Isolation of Frog Virus 3 from Pallid Sturgeon (*Scaphirhynchus albus*) Suggests an Interclass Host Shift

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Topics Covered

- Pallid Sturgeon Conservation within the Missouri River Basin
  - History of the decline & subsequent restoration effort
  - Impediments to recovery
    - Iridoviral epizootics
- 2009 Blind Pony State Fish Hatchery Epizootic
  - Disease Characterization
    - Case history
    - Gross & microscopic lesions
    - Virus isolation & virion architecture
    - Genetic characterization
    - Pathogenicity trial
- Ranavirus Host Fidelity
- Significance & Future Directions

Decline of Pallid Sturgeon within the Missouri River Basin

- Damming of the MRB led to PS decline
- 1990 listed as federally endangered
  - Aging adult population estimated at 250 w/o detectable recruitment
- Breeding program in 6 hatcheries across 3 management zones
  - Upper basin
  - Middle basin
  - Lower basin

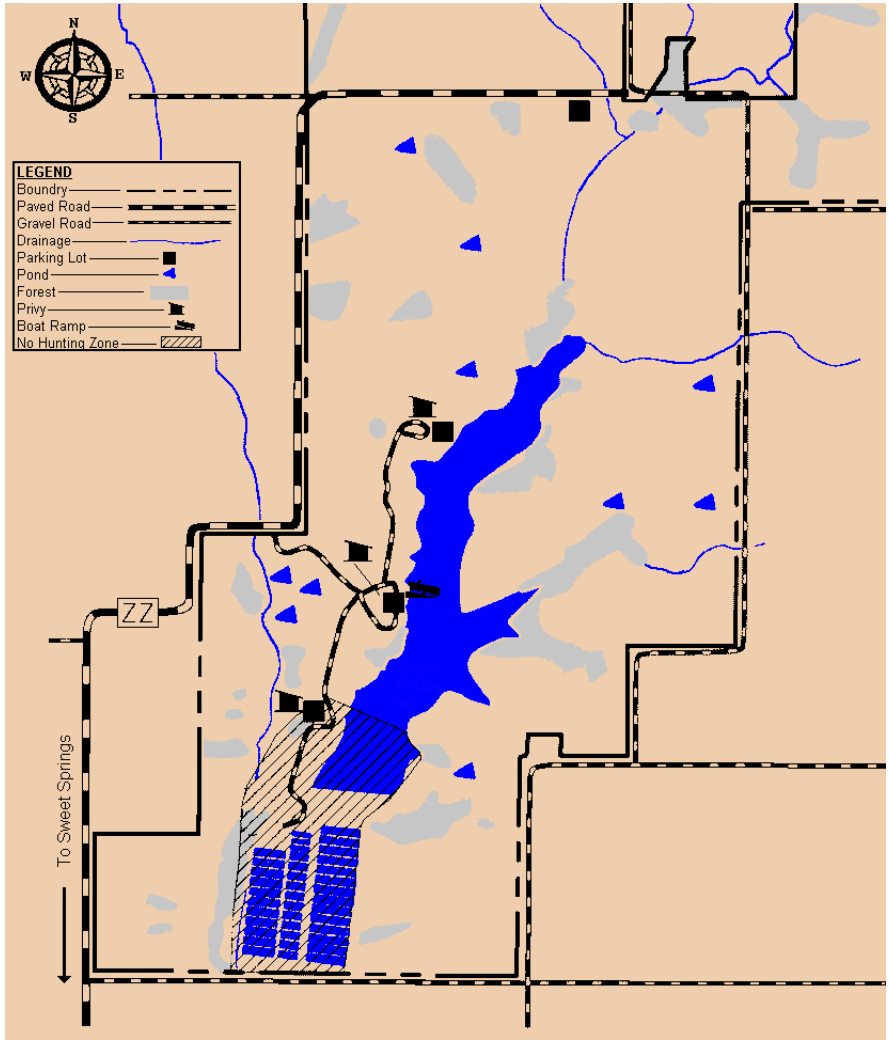
Pallid Sturgeon Restoration Effort

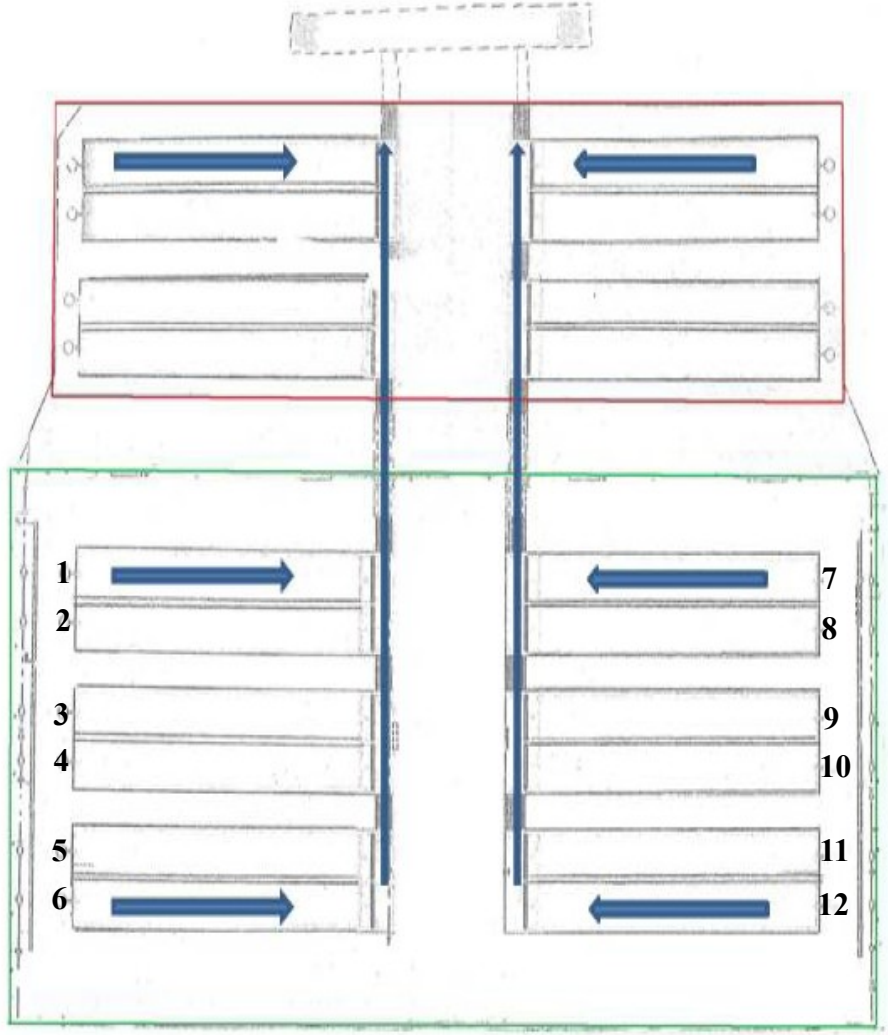
- Endangered adults are captured annually & transported to 1 of 6 hatcheries
- Adults spawned & then returned to the wild
- Progeny reared at 6 hatcheries for restocking 3 management zones

Blind Pony State Fish Hatchery (BPSFH)

Blind Pony State Fish Hatchery, Sweet Springs, MO

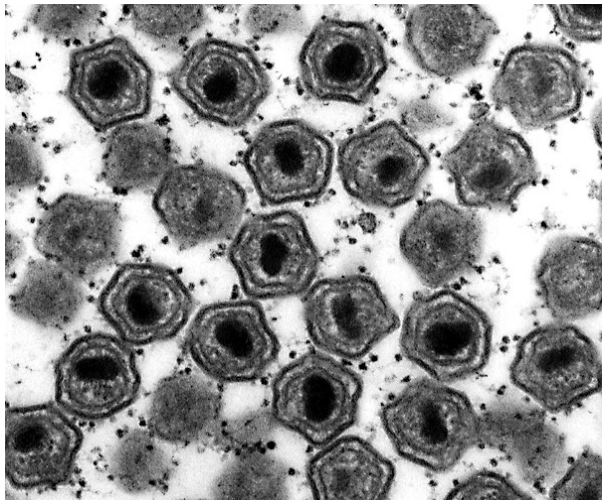
- Hatchery spawns & rears ~ 10,000 lake & 12,000 pallid sturgeon annually
- 12 raceways typically stocked at 2,000/raceway
- Raceways receive untreated H_2O by gravity from Blind Pony Lake Dam





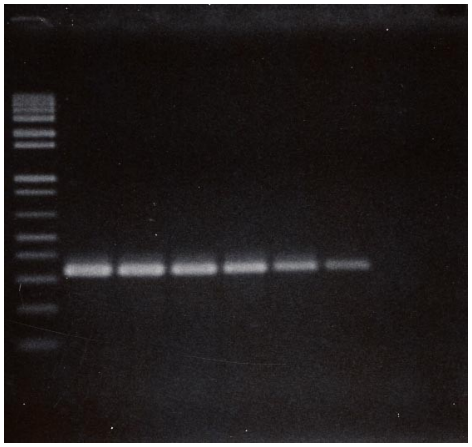
Pallid Sturgeon Restoration Effort - Impediments

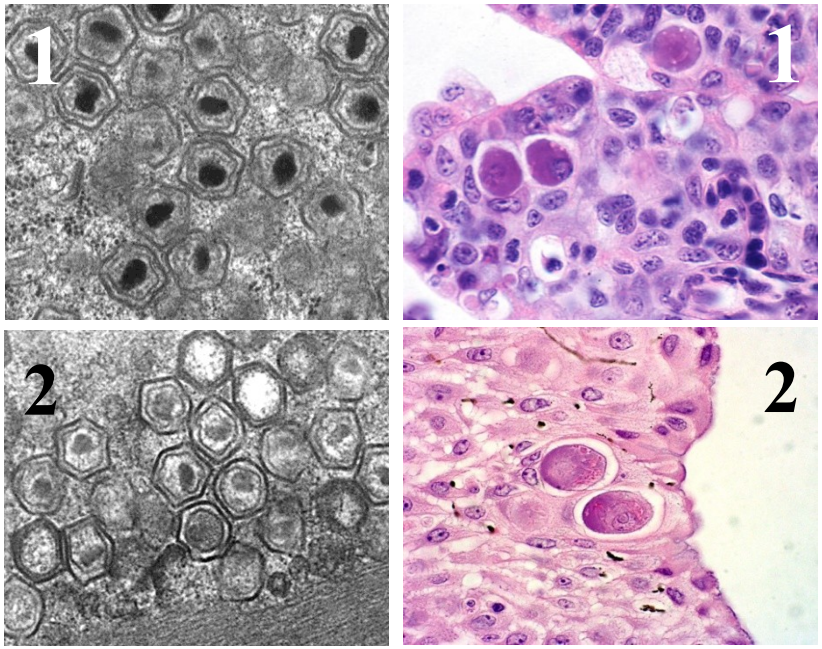
- Efforts complicated by nutritional deficiencies, water quality, & infectious etiologies
  - Significant iridovirus outbreaks since 1999…
- Family *Iridoviridae* characteristics
- Large dsDNA viruses infecting invertebrates & poikilothermic vertebrates
- Taxonomy
  - Genus *Iridovirus* (arthropod hosts)
  - Genus *Chloriridovirus* (dipteran host)
  - Genus *Lymphocystivirus* (fish hosts)
  - Genus *Megalocytivirus* (fish hosts)
  - Genus *Ranavirus* (fish, amphibian, and reptilian hosts)
  - Unassigned members
    * Pallid Sturgeon IV (PSIV), White Sturgeon IV (WSIV), Erythrocytic Necrosis Virus (ENV) et al…



Missouri River Sturgeon Iridovirus

1. Pallid (PSIV)] MRSIV
2. Shovelnose



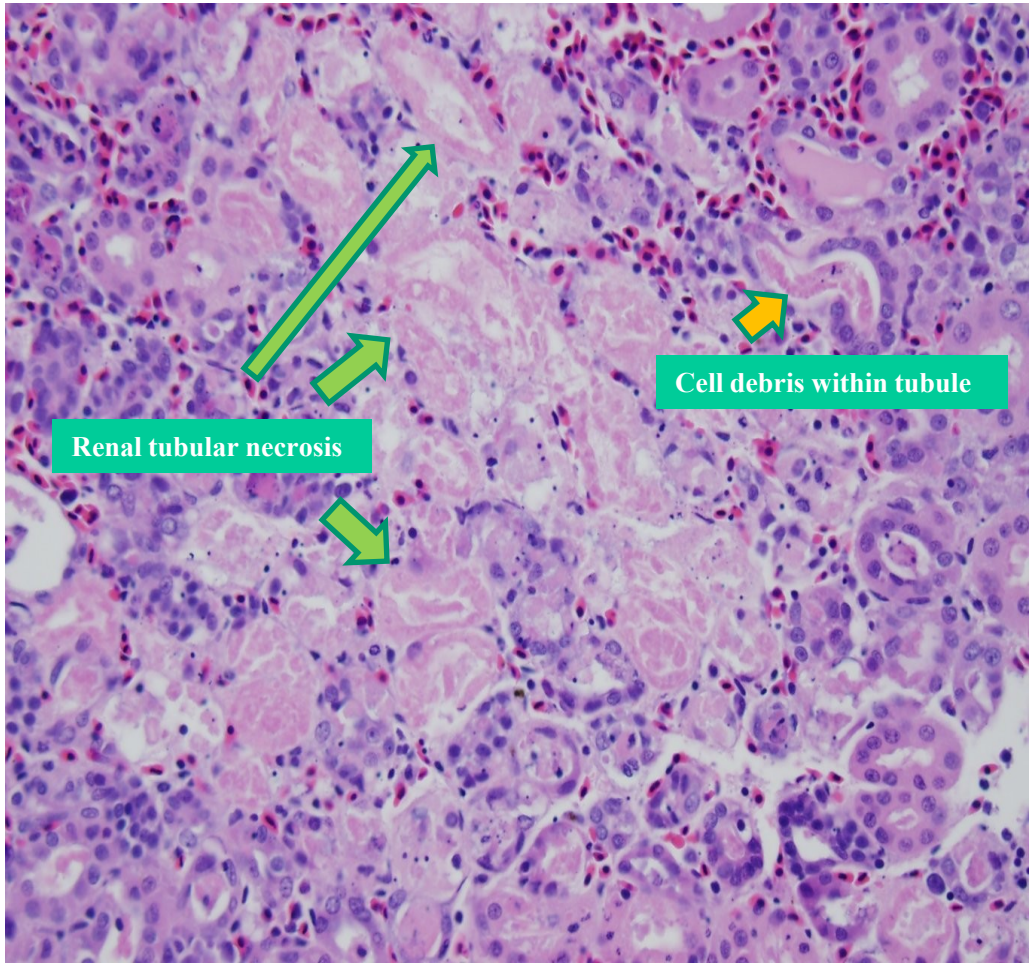


2009 Blind Pony State Fish Hatchery Epizootic

- July – September 3/9 raceways rearing YOY PS experienced heavy losses
- Mortality in affected raceways was 80-100% over the 3 month epizootic
- Mortality obs. between 60 -78 °F with higher mortality at warmer temps (550 morts/d)
- Samples submitted to the Bozeman Fish Health Center & UCD Fish Health Lab

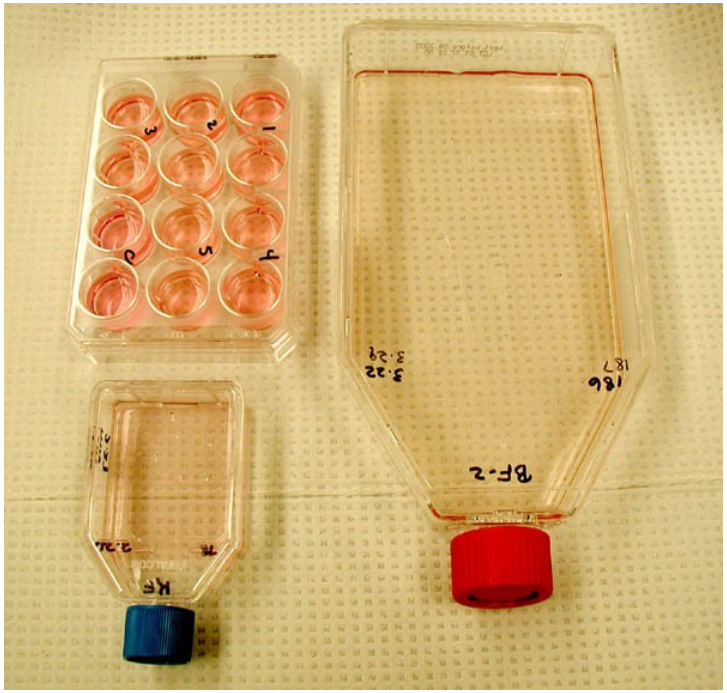
BPSFH PS Epizootic - Gross and Microscopic Lesions

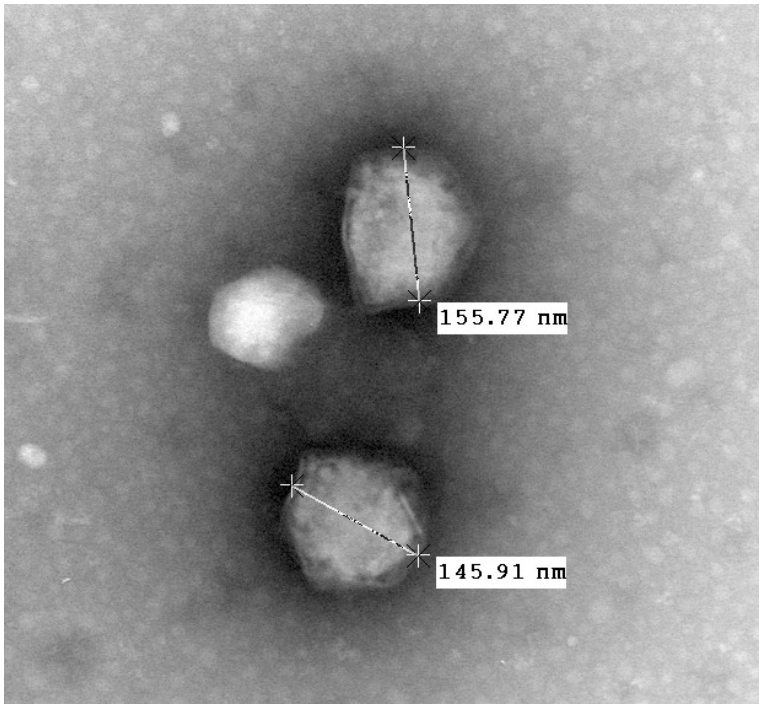
- Dying fish displayed external and internal hemorrhagic lesions
- Histology revealed necrosis of hematopoietic (K, S) et al. internal tissues in contrast to…
  - MRSIV that typically does not generate significant internal lesions



BPSFH PS Epizootic – Bacteriology, Cell Culture, SEM, PCR

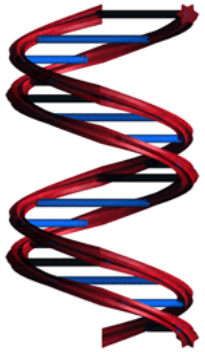
- Mixed pop. of bact. cultured but mort. continued despite repeated antib. Tx
- Replicating agent observed in CHSE & sturgeon cell lines
- Negative staining SEM revealed icosahedral particles (~ 150 nm) in contrast to…
  - MRSIV that has never been isolated and possesses larger virions (~ 250 nm)
  - Samples were negative (-) against MRSIV specific PCR assay





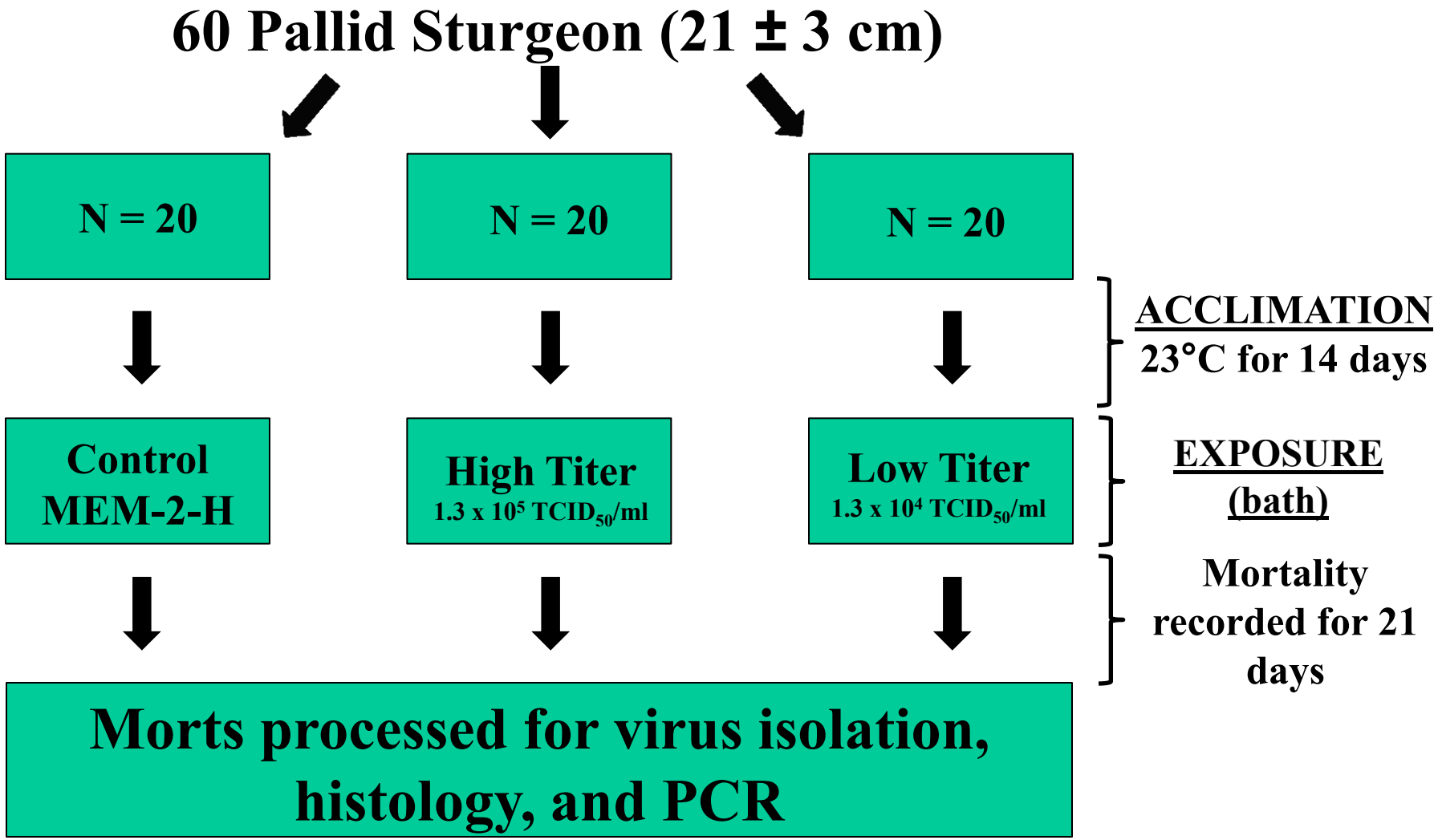
PS Isolate Genetic Characterization

- Degenerate PCR assay targeting a portion of the viral DNA polymerase (Hanson et al. 2006)
  - 100% identical to Frog Virus 3 (680 bps, Tan et al. 2004)
- Amplification of the full length Major Capsid Protein gene
  - 100% identical to Frog Virus 3 (1392 bps, Tan et al. 2004)
- Conclusion: Over 2,000 bps from two separate loci suggests PS represent a new fish host for Frog Virus 3 (FV-3) = interclass host shift!
- PS ranavirus (PSRV) the cause of the mortality???



Virus Pathogenicity Study

- PSRV tested against YOY PS in flow-through 30 L tanks



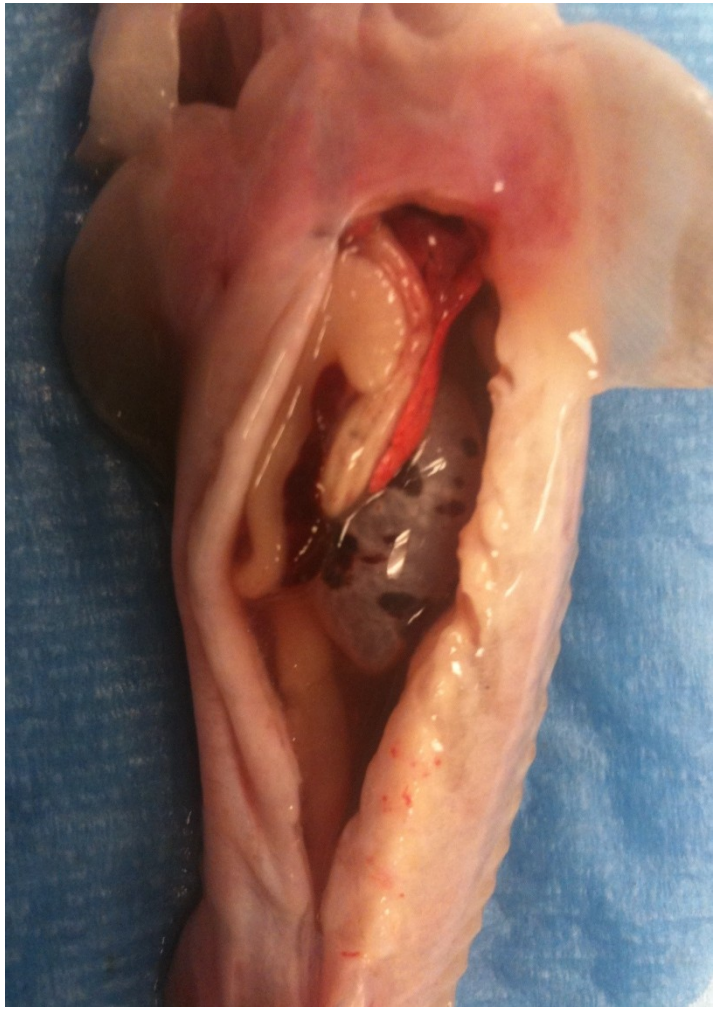
Virus Pathogenicity Study - Mortality

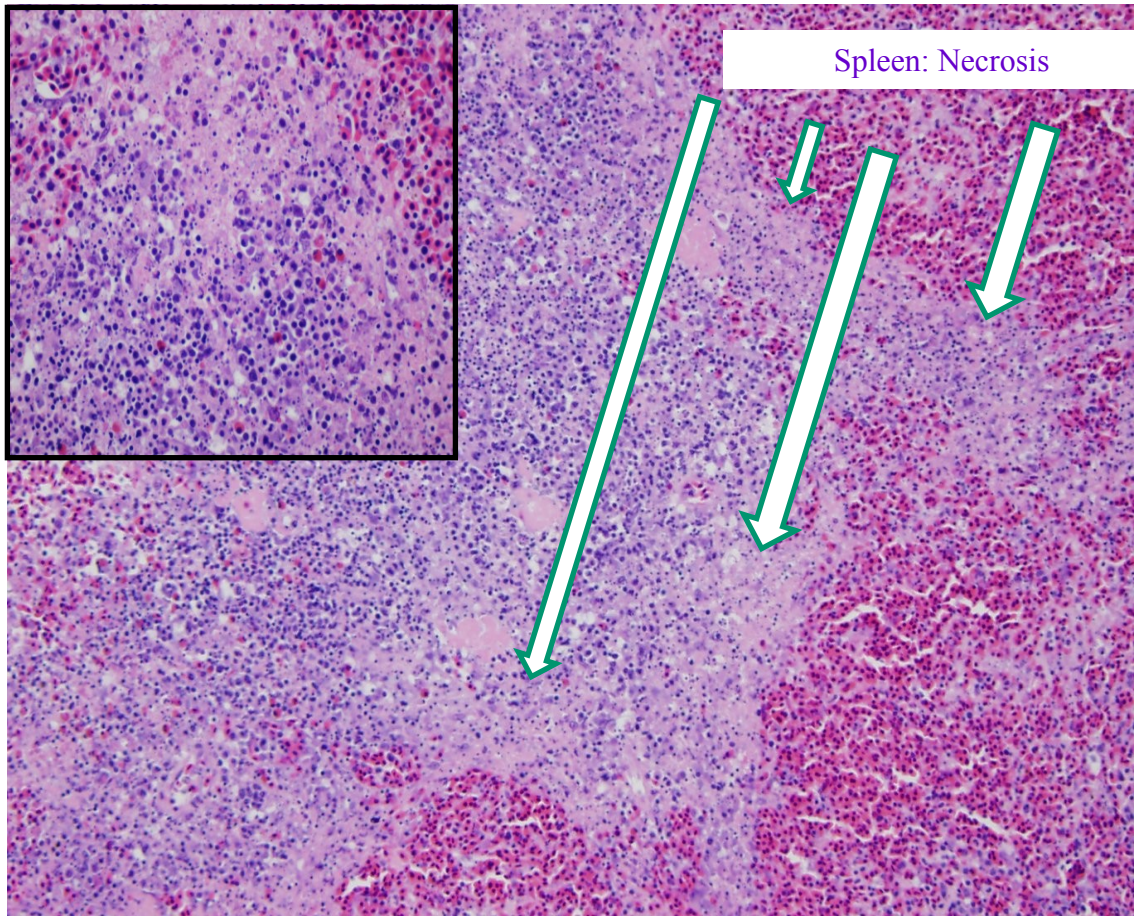
- 10% survival in low & high titer exposures vs. 100% in controls

- Qualitatively high titer treatment killed PS in less time

Virus Pathogenicity Study – Gross & Microscopic Lesions

- Reproduced previously observed necrosis of K, L, S, SB, Mes



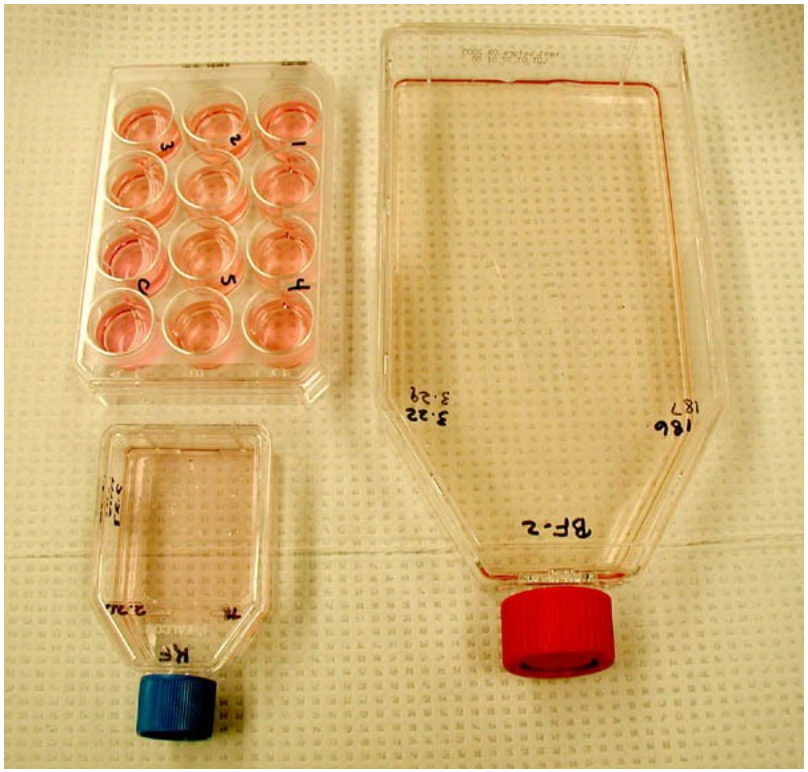


Virus Pathogenicity – Cell Culture

- Virus recovered from 36/36 morts, 2/4 virus exposed survivors, 0/5 controls

- High viral titers (3.1 x 10^7 – 6.7 x 10^8 $TCID_{50}$/ml) recovered from individual pooled tissue homogenates

- Koch's postulates fulfilled



2009 Blind Pony State Fish Hatchery Epizootic – Summary

- 2009 BPSFH PS epizootic attributed to a FV-3 like agent
- Induced gross & microscopic lesions, virion architecture, & in vitro characteristics typical of systemic IVs
- Virus isolated from hatchery PS in Sept, Oct, & Dec 2009 during & after active outbreak. PS lots destroyed.
- Oct 28 2009: 8 adult bullfrogs tested for ranavirus by PCR (all negative).
- Sept 07 2010: Given the untreated intake water 31 adult bullfrogs, 26 adult plains leopard frogs, & 4 plains leopard frog tadpoles from adjacent watersheds were cultured for virus (all negative) .

Mysterious 2001 BPSFH PS epizootic

- Epizootic began in July with rising water temperatures
  - All raceways experience 100% mortality
  - Samples negative (-) for MRSIV
  - Mixed bag of bact. cultured but mort. continued despite repeated antib. Tx
  - External & internal hemorrhagic lesions
  - Necrosis of haematopoietic tissues and mesentery
  - Virus isolated on several cell lines
  - Negative staining SEM revealed icosahedral particles (~ 160 nm)
  - Comparison of the 2001/2009 full MCP seq. revealed they are identical!!!
- PSRV continues to threaten conservation effort
- What is the source of the virus???
  - Sympatric amphibians contaminating hatchery intake H_2O in 01 & 09 (2x HS)
  - Adult population infected & pass vertically to progeny during manual spawning
  - Another hatchery reservoir?

Host Range of Frog Virus 3 like Agents

Frog Virus 3 like agents	Host Class	Host	Host common name	MCP % ID
Frog Virus 3 (FV3)	Amphibia	*Rana pipiens*	Leopard frog	/
Redwood Park Virus (RPV)	Amphibia	*Rana aurora*	Northern red-legged frog	100
Tadpole Edema Virus (TEV)	Amphibia	*Rana catesbeiana*	Bullfrog	99.6
Rana temporiana United Kingdom Virus (RUK)	Amphibia	*Rana temporaria*	European common frog	99.4
Rana gyrlio virus (RGV)	Amphibia	*Rana grylio*	Pig frog	99.2
Rana utricularia virus	Amphibia	*Rana utricularia*	Southern leopard frog	100
Rana clamitans virus	Amphibia	*Rana clamitans*	Green frog	100
Bufo bufo United Kingdom Virus (BUK)	Amphibia	*Bufo bufo*	European toad	99.2
Bufo marinus Venezuelan Iridovirus 1	Amphibia	*Bufo marinus*	Cane toad	99.2
Desmognathus quadramaculatus Virus	Amphibia	*D. quadramaculatus*	Blackbelly salamander	99.1
Box turtle virus 3 (TV3)	Reptilia	*Terrapene carolina*	Eastern box turtle	100
Tortoise virus 5 (TV5)	Reptilia	*Testudo horsfeldi*	Russian tortoise	99.1
Gopher tortoise virus	Reptilia	*Gopherus polyphemus*	Gopher toritoise virus	100
Burmese star tortoise virus	Reptilia	*Geochelone platynota*	Burmese star tortoise	100
Leopard tortoise Virus	Reptilia	*Geochelone pardalis*	Leopard tortoise	99.4
Softshell turtle iridovirus (STIV)	Reptilia	*Trionyx sinensis*	Chinese softshell turtle	99.7
Stickleback Virus (SBV)	Osteichthys	*Gasterosteus aculeatus*	Threespine stickleback	100
Pallid sturgeon Ranavirus (PSRV)	Osteichthys	*Scaphirhynchus albus*	Pallid sturgeon	100

Significance

- Ranaviruses are a global threat to both cultured & feral populations of poikilothermic vertebrates (fish, amphibians, & reptiles)

- RVs are especially concerning emerging pathogens given their high virulence & low host specificity (interclass host shifts/reservoirs)

- PSRV represents the first fully characterized example of a FV-3 isolate infecting a fish host

- These epizootics are especially concerning given the federally endangered status of pallid sturgeon

Future Directions

- Prevent future BPSFH PSRV epizootics
  - Install disinfection and chiller systems for incoming water
  - Sample a diversity of sympatric amphibians/life stages
  - Sample adult PS during manual spawning (reproductive products)

- Determine the pathogenicity of the PSRV isolates to other fish (sturgeon, LMB, CC, tilapia), amphibian, & reptilian spp.
  - PSRV exposed wood frog tadpoles succumbed within 7 days!

Future Directions

- Determine the pathogenicity of the PSRV isolates to reptilian spp.
  - Red-eared sliders up next…

- Functional phylogenomic analyses to compare PSRV to other RVs to determine genetic markers for pathogenicity & host fidelity
  - Sucrose pur. PSRV, WSIV, MRSIV, et al. submitted for 2nd Gen Seq

Future Directions

- Characterize other fish FV-3 like isolates
  - White sturgeon RV (1998 CA epizootic, closest relative Rana esculenta virus)
  - Et al…

Thanks for your attention!
Questions?

UF College of Veterinary Medicine
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