Amidst the Sixth Mass Extinction:

Factors Responsible for the Global Decline of Amphibians?

Bufo periglenes, CR

Hyla regilla, OR

Matthew J. Gray
University of Tennessee

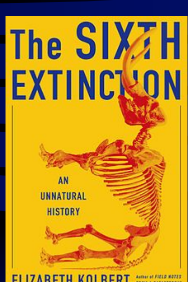
Outline

I. Wildlife & Amphibian Declines

II. Why Amphibians?

III. Factors Responsible for Declines

Sixth Mass Extinction



20-50% Flora & Fauna by 2100

Kolbert (2014)

Extinction rate is 100x higher than expected background rates

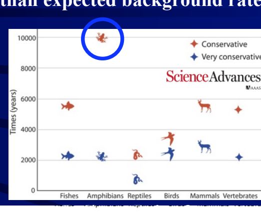
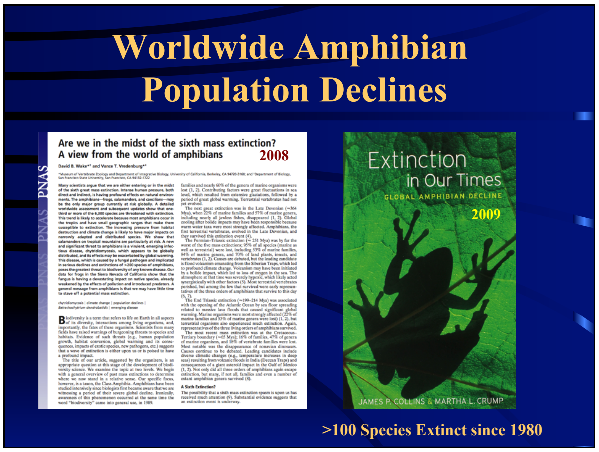


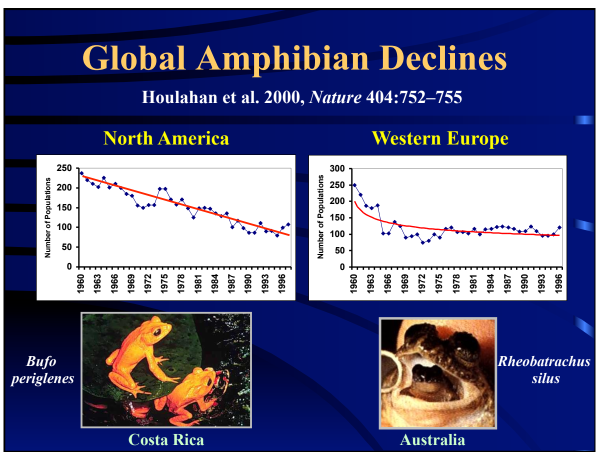
Fig. 2. Number of years that would have been required for the observed vertebrate species extinctions in the last 114 years to occur under a background rate of 2 E/MSY. Red markers, highly conservative

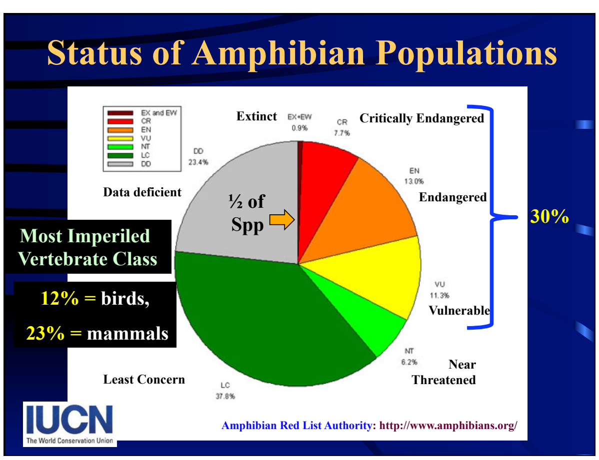
Accelerated modern human–induced species losses: Entering the sixth mass extinction

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
PNAS
Are we in the midst of the sixth mass extinction?
A view from the world of amphibians
2008
Extinction
in Our Times
GLOBAL AMPHIBIAN DECLINE
2009
JAMES P. COLLINS & MARTHA L. CRUMP
>100 Species Extinct since 1980


Global Amphibian Declines
Houlahan et al. 2000, Nature 404:752–755
North America
Western Europe
Number of Populations
Bufo periglenes
Costa Rica
Rheobatrachus silus
Australia


Status of Amphibian Populations
EX and EW
CR
EN
VU
NT
LC
DD
Extinct
EX+EW 0.9%
CR 7.7%
Critically Endangered
DD 23.4%
EN 13.0%
Data deficient
½ of Spp
Endangered
30%
Most Imperiled Vertebrate Class
12% = birds,
23% = mammals
VU 11.3%
Vulnerable
NT 6.2%
Near Threatened
Least Concern
LC 37.8%
IUCN
The World Conservation Union
Amphibian Red List Authority: http://www.amphibians.org/

Status of Amphibian Populations
(as of 2012)

Order	Total	EX	EW	CR	EN	VU	NT	LC	DD	% Threatened or Extinct
Anura Frogs & Toads	5,640	34	2	429	665	561	327	2,178	1,446	29.3
Caudata Salamanders & Newts	557	2	0	79	101	92	62	161	60	48.8
Gymnophiona Caecilians	177	0	0	1	1	4	0	53	118	3.4
Total	5,918	36	1	456	769	671	369	2,236	1,382	30.3

25%

CR, EN, or VU: Anura = 1655 spp
Caudata = 272 spp
Gymnophiona = 6 spp

Status of U.S. Amphibians

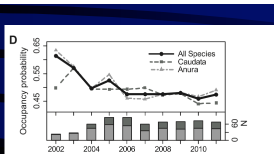
- 2 Species Extinct (*R. fisheri*; *P. ainsworthi*)
- 10 Endangered; 9 Threatened; 5 Awaiting
- CA = 8 Spp.; SW = 6 Spp.; SE = 6 Spp.
  (Chiracahua Leopard Frog, 80%)

- TN: 1 state-listed; 26 spp (30%)

Status of Amphibians: U.S.

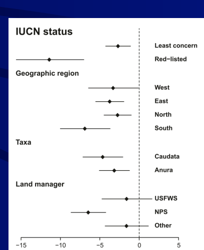
Trends in Amphibian Occupancy in the United States

Michael J. Adams[1]*, David A. W. Miller[2,3], Erin Muths[4], Paul Stephen Corn[5], Evan H. Campbell Grant[6], Larissa L. Bailey[7], Gary M. Fellers[8], Robert N. Fisher[9], Walter J. Sadinski[10], Hardin Waddle[11], Susan C. Walls[12]



Abstract

Though a third of amphibian species worldwide are thought to be imperiled, existing assessments simply categorize extinction risk, providing little information on the rate of population losses. We conducted the first analysis of the rate of change in the probability that amphibians occupy ponds and other comparable habitat features across the United States. We found that overall occupancy by amphibians declined 3.7% annually from 2002 to 2011. Species that are Red-listed by the International Union for Conservation of Nature (IUCN) declined an average of 11.6% annually. All subsets of data examined had a declining trend including species in the IUCN Least Concern category. This analysis suggests that amphibian declines may be more widespread and severe than previously realized.



Commonality of Being Uncommon

Southeastern United States

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

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)**
7) **Mississippi = 18 species (12 genera)**
8) **North Carolina = 41 species (15 genera)**
9) **South Carolina = 19 species (13 genera)**
10) **Tennessee = 26 species (14 genera)**

Why are Amphibians so Susceptible?

Late Devonian (370 mya)

Survived Three Previous Mass Extinction Events: Phenotypically Plastic

Exothermic vertebrates with a complex (biphasic) life cycle

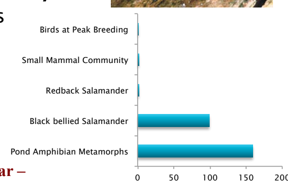
- **Thin, Permeable Skin that must remain Moist**
  1) Respiration
  2) Osmoregulation
- **Low Vagility (≤1 km)**
- **Long-lived (ca. 10 yr)**

Desiccation is a Lifelong Struggle

Absorb Compounds Readily

Who cares?

- Important part of the ecosystem
  - Huge amount of biomass
  - Prey
  - Predators
    - Pest (insect) populations
  - Nutrient cycling & seq.

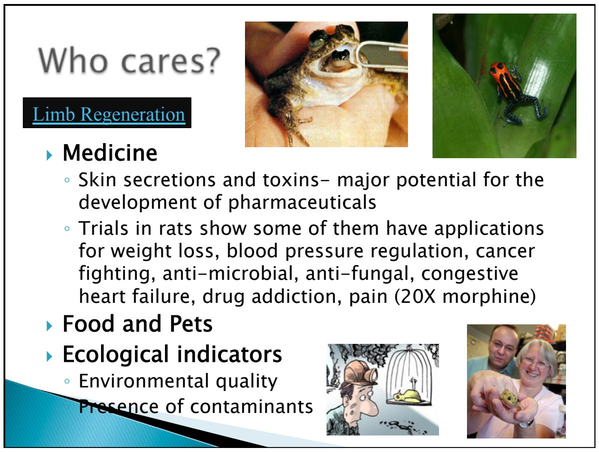


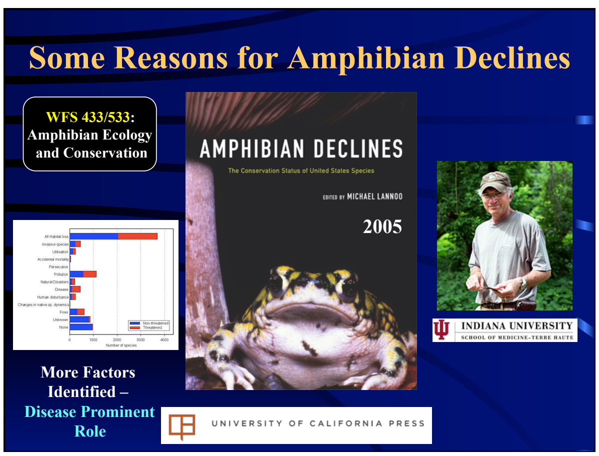
❖ **1000 frogs eat 5 million insects per year –**
Zoonotic pathogens (malaria, dengue, Zika, WNV, encephalitis)

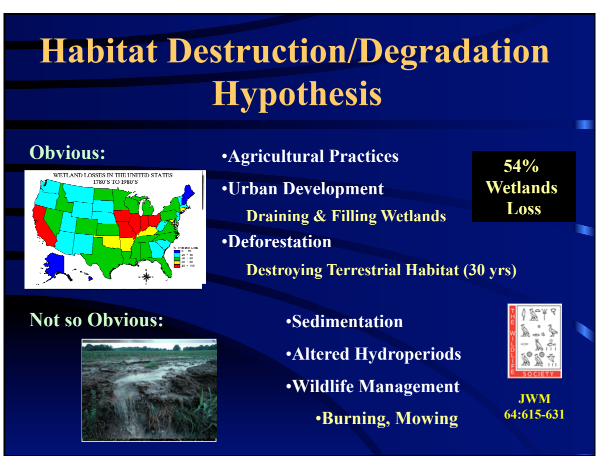
Herpetological Conservation and Biology 9(1):1−17.
Submitted: 19 December 2013; Accepted: 25 February 2014; Published: 13 July 2014.

AMPHIBIAN CONTRIBUTIONS TO ECOSYSTEM SERVICES

DANIEL J. HOCKING[1,2] AND KIMBERLY J. BABBITT[1]


Who cares?
Limb Regeneration
Medicine
Skin secretions and toxins– major potential for the development of pharmaceuticals
Trials in rats show some of them have applications for weight loss, blood pressure regulation, cancer fighting, anti-microbial, anti-fungal, congestive heart failure, drug addiction, pain (20X morphine)
Food and Pets
Ecological indicators
Environmental quality
Presence of contaminants


Some Reasons for Amphibian Declines
WFS 433/533: Amphibian Ecology and Conservation
AMPHIBIAN DECLINES
The Conservation Status of United States Species
EDITED BY MICHAEL LANNOO
2005
INDIANA UNIVERSITY
SCHOOL OF MEDICINE-TERRE HAUTE
More Factors Identified –
Disease Prominent Role
UNIVERSITY OF CALIFORNIA PRESS


Habitat Destruction/Degradation Hypothesis
Obvious:
WETLAND LOSSES IN THE UNITED STATES 1780'S TO 1980'S
•Agricultural Practices
•Urban Development
Draining & Filling Wetlands
•Deforestation
Destroying Terrestrial Habitat (30 yrs)
54% Wetlands Loss
Not so Obvious:
•Sedimentation
•Altered Hydroperiods
•Wildlife Management
•Burning, Mowing
JWM
64:615-631

Chemical Pollution Hypothesis

Point Source: Pollution originating from 1 point.

- **Effluent**: organic or industrial waste
- **Thermal**: electric plants

Non-point Source: Pollution originating from multiple points (e.g., field, parking lot).
Imp: Widespread

Chemicals & Effects: Relyea (2003, 2004, 2005, 2009)

- Nitrates & Ammonia: Direct mortality; Reduce growth
- Organophosphate Insecticides: Above plus malformations and altered behavior
- Atrazine: herbicide

(T. Hayes)

Introduced Predators & Competitors Hypothesis

Predators:

- Fish (eat everything)
  - ➡ Sport Fish (e.g. trout, bass)
- Bullfrogs (eat everything but adults)

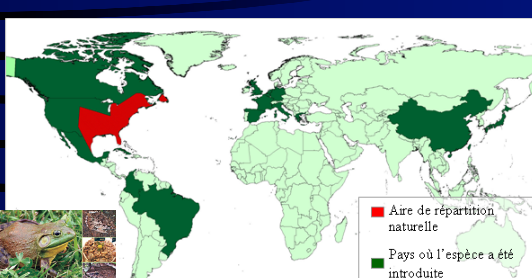
Copeia 1999:22-23
Copeia 1991:1-8
SARI Spec. Pub. 1

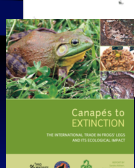
Competitors:

- Frogs
  - ➡ *Bufo marinus, L. catesbeianus*
- Mosquito Fish (*Gambusia*)

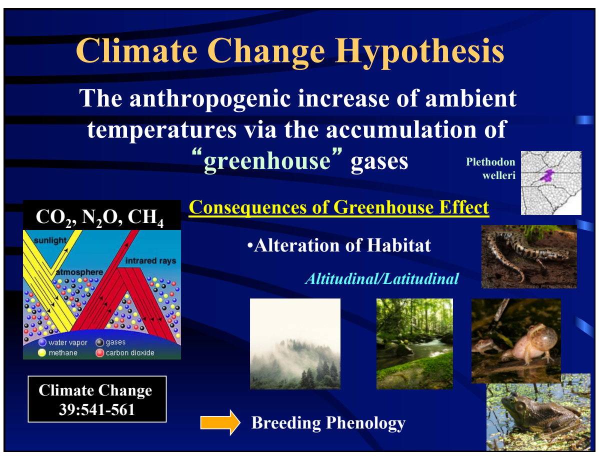
Conservation Biology 13:613-622
FROGLOG 15 & 17

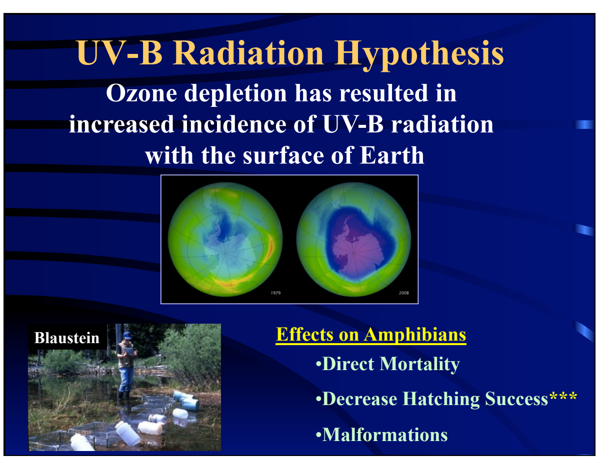
American Bullfrog Distribution






Commercial Exploitation Hypothesis
Major Concern in the SE Asia
Removal:
•200 million exported annually from Asia
•70 million exported annually from India
Environmentalist 10:39-41, 1990
Consumption, Pet Industry
Producers: Indonesia, China, Taiwan, Brazil
Consumers: EU (Belgium, France), USA: 23-72 metric tons
Release:
(Exotics or captive-reared specimens)
Bioscience 21:1027-1034
•9 million frogs (Rana)
shipped from biological supply
companies in the USA
CAROLINA
Charles D. Sullivan Co. Inc.
6685 Holt Road
Nashville, Tennessee 37211
Pathogen Pollution
Kolby et al. (2014), Schloegel et al. (2009), Cummingham et al. (2003)
Canapés to EXTINCTION

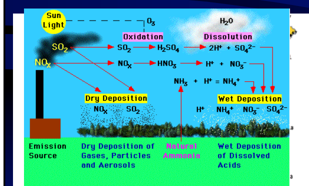

Climate Change Hypothesis
The anthropogenic increase of ambient temperatures via the accumulation of "greenhouse" gases
Plethodon welleri
CO_2, N_2O, CH_4
sunlight
atmosphere
infrared rays
water vapor
gases
methane
carbon dioxide
Consequences of Greenhouse Effect
•Alteration of Habitat
Altitudinal/Latitudinal
Climate Change 39:541-561
Breeding Phenology


UV-B Radiation Hypothesis
Ozone depletion has resulted in increased incidence of UV-B radiation with the surface of Earth
Blaustein
Effects on Amphibians
•Direct Mortality
•Decrease Hatching Success***
•Malformations

Acid Precipitation Hypothesis

The anthropogenic decrease in pH of precipitation via emissions of nitrogen oxides and sulfur dioxide and their oxidation and dissolution to acids

pH < 4



Effects on Amphibians

- Direct Mortality
- Delayed Hatching
- Reduced Mobility
- Reduced Larval GR & Size

Copeia 1986:454-466

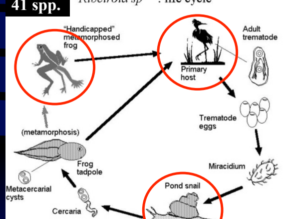
Food Web

Pathogenic Hypothesis: Parasites

Trematodes

(Ribeiroia ondatrae)

41 spp.



Effects on Amphibians

1) Cysts form in and around "limb-buds"

2) Limb Development

3) Malformations (mechanical)

→ High Nitrogen Sites (cattle)

Science 284:802-804

Pathogenic Hypothesis: Fungi

Chytrid *(KI-trid)* Fungus

Non-hyphal, Parasitic Fungus

Phylum: Chytridiomycota	Unicellular
Class: Chytridiomycetes	Most Haploid: Zoospores
Order: Chytridiales	*Batrachochytrium dendrobatidis*

Colonize Keratinized Epidermal Cells (Pelvic Patch)

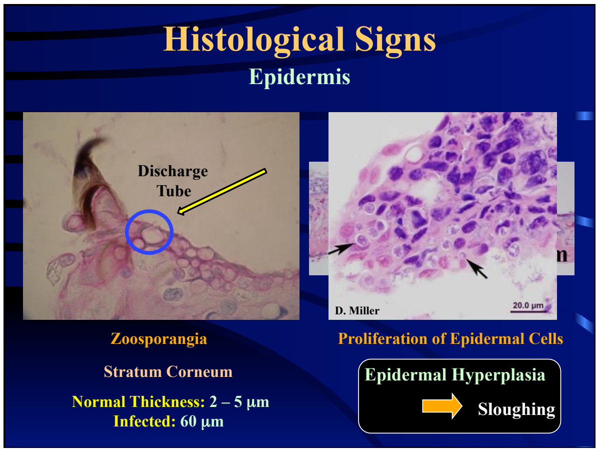
Proc. Natl. Acad. Sci. 95:9031-9036

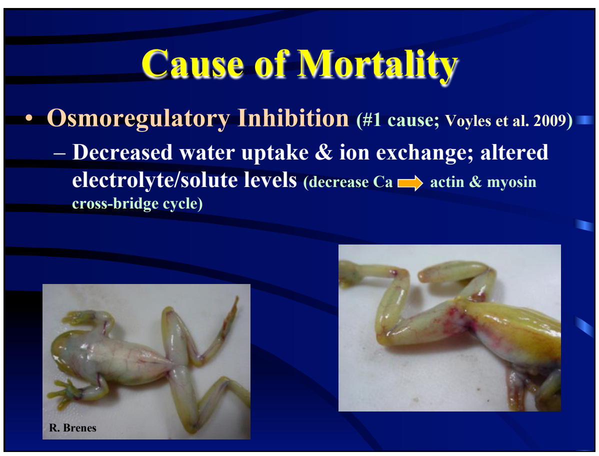
Effects on Amphibians

- 50-100% Mortality (adults: tropics)

Epidermal Hyperplasia → Sloughing

→ Interference w/ Cutaneous Respiration & Osmoregulation

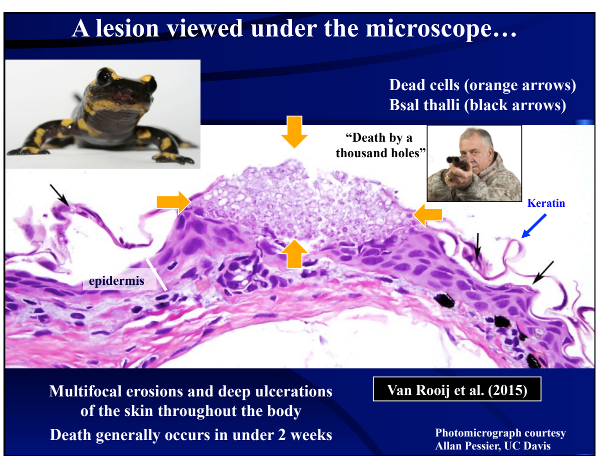

Histological Signs
Epidermis
Discharge
Tube
D. Miller
20.0 µm
Zoosporangia
Proliferation of Epidermal Cells
Stratum Corneum
Normal Thickness: 2 – 5 µm
Infected: 60 µm
Epidermal Hyperplasia
Sloughing

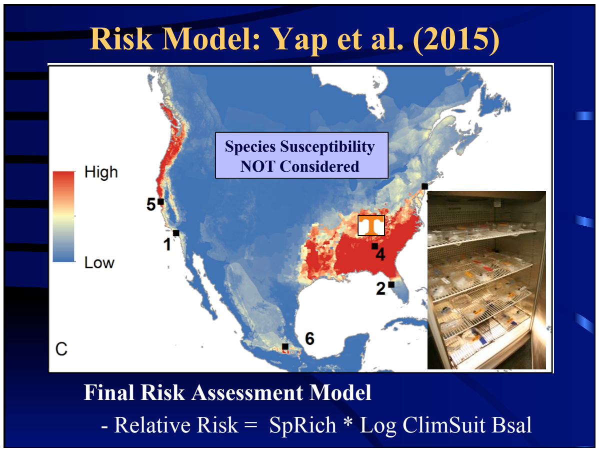

Cause of Mortality
• Osmoregulatory Inhibition (#1 cause; Voyles et al. 2009)
– Decreased water uptake & ion exchange; altered electrolyte/solute levels (decrease Ca → actin & myosin cross-bridge cycle)
R. Brenes

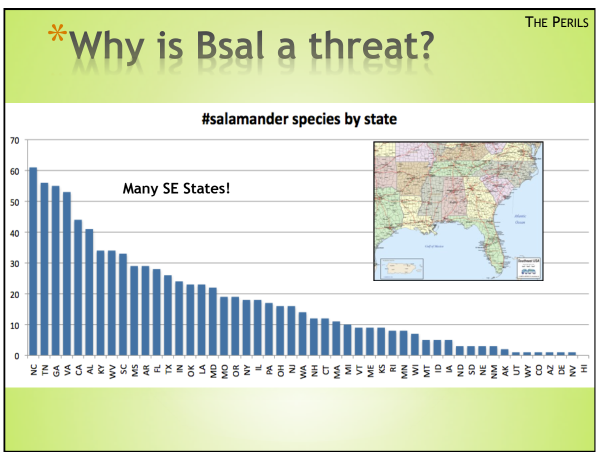

Photos = R. Brenes

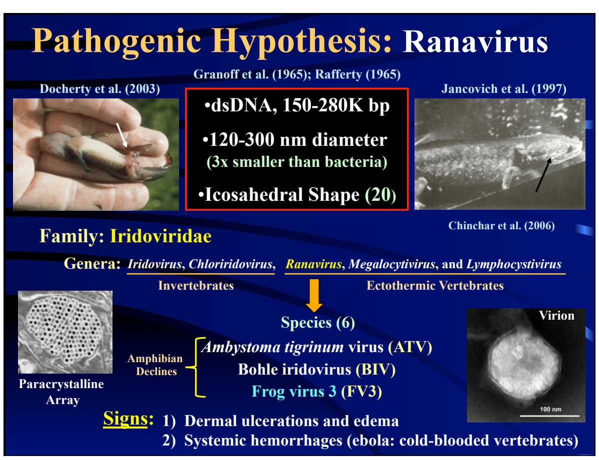

Batrachochytrium salamandrivorans:
Determining the Risk to North America
Robertville, Belgium
The Center for Wildlife Health
F. Pasmans, Ghent Univ.
Matthew J. Gray1, E. Davis Carter1, Jennifer A. Spatz1, J. Patrick Cusaac1,
Laura K. Reinert2, Louise Rollins-Smith2 and Debra L. Miller1,3
1UTIA Center for Wildlife Health
2Vanderbilt School of Medicine
3UTIA College of Veterinary Medicine
VANDERBILT
UTIA
INSTITUTE OF AGRICULTURE
THE UNIVERSITY OF TENNESSEE


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)
14 of 55 sites: 3 species
Present in:
wild salamanders in Asia (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
Ichthyosaura alpestris
Lissotriton vulgaris
Spitzen-van der Sluijs et al. (2016); EID

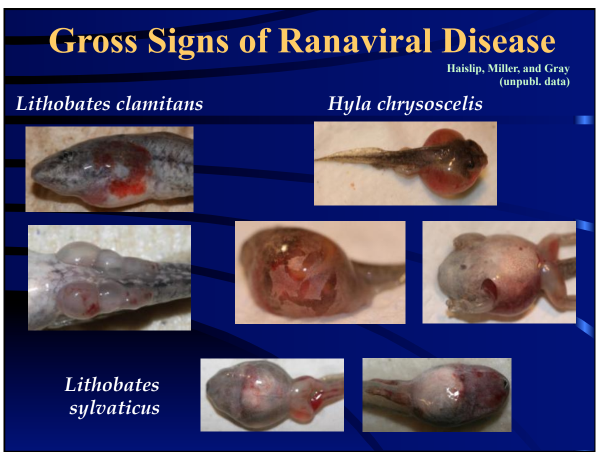

A lesion viewed under the microscope…
Dead cells (orange arrows)
Bsal thalli (black arrows)
“Death by a thousand holes”
Keratin
epidermis
Multifocal erosions and deep ulcerations of the skin throughout the body
Death generally occurs in under 2 weeks
Van Rooij et al. (2015)
Photomicrograph courtesy
Allan Pessier, UC Davis

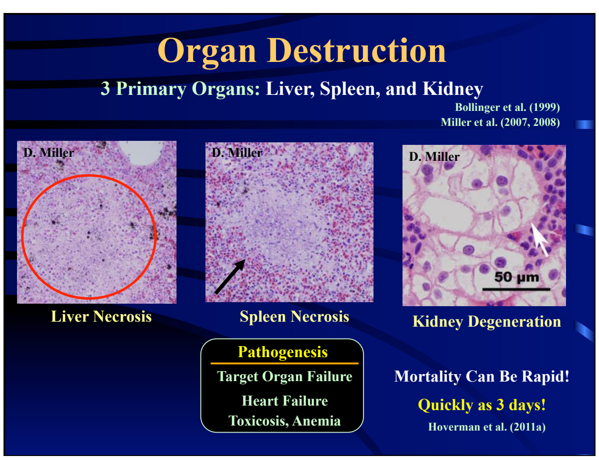

Risk Model: Yap et al. (2015)
Species Susceptibility NOT Considered
High
Low
C
Final Risk Assessment Model
- Relative Risk = SpRich * Log ClimSuit Bsal

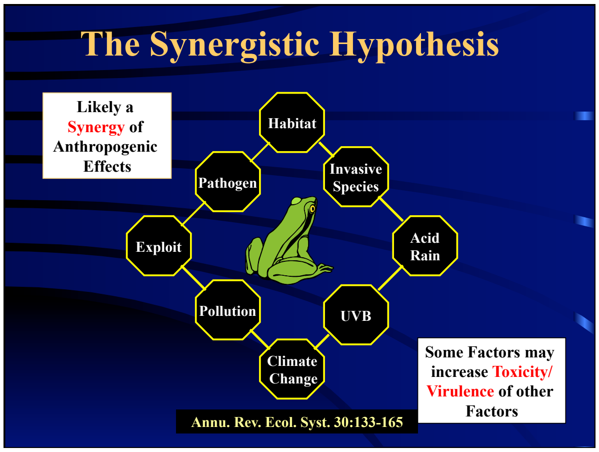

THE PERILS
Why is Bsal a threat?
#salamander species by state
Many SE States!


Pathogenic Hypothesis: Ranavirus
Granoff et al. (1965); Rafferty (1965)
Docherty et al. (2003)
Jancovich et al. (1997)
•dsDNA, 150-280K bp
•120-300 nm diameter
(3x smaller than bacteria)
•Icosahedral Shape (20)
Chinchar et al. (2006)
Family: Iridoviridae
Genera: Iridovirus, Chloriridovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus
Invertebrates
Ectothermic Vertebrates
Species (6)
Amphibian Declines
Ambystoma tigrinum virus (ATV)
Bohle iridovirus (BIV)
Frog virus 3 (FV3)
Paracrystalline Array
Virion
Signs: 1) Dermal ulcerations and edema
2) Systemic hemorrhages (ebola: cold-blooded vertebrates)


Maine 2013 Die-off
The Boston Globe
1000 carcasses/m²
≥200,000 dead
qPCR Confirmed
Wheelwright et al.
(2014)
<24 hrs
6/14/13
6/15/13


Gross Signs of Ranaviral Disease
Haislip, Miller, and Gray
(unpubl. data)
Lithobates clamitans
Hyla chrysoscelis
Lithobates sylvaticus


Organ Destruction
3 Primary Organs: Liver, Spleen, and Kidney
Bollinger et al. (1999)
Miller et al. (2007, 2008)
D. Miller
D. Miller
D. Miller
50 µm
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)


The Synergistic Hypothesis
Likely a Synergy of Anthropogenic Effects
Habitat
Invasive Species
Acid Rain
UVB
Climate Change
Pollution
Exploit
Pathogen
Some Factors may increase Toxicity/ Virulence of other Factors
Annu. Rev. Ecol. Syst. 30:133-165


Questions??
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865-974-2740
KNOWLEDGE • COMPASSION • DISCOVERY •