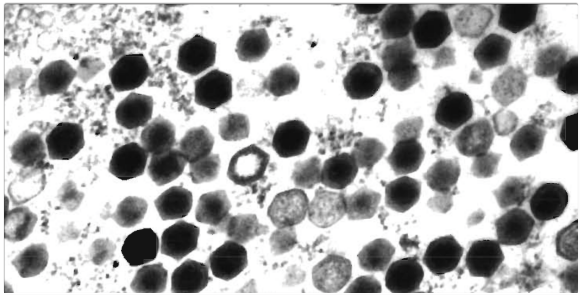


RANAVIRUS PERSISTENCE

JESSE BRUNNER
WASHINGTON STATE UNIVERSITY

TWO KEY QUESTIONS RELATED TO PERSISTENCE:

1. How do these lethal viruses stick around between epidemics (=years) when:
   A. they seem to kill most all of their hosts
   B. their hosts are often seasonally abundant (e.g., pond-breeding amphibians)?
2. Can ranaviruses be transmitted from the environment at appreciable rates?

PERSISTENCE IN THE ENVIRONMENT

- **Water**
  - liquid or frozen
- **Substrate or soil**
  - wet or dried
- **Carcasses**
  - fresh or frozen
- **Stuck to fomites** (e.g., aquatic invertebrates)

PERSISTENCE IN (SEMI-) STERILE CONDITIONS

- No loss of EHNV titer ≥97 days at 15°C *in distilled water* (Langdon et al. 1989)
- EHNV survived 155-200 days at 15°C *in sterile tissue culture on sterile petri dishes* (Langdon et al. 1997)
- Amphibian & Reptile RVs had T-90s of 9-11 days when *dried on sterile, stainless steel discs* (Nazir et al. 2012)

PERSISTENCE IN MORE NATURAL CONDITIONS

- LMBV lost 90% of its infectivity (T-90) in water* after 2 days (Brunner and Grizzle 2003), but remained detectable for 7 days

* kind of water not stated… presumably hatchery

PERSISTENCE IN WATER

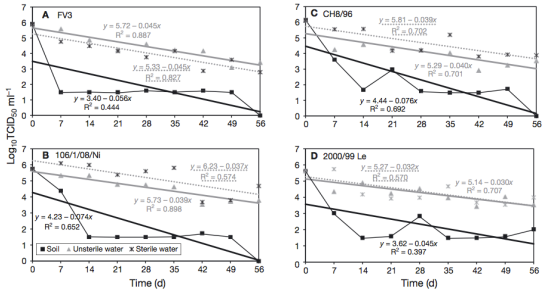
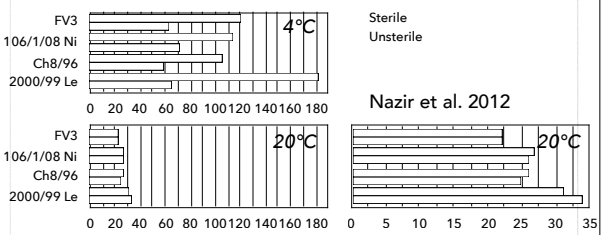
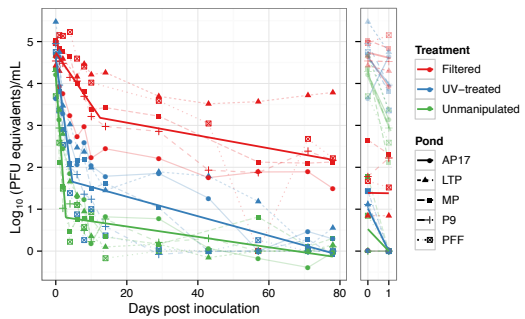


Fig. 3. Inactivation kinetics of amphibian and reptilian ranaviruses in unsterile (gray equation, solid gray line) and sterile (gray equation, dotted gray line) pond water and soil (black equation, solid black line) at 20°C. Linear regression models were calculated for inactivation in unsterile and sterile pond water. Inactivation in soil was non-linear

PERSISTENCE IN WATER

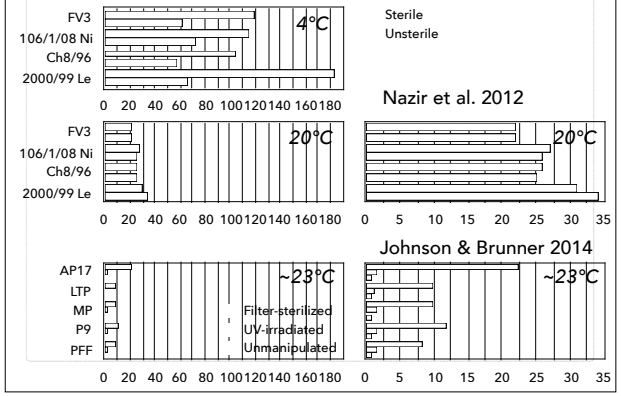


PERSISTENCE IN WATER



Johnson & Brunner et al. 2012

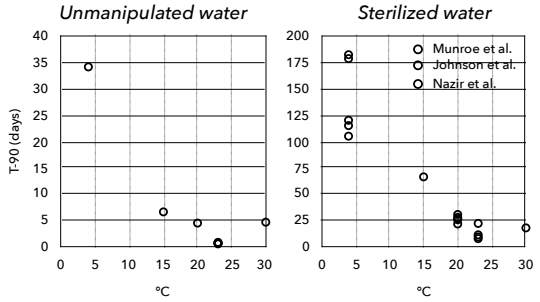
PERSISTENCE IN WATER



Nazir et al. 2012

Johnson & Brunner 2014

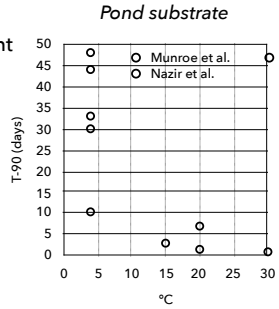
PERSISTENCE IN WATER



Temperature, microbial community matter a great deal

PERSISTENCE IN SOIL OR SUBSTRATE

ATV-spiked pond sediment was <u>dried</u> & rehydrated it was not infectious to salamander larvae (Brunner et al. 2007)



Temperature & drying(?) matter a great deal

FROZEN IN THE ENVIRONMENT

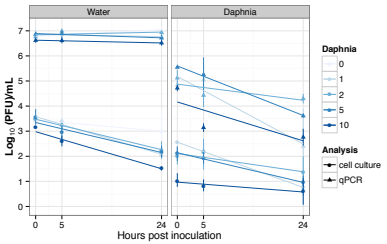
EHNV persists in frozen fish (–20°C and –70°C) for ≥ 2 years & ≥ 7 days at 4°C (Langdon 1989)

LMBV persists in frozen tissues for 155 days (Plumb and Zilberg 1999)

ATV has been detected in frozen carcasses (D. Schock, pers. comm.)

PERSISTENCE IN THE ENVIRONMENT

- **Water**
  - liquid or frozen
- **Substrate or soil**
  - wet or dried
- **Carcasses**
  - fresh or frozen
- **Stuck to fomites** (e.g., aquatic invertebrates)



Johnson & Brunner 2014

PERSISTENCE BETWEEN EPIDEMICS?

Possibly in:

- **Frozen in water or carcasses**
- **In cold water (?)**

Probably not in

- **Substrate or soil**

TRANSMISSION WITHIN EPIDEMICS?

Possibly in:

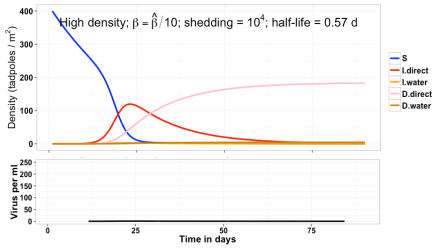
- **water**
- **carcasses**
- **substrate or soil**

The question is, How important is this?

WATER-BORNE TRANSMISSION

- Add term for concentration-specific transmission from water
  - **Probability of infection** from LD_{50} study in Warne et al. 2011
- Add terms for accumulation and loss of virus in water
  - **Viral shedding**: rough estimates range from 10^2 to 10^4 pfu/day in lab experiments with *Ambystoma nebulosum* (Storfer et al. *in prep*, Brunner *unpublished data*)
  - **Half-life of ranaviruses** ranges from
    - 9.65 days in "unsterile" pond water at 20°C (Nazir et al. 2011)
    - 0.57 days in pond water at 20–24°C (Johnson & Brunner *in prep*; see poster)

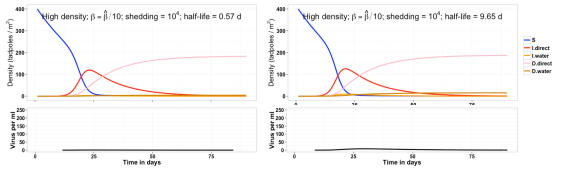
WATER-BORNE TRANSMISSION



- Very few tadpoles infected from the water (even with lower transmission)

WATER-BORNE TRANSMISSION

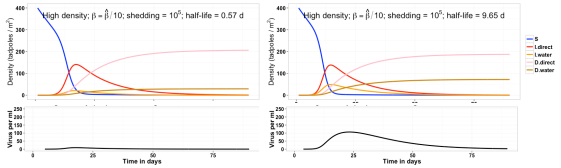
- Does a longer half-life of Rv in water help?



  - Even with very long persistence times, water-borne transmission contributes very few infections

WATER-BORNE TRANSMISSION

- What about a greater shedding rate?



Even with a

- *low rate of direct transmission*,
- *long persistence time*, &
- *high shedding rate*

water-borne transmission is <u>still</u> minor source of infection compared to direct contacts

PERSISTENCE IN HOSTS– RESERVOIRS

Sublethally-infected hosts (i.e., carriers) are common

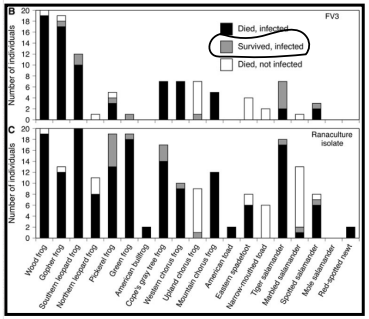
- **survivors of otherwise lethal infections (*intra*specific reservoirs)**
- **tolerant species (*inter*specific reservoirs)**

PERSISTENCE IN HOSTS– RESERVOIRS

Sublethally-infected hosts (i.e., carriers) are common

- **tolerant species (*inter*specific reservoirs)**
  - ***Hayden et al. 2002 is a good reference for conceptual problem of IDing reservoirs***
- **survivors of otherwise lethal infections (*intra*specific reservoirs)**

PERSISTENCE IN HOSTS– RESERVOIRS



***Resistance** = ability to prevent or clear infection*
***Tolerance** = ability to minimize fitness consequence of infection*

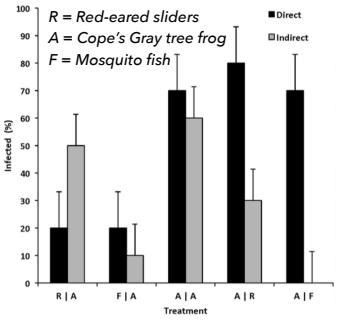
Large differences among species in both resistance and tolerance

Hoverman, J. T., M. J. Gray, N. A. Haislip, and D. L. Miller. 2011. Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. EcoHealth 8:301-319.

PERSISTENCE IN HOSTS– RESERVOIRS

Transmission is possible between species, even classes!

Open question is how and how often does this occur?



Brenes et al. 2014

DURATION OF CARRIER STATES

Notophthalmus viridescens developed persistent (≥81 days) infections with T6-T20 (FV3-like) (Clark et al. 1969)

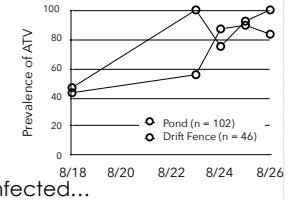
FV3 persists in peritoneal leukocytes for ≥ 3 weeks in *Xenopus laevis* (Morales et al. 2010)

Evidence of carrier state in EHNV infections is mixed, but likely in redfin perch (reviewed in Whittington et al. 2010)

Ambystoma tigrinum larvae maintained persistent, transmissible ATV infections for ≥ 5 months (Brunner et al. 2004)

Over 1/3 of adult male Rana sylvatica returning to ponds to breed harbored sublethal FV3 infections (Crespi et al. 2015)

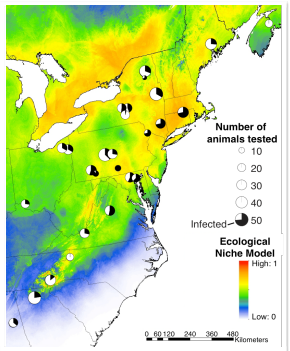
ATV PERSISTENCE AT DOT



Metamorphs leave ponds infected…

and adults return to ponds infected

DURATION OF CARRIER STATES



Male wood frogs (*Lithobates sylvaticus*) coming to to breed

RV found in:

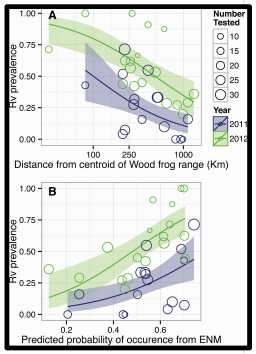
- 25/27 ponds over 2 years
- 39% of all 753 frogs tested

Crespi, E. J., L. J. Rissler, N. M. Mattheus, K. Engbrecht, S. I. Duncan, T. Seaborn, E. M. Hall, J. D. Peterson, and J. L. Brunner. 2015. Geophysiology of wood frogs: landscape patterns of prevalence of disease and circulating hormone concentrations across the eastern range. Integrative and Comparative Biology 55:602-617.

DURATION OF CARRIER STATES

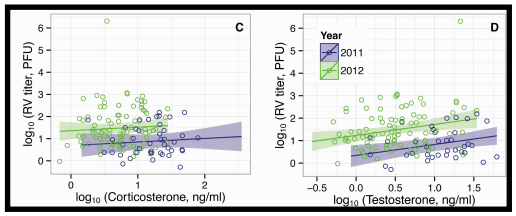
Two surprises:

1. High prevalence
   - wood frogs are a highly susceptible species
   - So how do so many adults survive with RV infections?
2. Infections more common in *core* of range
   - expected animals on edge to be in worse shape, less able to defend against infection
   - maybe more likely to survive with infection?



Crespi, E. J., L. J. Rissler, N. M. Mattheus, K. Engbrecht, S. I. Duncan, T. Seaborn, E. M. Hall, J. D. Peterson, and J. L. Brunner. 2015. Geophysiology of wood frogs: landscape patterns of prevalence of disease and circulating hormone concentrations across the eastern range. Integrative and Comparative Biology 55:602-617.

DURATION OF CARRIER STATES



RV titer in frogs not related to CORT
…but increases with Testosterone

- Maybe T causes (some) infections to recrudesce?

Crespi, E. J., L. J. Rissler, N. M. Mattheus, K. Engbrecht, S. I. Duncan, T. Seaborn, E. M. Hall, J. D. Peterson, and J. L. Brunner. 2015. Geophysiology of wood frogs: landscape patterns of prevalence of disease and circulating hormone concentrations across the eastern range. Integrative and Comparative Biology 55:602-617.

TAKE HOME MESSAGES

- **RVs can persist for short to long duration in the environment**
  - **Many details to be sorted out**
    - **temperature, ice, carcasses, microbes and detritivores**
  - **But clear potential for persistence between epidemics AND movement between ponds!**
- **RVs can persist in carrier state**
  - **both inter- and intraspecific reservoirs possible**
  - **Details of how RV gets back into a population are unknown, BUT larger potential for movement in carriers**

REFERENCES

- Brenes, R., M. J. Gray, T. B. Waltzek, R. P. Wilkes, and D. L. Miller. 2014. Transmission of ranavirus between ectothermic vertebrate hosts. PLoS One 9:e92476.
- Brunner, J. L., D. M. Schock, J. P. Collins, and E. W. Davidson. 2004. The role of an intraspecific reservoir in the persistence of a lethal ranavirus. Ecology 85:560-566.
- Brunner, J. L., A. Storfer, M. J. Gray, and J. T. Hoverman. 2015. Ranavirus ecology and evolution: from epidemiology to extinction. Pages 71-104 in Gray, M. J., and V. G. Chinchar, editors. Ranaviruses: Lethal pathogens of ecothermic vertebrates. Springer International Publishing,
- Clark, H. F., C. Gray, F. Fabian, R. Zeigel, and D. T. Karzon. 1969. Comparative studies of amphibian cytoplasmic virus strains isolated from the leopard frog, bullfrog, and newt. Pages 310-326 in Mizell, M., editors. Biology of Amphibian Tumors. Springer-Verlag, New York, New York, USA.
- Crespi, E. J., L. J. Rissler, N. M. Mattheus, K. Engbrecht, S. I. Duncan, T. Seaborn, E. M. Hall, J. D. Peterson, and J. L. Brunner. 2015. Geophysiology of wood frogs: landscape patterns of prevalence of disease and circulating hormone concentrations across the eastern range. Integrative and Comparative Biology 55:602-617.
- Grizzle, J. M., and C. J. Brunner. 2003. Review of largemouth bass virus. Fisheries 28:10-14.
- Haydon, D. T., S. Cleaveland, L. H. Taylor, and M. K. Laurenson. 2002. Identifying reservoirs of infection: A conceptual and practical challenge. Emerging Infectious Diseases 8:1468-1473.
- Johnson, A. F., and J. L. Brunner. 2014. Persistence of an amphibian ranavirus in aquatic communities. Diseases of Aquatic Organisms 111:129-138.
- Langdon, J. S. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, Perca fluviatilis L., and 11 other teleosts. Journal of Fish Diseases 12:295-310.
- Morales, H. D., L. Abramowitz, J. Gertz, J. Sowa, A. Vogel, and J. Robert. 2010. Innate immune responses and permissiveness to ranavirus infection of peritoneal leukocytes in the frog Xenopus laevis. Journal of Virology 84:4912-4922.
- Munro, J., A. E. Bayley, N. J. McPherson, and S. W. Feist. 2016. Survival of Frog Virus 3 in freshwater and sediment from an English lake. Journal of Wildlife Diseases 52:138-142.
- Nazir, J., M. Spengler, and R. E. Marschang. 2012. Environmental persistence of amphibian and reptilian ranaviruses. Diseases of Aquatic Organisms 98:177-184.
- Whittington, R. J., J. A. Becker, and M. M. Dennis. 2010. Iridovirus infections in finfish - critical review with emphasis on ranaviruses. Journal of Fish Diseases 33:95-122.