Temperature Coupling: Relationships with Ranavirus Pathogenicity



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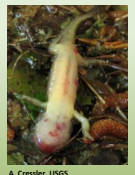
Die-offs in Summer

GSMNP: Cades Cove

Ken Dodd (USGS), Jamie Barichivich (USGS), and Megan Todd-Thompson (UT)



A. Cressler, USGS

M. Niemiller, Yale Univ.

A. Cressler, USGS

May 1999, 2000, 2009, 2012, 2013: Green et al. (2002), Todd-Thompson (2010)

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

Virus Replication increases with Temperature
12 – 32 C (in vitro) Chinchar (2002)

Ranavirus Landscape Prevalence

Tennessee Ponds
Hoverman et al. (2011)

Green Frog, Bullfrog, Pickerel Frog, Eastern Newt, Tiger and Spotted Salamanders



Ranavirus Distribution: 83% of Ponds Sampled

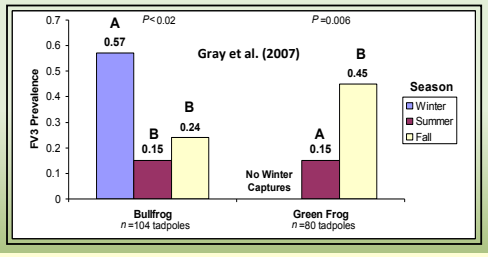
Greatest Prevalence and a Die-off in Autumn

Widespread Occurrence of Ranavirus in Pond-Breeding Amphibian Populations

Jason T. Hoverman,[1,2] Matthew J. Gray,[2] Debra L. Miller,[2,3,4] and Nathan A. Haislip[2,5] 2011

EcoHealth
2011 International Association for Ecology and Health

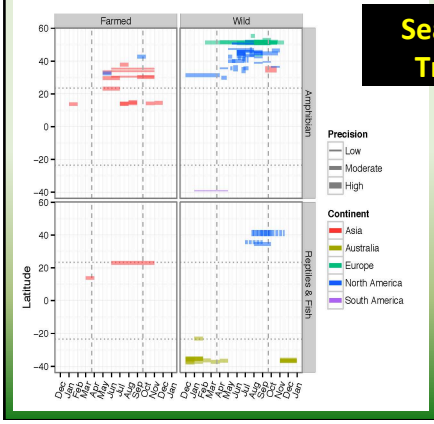
Seasonal Trends in Prevalence



- Increase in pathogenicity of the ranavirus ATV at colder temperatures
  Rojas et al. (2005)

- Decrease in immune function of ectothermic vertebrates at colder temperatures
  Raffel et al. (2006)

Seasonal Trends



Brunner et al. (2015): Rv Book

n = 109 events, 40 publications

Most die-offs occur in summer during warmer months

Does Temperature Play a Role in the Emergence of Ranaviruses?

- **Seasonal Trends**:
  - Density dependent factors
  - Changes in natural (predator density, development) or anthropogenic stressors (nitrogen concentration)
- **Water Temperature's Role:**
  - Viral Replication vs. Immune Function Chinchar (2002), Raffel et al. (2006)
  - Function as a Natural Stressor Long et al. (2012)
    - Fish: regulation of transcription, nucleosome assembly, chromatin organization and protein folding

Competing Hypotheses

- **Virus Replication Hypothesis**
  - Ranavirus replication increases with temperature up to 32 C
  - Caveat: Immune function in ectotherms also increases with temperature

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

Objective

Test for Differences in Pathogenicity of Ranavirus at Two Temperatures (10 and 25 C) among Four Amphibian Species

(two spring breeding and two summer breeding species)

Indices of Pathogenicity:

- **Percent Mortality**
- **Infection Prevalence**

Species Tested

- **Early spring breeders**
  - Spotted Salamander
  - Wood Frog

Larvae Metamorphose Prior to June

- **Summer breeders**
  - Cope's Gray Tree Frog

Larvae Metamorphose Prior to Sept

  - Green Frog

Larvae Overwinter & Metamorphose following Summer




Experimental Design

- **Two environmental chambers**
  - Low temperature (10°C = 50°F)
  - High temperature (25°C = 77°F)

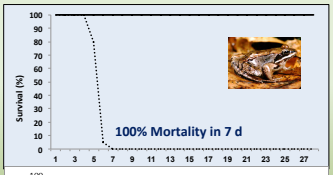
Acclimated 1 wk

- **Two treatments in a RBD** (n = 20/trt)
  - Exposed: 10^3 PFU/mL of FV3-like isolate
  - Control: Virus culture media (MEM)
- **2-L containers**
- **3-d Exposure**
- **28 days**

CONVIRON
Building Partnerships : Creating Solutions

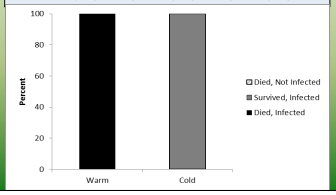
Wood Frog
Survival and Infection Prevalence

No Control Mortality



Clinical

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

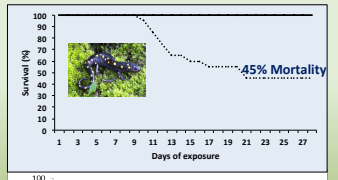


Subclinical

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

Spotted Salamander
Survival and Infection Prevalence

No Control Mortality

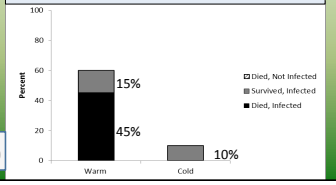


Clinical

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

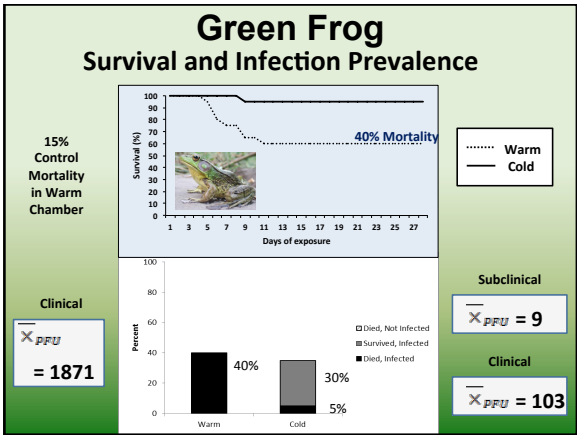
Subclinical

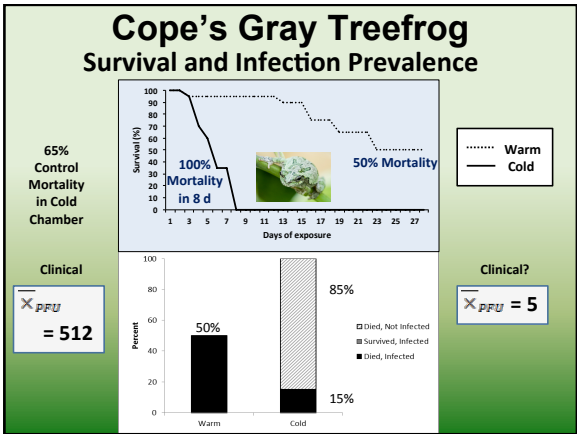
$\bar{x}_{PFU}$ = 1700

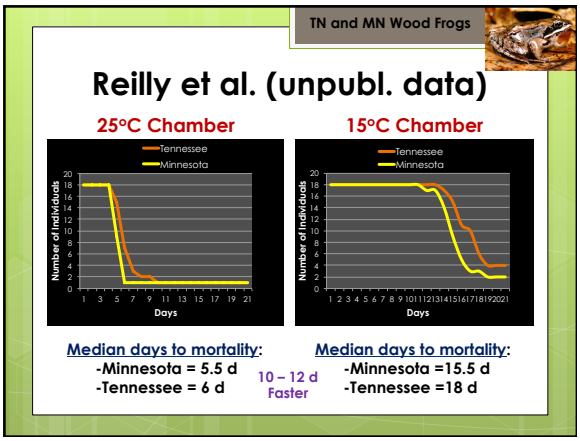


Subclinical

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


Green Frog
Survival and Infection Prevalence
15% Control Mortality in Warm Chamber
40% Mortality
Warm
Cold
Survival (%)
Days of exposure
Clinical
$\bar{x}_{PFU}$ = 1871
Percent
40%
30%
5%
Died, Not Infected
Survived, Infected
Died, Infected
Warm
Cold
Subclinical
$\bar{x}_{PFU}$ = 9
Clinical
$\bar{x}_{PFU}$ = 103


Cope's Gray Treefrog
Survival and Infection Prevalence
65% Control Mortality in Cold Chamber
100% Mortality in 8 d
50% Mortality
Warm
Cold
Survival (%)
Days of exposure
Clinical
$\bar{x}_{PFU}$ = 512
Percent
50%
85%
15%
Died, Not Infected
Survived, Infected
Died, Infected
Warm
Cold
Clinical?
$\bar{x}_{PFU}$ = 5


TN and MN Wood Frogs
Reilly et al. (unpubl. data)
25°C Chamber
15°C Chamber
Tennessee
Minnesota
Number of Individuals
Days
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

Hypothesis Support

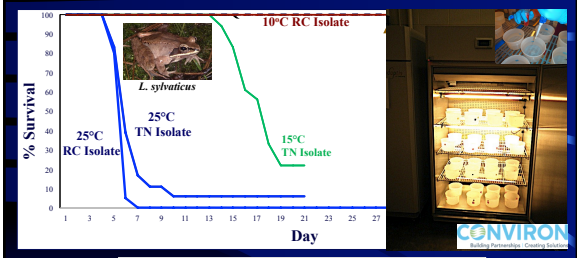
- **Virus Replication Hypothesis**
  - Mortality Greater in Warm:
    - Wood frogs, spotted salamanders, and green frogs
  - Infection Greater in Warm: $\overline{\times}_{PFU}$ **775X greater in warm**
    - All species (wood frog: 100% infection in both)
- **Morbidity-Infection Threshold (Wood Frogs)**
  - 10 C = 100% infection, no mortality
  - 15 C = 80 – 90% mortality (Reilly et al.)

In vitro 12 – 32°C

Chinchar (2002)

Temperature Dependency

Survival: Brand, Chaney, Reilly et al. (in review)

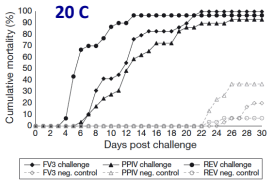


FV3 *In Vitro* Replication Stops at 12 C (Chinchar 2002)

FV3-like ranaviruses appears to become pathogenic at 12 C (54 F) but infection *in vivo* can occur at lower temperatures.

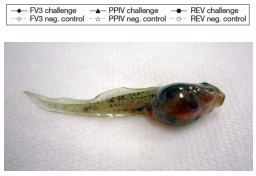
Temperature: Common frog tadpoles

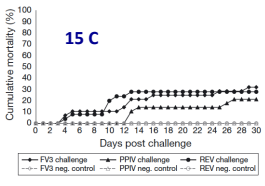
Bayley et al (2013)



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

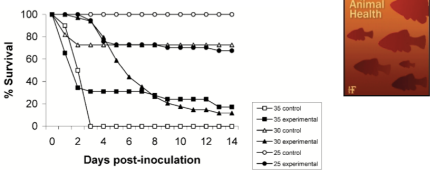
Vol. 103: 175–183, 2013
doi: 10.3354/dao02574
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published April 11

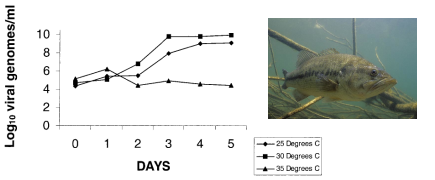




Temperature: LMBV (SSRV)

Grant et al. (2003)



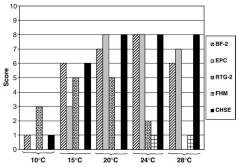


Temperature: Ranavirus Replication

Ariel et al. (2009)

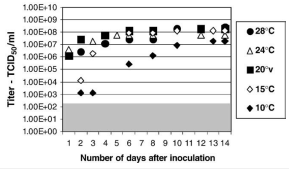
Time to reach titer of >8.6 x 10^6 $TCID_{50}$/ml –

Across 6 ranavirus isolates

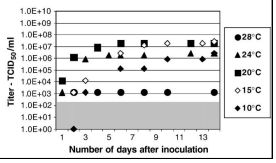


Aquaculture 294 (2009) 159–164

EHNV:



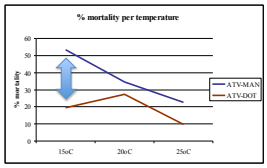
SERV: short-finned eel (cold water host!)



Temperature: ATV & Tiger Salamanders

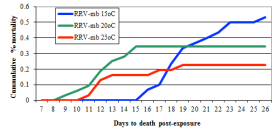
Schock et al (unpubl. data)

MAN = Manitoba
DOT = Arizona



RRV-mb Cummulative Mortality - Temp Experiment

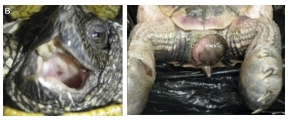
RRV = Saskatchewan

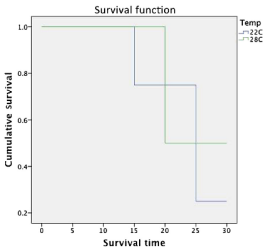


Temperature: FV3 & Red-eared Sliders

Allender et al (2013)

J. Comp. Path. 2013, Vol. 149, 356–367

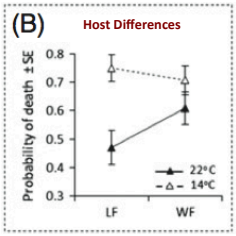


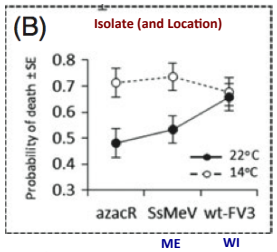


Tissue	Value	22°C		28°C	
Heart*	Mean/median‡	2.92×10^{10}		1.27×10^{92}	
	95%CI/10–90% percentiles‡	1.58×10^{10}	4.36×10^{10}	7.05×10^{6}	1.27×10^{92}
	Min/max	1.89×10^{10}	3.72×10^{10}	7.05×10^{6}	2.54×10^{9}
Liver†	Mean/median‡	2.15×10^{9}		1.70×10^{72}	
	95%CI/10–90% percentiles‡	1.13×10^{9}	3.16×10^{9}	1.87×10^{6}	1.70×10^{72}
	Min/max	1.32×10^{9}	2.85×10^{9}	1.87×10^{6}	3.21×10^{7}
Spleen	Mean/median‡	2.23×10^{102}		5.44×10^{72}	
	95%CI/10–90% percentiles‡	6.43×10^{9}	1.4×10^{11}	1.52×10^{8}	5.44×10^{82}
	Min/max	6.43×10^{9}	1.77×10^{11}	1.52×10^{8}	9.36×10^{8}

Complexities of Temperature

Echaubard et al. (2014) Evolutionary Applications





Environmental dependency of amphibian–ranavirus genotypic interactions: evolutionary perspectives on infectious diseases

Pierre Echaubard,[1] Joel Leduc,[1] Bruce Pauli,[2] V. Gregory Chinchar,[3] Jacques Robert[4] and David Lesbarrères[1]

Summary

- **Ranavirus pathogenicity is temperature dependent**
  - Interaction between optimal replication temperature and temperature-dependent host immune responses
  - Virus replication hypothesis may be supported, but also may be related to macrophage dissemination (Grayfer et al., 2015)
  - Temperatures may stress ectothermic vertebrates, but also may kill the host for other reasons when outside of their TNZ
- **Temperature relationships are not consistent**
  - Vary depending on ranavirus species/isolate and host species
  - New hypothesis: Pathogenicity may be affected by the environment (location); thermal optimum of Rv may be coupled with historical conditions in the host's habitat.


Questions??
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