Ranaviruses:
Cold Blooded Killers!




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11:15 a.m.
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Austin Peay State University

Presentation Contributors

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Danna Schock, University of Calgary
Amber Teacher, University of Helsinki
Megan Todd-Thompson, University of Tennessee
Yumi Une, Azabu University

Outline

I. Emerging Infectious Diseases

II. Are Ranaviruses a Threat?

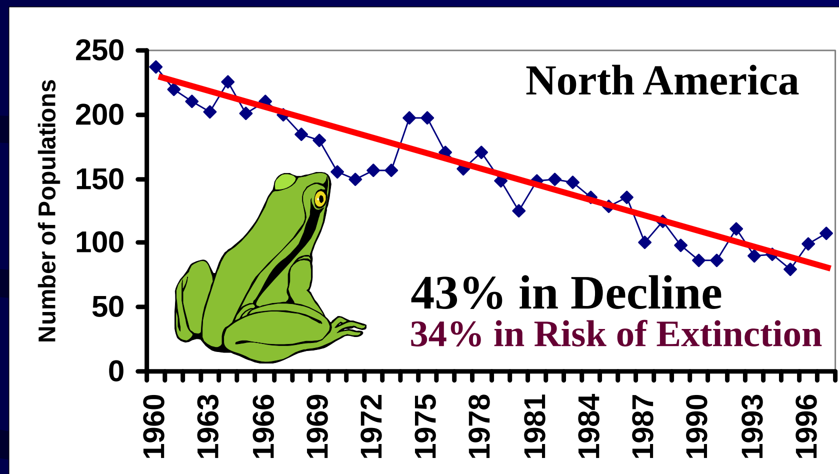
III. Ranavirus Pathology and Ecology

IV. Future Research Directions

Amphibian Declines and Emerging Infectious Diseases

Science
306:1783-1786

EID 5:735-748



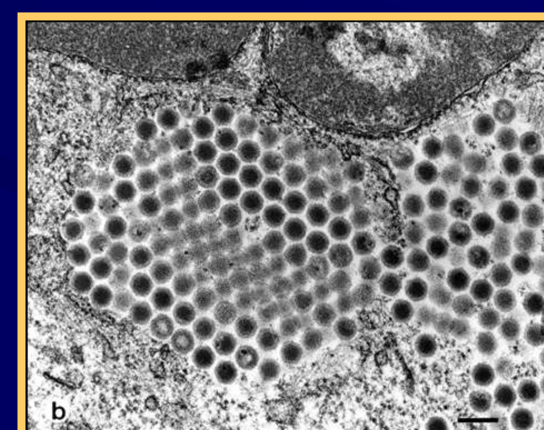
Nature
404:752-755

Biotropica
37:163-165

Chytrid Fungus

Adults: >95% (Europe)

Larvae: 80-100%



Ranaviruses

Commonly Asserted

Ranavirus Die-offs are not Widespread

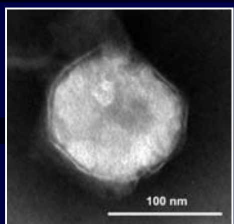
Ranavirus only affect Common Species

No Evidence that Ranaviruses are Capable of Causing Declines

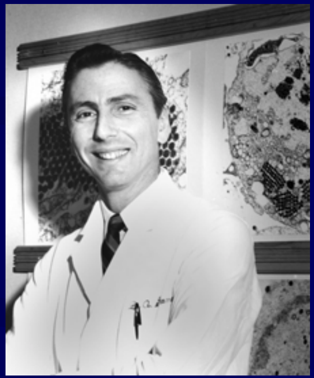
Does the current knowledge of the ranavirus host-pathogen system support these claims?

History of Ranavirus Die-offs

First Isolated:



- Dr. Allan Granoff
- St. Jude Hospital
- *Rana pipiens* (1962)

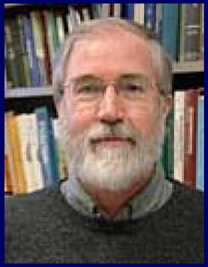


First Large-scale Die-offs:

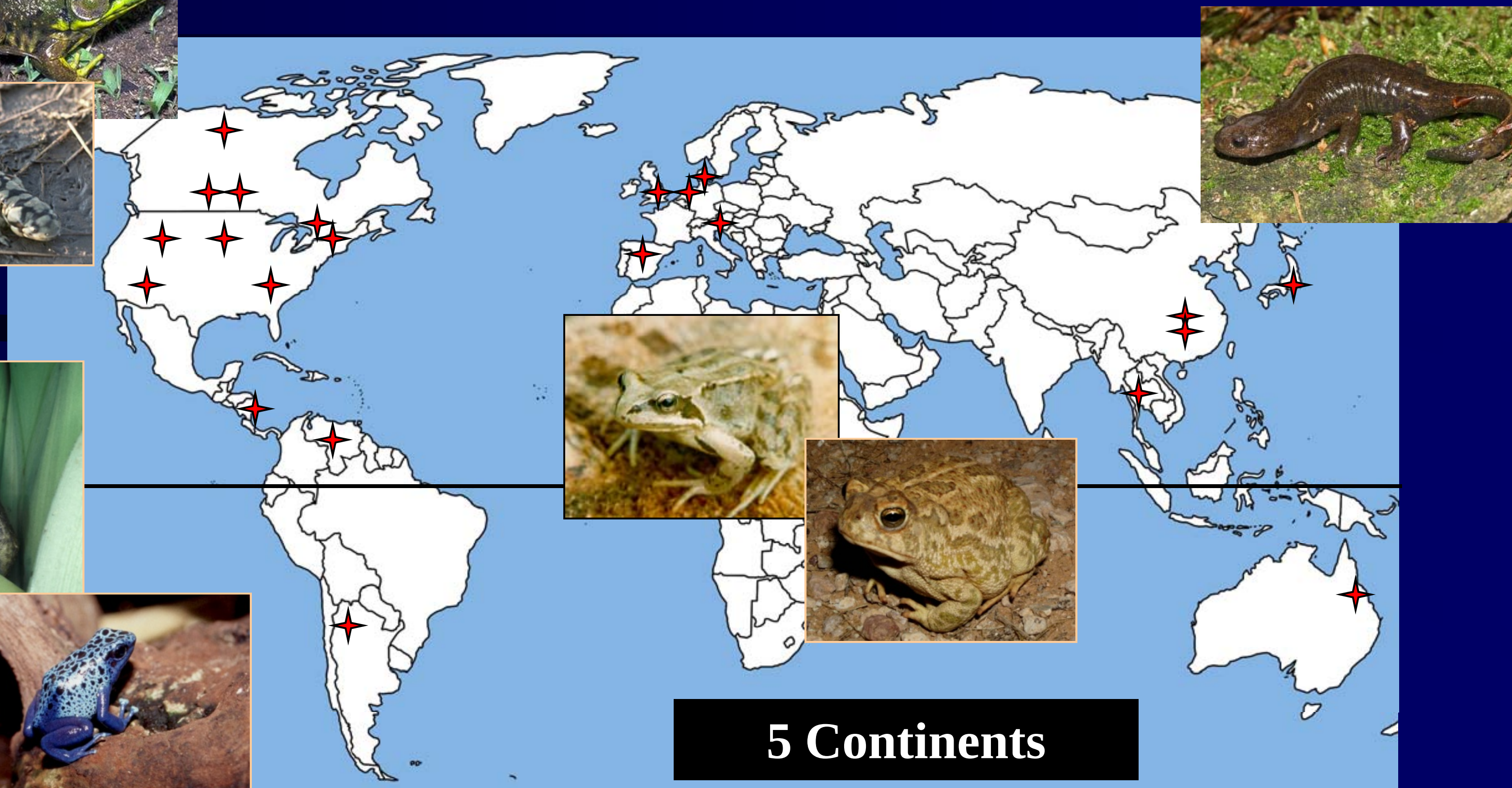
- Dr. Andrew Cunningham
- Institute of Zoology, ZSL
- *Rana temporaria* (1992)

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*

Uncommon

>30 States & 20 Spp; 5 Provinces

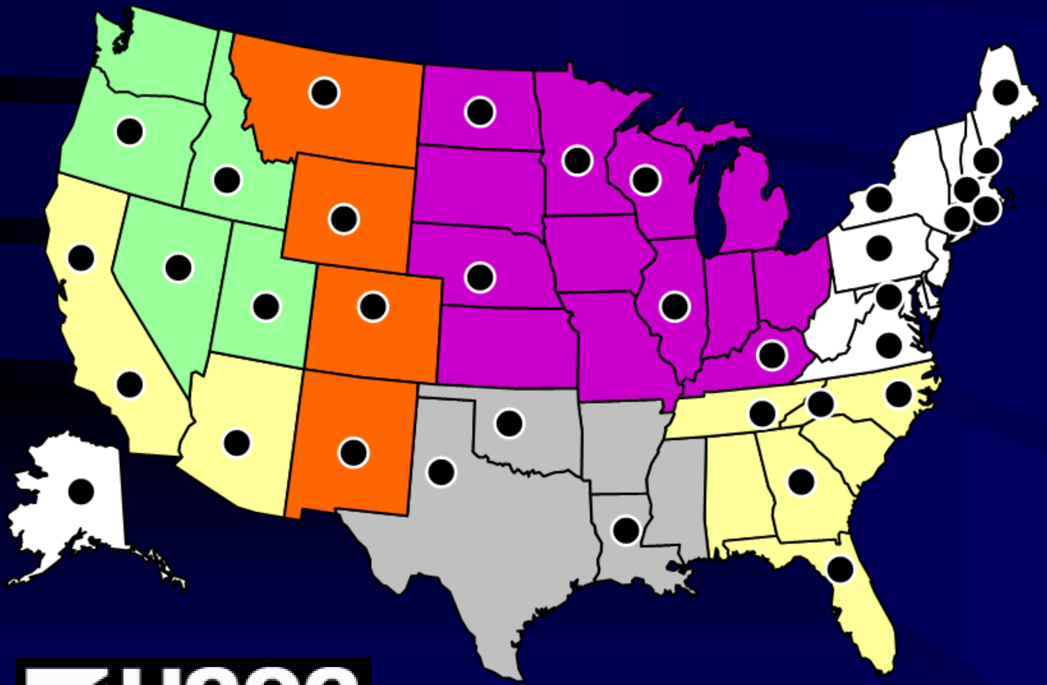
Families

Ranidae
Hylidae
Bufonidae
Ambystomatidae
Salamandridae

Lithobates sylvaticus

Are Ranavirus Die-offs Widespread?

YES, Ranavirus Die-offs are Widespread!



ARMI 2006

(110; 34 states)

43% = *Ranavirus*

16% = fungi

10% = protozoan

USGS science for a changing world

D. Green, unpubl. data

Common Species Only?

Case Examples

Japan: Azabu University

Dr. Yumi Une

Introduced American Bullfrogs:

Sept 2008 & 2009

Asian Salamanders: **Hynobiidae**

Hynobius nebulosus

Experimental Challenges

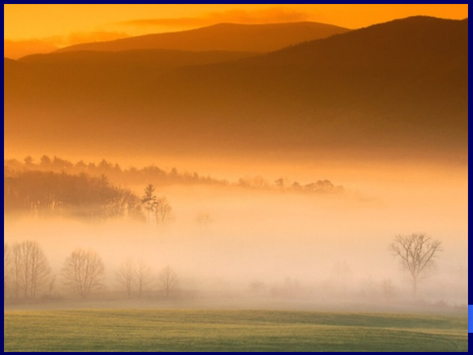
11 Species (7: 100%)

1. Hynobius nebulosus
2. Hynobius lichenatus
3. Hynobius nigrescens
4. Hynobius tokyoensis
5. Cynops ensicauda
6. Cynops pyrrhogaster
7. Rana (Pelophylax) porosa porosa

Case Examples

North America

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



A. Cressler, USGS

M. Niemiller, UT

A. Cressler, USGS

GSMNP, Cades Cove
Gourley Pond

May 2009

D. Green, USGS

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

Southern Appalachia:

What about Plethodontids?

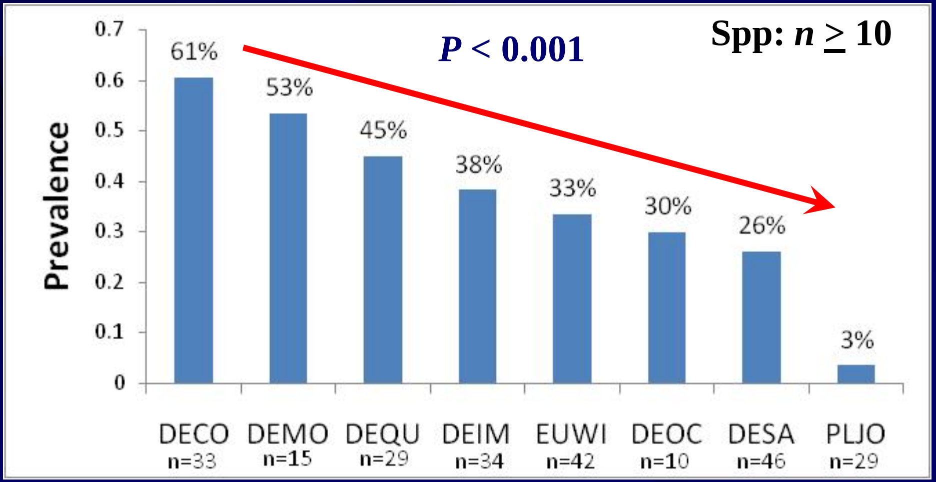
2007-2009

12 Species & 4 Genera:

>97% MCP similarity with the ranavirus FV3

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



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

26 Species of Concern (14 genera)

Desmognathus aeneus
Desmognathus welteri
Desmognathus wrighti
Eurycea junaluska
Gyrinophilus palleucus
Gyrinophilus gulolineatus
Pseudotriton montanus
Plethodon dorsalis
Plethodon richmondi
Plethodon wehrlei
Plethodon welleri
Plethodon yonahlossee
Plethodon aureolus
Plethodon jordani

Cryptobranchus alleganiensis
Siren intermedia
Aneides aeneus
Ambystoma barbouri
Ambystoma talpoideum
Hemidactylium scutatum

Hyla gratiosa
Hyla versicolor
Acris gryllus
Pseudacris brachyphona
Rana areolata
Rana capito
Scaphiopus holbrookii





Species of Concern: Uncommon

Southeastern United States

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

Species of Concern: **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)**

Capable of Causing Local Extirpations?

Evidence of Local Extinction

Wetlands
23:278-290

Dr. Jim Petranka

Tulula Wetland Complex, NC

Biological Conservation
138:371-380

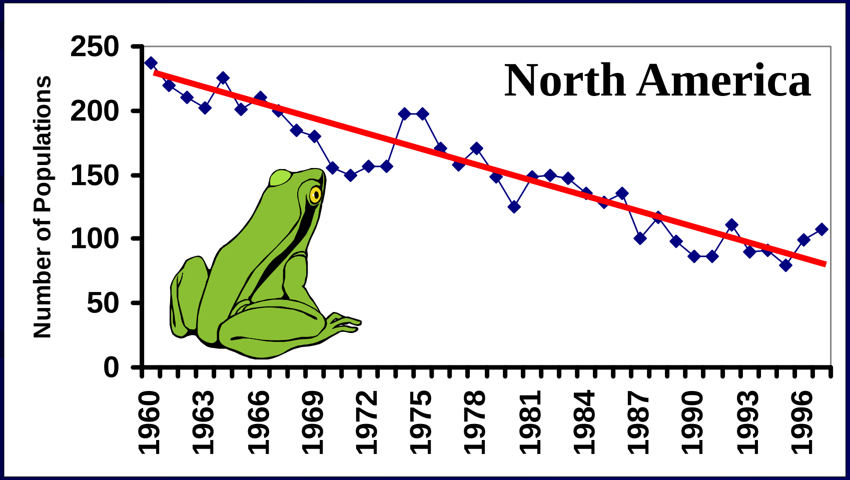
1998-2006

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?

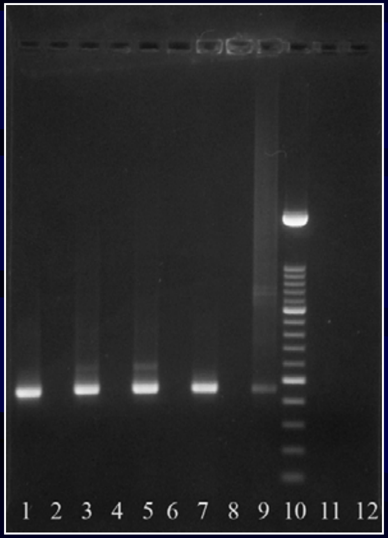


YES, Recurring die-offs → **Local Population Extinction**

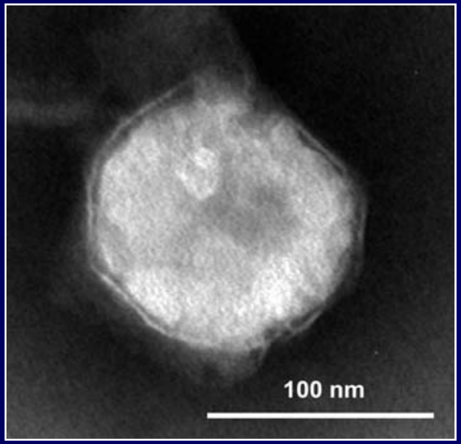
Greatest Threat: •Rare species
•Isolated Populations (no rescue effect)

Common Species: •Keep common species common!

Pathology and Ecology of Amphibian Ranaviruses



A. Cressler, USGS



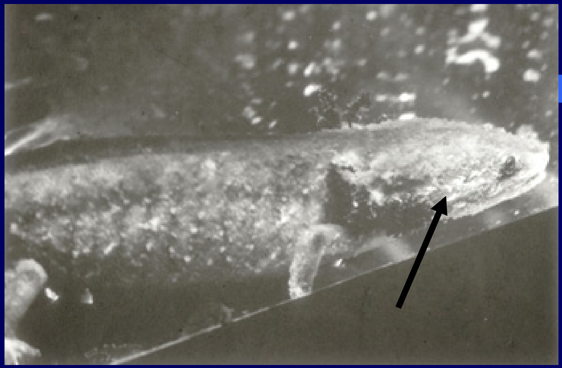
Ranavirus Characteristics

Docherty et al. (2003)

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

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

Jancovich et al. (1997)

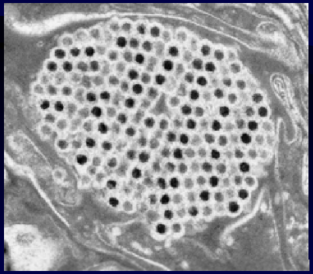


Chinchar et al. (2006)

Family: Iridoviridae

Genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Megalocytivirus*, and *Lymphocystivirus*

Invertebrates (*Iridovirus*, *Chloriridovirus*) — Ectothermic Vertebrates (*Ranavirus*, *Megalocytivirus*, *Lymphocystivirus*)

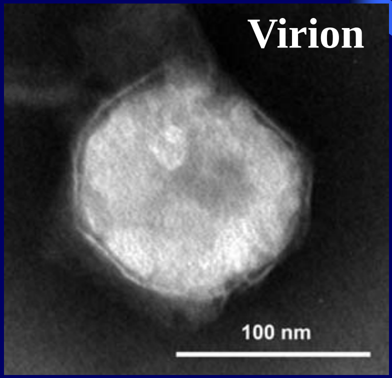


Paracrystalline Array

Species (6)

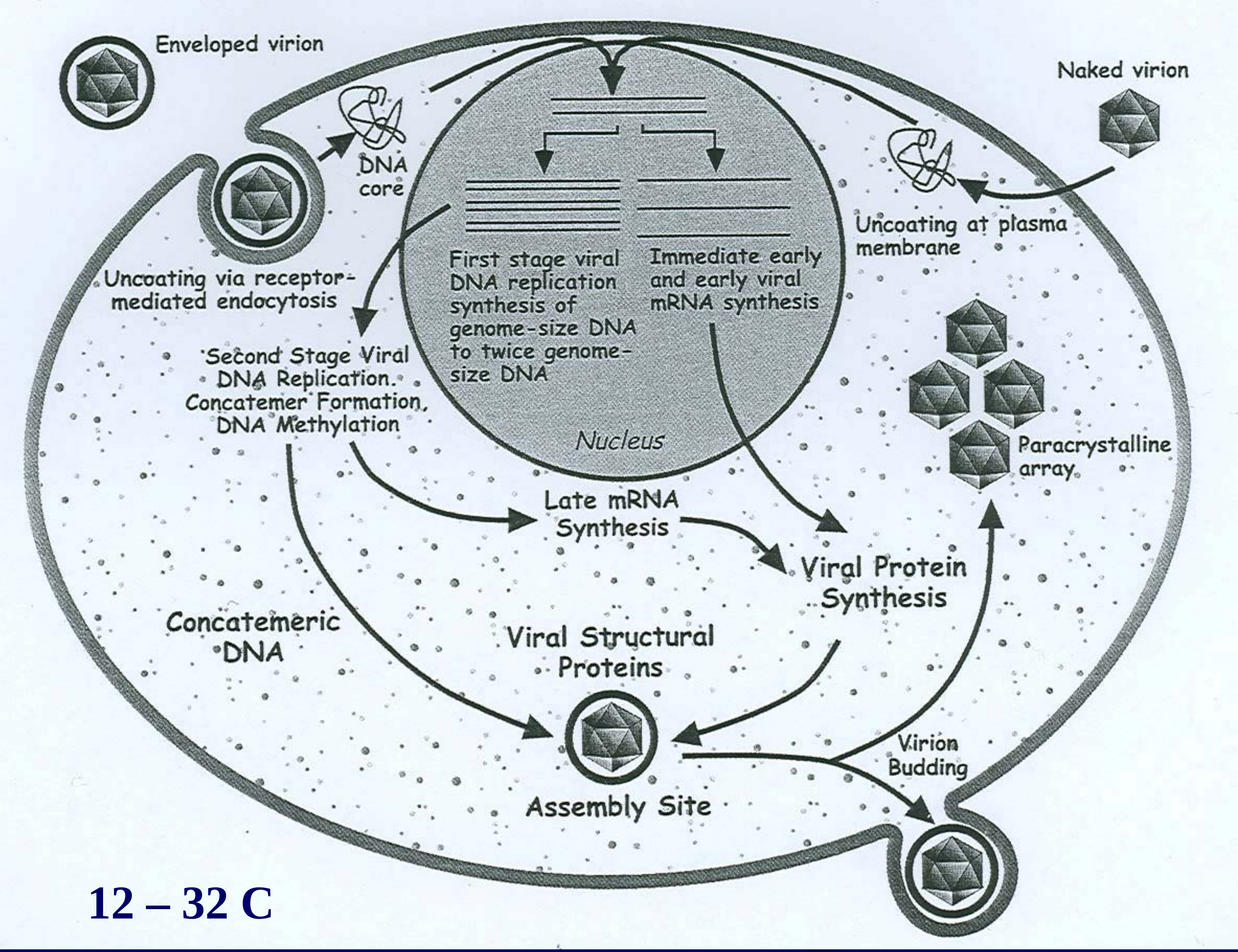
Amphibian Declines:

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



Ranavirus Replication Cycle

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

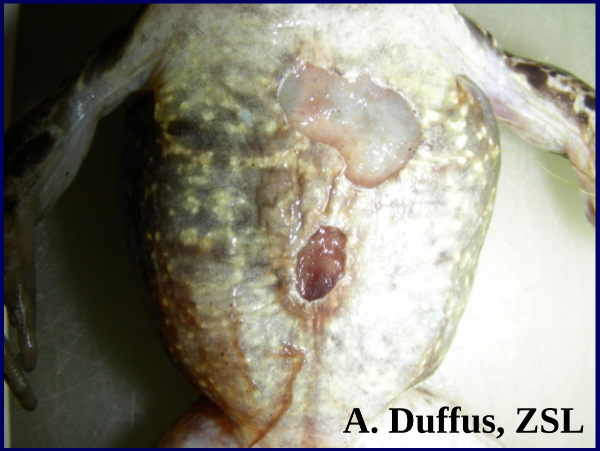


Protein synthesis within hours of infection

Cell death occurs within 6 – 9 hrs PI

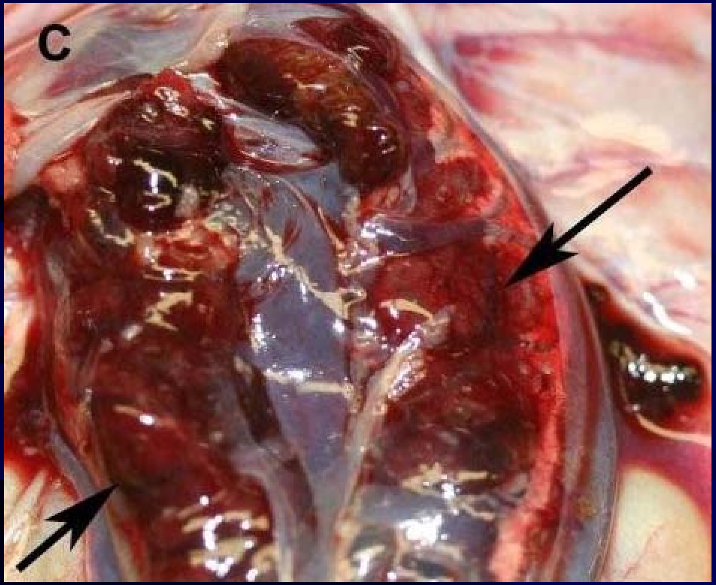
Ranavirus: Gross Signs

Edema, Erythema, Hemorrhages, Ulcerations



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

Humans
Ebola,
Anthrax,
Elephantiasis

There is no Cure!

Friday

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

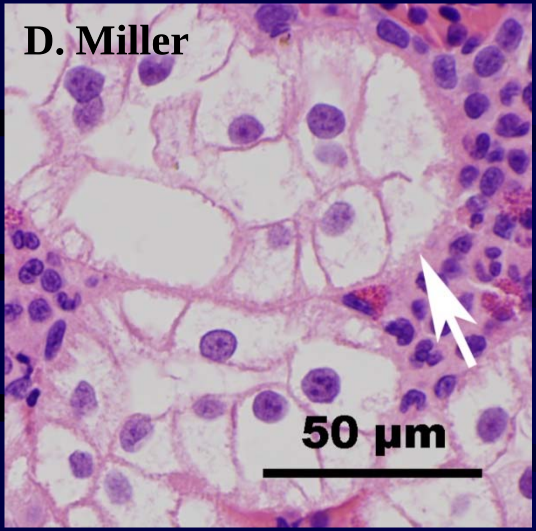
Sunday

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

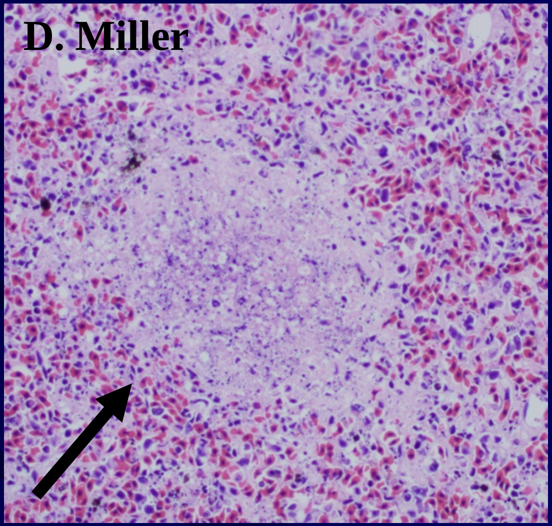
Ranavirus: Histopathological Signs

3 Primary Organs: Kidney, Liver and Spleen

Chinchar (2002),
Chinchar et al. (2003)



Kidney Degeneration



Spleen Necrosis

Viral Inclusions

Pathogenesis

Target Organ Failure

Heart Failure

Toxicosis, Anemia

Routes of Transmission

Oral inoculation

Ingestion

3 – 7 days

Necrophagy

Cannibalism

Water Bath
Contaminated Sediment

Time to signs: 1 – 2 weeks

Time to mortality: 1 – 3 weeks

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

Horizontal vs. Vertical:

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

Environmental Persistence (2-4 weeks)

Ectothermic Reservoirs

Reptiles and Fish



Are Amphibian Ranaviruses Lethal to other Ecothermic Vertebrates?

Reptiles:

Gopherus polyphemus ,Testudo hermanni, Terrapene carolina carolina, Trionyx sinensis, Uroplatus fimbriatus*, and *Chondropython viridis

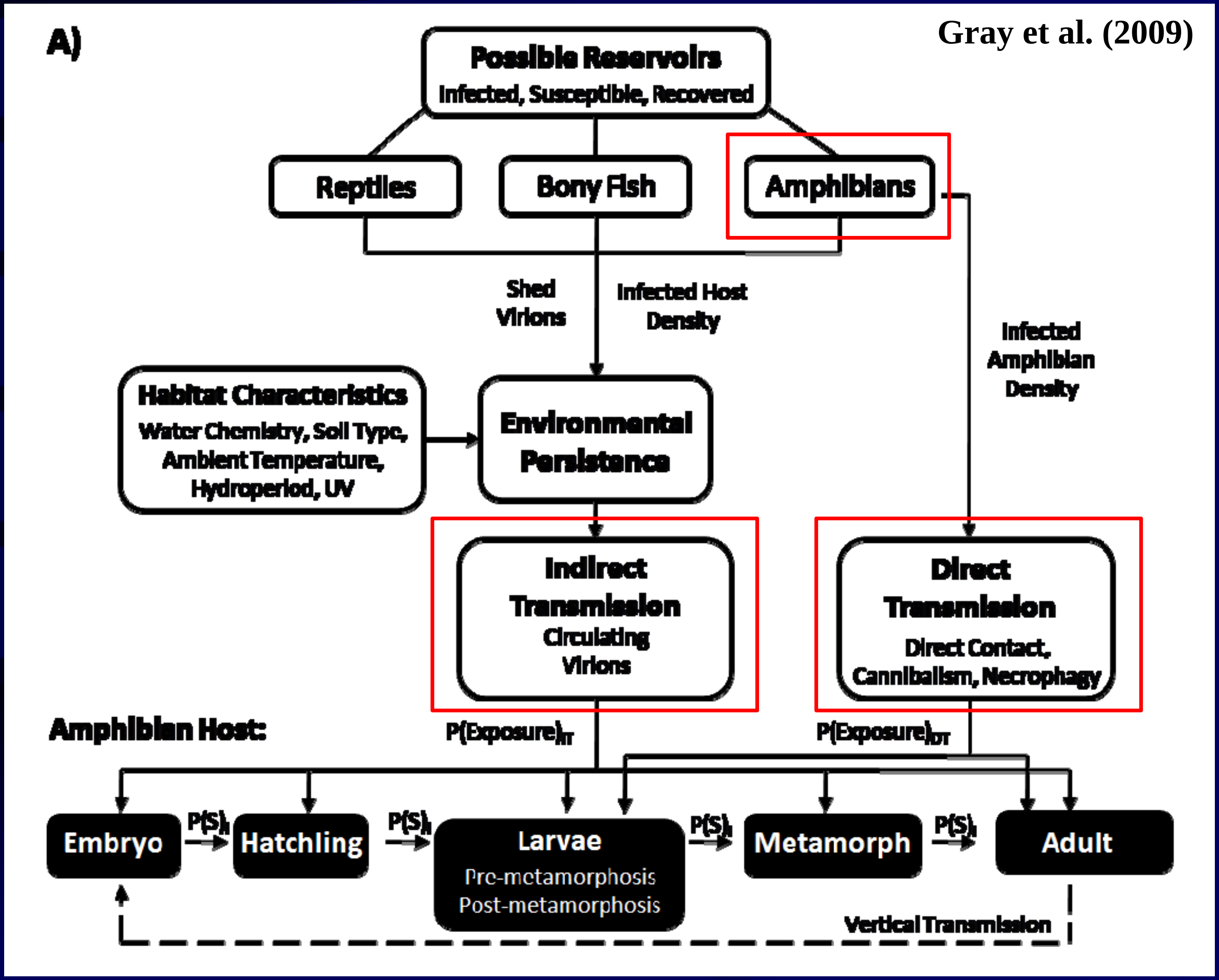
(Marschang et al. 1999, 2005; Hyatt et al. 2002; Allender et al. 2006)

Fish:

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


Blind Pony Hatchery

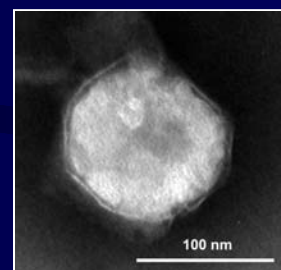
Ranavirus Ecology



Red = Well Studied

N. Haislip, University of Tennessee

Variation in Susceptibility to *Ranavirus* Among Species and Tadpole Developmental Stages

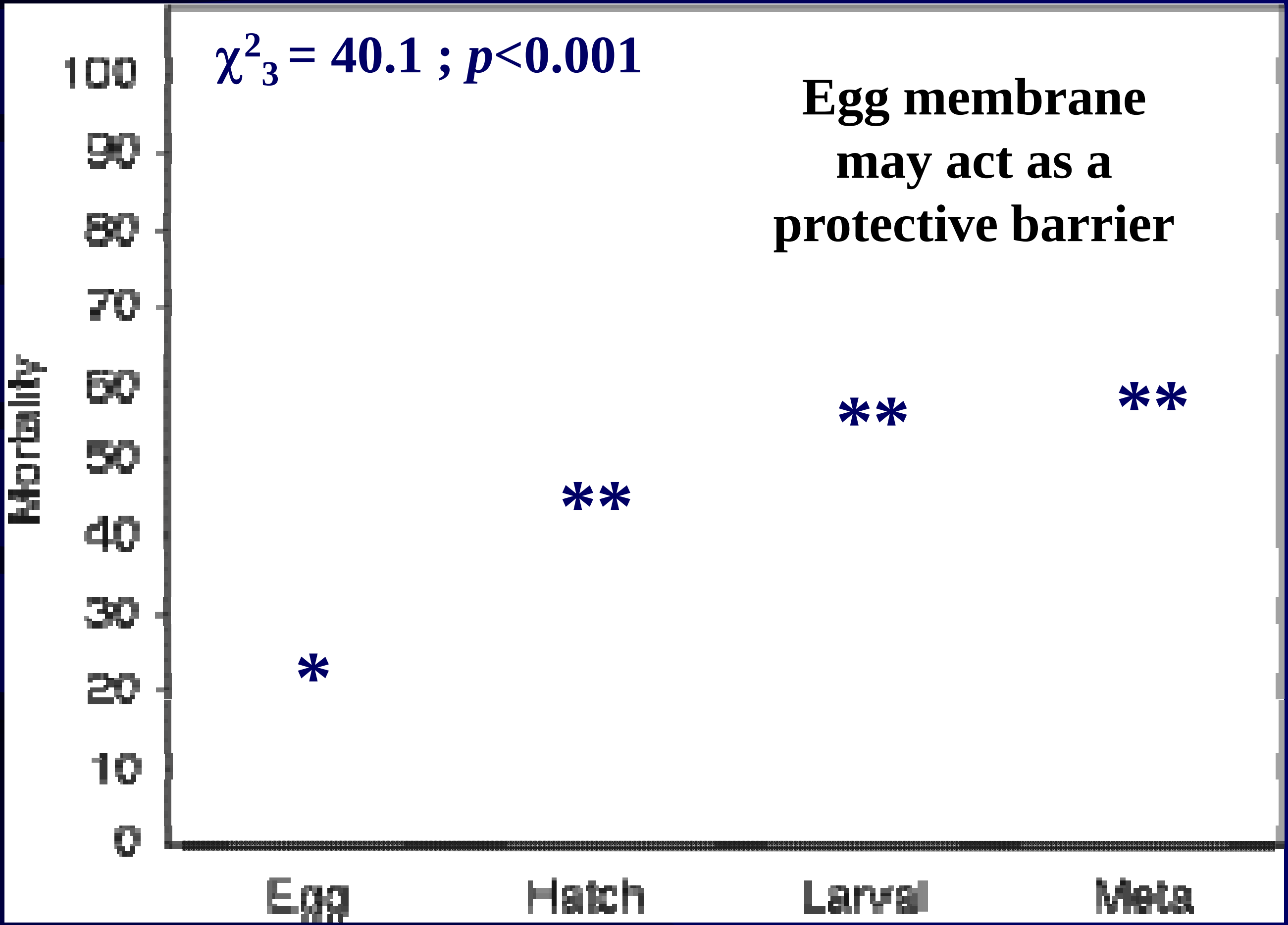


Nathan A. Haislip

M.S. Candidate

Stage Comparison

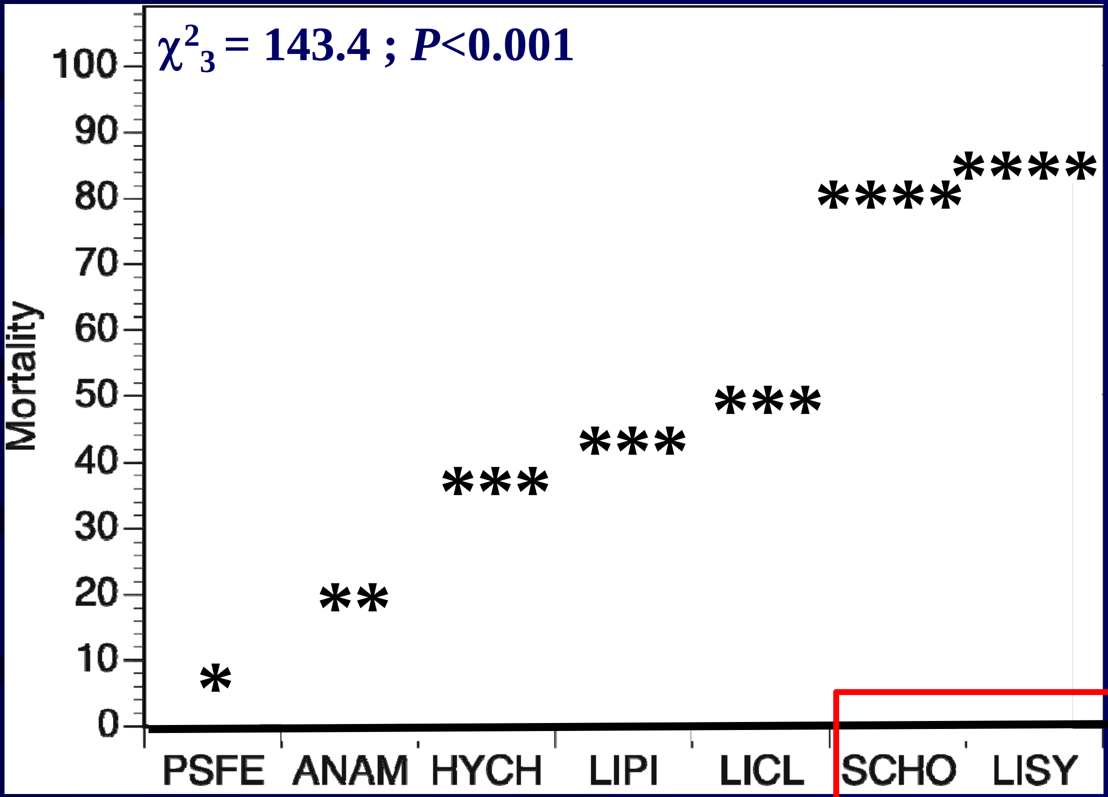
Across All Species



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

Species Comparison

Across All Development Stages



Logistic Analyses Species*Stage Interactions

Rapid Developers

ML Estimate:

A. americanus – 4X > *P. feriarum*

H. chrysoscelis – 10X > *P. feriarum*

L. pipiens – 13X > *P. feriarum*

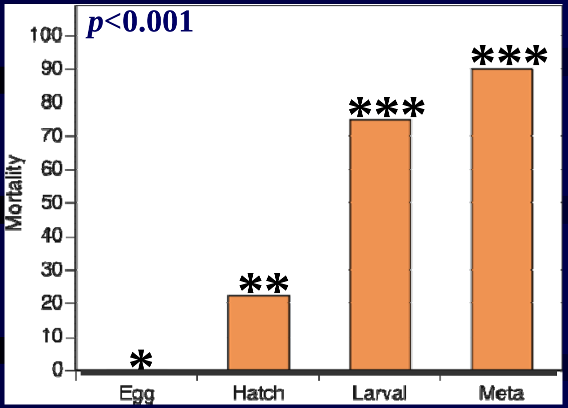
L. clamitans – 1.3X > *L. pipiens*, 16X > *P. feriarum*

S. holbrookii – 65X > *P. feriarum*

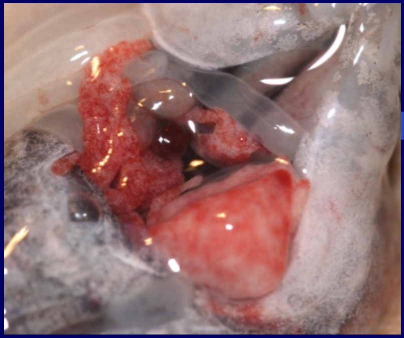
L. sylvaticus – 7X > *L. pipiens*

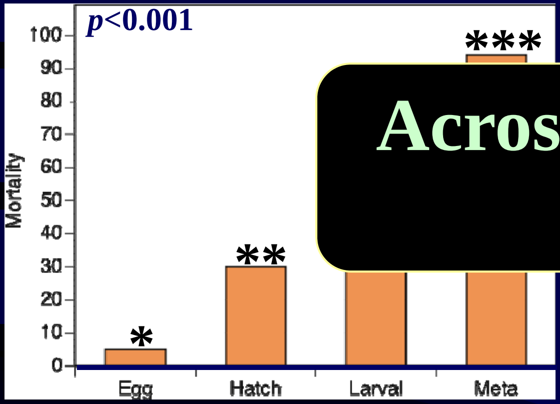
– 70X > *P. feriarum*

Ranids



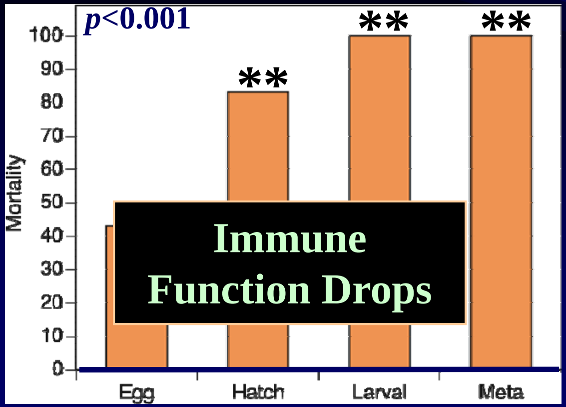
Lithobates clamitans





Lithobates pipiens

Across Ranids, Metamorph Stage - Most Susceptible

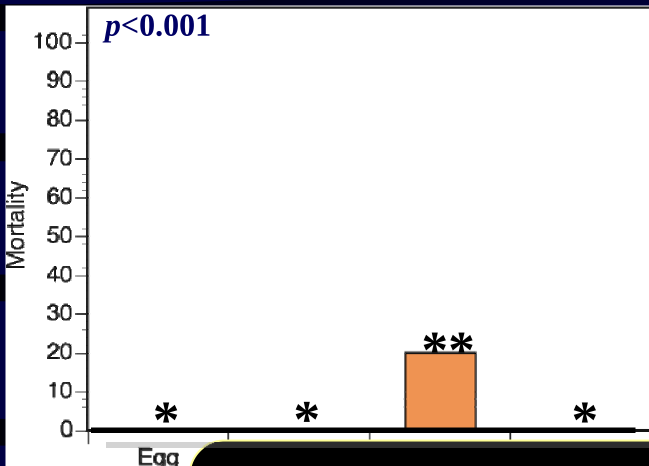


Immune Function Drops

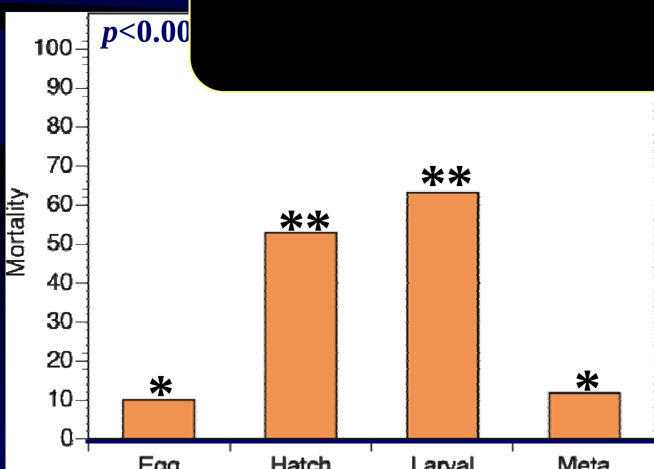
Lithobates sylvaticus

Hylids

Pseudacris feriarum

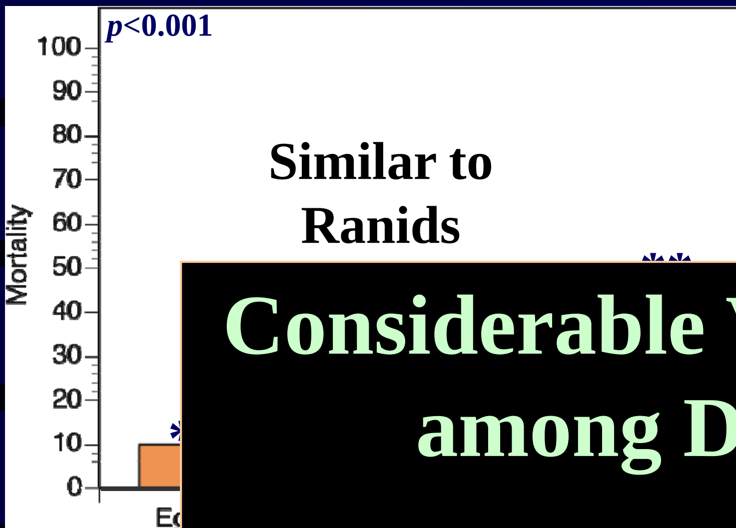


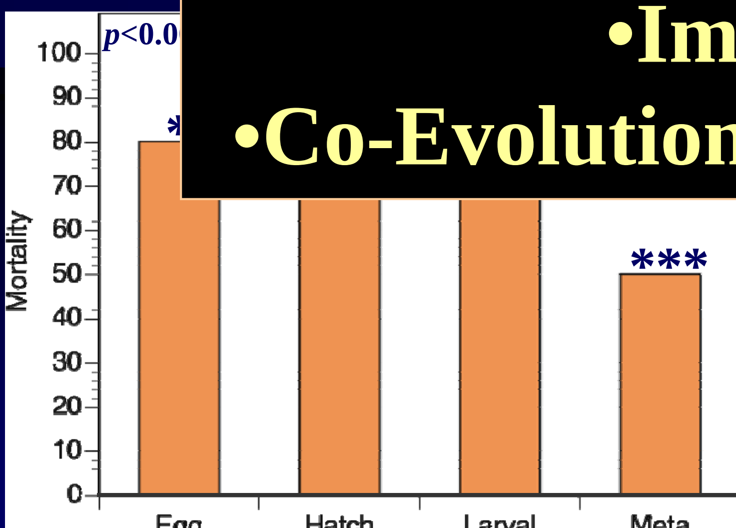
For HYCH, Hatchling and Larval Stages - Most Susceptible

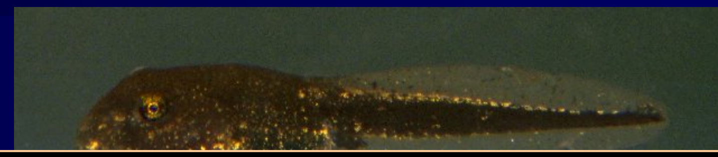


Toads

Anaxyrus americanus



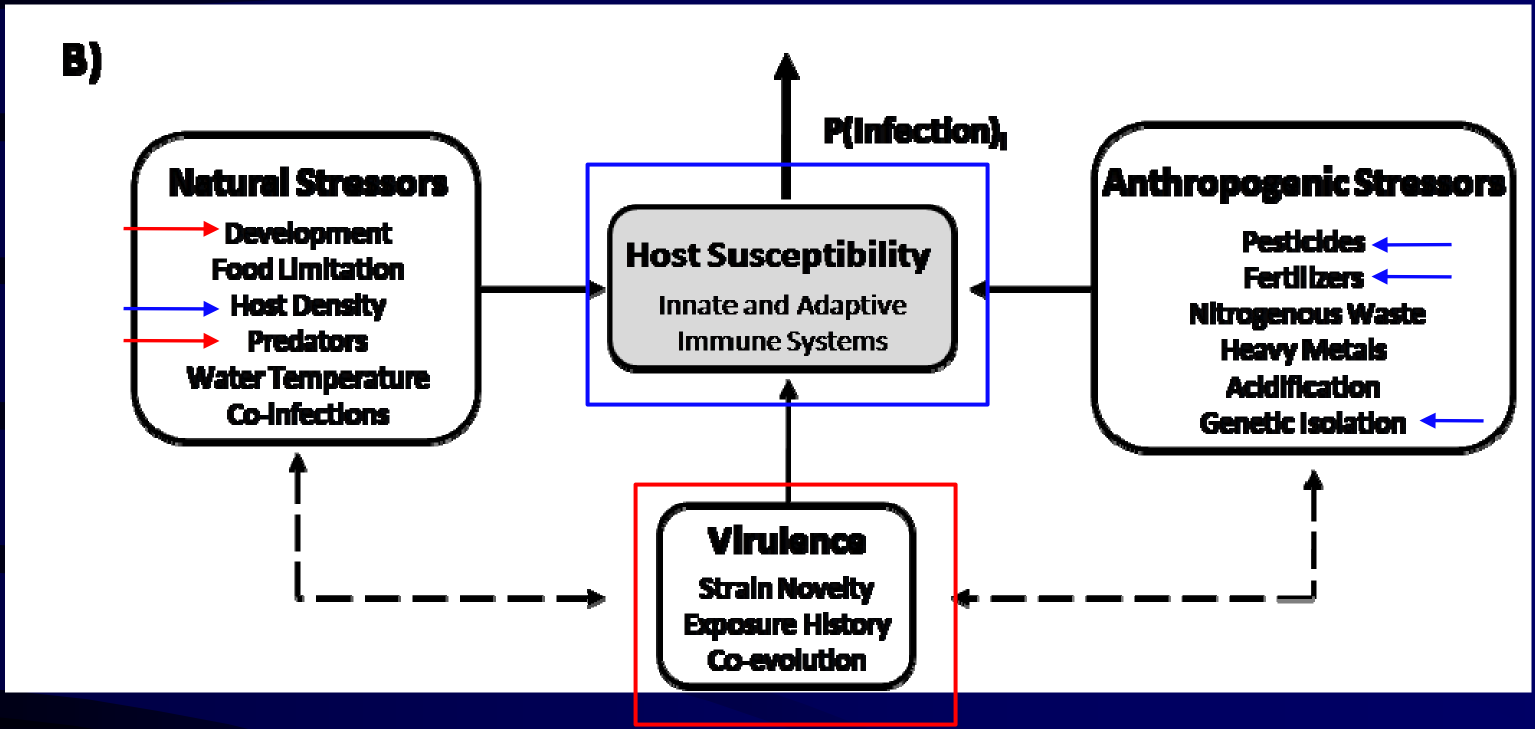




Considerable Variation in Susceptibility among Developmental Stages

- **Immune Function**
- **Co-Evolutionary or Ecological Factors**

Ranavirus Ecology



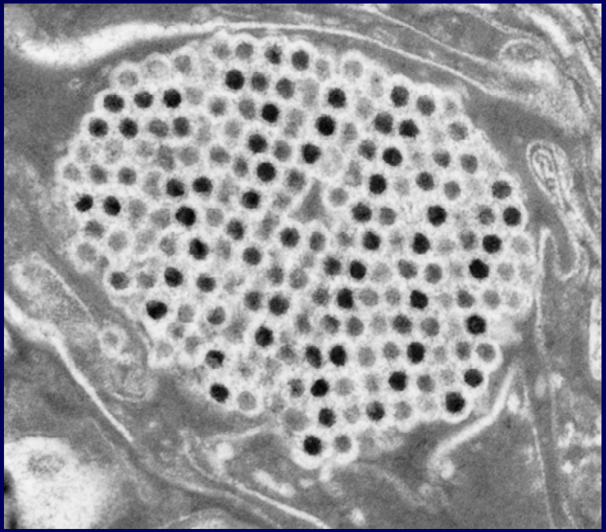
Red = Well Studied

Blue = More Studies Needed

No Color = Very Little Information

Gray et al. (2009)

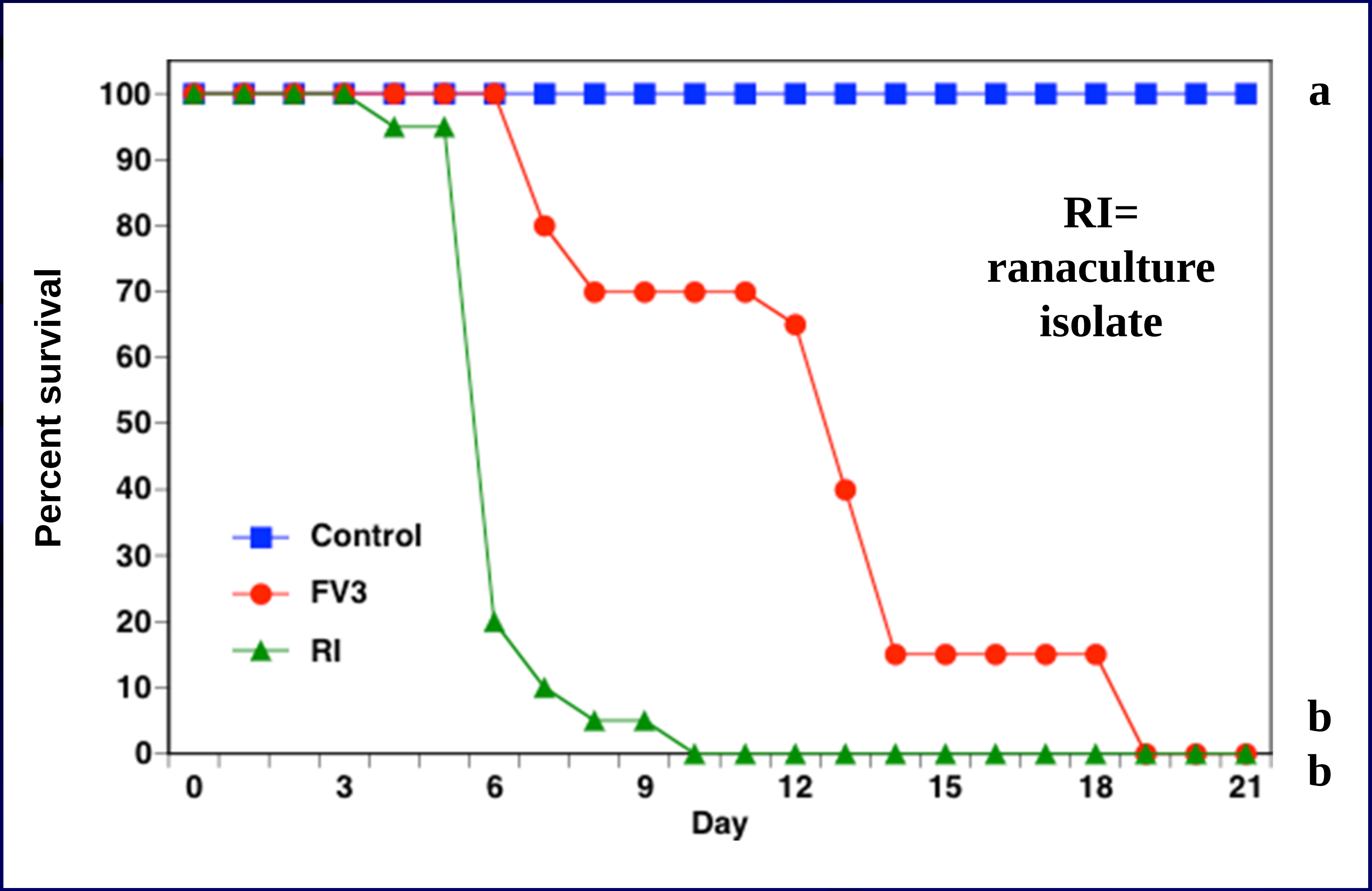
Pathogenicity of a Ranaculture *Ranavirus* Isolate



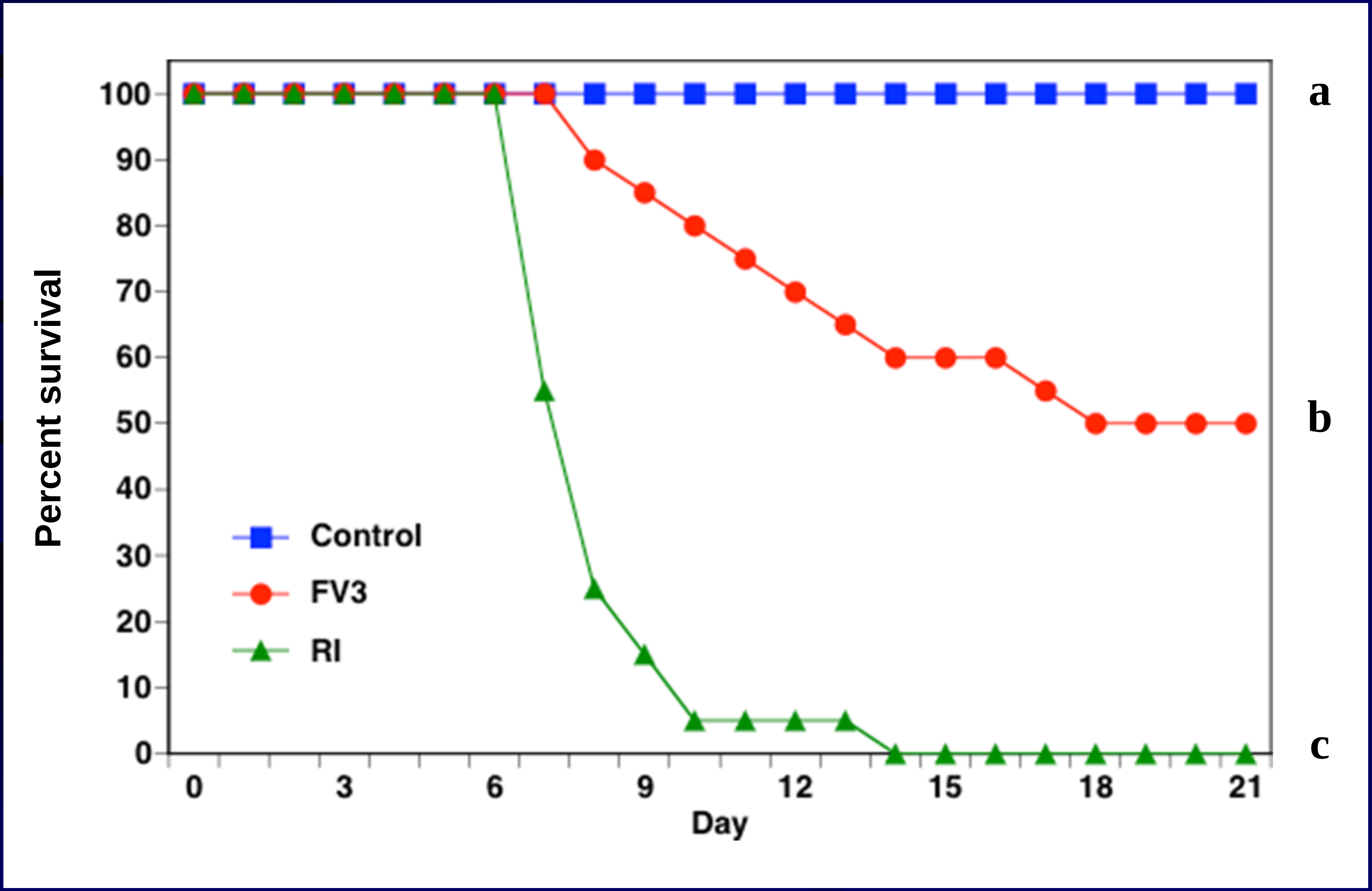
Jason T. Hoverman
Post-doctoral Research Associate

Wood Frog

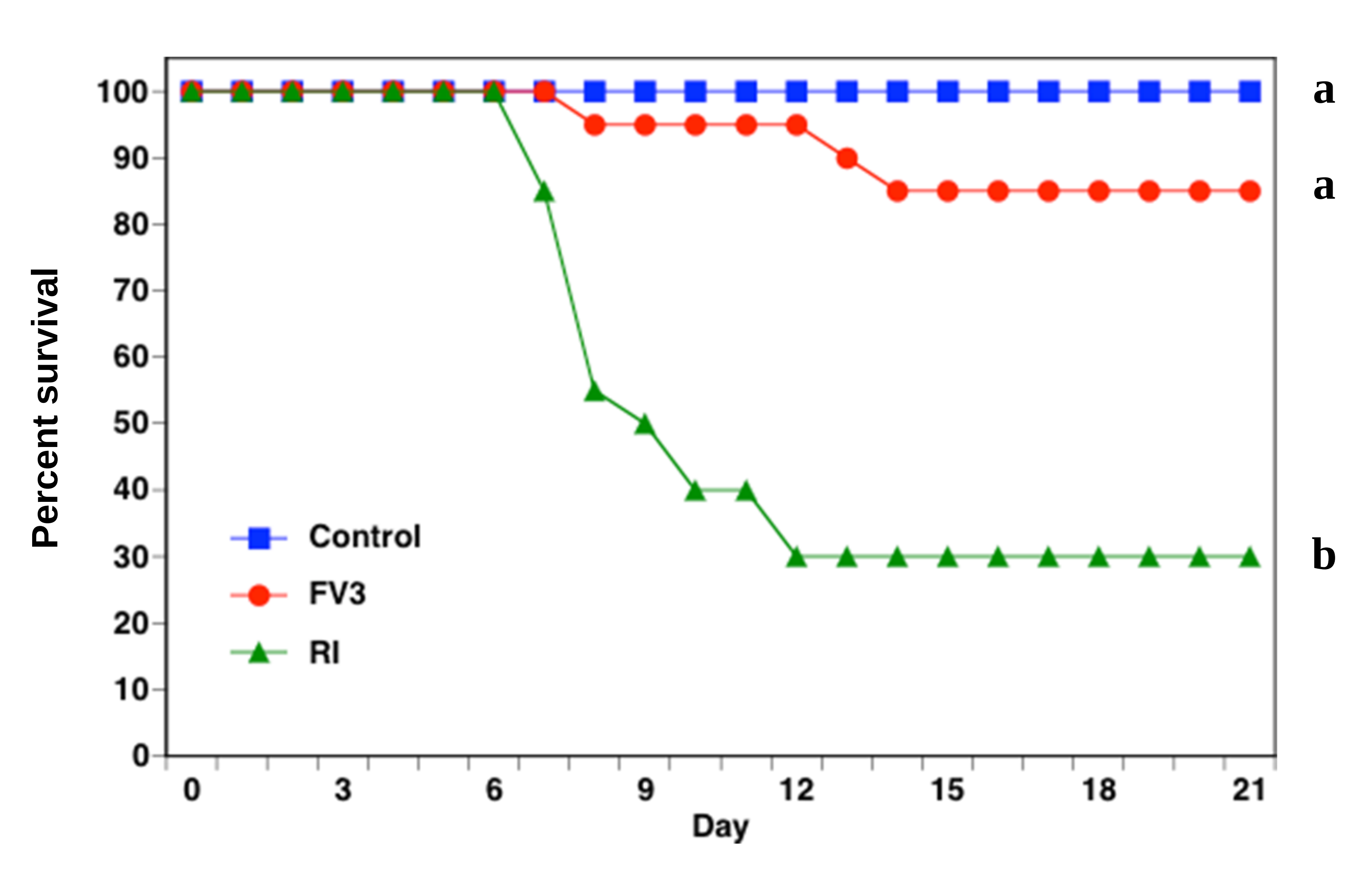
Pearson's chi-square test



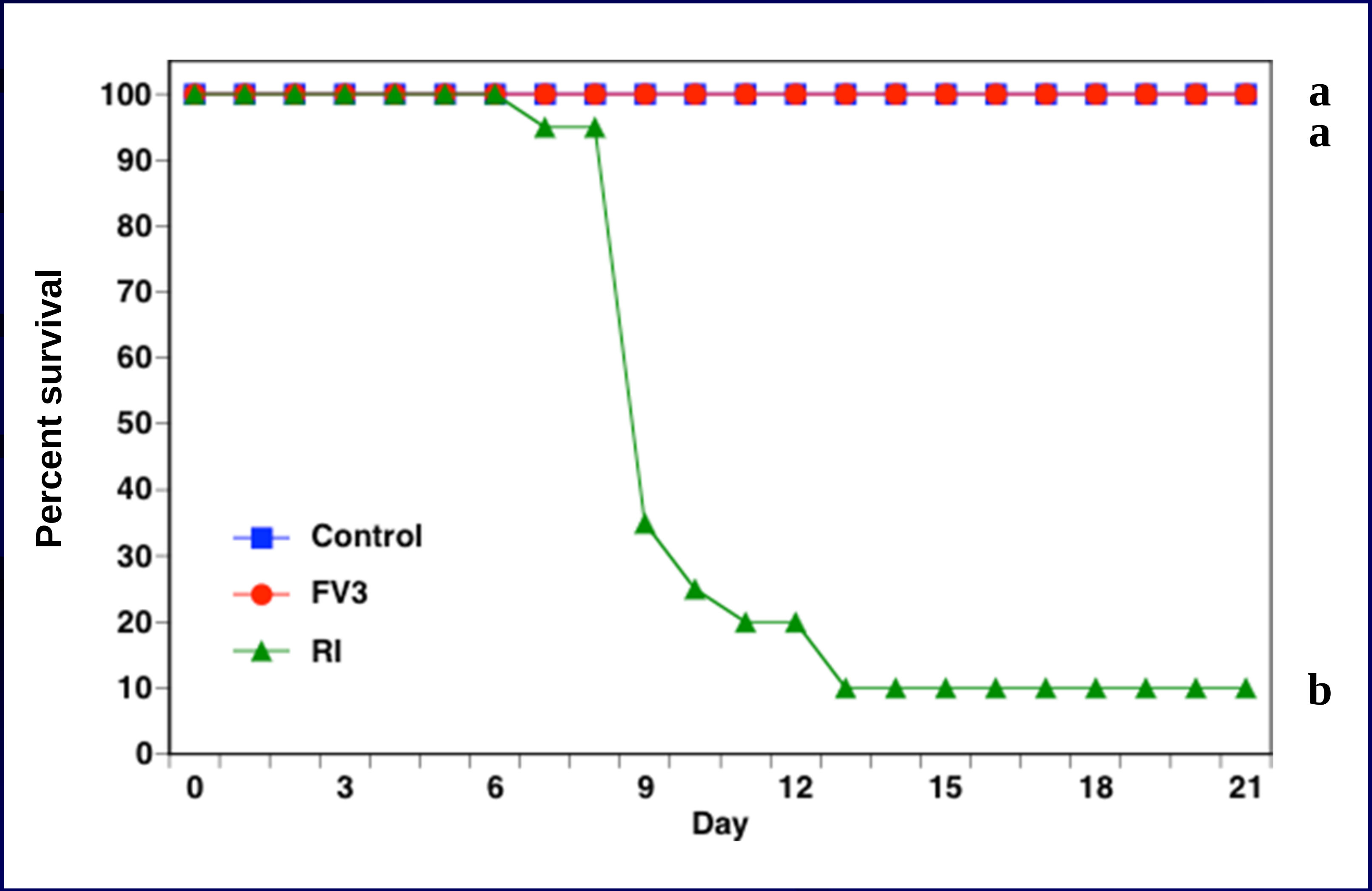
Southern Leopard Frog



Pickerel Frog

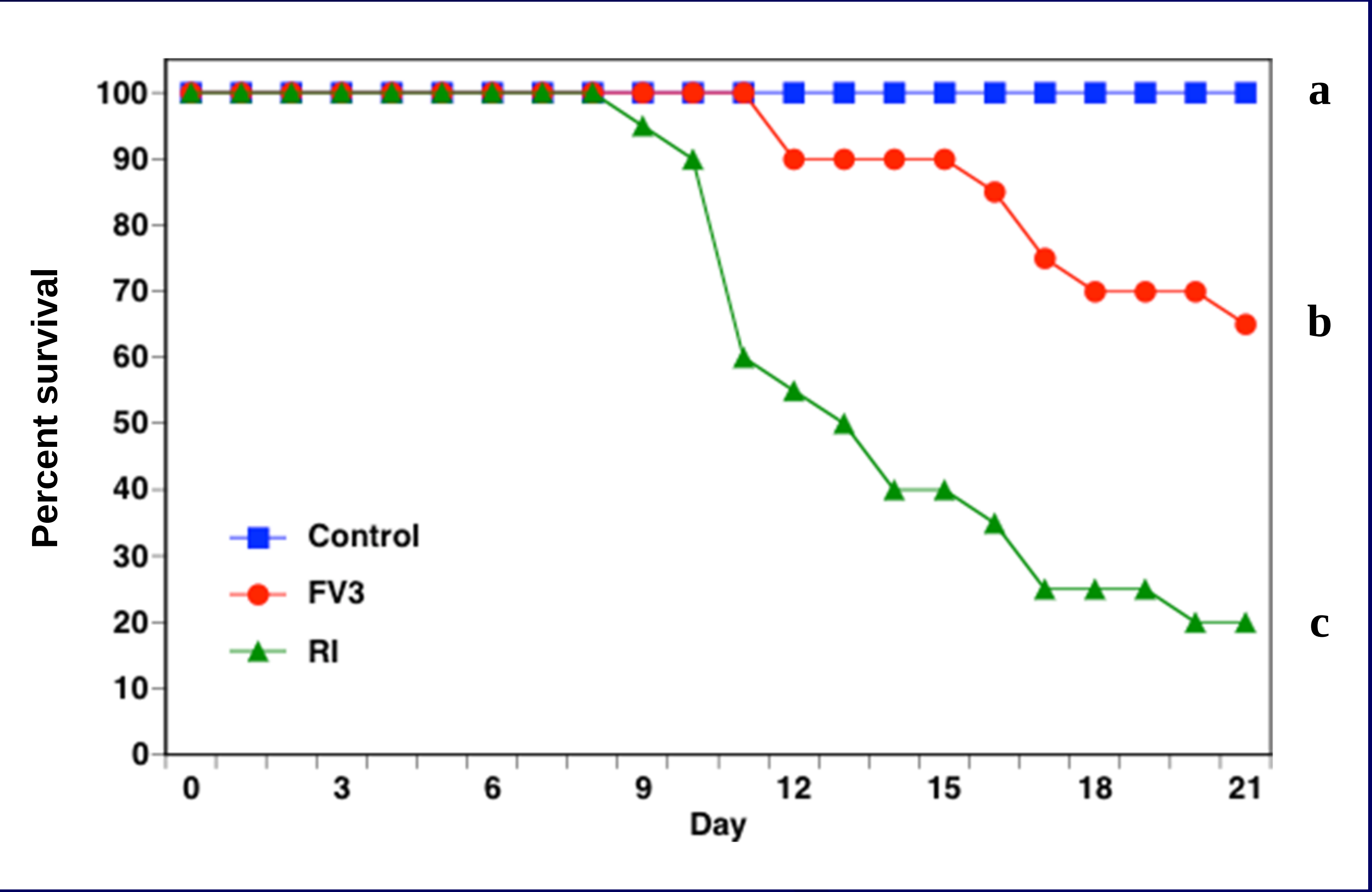


Green Frog

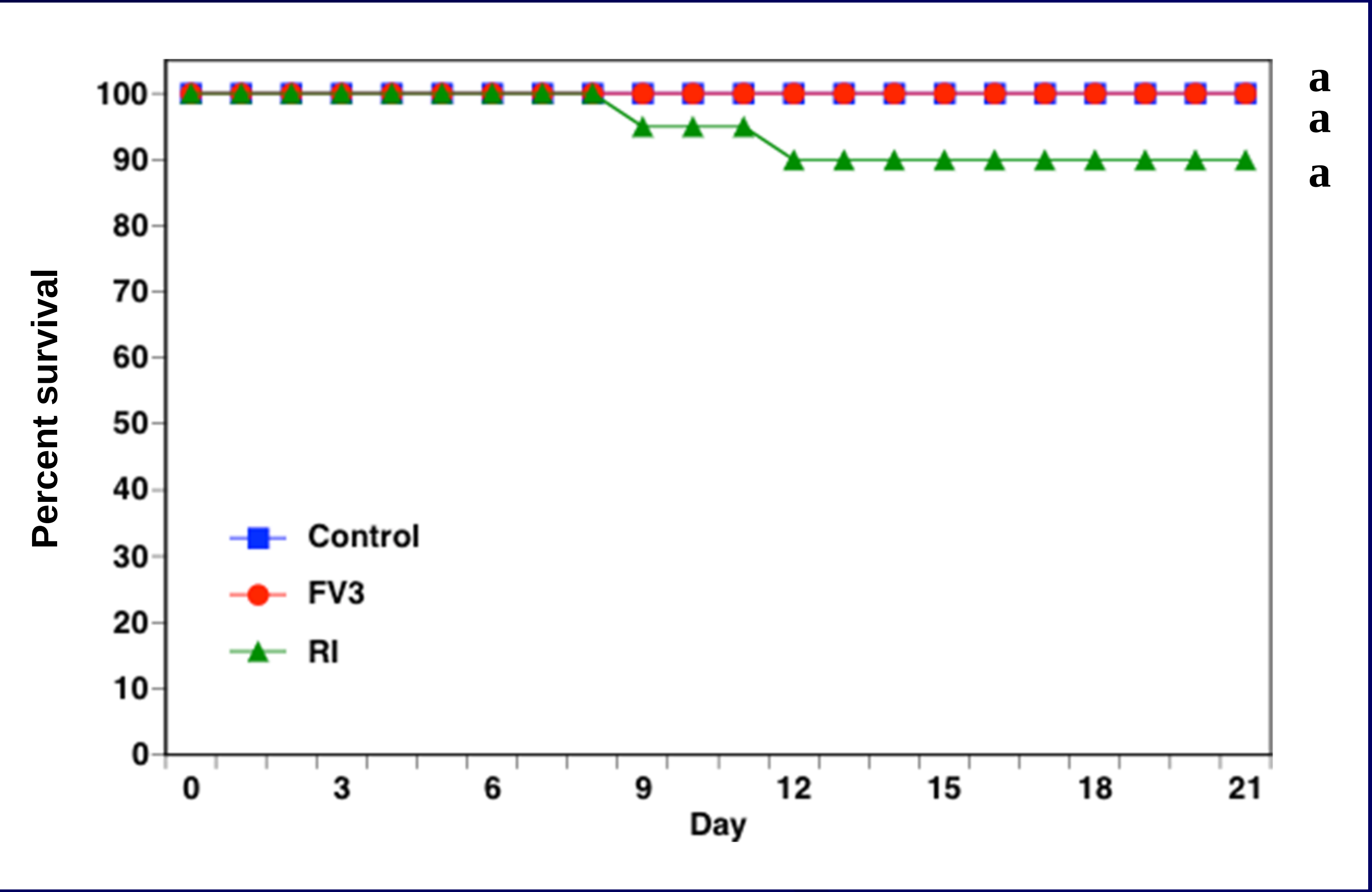


100
90
80
70
60
50
40
30
20
10
0
Percent survival
0
3
6
9
12
15
18
21
Day
Control
FV3
RI
a
a
b

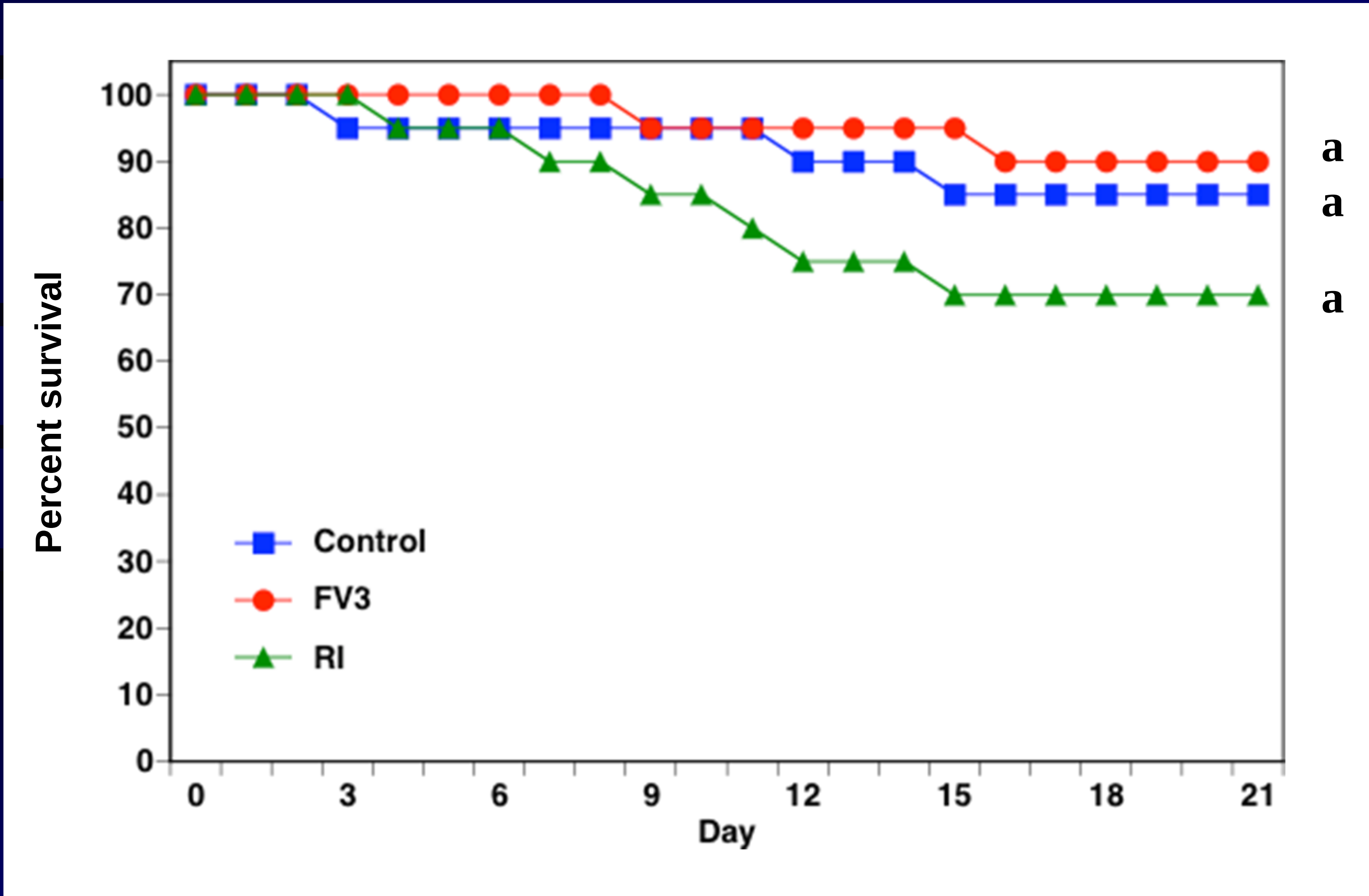
Cope's Gray Treefrog



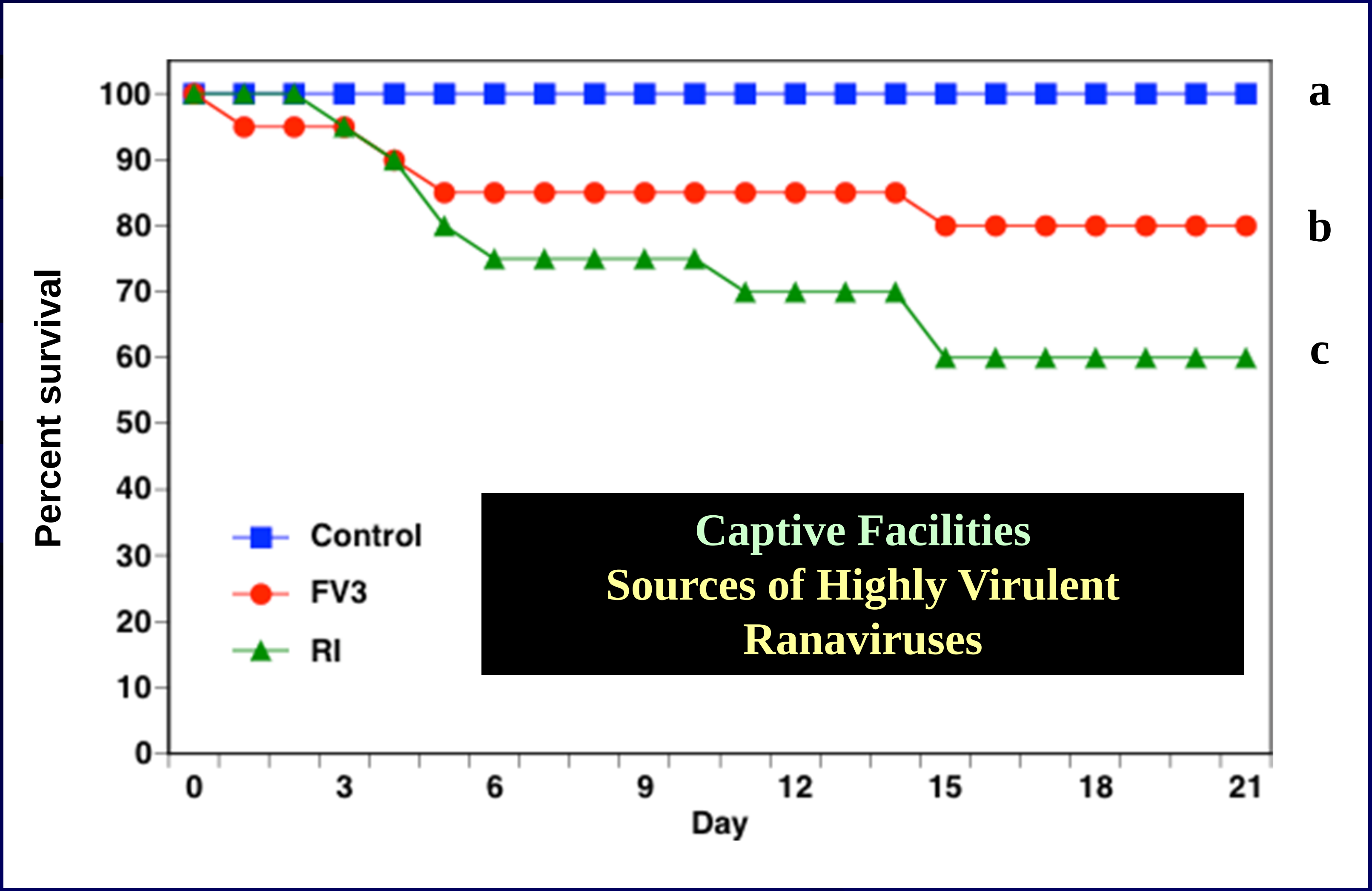
American Toad



Eastern Narrow-mouthed Toad



Eastern Spadefoot



Factors Contributing to Emergence

Pathogen Pollution: (Cunningham et al. 2003)

Anthropogenic introduction of novel strains to naïve populations

Picco et al. (2007)

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

Schloegel et al. (2009)

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

A. tigrinum

1) Herbicide (Atrazine)
   Fertilizer (sodium nitrate)
   → Leukocytes ↓ → ATV Susceptibility ↑ / Inconclusive

2) Cattle Land Use: FV3 Prevalence → 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

Notifiable Diseases

Certification for Shipment

Schloegel et al. (2010)

International Transport of Animals

Disinfection:

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

$50/ bottle

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

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



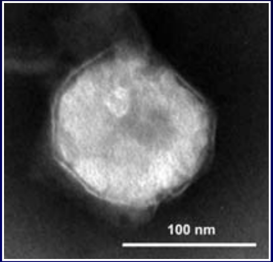


2) Pesticides: Atrazine, Carbaryl

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






i = species

j = age class

Pathogen Ecology

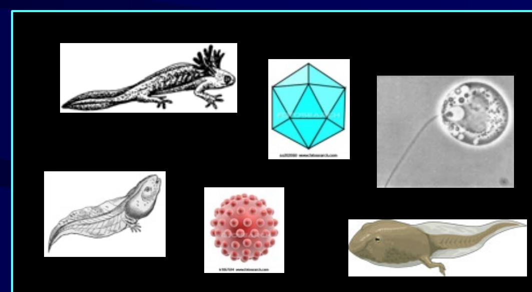
k = pathogen

l = wetland

Spatially Structured Breeding Sites

$EM(t)_{ijkl}$

Host-Pathogen Community



$I(t)_{ijkl}$

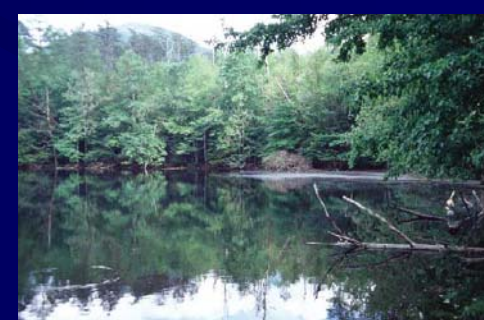
$I(t|k)_{ijkl}$

$S(t)_{ijkl}$

$S(t|I)_{ijkl}$

$IM(t)_{ijkl}$

$P(N_t)_{il} > 0$



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Questions??

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