# Ecology of Ranaviruses:
## A State of Understanding




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UF Infectious Diseases and Pathology

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Unpublished Data

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## Outline

I. Ranavirus-Host Characteristics

II. Ecology: Species to Communities

III. Effects of Stressors

IV. Commercial Trade & Pathogen Pollution

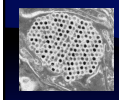
# Ranavirus Characteristics


- 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

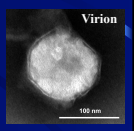


Paracrystalline Array

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)

# How Does Ranavirus Infect A Host?

## Routes of Transmission

**Indirect Transmission**


Skin, Gills, Intestines (epithelial cells)

**Direct Contact**

One Second Skin Contact

**Ingestion**

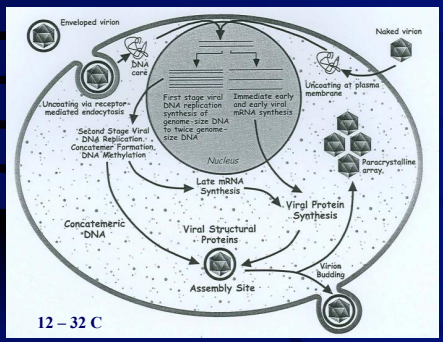
Incidental, Necrophagy, Cannibalism, Predation

(Mortality 2X Faster)

Brunner et al. (2004), Harp & Petranka (2006), Brunner et al. (2007), Hoverman et al. (2010)

# Ranavirus Replication Cycle

Chinchar (2002), Chinchar et al. (2006), Robert et al. (2011)




Viral Transcription within 3 hours of exposure

Cell death occurs within 6 – 9 hrs PI

## Gross Signs of Infected Amphibians

Edema, Erythema, Hemorrhages, Ulcerations

A. Duffus, Gordon

N. Haislip, UT

D. Green, USGS

## Signs Vary Among Species

Haislip, Miller, and Gray (unpubl. data)

*Lithobates clamitans*

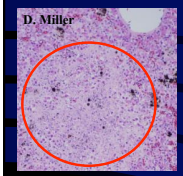
*Hyla chrysoscelis*

*Lithobates sylvaticus*

## Organ Destruction

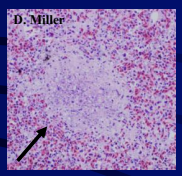
3 Primary Organs: Liver, Spleen, and Kidney

Bollinger et al. (1999)
Miller et al. (2007, 2008)



D. Miller

Liver Necrosis



D. Miller

Spleen Necrosis


D. Miller

Kidney Degeneration

**Pathogenesis**

Target Organ Failure
Heart Failure
Toxicosis, Anemia

Mortality Can Be Rapid!

Quickly as 3 days!

Hoverman et al. (2011a)

## Maine 2013 Die-off

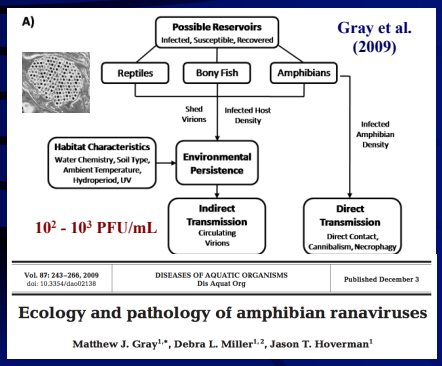
1000 carcasses/m$^2$
≥200,000 dead
qPCR Confirmed

Wheelwright et al. (in review)

<24 hrs

6/14/13 6/15/13

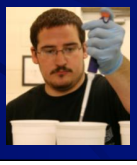
## What Mechanisms Lead to Outbreaks?



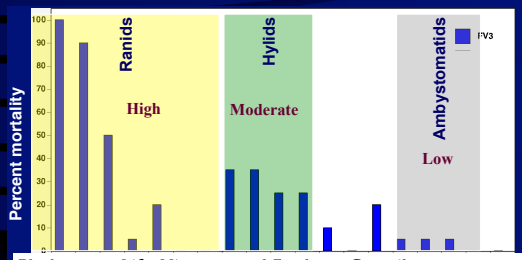

Gray et al. (2009)

## Species Challenges
### FV3-like Ranaviruses

The FUTURE of Veterinary Medicine



## Single-species FV3-like Challenges




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

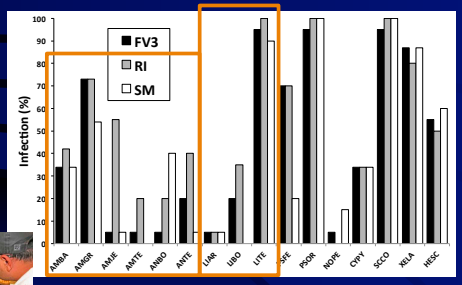
Hoverman et al. (2011a): 19 Species Tested

## Single-species FV3-like Challenges

15 Additional Species

### Amphibians

Brenes (2013)



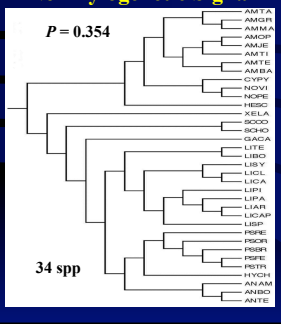

Ambystomatidae Ranidae

## Life History and Phylogeny

### Amphibians

Brenes (2013)

No Phylogenetic Signal




All Three Isolates

- Fast development hatching time*
- Low aquatic index
- Breeding habitat (temporal)*
- Breeding time (spring)

Smoky Mountains Isolate

- Distance: Population & Isolate

Co-evolution

Storfer et al. (2007)

## Single-species FV3-like Challenges

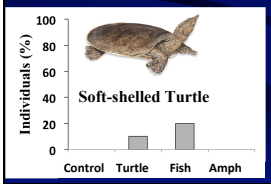
### Chelonians

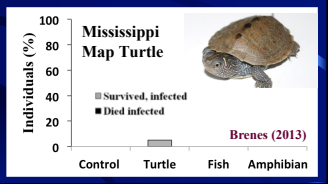
*Terrapene carolina, T. ornata, Elseya latisternum, Emydura krefftii , Trachemys scripta*

**Greatest infection and morbidity with IP injection or oral inoculation.**

**Water bath exposure sufficient for transmission with some species.**

Ariel (1997), Johnson et al. (2007), Allender (2012), Waltzek, Gray, Miller (unpubl. data)




## Single-species FV3-like & ATV Challenges

### Fishes

**No Transmission:**

*Cyprinus carpio, Carassius auratus, Lepomis cyanellus*

Jancovich et al. (2001), Bang Jensen et al. (2011a)

**Low Transmission:**

*Amelurus melas, Esox luscious, Sander lucioperca, Micropterus salmoides*

Gobbo et al. (2010), Bang Jensen et al. (2009, 2011b), Picco et al. (2010)

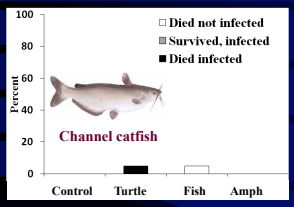
**High Mortality:**

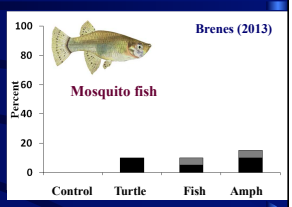
*Scaphirhynchus albus*

Waltzek et al. (in review; DAO)

## Single-species FV3-like Challenges

### Fishes



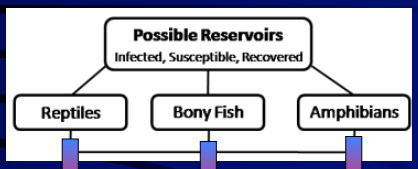




**No Transmission:** tilapia, bluegill and fathead minnow

**Limitation:** Density dependence (transmission/stressor)

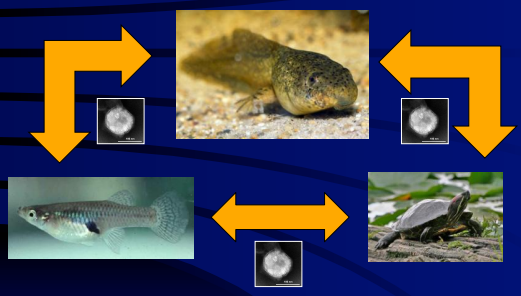
## Reservoirs or Amplification Hosts?

### FV3-like Ranaviruses




| Low Mortality (Subclinical) | Low Mortality (Subclinical) | Low – High Mortality (Subclinical & Clinical) |
|---|---|---|
| Reservoir | Reservoir | Reservoir or Amplification |

## Can Interclass Transmission Occur?



Host range, host specificity and hypothesized host shift events among viruses of lower vertebrates **Bandin & Dopazo (2011)**

VETERINARY RESEARCH

## Evidence from the Wild

**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 (2013)**

## Evidence of Interclass Transmission

### Bayley et al. (2013)

Pike-perch Iridovirus

Common Frog Tadpoles

**Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts**

| Vol. 103: 171–183, 2013<br>doi: 10.3354/dao02574 | DISEASES OF AQUATIC ORGANISMS<br>Dis Aquat Org | Published April 11 |
|---|---|---|

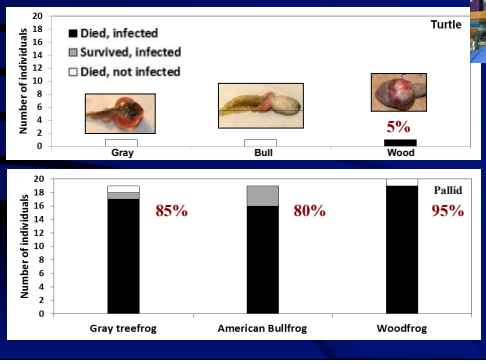
Frog Virus 3 → Pike, Pike-perch, Black Bullhead

Bang Jensen 2009, 2011; Gobbo et al. 2010

## Evidence of Interclass Transmission

0% mortality in controls

### Waltzek, Gray, and Miller




## Evidence of Interclass Transmission

0% mortality in controls

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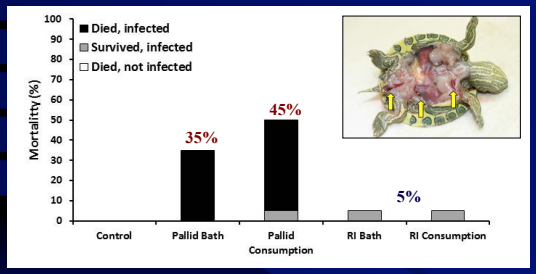
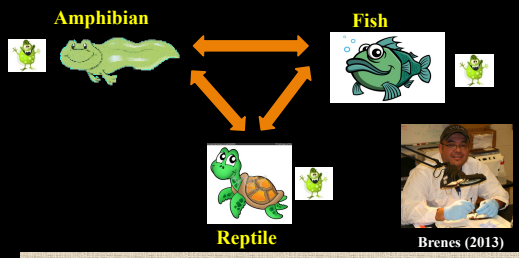



**Pallid Isolate Caused Mortality; Bullfrog Isolate Resulted in Infection**

## Interclass Transmission

### Sympatric Ectothermic Vertebrate Species



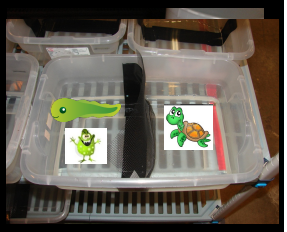



Brenes (2013)

**Can ranavirus move among host species?**

## Experiment

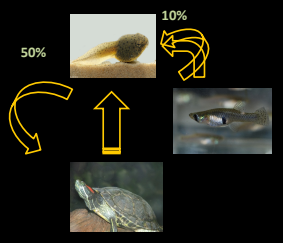
- Direct exposure
  - Exposed to $10^3$ PFU/mL
  - 3 days
- 12-L containers divided in half by a 2000 µm plastic mesh
- Different species in each side of the container



## Turtle and Fish Results

Brenes et al. (in review; PLoS ONE)

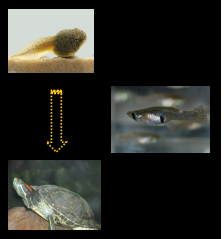
- All classes tested can transmit the virus
- Turtles infected tadpoles
  - 50% mortality
- Fish infected tadpoles
  - 10% mortality



# Amphibian Results

Brenes et al. (in review; PLoS ONE)

- Amphibians transmitted to turtles but not fish
- No mortality of turtles or fish exposed to infected tadpoles
- Supporting that turtles and fish may be reservoirs of ranavirus
- Amphibians may be amplifying species



# Superspreaders and Amplifying Species

Paull et al. (2012)

Persistence | Dispersal | Host Community | Contact Rate

Disease Hotspots

•Gahl and Calhoun (2010)
•Uycharaet al. (2010)
•Brunner et al. (2011)

•Green et al. (2002)
•Petranka et al. (2003)
•Harp and Petranka (2006)

Superspreading Individuals: Shedding Rate | Contact

Amplification Species: Susceptibility | Contact Rate

REVIEWS REVIEWS REVIEWS

From superspreaders to disease hotspots: linking transmission across hosts and space

Sara H Paull[1*], Sejin Song[1], Katherine M McClure[1,2], Loren C Sackett[1], A Marm Kilpatrick[3], and Pieter TJ Johnson[1]

2012

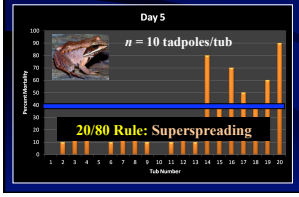
Frontiers in Ecology and the Environment 10:75-82

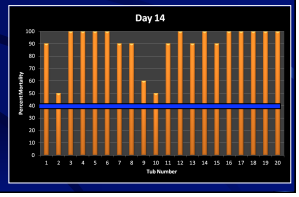
# Ranavirus Superspreaders

Reilly, Gray, & Miller (unpubl. data)

6 hrs cohabitation

3-day $10^3$PFU/mL



## Community Level Transmission

Brenes, Gray, & Miller (unpubl. data)

Inoculated in Lab
$10^3$ PFU/mL FV3
Exposure Order

Appalachian: Wood frog, chorus frog, spotted salamander
Coastal Plains: Gopher frog, chorus, southern toad

**Does Exposure Order or Composition Matter?**

## Exposure Order Matters

Brenes (2013)

**Design**

*n* = 5 pools/trt
10 larvae/spp
60 days

**Exposure Treatments**

Only Wood Frogs
Only Chorus Frogs
Only Spotted Salamanders
Control

**Appalachian Community**

| | | | | |
|---|---|---|---|---|
| Wood Frogs | 100% (high) | 43% | Chorus Frogs | 52% |
| | | 12% | Spotted Salam | |
| Chorus Frogs | 44% (mod) | 72% | Wood Frogs | 40% |
| | | 3% | Spotted Salam | |
| Spotted Salam | 6% (low) | 24% | Chorus Frogs | 16% |
| | | 18% | Wood Frogs | |

## Community Composition Matters

Brenes (2013)

**Design**

*n* = 5 pools/trt
10 larvae/spp
60 days

**Exposure Treatments**

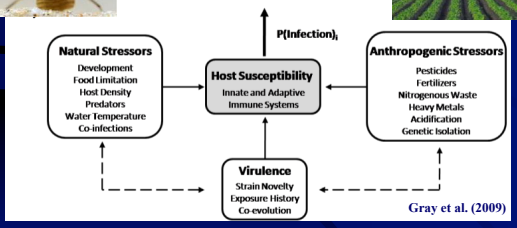
Only Gopher Frogs
Only Chorus Frogs
Only Southern Toad
Control

**Gulf Coastal Plain, USA**

| | | | | |
|---|---|---|---|---|
| Gopher Frogs | 100% (high) | 52% | Chorus Frogs | 62% |
| | | 34% | Southern Toad | |
| Chorus Frogs | 78% (high) | 70% | Gopher Frog | 68% |
| | | 58% | Southern Toad | |
| Southern Toad | 76% (high) | 32% | Chorus Frogs | 62% |
| | | 80% | Gopher Frog | |

## Impacts of Stressors



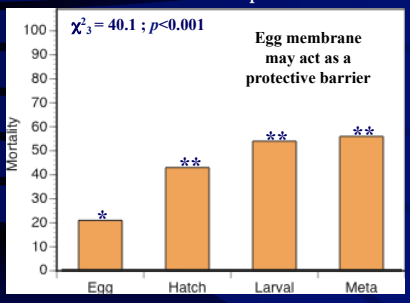



Gray et al. (2009)

## Impacts of Development

Across Seven Species

Haislip et al. (2011)



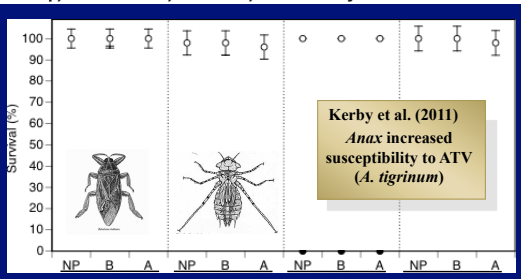

ML Estimate: Hatchling – 3X > Embryo
Larval – 4X > Embryo
Metamorph – 5X > Embryo

PLoS one
July 2011 | Volume 6 | Issue 7 | e22307

## Natural stressors and disease risk: does the threat of predation increase amphibian susceptibility to ranavirus?

Can. J. Zool. **90**: 893–902 (2012)

N.A. Haislip, J.T. Hoverman, D.L. Miller, and M.J. Gray




Kerby et al. (2011)
*Anax* increased susceptibility to ATV (*A. tigrinum*)

## Competing Temperature Hypotheses

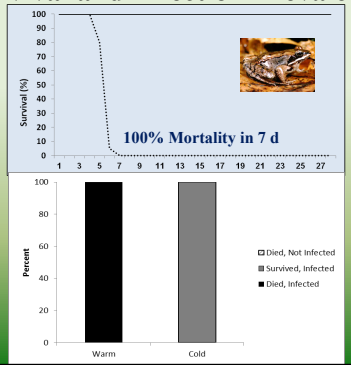
- **Virus Replication Hypothesis** Bayley et al. (2013)
  - Ranavirus replication increases with temperature up to 32 C **High Pathogenicity at Higher Temperatures**
- **Temperature Induced Stress Hypothesis**
  - Early Spring Breeding Species:
    - Stressed by Warm Temp
  - Summer Breeding Species:
    - Stressed by Cold Temp

**Pathogenicity is Species-specific and Related to Typical Water Temperature Experienced During Tadpole Development**

M. Brand

## Wood Frog
### Survival and Infection Prevalence

No Control Mortality




Warm
Cold

Brand et al. (unpubl. data)

Clinical

$\bar{x}_{PFU}$ = 152484

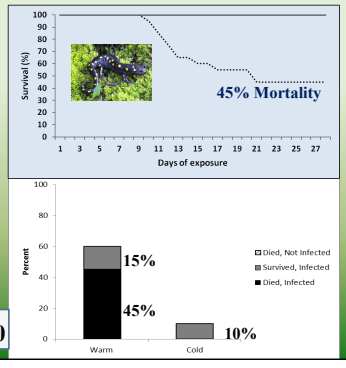



Subclinical

$\bar{x}_{PFU}$ = 84

## Spotted Salamander
### Survival and Infection Prevalence

No Control Mortality




Warm
Cold

Brand et al. (unpubl. data)

Clinical

$\bar{x}_{PFU}$ = 6837

Subclinical

$\bar{x}_{PFU}$ = 1700

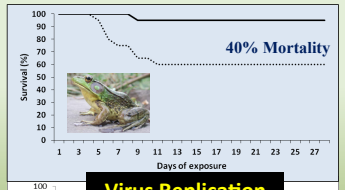



Subclinical

$\bar{x}_{PFU}$ = 10

# Green Frog
## Survival and Infection Prevalence

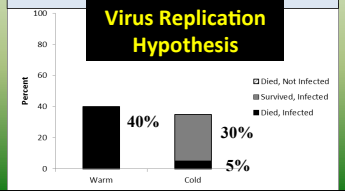
15% Control Mortality in Warm Chamber




Warm
Cold

Brand et al. (unpubl. data)

Virus Replication Hypothesis




Clinical
$\overline{\times}_{PFU} = 1871$

Subclinical
$\overline{\times}_{PFU} = 9$

Clinical
$\overline{\times}_{PFU} = 103$

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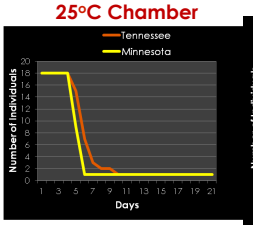
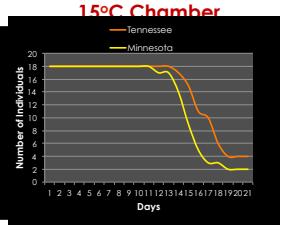
TN and MN Wood Frogs

## Reilly, Gray and Miller(unpubl. data)

25°C Chamber




15°C Chamber




Median days to mortality:
-Minnesota = 5.5 d
-Tennessee = 6 d

10 – 12 d Faster

Median days to mortality:
-Minnesota =15.5 d
-Tennessee =18 d

In Vitro Replication Stops at 12 C (Chinchar 2002)

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# Factors Contributing to Emergence

**Anthropogenic Stressors:** Forson & Storfer (2006); Gray et al. (2007); Greer et al. (2008); Kerby et al. (2011)

*A. tigrinum*

1) Herbicide (Atrazine)
Insecticide (Carbaryl) → ATV Susceptibility ↑

2) Cattle Land Use: Prevalence → Green Frogs and Tiger Salamanders

Other Possible Stressors: Pesticide Mixtures, Nitrogenous Waste, Endocrine Disruptors, Acidification, Global Warming, Heavy Metals

**Pathogen Pollution:** (Cunningham et al. 2003)

Anthropogenic introduction of novel strains to naïve populations

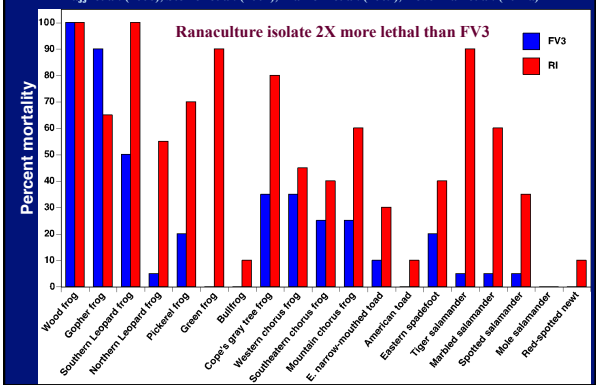
Picco et al. (2007)

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

Schloegel et al. (2009)

# Risk of Pathogen Pollution

Majji et al. (2006), Storfer et al. (2007), Mazzoni et al. (2009), Hoverman et al. (2011a)

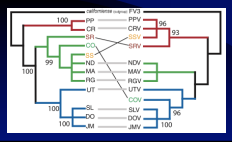


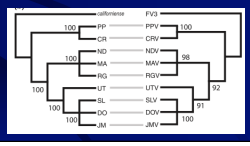

# Commercial Trade and Emergence

Drs. Andrew Storfer and Angela Picco

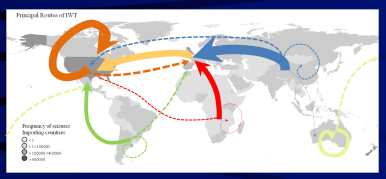
Storfer et al. (2007), Picco & Collins (2008)

- 85% bait shops had ≥1 infected salamander
- 32% prevalence (n = 2228)
- Anglers: used (26-73%) and released (26 – 67%)
- Different ATV strains are being transported
- Phylogenetic Concordance Analysis
  - Lack of co-evolution: host-pathogen phylogenies
  - Complete concordance when adjusted for human trade
- Emergence: pathogen pollution


# Global Trade of Ranavirus Hosts




Kristine Smith, DVM

From 2000-2006, the U.S. imported >1.5 billion individual animals (fish & wildlife; Smith et al. 2009)

- 90% fish, 2% amphibians, 1% reptiles
- 25 million live amphibians imported to U.S./year

Ranavirus Positive

- Hong Kong = 89%
- Dominican Republic = 70%
- Madagascar = 57%

Smith et al. (unpubl. data)

EcoHealth Alliance

## 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); Gold et al (2014)

$75/ bottle

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

## What do we Know?

- Ranavirus are Multi-species Pathogens
- Amphibians with fast-developing larvae most susceptible
- Interclass Transmission can occur
- Community Composition matters
- Amplification: amphibians; Reservoirs: all classes
- Transmission is efficient – Multiple Routes
- Environmental Persistence is long
- Pathogenicity might increase in Warm Temperatures
- Anthropogenic Stressors and Pathogen Pollution contribute to Ranavirus Emergence
- Enforcing OIE regulations and Biosecurity is Essential

**Ranaviruses represent a significant threat to the global biodiveristy of ectothermic vertebrates**

## Global Ranavirus Consortium

http://fwf.ag.utk.edu/mgray/ranavirus/ranavirus.htm

Symposia
Discussion Groups
Website
Reporting System
Outreach Resources
Springer eBook

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

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Questions??
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