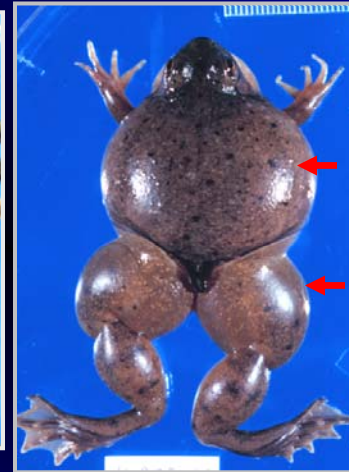


# Comparative Pathology of Ranavirus Infections in Wild Amphibians

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## Lethal Ranaviral Infections, USA

### Epizootiology:

- Kills larvae & larvae in metamorphosis
- Mortality rate often >95% of larvae
- Onset is sudden (explosive)
- Seldom affects adult amphibians in USA

### Hosts:

#### Frogs

True frogs

Chorus frogs

Treefrogs

#### Toads

True toads

Clawed toad?

Spadefoots?

#### Salamanders

Mole sal's

Newts

Unknown: Woodland salamanders, Sirens, Caecilians



## Hosts of Lethal Ranaviral Infections by Genera, USA

**Anurans: 4 genera, 18 spp.**

**Caudates: 2 genera, 6 spp.**

### **Genera:**

**Ambystoma**

**Bufo**

**Hyla**

**Notophthalmus**

**Pseudacris**

**Rana**



## Lethal Ranaviral Infections, USA

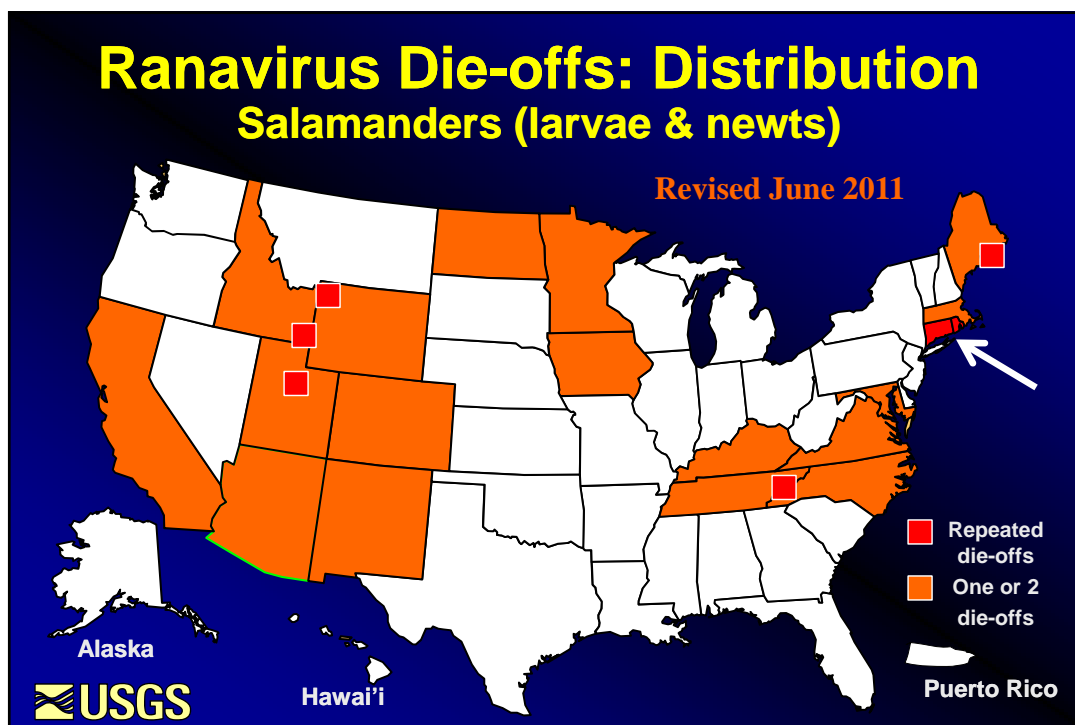
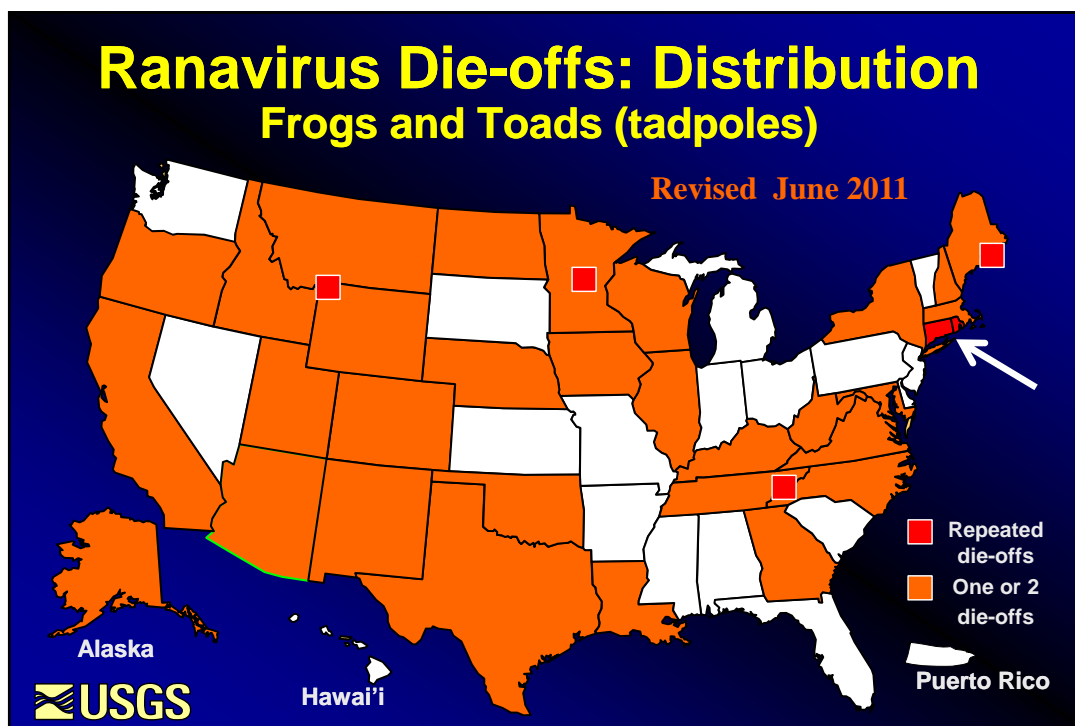
### **Findings by National Wildlife Health Center:**

- Cases: ~ 300 die-offs or disease outbreaks since 1996
- Ranavirus cases: >75
- Isolates of ranaviruses: >175 (+ 70 wild research frogs)

### **Hosts:**

- 85-90% of ranaviral die-offs occur in larvae in USA
- Ranaviral infections in PM amphibians are uncommon (exceptions are *R. luteiventris*, newts & a few treefrogs)





## Ranavirus Die-Offs



**Larval Tiger Salamanders:**  
**Yellowstone NP, WY**  
Photo by:  
Dr Sophie St Hilaire



**Larval Tiger Salamanders:**  
**Caribou-Targhee NF, ID**  
Photo by:  
Devon Green, NFS



## Field (gross) Signs of Ranavirus Infection

### Larvae show 4 characteristic abnormalities:

- Reddening or hemorrhages in ventral skin (near vent)
- Bloating or fluid accumulation under skin
- Skin ulcers are uncommon: pinpoint or large
- Live larvae are weak, swim poorly, may float upside down

(Hemorrhages may occur anywhere on body, including eyes and internal organs)



## Field (gross) Signs



**Larval Tiger Salamanders:**  
Caribou-Targhee NF, ID  
Photo by:  
Devon Green, NFS

## Field Signs: Skin Hemorrhages

*A. maculatum*



*A. tigrinum*

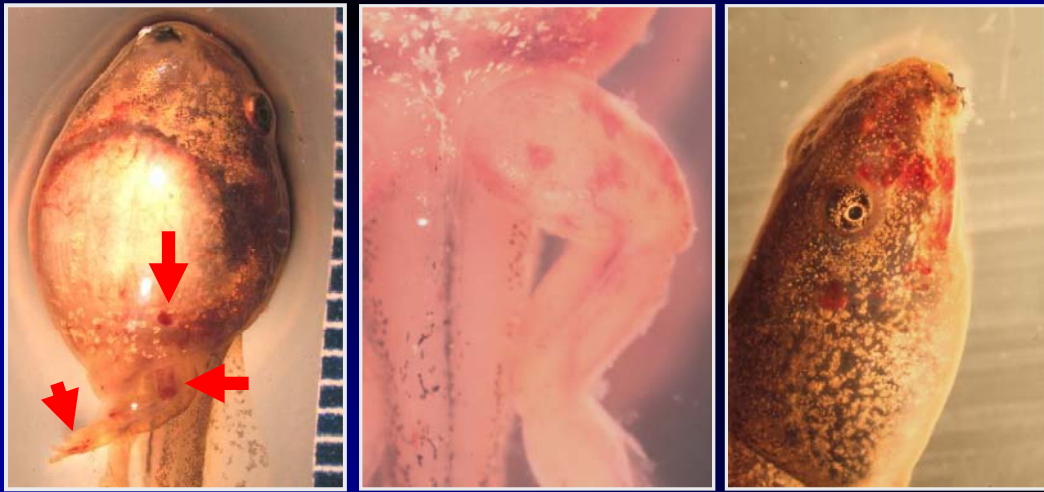


*A. opacum*





## Field Signs: Skin Hemorrhages (tadpoles)

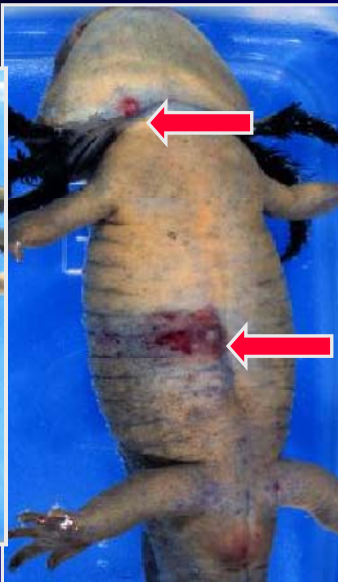


## Field (gross) Signs: Skin Ulcers

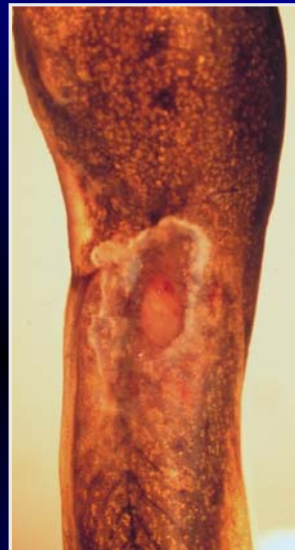
*A. tigrinum*



USGS



*R. catesbeiana*



## Field (gross) Signs: Tadpole Skin Ulcers

*R. catesbeiana*, 22704

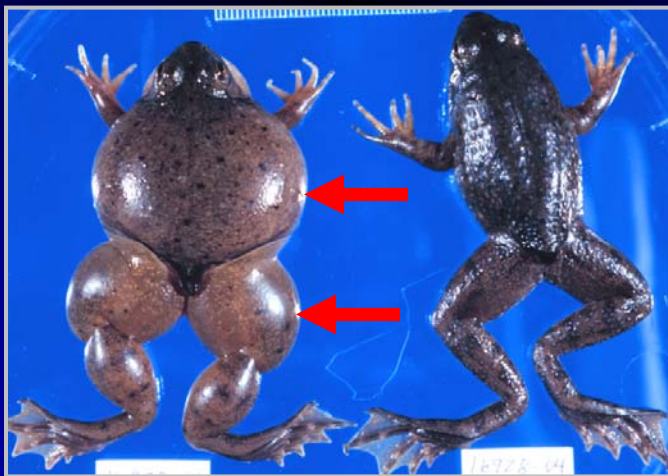


*R. luteiventris*, 20426



## Field (gross) Signs: Skin Edema (due to excess fluid accumulation under skin in lymphatic sacs)

Bloated Bullfrog



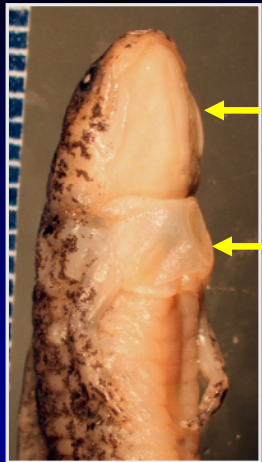
Normal Bullfrog

Larval Bullfrog



## Field (gross) Signs: Skin Edema (due to excess fluid accumulation under skin in lymphatic sacs)

*A. maculatum*



*A. macrodactylum*



USGS

## Internal Gross Findings

Tiger salamanders (*Ambystoma tigrinum*)



**Liver:** Petechiae & Enlargement

**Spleen:** Diffuse hemorrhage, enlargement or miliary necrosis

**Body cavity:** Hydrocoelom

**Stomach & Intestine:**

Hemorrhage—petechial or diffuse

**Mesonephroi:** glomerular petechiae

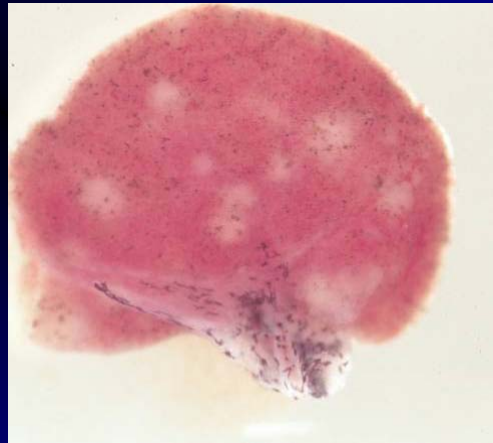




## Internal Findings



Bullfrogs (*Rana catesbeiana*)  
with palatal ulcer and white foci  
of necrosis in spleen



## Histological Features of Ranavirus

### Organs affected:

Epidermis, dermis, blood vessels, liver, spleen, mesonephroi,  
stomach, intestine

### Occasionally affected organs:

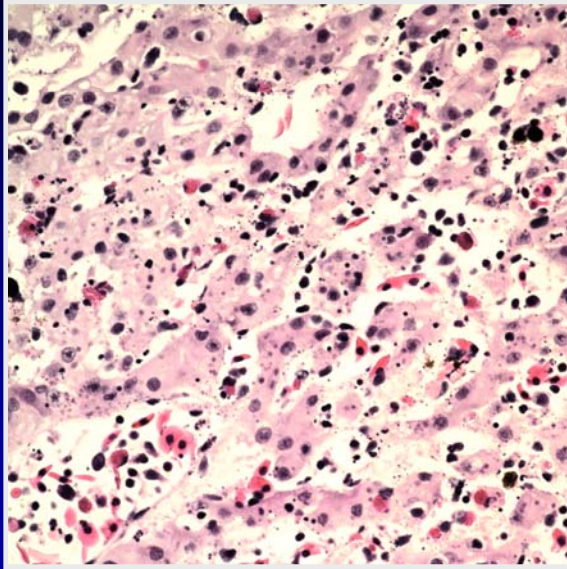
Pancreas, lungs, gills

### Abnormalities:

Necrosis of endothelium, macrophages, lymphocytes, liver  
cells, pancreatic acini, GI tract epithelium, renal glomeruli,  
renal interstitial myeloid cells ("bone marrow")

Hemorrhages: dermis, muscles, eyes, many visceral organs

## Histological Findings: Liver



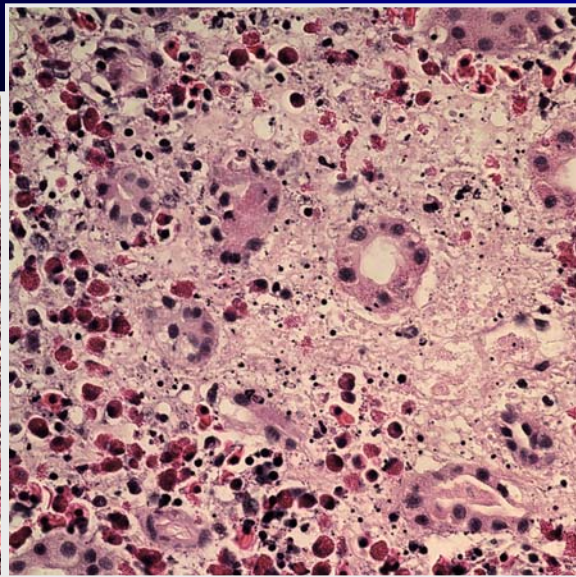
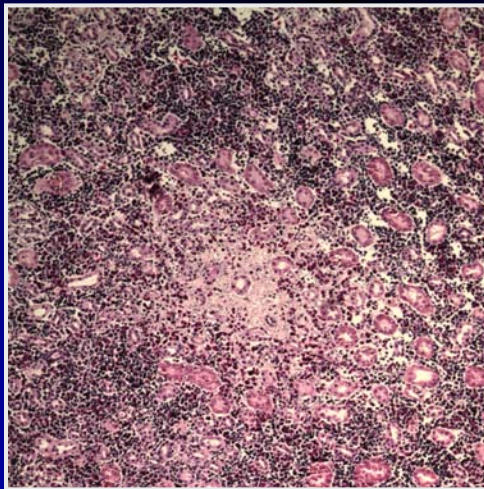
**Early Changes:** Necrosis of endothelium lining sinusoids; swelling of liver cells

**Advanced Changes:** Necrosis of liver cells and macrophages of PMAs

*Rana heckscheri*, larva, GA, 19709

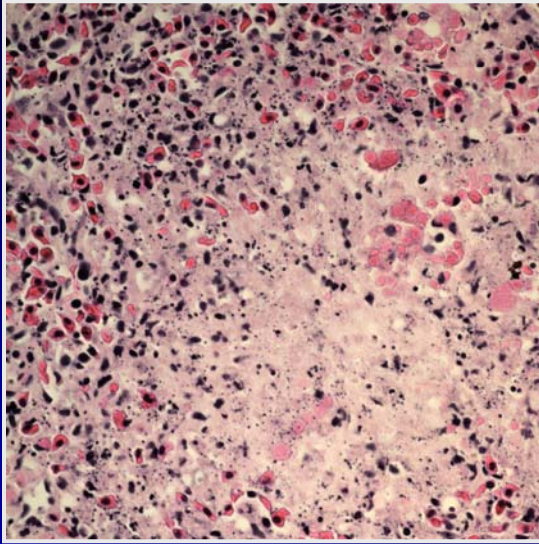
## Histological Findings: Mesonephroi (“kidneys”): Necrosis of glomeruli and interstitial cells, rarely tubules

*Rana heckscheri*, larva, GA, 19709





## Histological Findings: Spleen



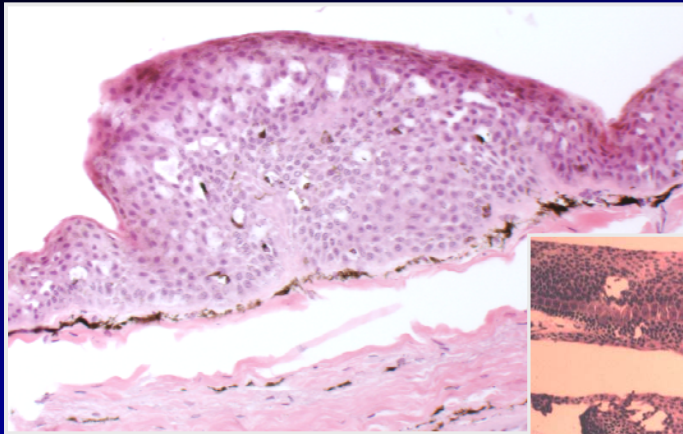
**Early Changes: Multiple tiny foci of necrosis**

**Advanced Changes: Necrosis is coalescing or diffuse**

**Viral inclusion bodies are rarely seen in spleen**

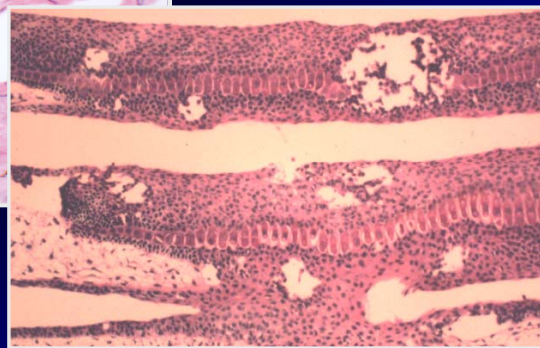
*Rana heckscheri*, larva, GA, 19709

## Histological Findings: Epidermis (skin)



*Bufo boreas*, skin papule

**Early Changes: papules (swollen cells), necrosis of cells, spreading necrosis to form vesicles , ending with ulcers of skin**



*R. warszewitschii* , oral disc vesicles

## Key Histological Feature:

**Basophilic intra-cytoplasmic inclusion bodies (ICIB)**

**Most easily found in cells of:**

Epidermis (skin) and liver

**Occasionally found in cells of:**

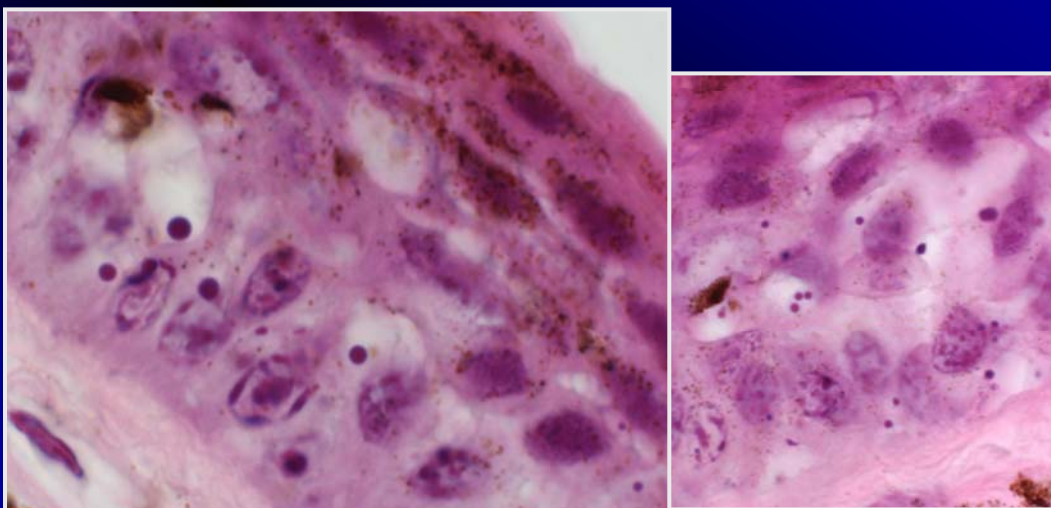
Pancreas, mesonephroi, stomach and intestine

**Rarely found in cells of:**

Blood vessels, glomeruli, renal interstitium, spleen



## Ranaviral ICIB in Skin

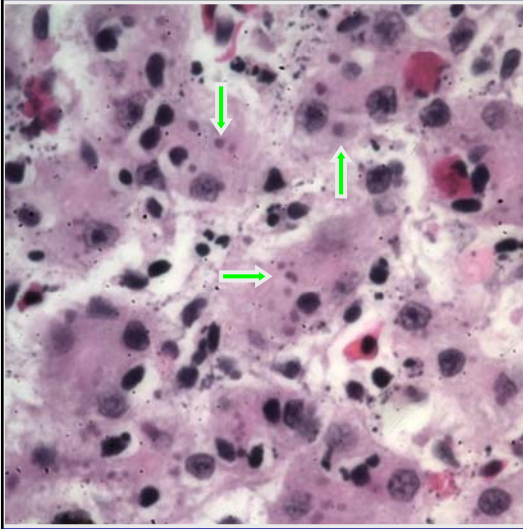


*Bufo boreas*, 19780-01, AK

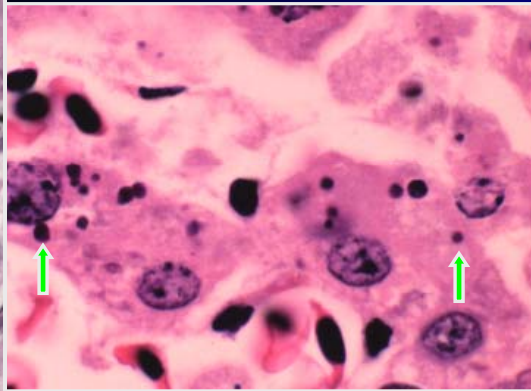


# Ranaviral ICIB in Liver

*Rana heckscherii*



*Ambystoma tigrinum*

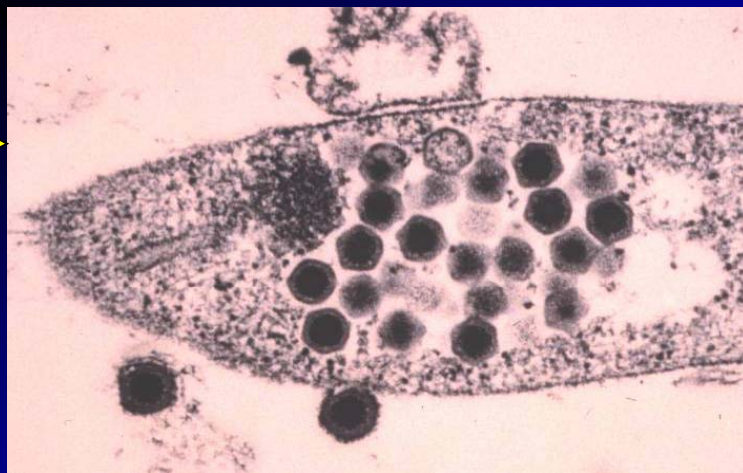


## Ranavirus: Ultrastructure

Ranavirus particles are icosahedral, c.160nm, and strictly cytoplasmic



Vesicles in  
tooththrows



## **Ranaviruses In USA: Summary**

- 1. Kills massive numbers of larvae; rarely affects adult amphibians**
- 2. Skin hemorrhages, ulcers & bloating are key field (external) abnormalities**
- 3. Diagnosis is made by**
  - Culture / isolation of virus in lab, or**
  - Histology of tissues**
- 4. Key histological features are ICIB with widespread necrosis of liver, spleen, mesonephroi and blood vessels**



# **END**

## **Acknowledgments:**

**Contributors, field biologists nationwide (TNTC)**

**NWHC Virologists & Technicians:** Hon Ip, Doug Docherty, Renee Long, Tina Egstad, Katy Burns, et.al

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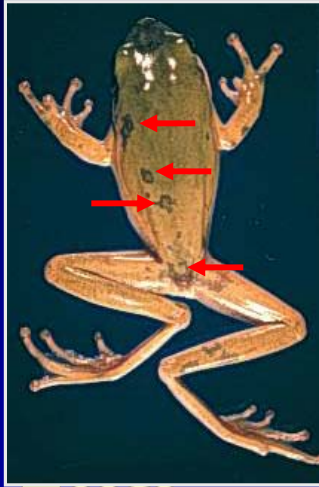
**NWHC Microbiologists:** David Blehert, Brenda Berlowski

**NWHC Parasitologists:** Rebecca Cole, Skip Sterner



## **Ranavirus: Gross Findings (Adult Treefrogs)**

**Green treefrog**



- 1. Sporadic outbreaks of epidermal ulceration (mostly in dorsal skin)**
- 2. Low mortality (assumed)**
- 3. May be reported in captive (zoo) frogs**
- 4. Virus isolated from skin ulcers only (not viscera)**
- 5. Somewhat resembles reported herpesviral infections in Europe**

## **Field Signs: 22199-03 RaBla Nebr**

