits Tested using Program COMPARE (categorical) and "Caper" (continuous) in R Software

Hoverman et al. (in press)

Logistic Regression

Mortality and Infection Prevalence Among Species and Isolates

SAS: $\alpha = 0.05$

Chapter 2 Methods

Pathogen Transmission and Ecosystem Function Experiments

Aquatic Mesocosms

Experimental Amphibian Community

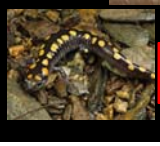
- Typical Spring Breeding Community in East TN
- Vary in Susceptibility

Does the presence of wood frogs (superspreader species) increase mortality of other species?

Can low susceptible species initiate an outbreak?

High: Wood frog

Medium: Chorus Frog



Low: Spotted salamander

Aquatic Mesocosms

- JARTU Mesocosm Site
- 324-L wading pools with 70% shade cloth lids
- *n* = 25 pools, 5 pools per treatment
- Each pool = 10 individuals/species
- Duration = 8 weeks

Treatments

- Completely Randomized Design
  - Highly Susceptible Species Exposed Only; Others Not
  - Mod. Susceptibility Exposed
  - Low Susceptibility Exposed
  - All Species Exposed
  - Control

- Controlled Exposure
  - 2-L containers
  - 10^3 PFU/mL
  - Isolate = FV3
  - Control = MEM Eagle media
  - Exposure = 3 days then distributed to mesocosms

(Same Procedures as Chapter I)

Mesocosms: Aquatic Ecosystems

- To prepare mesocosms:
  - Week 1: Age water
  - Week 2: Green water
    97 g leaf litter
    8 g rabbit chow
  - Week 3: Glaze tiles
  - Week 4: Invertebrates
  - Week 6: Tadpoles

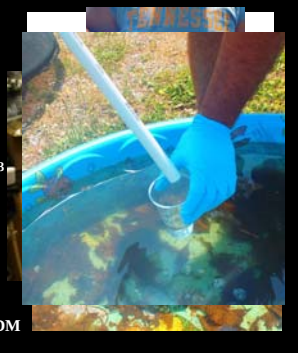
Adapted from Relyea and Diecks (2008)

Mortality and Ecosystem Responses

- Amphibians:
  - Daily for deaths and metamorphs

Once a week

- Water quality: O_2, NH_4, NO_3
- Zooplankton
- Phytoplankton
- Periphyton
- Laboratory Processing
  - Abundance (zooplankton, diatoms), Chlorophyll A, AFDM



Analyses

- Percent Mortality and Metamorphosed per Species (end of experiment)
  - Kruskal-Wallis Test
  - Odds Ratio Statistics (likelihood of survival: control)
- Ecosystem Variables and Water Quality
  - Multivariate Ordinations (trophic interactions)
  - Repeated Measures ANOVA (weekly trends)

SAS: $\alpha = 0.05$

Acknowledgments

- UT Institute of Agriculture
  - Access and Diversity Fellowship (UTIA AgResearch)
  - Hazelwood Scholarship (CASNR)
  - UT-ESPN Scholarship (CASNR)
- Advisors
  - Dr. Matt Gray and Dr. Debra Miller
- Drs. Melissa Kennedy and Becky Wilkes (CVM)
- Dr. Jennifer Franklin
- Dr. Eric Drumm and Melanie Steward (BESS)
- Gerry Middleton
- Becky Hardman and Lauren Henderson
- Chris Lituma
- Technicians (Sean Roon and Seth Brown)
- Numerous Volunteers

References

- Benke, A. C. 1996. Secondary production of macroinvertebrates.In F. R. Haver and G. A. Lamberti, editors. Methods in stream ecology. Academic Press, Inc., San Diego, CA.
- Collins, J. P. and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions **2003**:89-98.
- Colon-Gaud, C., M. R. Whiles, R. Brenes, S. S. Kilham, K. R. Lips, C. M. Pringle, S. Connelly, and S. D. Peterson. 2010a. Potential functional redundancy and resource facilitatio between tadpoles and insect grazers in tropical headwater streams. Freshwater Biology **55**:2077-2088.
- Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, and A. D. Huryn. 2008. Changes in Stream Primary Producer Communities Resulting from Large-Scale Catastrophic Amphibian Declines: Can Small-Scale Experiments Predict Effects of Tadpole Loss? Ecosystems **11**:1262-1276.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Wildlife ecology - Emerging infectious diseases of wildlife - Threats to biodiversity and human health. Science **287**:443-449.
- De Voe, R., K. Geissler, S. Elmore, D. Rotstein, G. Lewbart, and J. Guy. 2004. Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (Terrapen carolina carolina). Journal of Zoo and Wildlife Medicine **35**:534-543.
- Duffus, A. L. J., B. D. Pauli, K. Wozney, C. R. Brunetti, and M. Berrill. 2008. Frog virus 3-like infections in aquatic amphibian communities. Journal of Wildlife Diseases **44**:109-120.
- Gobbo, F., E. Cappellozza, M. R. Pastore, and G. Bovo. 2010. Susceptibility of black bullhead Ameiurus melas to a panel of ranavirus isolates. Diseases of Aquatic Organisms **90**:167-174.
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**:183-190.
- Gray, M. J., D. L. Miller, and J. T. Hoverman. 2009. Ecology and pathology of amphibian ranaviruses. Diseases of Aquatic Organisms **87**:243-266.
- Greer, A. L., M. Berrill, and P. J. Wilson. 2005. Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. Diseases of Aquatic Organisms **67**:9-14.
- Hoverman, J. T., M. J. Gray, N. A. Haislip, and D. L. Miller. In Review. Phylogeny and ecology impact amphibian susceptibility to ranaviruses. Ecohealth.
- Hyatt, A. D., M. Williamson, B. E. H. Coupar, D. Middleton, S. G. Hengstberger, A. R. Gould, P. Selleck, T. G. Wise, J. Kattenbelt, A. A. Cunningham, and J. Lee. 2002. First identification of a ranavirus from green pythons (Chondropython viridis). Journal of Wildlife Diseases **38**:239-252.
- IUCN. 2011. IUCN Red List of Threatened Species.
- Jancovich, J. K., M. Bremont, J. W. Touchman, and B. L. Jacobs. 2010. Evidence for Multiple Recent Host Species Shifts among the Ranaviruses (Family Iridoviridae). Journal o Virology **84**:2636-2647.
- Lips, K. R., P. A. Burrowes, J. R. J. Mendelson, and G. Parra-Olea. 2005. Amphibian Declines in Latin America: Widespread Population Declines, Extinctions, and Impacts. Biotropica **37**:163-165.
- Mao, J. H., D. E. Green, G. Fellers, and V. G. Chinchar. 1999. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. Virus Research **63**:45-52
- Miller, D. L., S. Rajeev, M. J. Gray, and C. A. Baldwin. 2007. Frog virus 3 infection, cultured American bullfrogs. Emerging Infectious Diseases **13**:342-343.
- Ranvestel, A. W., K. R. Lips, C. M. Pringle, M. R. Whiles, and R. J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biology **49**:274-285.
- Relyea, R. A. and N. Diecks. 2008. An unforeseen chain of events: Lethal effects of pesticides on frogs at sublethal concentrations. Ecological Applications **18**:1728-1742.