

### Ranavirus Pathology in Chelonians

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### Ranavirus epidemiology

- Disease events are often clustered in local epizootics
  - Some occur on annual basis
- Several sources report a significant threat to biodiversity
  - Population density mortality in salamander studies
- Environmental factors change prevalence
  - *Restoration efforts*

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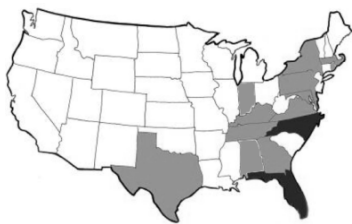
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### Chelonian cases after 2004



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Numerous cases

State	Species	Reference
Florida	Gopher tortoise	Westhouse et al.
	Florida Box turtle	Johnson et al.
North Carolina	Eastern box turtle	DeVoe et al., Allender et al.
Tennessee	Eastern box turtle	Allender et al.
Pennsylvania	Eastern box turtle	Johnson et al.
	Snapping turtle	USGS
Maryland	Eastern box turtle	USGS, Mao?
	Tortoise	Mao?
Rhode Island	Painted turtle	USGS
Kentucky	Eastern box turtle	Ruder et al.
Georgia	Burmese Star tortoise	Johnson et al.
New York	Eastern Box turtle	Johnson et al.
Texas	Eastern box turtle	Johnson et al.
Massachusetts	Eastern box turtle	Allender
Virginia	Eastern box turtle	Johnson pers. comm.
Indiana	Eastern box turtle	Johnson pers. comm.

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An Outbreak



**The News-Gazette**  
 JEFFERSON COUNTY, ILLINOIS  
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**US scientists worried about cause of turtle deaths**  
 Tue, 08/25/13 7:58am Tracy Moore

JEFFERSON COUNTY, Ill. — Wildlife conservationists in Jefferson County are worried about the cause of several dead turtles that have been found in the county.

The staff biologist and his team are currently investigating the cause of the deaths. The staff biologist is currently investigating the cause of the deaths.

Jefferson County Conservation District staff biologist is currently investigating the cause of the deaths.

According to the staff biologist, the cause of the deaths is currently unknown.

**O'BRIEN**  
 Local News Coverage

www.ohnews.com

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Transmission

- Role of temperature well-established in development of clinical signs from iridoviruses
  - Epizootic Hematopoietic Necrosis virus in red perch
    - 11 day incubation at 19-21C
    - No disease below 12C
  - EHN in white sturgeon
    - Higher cumulative mortality and longer disease course at lower temperatures
    - Higher daily mortality and secondary infections at higher temperatures
  - Tiger salamanders with ATV
    - Survived infection at 26C
    - All or most died at 18C or 10C

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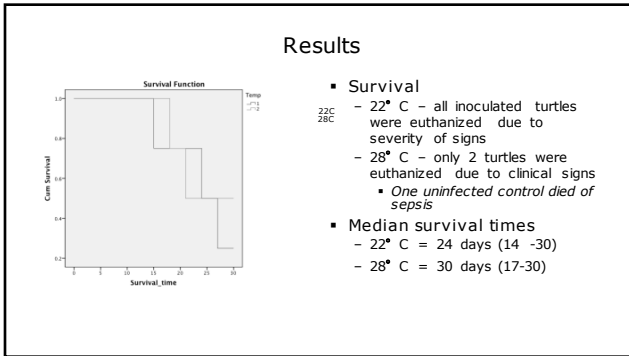
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### Results

Time	22° C Weight	28° C Weight
Pre-inoculation	1693.5625*	2063.1250#
Initial post-inoculation	1692.50*	2082.50#
Terminal	1802.50*	2159.50#

- \*Significant increase over time (F=11.1, p=0.045)
  - Significant difference between control and inoculated turtles (p=0.035)
- #Significant increase over time (F=7.13, p=0.026)

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### Results

Time	Sample	Viral copies 22° C	Viral copies 28° C
Pre-inoculation	Whole Blood	0	0
	Oral swab	0	0
	Cloacal swab	0	0
Initial post-inoculation	Whole Blood	0	0
	Oral swab	0	0
	Cloacal swab	0	0
Terminal	Whole Blood	1.23 x 10 <sup>7</sup>	2.45 x 10 <sup>3</sup>
	Oral swab	7.23 x 10 <sup>6</sup>	0
	Cloacal swab	1.37 x 10 <sup>6</sup>	1.15 x 10 <sup>3</sup>

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### Results

- **Lethargy and leg swelling**
  - Present in all 6 infected turtles at both temperatures
- **Nasal discharge, ocular discharge, and oral plaque**
  - Present in all 4 infected turtles at 22° C and none of the infected turtles at 28° C
- **Skin abscess**
  - Present in 3 of 4 positive turtles at 22° C and 1 of 2 positive turtles at 28° C
- **Uninfected controls**
  - Lethargy observed in one animal
  - Leg swelling observed in two animals

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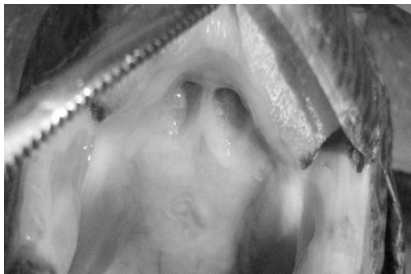
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**Results**

Tissue	Parameter	22C	28C
		Viral Copies	Viral Copies
Tongue	Mean/median*	1.25 x 10 <sup>9</sup> **	5.94 x 10 <sup>6</sup> *
Skeletal Muscle	Mean/median*	3.7 x 10 <sup>10</sup> **	3.64 x 10 <sup>8</sup> *
Lung	Mean/median*	6.29 x 10 <sup>9</sup> **	5.01 x 10 <sup>9</sup> *
Heart <sup>#</sup>	Mean/median*	2.92 x 10 <sup>10</sup>	1.27 x 10 <sup>9</sup> *
Liver <sup>^</sup>	Mean/median*	2.15 x 10 <sup>9</sup>	1.70 x 10 <sup>7</sup> *
Spleen	Mean/median*	2.23 x 10 <sup>10</sup> **	5.44 x 10 <sup>7</sup> *
Ovary	Mean/median*	8.93 x 10 <sup>9</sup> **	9.06 x 10 <sup>6</sup> *
Kidney	Mean/median*	3.46 x 10 <sup>10</sup> **	2.54 x 10 <sup>8</sup> *

# Significant difference between environmental temperatures, p=0.012  
 ^ Significant difference between environmental temperatures, p=0.011

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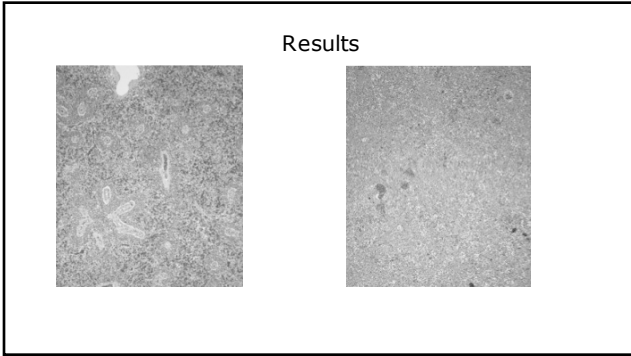
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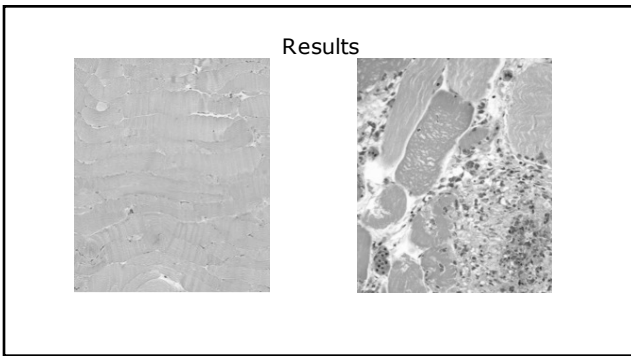
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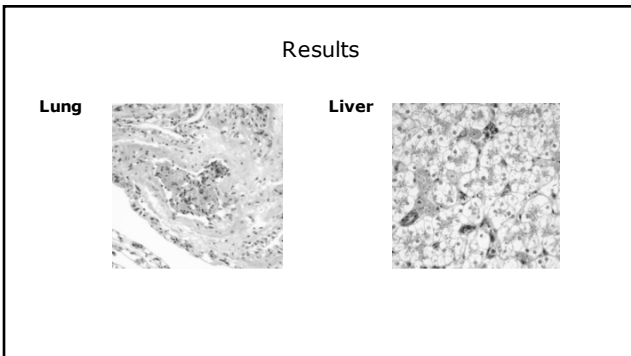
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### Discussion

- Mortality
  - 22° C
    - Significant association between inoculation and disease (p=0.014)
  - 28° C
    - No significant association between inoculation and disease (p=0.214)
- Non-significant mortality was seen at lower temperature (100%) than higher temperature (50%)
  - Power = 0.34
- Clinical signs
  - Weight gain (p=0.045; p=0.026)
  - Nasal discharge (p=0.018)
  - Ocular discharge (p=0.018)
  - Lethargy (p=0.002)
  - Inoculation site swelling (p=0.007)

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### Animals

- Four species
  - Red-eared slider
  - Mississippi map turtle
  - False map turtle
  - River Cooter
- 24 individuals of each species
  - 12 housed at 22C, 12 housed at 27C
- Inoculated and Control groups
  - 8 inoculated and 4 control for each temperature and species




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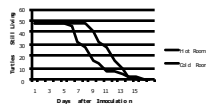
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### Results - Juveniles

- 100% mortality in all inoculated individuals of all species at both temperatures
- Survived 6 to 16 days
- Only clinical sign was lethargy and was present for 12-48 hours prior to death/euthanasia
- No control animal demonstrated clinical signs or tested positive for FV3




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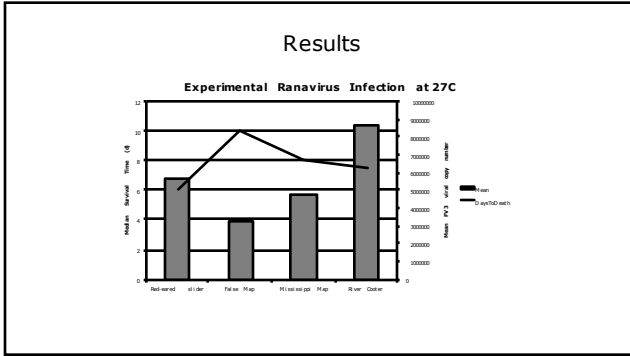
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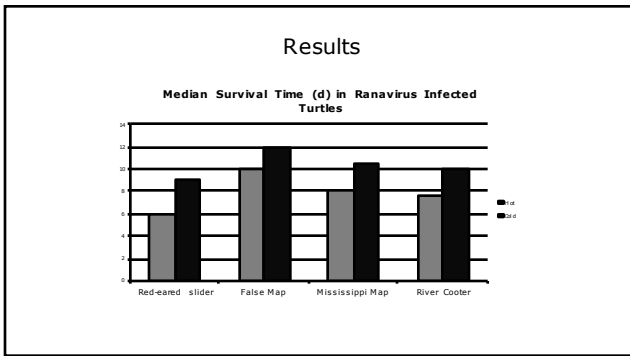
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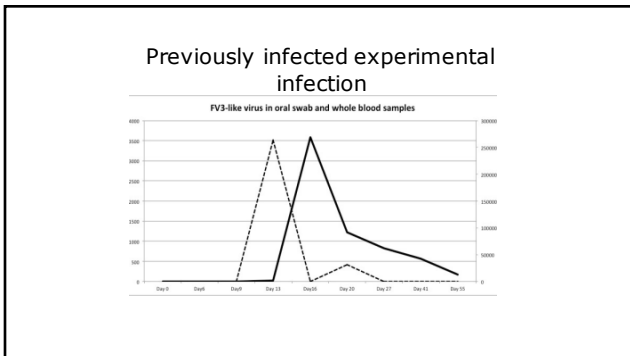
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		Mean/median*	95% CI 10-90% percentiles*		Min	Max
PCV (%)	Pre-inoculation	21*	9	22.13*	9	22.5
	Post-inoculation	24.7	14.7	34.6	7	35
	Terminal	19.1	10.3	27.8	3	26
TS <sup>h</sup> (mg/dl)	Pre-inoculation	3.8	2.8	4.7	2.3	5
	Post-inoculation	3.8	2.6	5	2	5
	Terminal	2.1	1.4	2.9	1	3
WBC (cells/ul)	Pre-inoculation	4544.5	3364.7	5724.3	2400	5628
	Post-inoculation	7389.3	1668.6	13110.1	1203	16264
	Terminal	4073*	1232	6283.4*	1232	12623
Heterophils (cells/ul)	Pre-inoculation	1388.5	1016.6	1760.5	933.9	1916
	Post-inoculation	2429.3	813.9	4044.7	346	4091
	Terminal	1537	620.8	2453.3	344.9	2777.2
Lymphocytes (cells/ul)	Pre-inoculation	870.3	184.5	1556.1*	223	1799.4
	Post-inoculation	944.1*	223	1594.6*	0	3764
	Terminal	372*	24.6	1569.6*	24.6	4544.4
Monocytes (cells/ul)	Pre-inoculation	654.9	314.2	995.5	272	1118
	Post-inoculation	910*	204.8	3167.3*	204.8	3600
	Terminal	805.1	457.1	1153.2	344.9	1205

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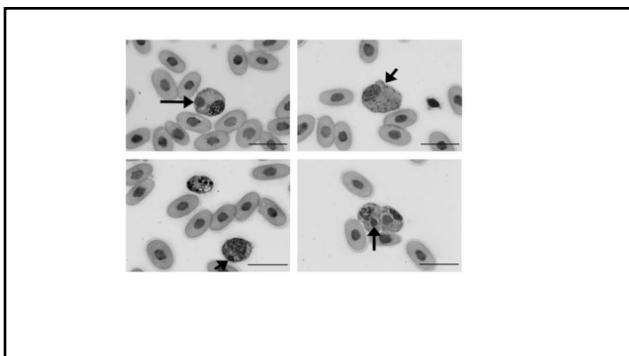
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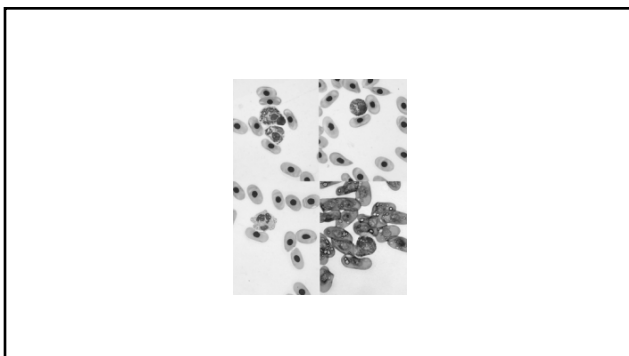
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Discussion

- Hematology
  - Transient elevation of WBC in FV3-infected red-eared sliders
    - Occurs earlier post-inoculation in 28C trial
  - Total solids decreased in terminal sample
  - Trend toward reduction in lymphocytes
    - Observed power = 0.05 through 0.448
  - Inclusions not reliable method to diagnose ranavirus infection

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Ranavirus outbreak

	Number of infected turtles	Mortality rate
Total study population	22	50%
RV negative	3	0%
HV & Myco co-infected	2	0%
RV positive (including all co-infections)	19	42%
RV alone	4	50%
RV & Myco	5	60%
RV & HV	2	50%
RV, HV & Myco	8	37%

<sup>a</sup>RV, Ranavirus; HV, herpesvirus; Myco., *Mycoplasma* sp.

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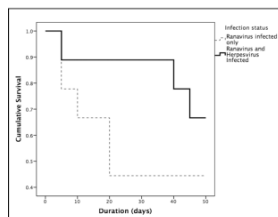
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Ranavirus outbreak




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Conclusions

- Ranavirus causes high morbidity and mortality in affected species
- Temperature plays a role in the pathogenesis and severity of clinical and histopathological signs
- Chelonians surviving from ranavirus are to be considered lifelong carriers of the disease

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Questions

- Thank You!!

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