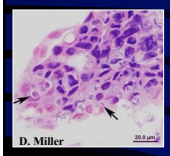
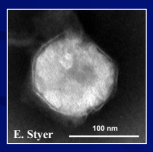
Role of Emerging Infectious Diseases in Amphibian Population Declines: Chytrid Fungi and *Ranavirus*





Matthew J. Gray
WFS 433/533: 11 April 2017
Institute of Agriculture
Center for Wildlife Health

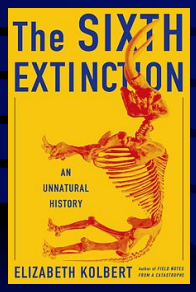
Outline

I. Amphibian Declines and EIDs

II. Chytridiomycosis (*Bd* and *Bsal*)

III. Ranaviral Disease

Sixth Mass Extinction



20-50% Flora & Fauna by 2100

Kolbert (2014)

Extinction rate is 100x higher than expected background rates

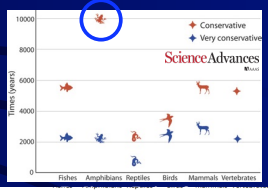


Fig. 2. Number of years that would have been required for the observed vertebrate species extinctions in the last 114 years to occur under a background rate of 2 E/MSY. Red markers, highly conservative

Accelerated modern human–induced species losses: Entering the sixth mass extinction

Gerardo Ceballos,[1]* Paul R. Ehrlich,[2] Anthony D. Barnosky,[3] Andrés García,[4] Robert M. Pringle,[5] Todd M. Palmer[6]

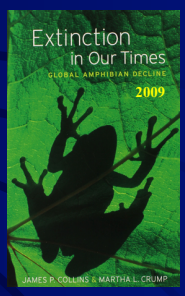
2015

Worldwide Amphibian Population Declines


Are we in the midst of the sixth mass extinction? A view from the world of amphibians 2008

David B. Wake* and Vance T. Vredenburg*

PNAS · PNAS

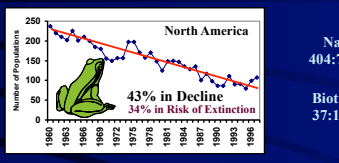


>100 Species Extinct since 1980

Amphibian Declines and Emerging Infectious Diseases

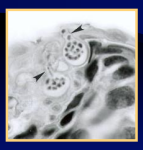
Science 306:1783-1786

EID 5:735-748



Nature 404:752-755

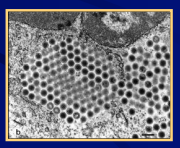
Biotropica 37:163-165



Chytrid Fungi

Adults: >95% (Europe)

Larvae: 80-100%



Ranaviruses

Frog Chytrid Fungus

Batrachochytrium dendrobatidis

Boreal toad

Wyoming toad

CA red-legged frog

Chiricahua leopard frog?

Mountain yellow-legged frog

(*Rana muscosa*)

>200 spp in decline

- Some Species: Highly Pathogenic
- Mostly Tropical at High Elevations
- Western United States

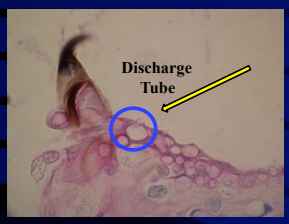
The pathogen

Phylum: Chytridiomycota
Class: Chytridiomycetes
Order: Chytridiales

- *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

Histological Signs

Epidermis



Zoosporangia

Stratum Corneum

Normal Thickness: 2 – 5 µm
Infected: 60 µm

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

Matthew J. Gray[1], E. Davis Carter[1], Jennifer A. Spatz[1], J. Patrick Cusaac[1], Laura K. Reinert[2], Louise Rollins-Smith[2] and Debra L. Miller[1,3]

[1]UTIA Center for Wildlife Health
[2]Vanderbilt School of Medicine
[3]UTIA College of Veterinary Medicine

*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)
  - *wild salamanders in Asia
  - *museum records in Asia >150 yrs

Unknown to occur in North America

14 of 55 sites: 3 species

Ichthyosaura alpestris
Lissotriton vulgaris

Spitzen-van der Sluijs et al. (2016); EID

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)
Bsal thalli (black arrows)

"Death by a thousand holes"

Keratin

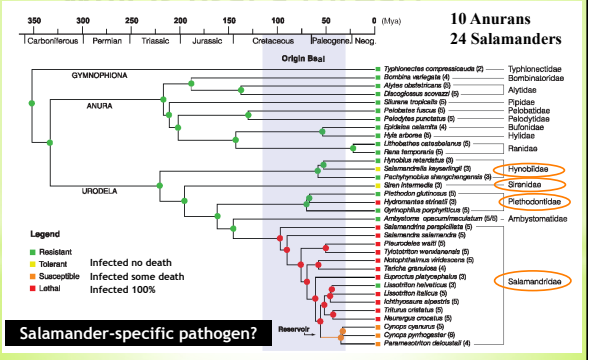
epidermis

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

Van Rooij et al. (2015)

Photomicrograph courtesy Allan Pessier, WSU

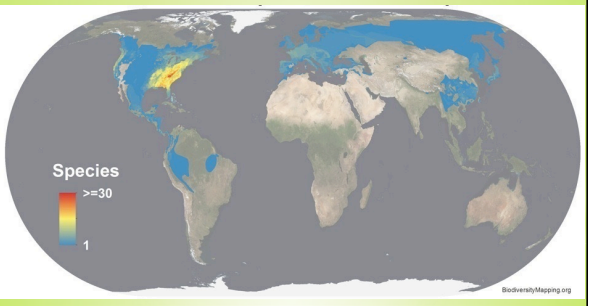
*Why is Bsal a threat?



Martel et al. 2014. Science

*Why is Bsal a threat?

•50% of salamander species occur in North America

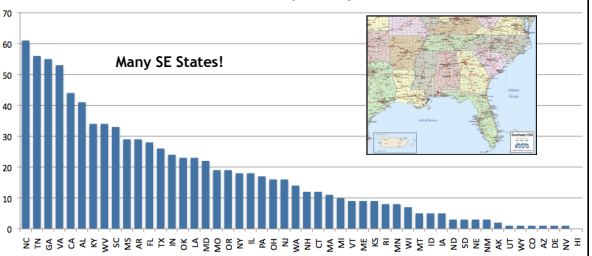


Jenkins et al. 2013. PNAS.
http://www.biodiversitymapping.org/amphibians.htm

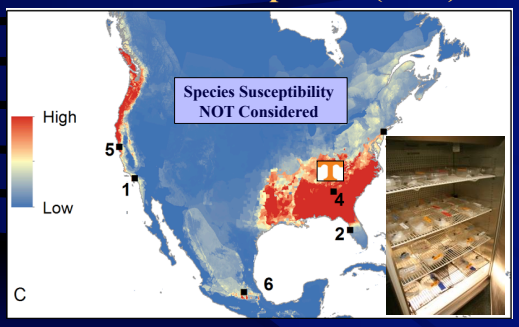
*Why is Bsal a threat?

THE PERILS

#salamander species by state



Risk Model: Yap et al. (2015)



Final Risk Assessment Model

- Relative Risk = SpRich * Log ClimSuit Bsal

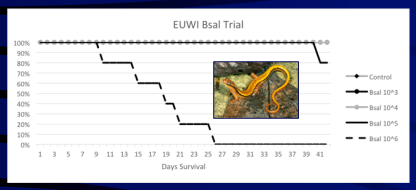
Study Animals

Salamanders (12; 6) **Frogs (4; 2)**

Lithobates sylvaticus, L. chiricahuensis, L. catesbeianus, Hyla chrysoscelis

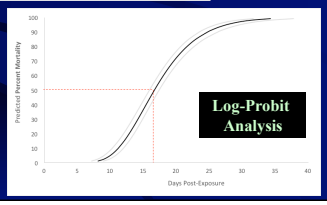
Ambystoma opacum, A. laterale, Aneides aeneus, Desmognathus ocoee, D. aeneus, D. monticola, Plethodon shermani x P. teyahalee, P. metcalfi, Hemidactylium scutatum, Necturus maculosus, Cryptobranchus alleganiensis, and Eurycea wilderae

Survival and Time to Death : EUWI

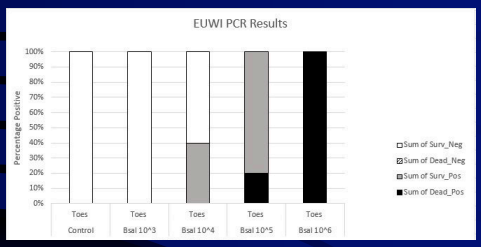


5 x 10^6
10-27 days
5 x 10^5
41 days

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

Endangered	Vunerable	Least Concern	Data Deficient	Region
			Eurycea aquatica	AL, GA, TN
		Eurycea bislineata		Eastern US
			Eurycea chamberlaini	NC, SC
	Eurycea chisholmensis			TX
		Eurycea cirrigera		Eastern US
		Eurycea guttolineata		Southeastern US
	Eurycea junaluska			TN, NC
	Eurycea latitans			TX
		Eurycea longicauda		Eastern US
		Eurycea lucifuga		South-central US
		Eurycea multiplicata		MO, AR, OK
	Eurycea nana			TX
Eurycea naufragia				TX
	Eurycea neotenes			TX
			Eurycea pterophila	TX
		Eurycea quadridigitata		Southeastern US
	Eurycea rathbuni			TX
			Eurycea robusta	TX
	Eurycea sosorum			TX
		Eurycea spelaea		MO, AR, OK
			Eurycea subfluvicola	AR
Eurycea tonkawae				TX
	Eurycea tridentifera			TX
			Eurycea troglodytes	TX
	Eurycea tynerensis			OK, AR, MO
	Eurycea wallacei			FL
	Eurycea waterlooensis			TX
		Eurycea wilderae		Southern Appl

28 Species in USA. 43% are in decline

Ecology and Pathology of Ranaviruses




Matthew J. Gray[1] and Debra L. Miller[1,2]

University of Tennessee
[1]Center for Wildlife Health
[2]CVM Department of BDS

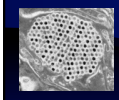
Ranavirus Characteristics

- 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

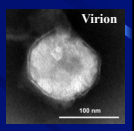


Paracrystalline Array

ICTV (2012)

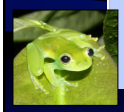
Species (6)

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



Chinchar et al. (2011)

Global Distribution of Ranavirus Cases: Amphibians



Duffus et al. (2015)

6 Continents: 1965, 1992/97

All Latitudes, All Elevations

18 Families: Alytidae, Ranidae, Hylidae, Bufonidae, Centrolenidae, Craugastoridae, Dendrobatidae, Discoglossidae, Leptodactylidae, Pipidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae

>100 Species

Case Example

Re-occuring Die-offs

Jamie Barichivich (USGS) and Megan Todd-Thompson (UT)

A. Cressler, USGS

M. Niemiller, UT

A. Cressler, USGS

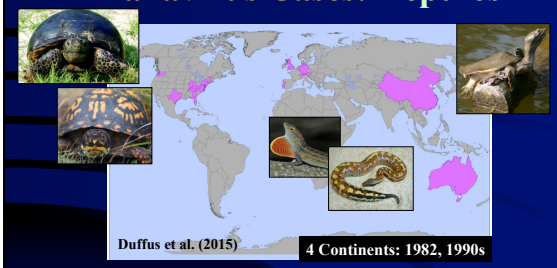
May 1999, 2000, 2009, 2012

GSMNP, Cades Cove
Gourley Pond

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

Green et al. (2002)

Global Distribution of Ranavirus Cases: Reptiles



Duffus et al. (2015)

4 Continents: 1982, 1990s

Most FV3-like Ranaviruses: Captivity

12 Families: Agamidae, Anguidae, Boidae, Dactyloidae, Emyidae, Gekkonidae, Iguanidae, Lacertidae, Pythonidae, Testudinidae, Trionychidae, Varanidae

>30 Species

Ranaviral Disease in Eastern Box Turtles

13 February 2012

The Washington Post Make us your start page

POSTLOCAL

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.

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.

Ranaviral Disease in Eastern Box Turtles

Charleston Gazette-Mail

HOME NEWS BUSINESS OPINION SPORTS LIFE A&E OUTDOORS BLOGS OBITUARIES

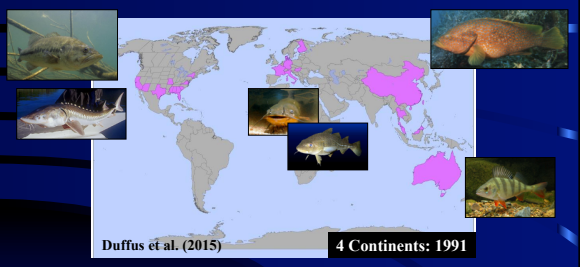
W.Va. turtle die-off linked to ranavirus disease

Steward Co., TN, Sept. 2015 John Hewlett

Clendenin, WV July 2012

Kenny Kemp Archibald among the shells and skeletons of some of the 28 turtles found dead on his property this year.

Global Distribution of Ranavirus Cases: Fishes



Duffus et al. (2015)

4 Continents: 1991

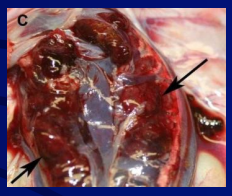
Most non-FV3-like Ranaviruses

22 Families: Acipenseridae, Anguillidae, Centrarchidae, Channidae, Cobitoidae, Cyprinidae, Eleotridae, Esocidae, Gadidae, Gasterosteidae, Ictaluridae, Labridae, Latidae, Lutjanidae, Moronidae, Percidae, Poeciliidae, Salmonidae, Sciaenidae, Scophthalmidae, Serranidae, Siluridae

>50 Species

Ebola of Ectothermic Vertebrates

Hemorrhages



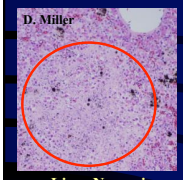
Friable Spleen:

"Apparent tropism for vascular endothelium"
R. Whittington

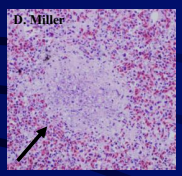
Organ Destruction

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!

Hoverman et al. (2011a)

Potential Major Factor Contributing to Ranavirus Emergence

L. catesbeianus

Kristine Smith, DVM

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

- Hong Kong = 56% infected

PLOS ONE

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

2014

Jonathan E. Kolby[1,2*], Kristine M. Smith[2], Lee Berger[1], William B. Karesh[2], Asa Preston[3], Allan P. Pessier[3], Lee F. Skerratt[1]

Captive Conditions

https://www.youtube.com/watch?v=f2q5jqdsTeA

The perfect cauldron for virulence evolution!

- Abundant Hosts
- Multiple Strains
- Immunocompromised

Bullfrog Die-off: Alapaha, GA

DISEASES OF AQUATIC ORGANISMS

1998

Rana ... novel

Expt	FV3	RCV-Z	MEM
1	1/10 (10%)	10/10 (100%)	0/10 (0%)
2	0/10 (0%)	5/10 (50%)	0/10 (0%)

Sai M ... ryan[1],

Bullfrog Die-off: Alapaha, GA

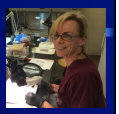
EMERGING INFECTIOUS DISEASES

The University of Georgia

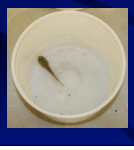
Volume 13, Number 2—February 2007 **Miller et al. (2007)**

Letter

Frog Virus 3 Infection, Cultured American Bullfrogs

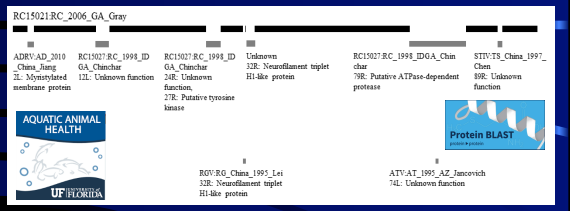


The FUTURE of Veterinary Medicine



Possible Mechanism: Recombination

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



AQUATIC ANIMAL HEALTH

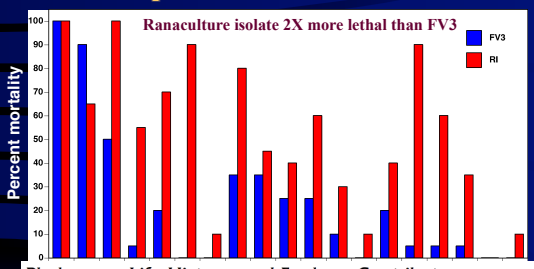
UF UNIVERSITY of FLORIDA

Protein BLAST

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

Jason T. Hoverman,[1,2] Matthew J. Gray,[2] Nathan A. Haislip,[2,4] and Debra L. Miller[2,3,5]

EcoHealth

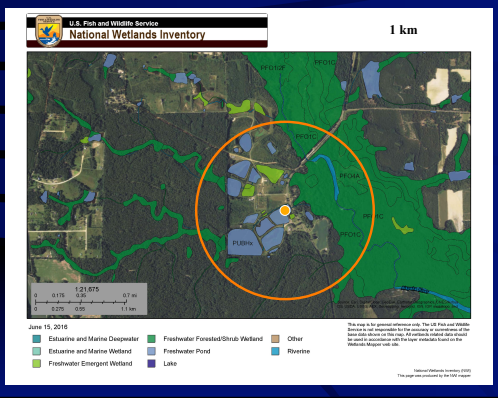
Hoverman et al. (2011a): 19 Species Tested

Opportunity for Spillover

Wastewater Rarely Decontaminated; Poor Animal Contaminant

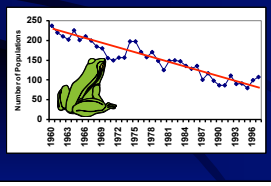
Guaranteed Transmission

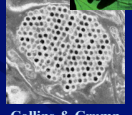
Site of Highly Virulent Ranavirus



Are Ranaviruses Capable of Causing Local Extirpations and Species Declines?

Muths et al. (2006)





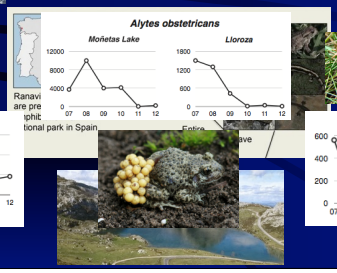
Collins & Crump (2009)

Evidence of Local Extinction

Dr. Stephen Price
University College London
Picos de Europa National Park
2007-2012

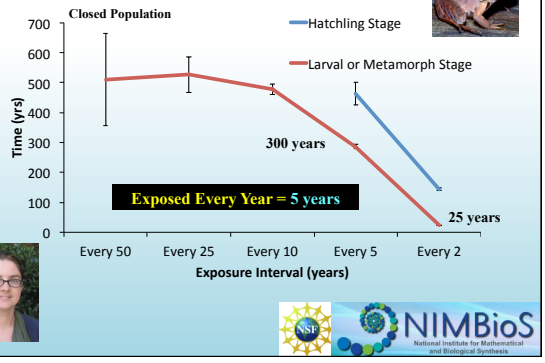
Current Biology
24:2586-2591

Ranavirus die-offs with six species



Time to Extinction

Earl and Gray (2014)



Global Ranavirus Consortium, Inc.

http://www.ranavirus.org/

Website
Symposia
Reporting System
Springer eBook
Ranavirus Course

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

Bylaws Approved

GRC@LISTSERV.UTK.EDU

Membership in 2015


Questions??
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mgray11@utk.edu
http://www.ranavirus.org/
https://ag.tennessee.edu/cwh/Pages/default.aspx