Amphibian ranavirus transmission and persistence

With an emphasis on ecological relevance

Jesse Brunner

Washington State University

Amphibian ranaviruses

1) Transmission

- Routes of transmission – *How* does ranavirus get around?
- Function form of transmission – How does transmission scale with density?

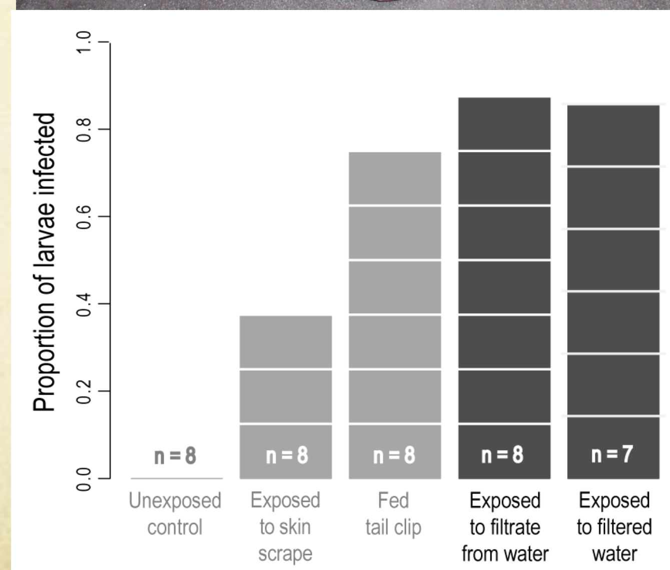
2) Persistence

- Environmental persistence
- Persistent infections
- Biotic reservoirs

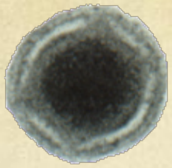
3) Future directions

Routes of transmission: *via water*

- Essentially every dose-response study with ranavirus
  - BIV Cullen et al. 1995, Cullen & Owens 2002
  - ATV Brunner et al. 2005
  - FV3 Pearman et al. 2004, Hoverman et al. 2010, Warne et al. 2011
  - RUK Cunningham et al. 2007
  - LMBV Grant et al. 2003
- Small particles (filtered water) and chunky bits (filtrate) are both very infectious (Brunner et al. 2007)



Brunner et al. 2007



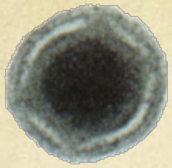
Routes of transmission: *via water*

Harp & Petranka (2006) added water (~2L) and pond substrate (~0.3kg) from ponds undergoing die-offs to kiddie pools with wood frog tadpoles

TABLE 1. Results of polymerase chain reaction assays for ranavirus detection in surviving wood frog tadpoles from Experiment 3.[a]

Experimental treatment			
Sediment	Water	No. of samples (% positive)	Pools (% positive)
Exposed	Unexposed	11 (45%)	83%
Exposed	Exposed	9 (44%)	67%
Unexposed	Unexposed	6 (0%)	0%
Unexposed	Exposed	6 (0%)	0%

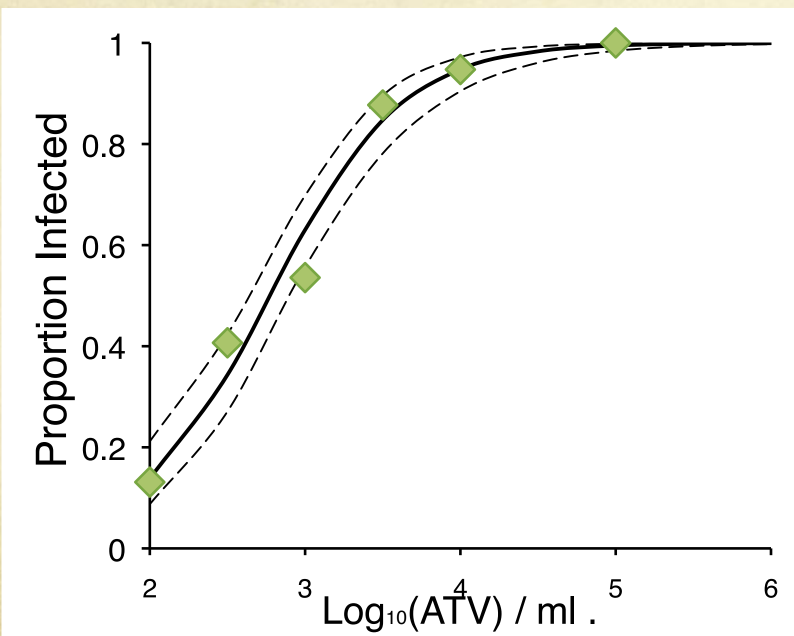
[a] Treatments reflect all combinations of exposed or unexposed sediment and water. Exposed samples were collected during an active ranavirus outbreak and unexposed samples from a pond without ranavirus. Data represent the number of samples analyzed, the percentage of samples that were positive (in parentheses), and the percentage of pools (n=6 per treatment) that were positive for ranavirus. Each sample reflects a pooled analysis of five tadpoles, and from one to three samples (5–15 tadpoles) were analyzed per pool.



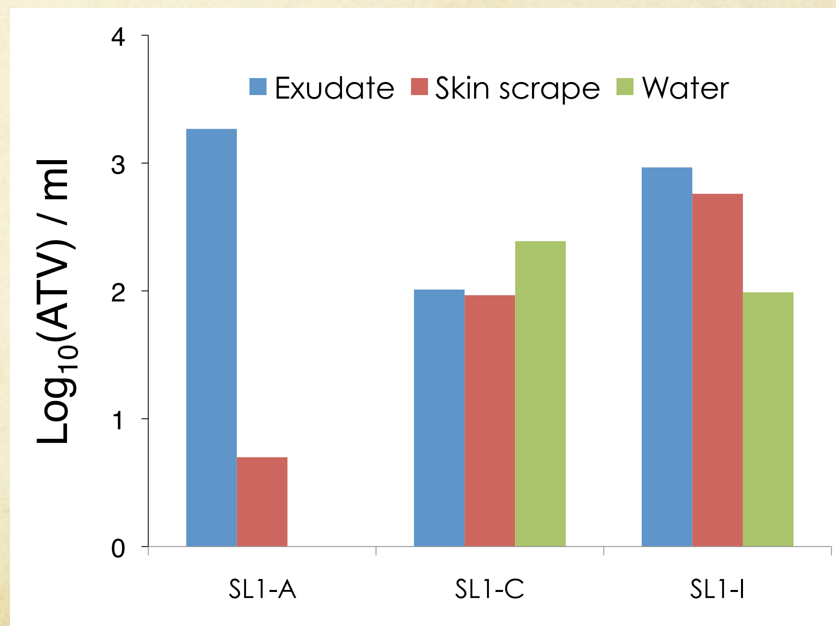
Routes of transmission: *via water*

Are infectious levels of virus in water reached in nature?

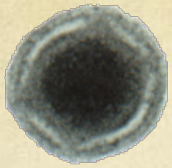
- Measured ATV concentration in exudate, scrapes, and water (~500mL) a week after IP injection
- In a well-mixed, 10m diameter pond 1m deep, would need 157,000 sick larvae shedding to reach 10^2pfu/ml!



Brunner et al. 2005

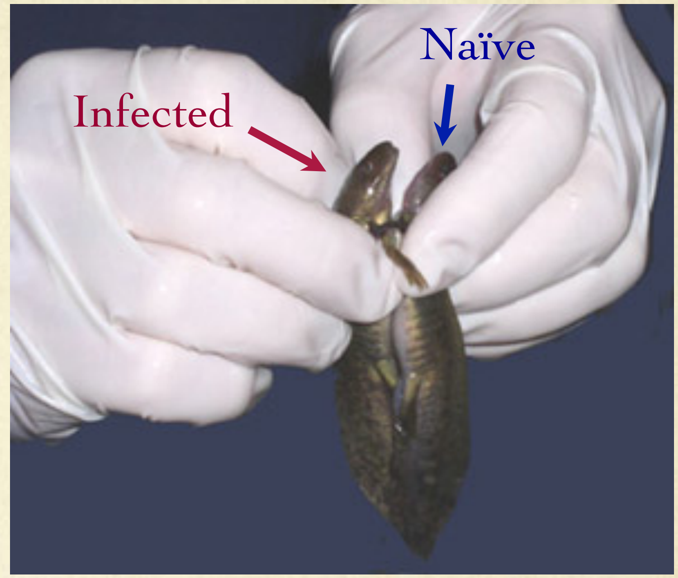


Brunner, unpublished data



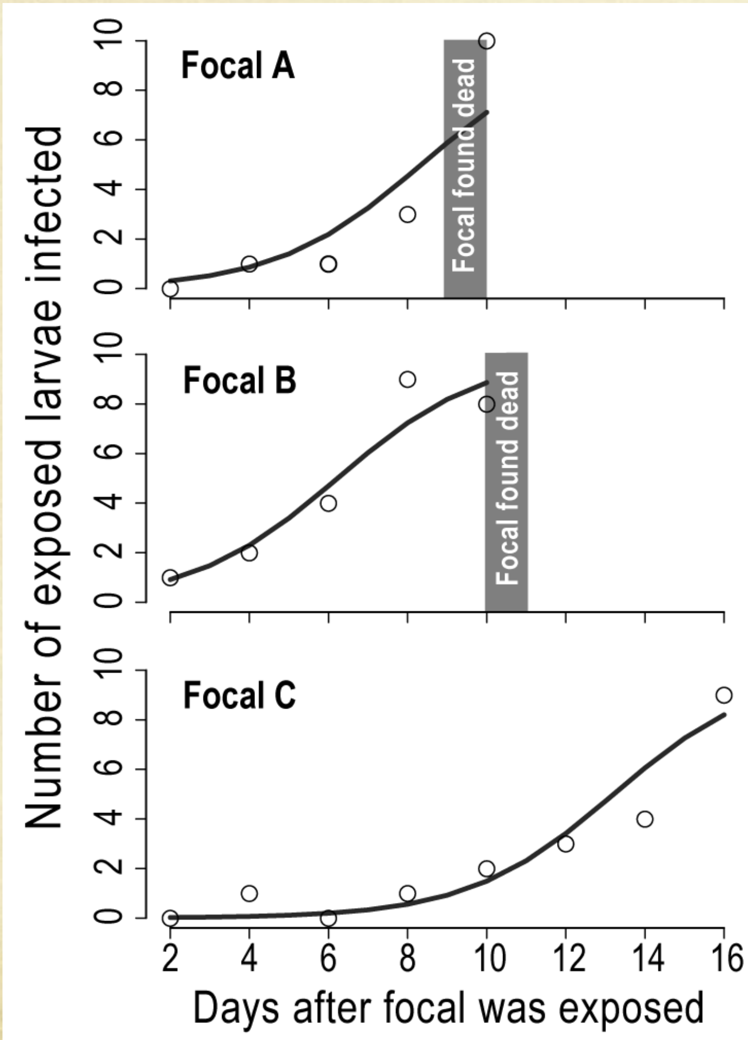
Routes of transmission: *direct contact*

- ATV: one second, belly-to-belly contact caused infection in 18/21 *Ambystoma tigrinum* larvae (Brunner et al. 2007)
- BIV: 5/8 *Limnodynastes terraereginae* metamorphs co-housed with IP-injected frogs were infected (Cullen et al. 1995)[*]

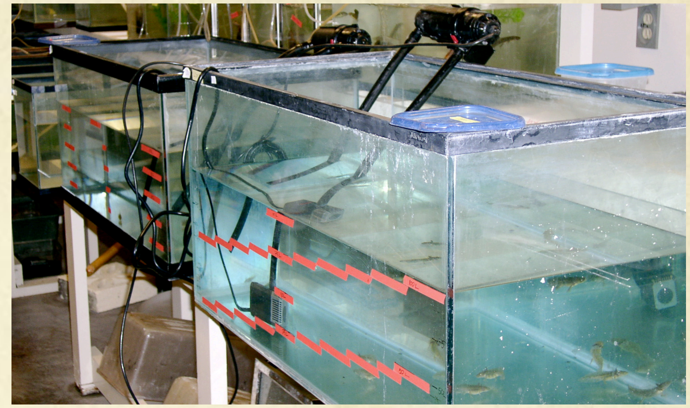


[*]but not *L. caerulea* or *Cophixalus ornatus* adults (Cullen & Owen 2002)

Routes of transmission: *direct contact*



Brunner et al. 2007

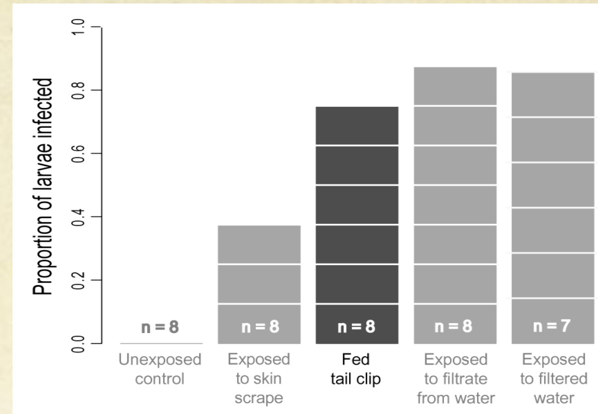


- Infected *A. tigrinum* larvae become more infectious through time
- Carcasses are very infectious

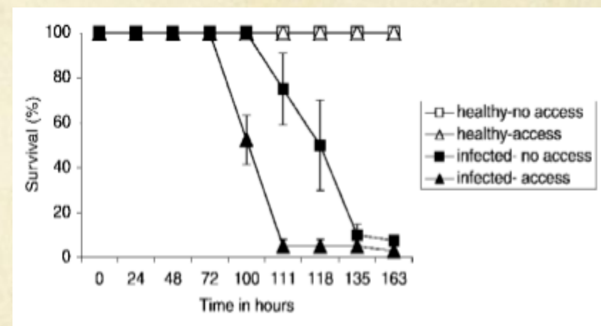
Routes of transmission: *consumption*

- Bits & pieces (nipping, biting)
  - Fed tail clips form ATV-exposed larvae (Brunner et al. 2005)
  - Orally inoculated 3 anuran spp with FV3 (Hoverman et al. 2010)
- Cannibalism

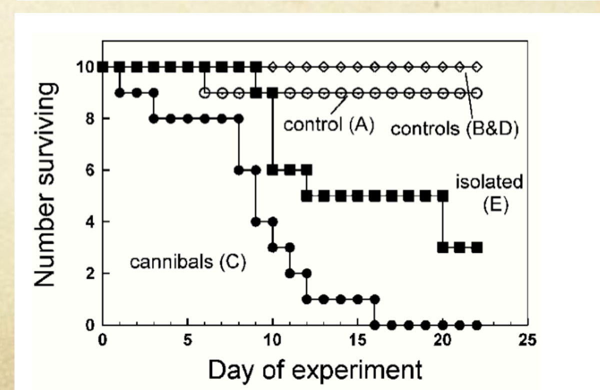
- Necrophagy/scavenging
  - Tadpoles with access to FV3-infected carcasses get sick and die faster (Harp & Petranka 2006, Pearman et al. 2004)



Brunner et al. 2007



Harp & Petranka 2006



Pearman et al. 2004

Routes of transmission: *vertical*

- 60% of wood frog tadpoles raised from eggs in lab "weakly positive" for FV3-like virus (Greer et al. 2005)
- FV3-contaminated wood frog eggs: 4/5 field-collected & 1/3 laid in captivity (Duffus et al. 2008)
  - Only 1/59 tadpoles tested from these four clutches was positive by PCR

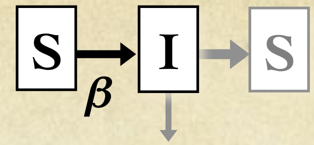
Contamination or true vertical transmission?

Vertical transmission is rare

unimportant for epidemic dynamics

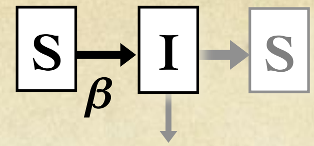
potentially important for year-to-year persistence

The functional form of the transmission term



$$contacts \times \left(I/N\right) \times P(\text{inf} \mid contact) \times S$$

The functional form of the transmission term



$$contacts \times (I/N) \times P(\inf \mid contact) \times S$$

Contact rate increases with density

$$\beta IS$$

- Disease fades out before host goes extinct
- Culling is an effective control measure

Contact rate is constant (density-independent)

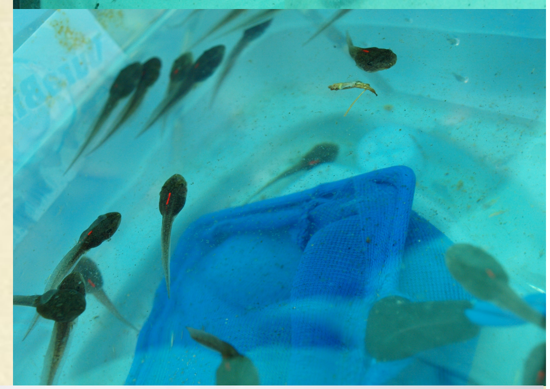
$$\beta (I/N) S$$

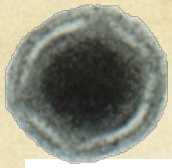
- Transmission continues as host goes extinct
- Culling will not control disease

Add infected and susceptible animals to pools

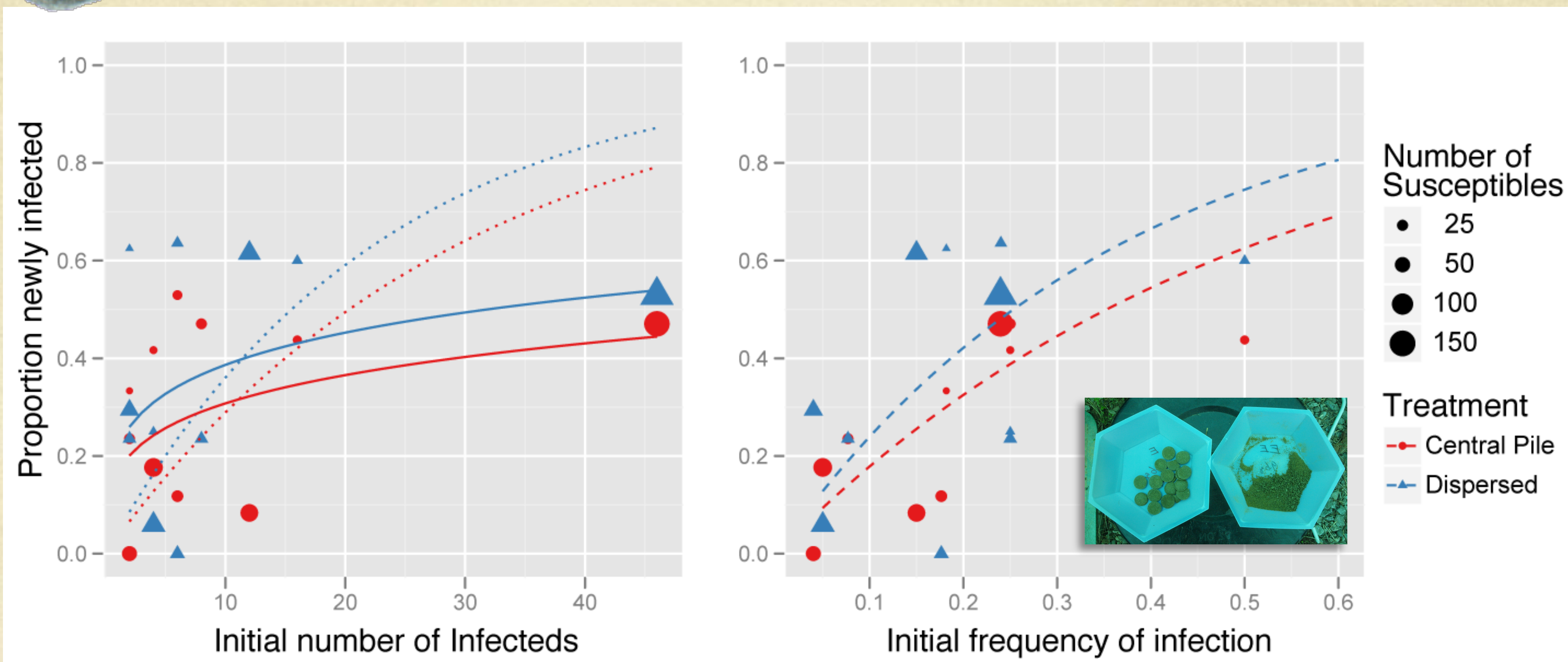
Wait 24h and see how many were infected

Fit transmission terms to data





Form of the transmission term



Transmission model	df	Central pile ΔAICc	Central pile weight	Dispersed ΔAICc	Dispersed weight
Constant βS	1	17.3	0	3.6	0.09
Density βIS	1	35.8	0	26.1	0
Frequency $\beta (I/N)S$	1	0	1.0	0	0.52
Power $\beta I^q S$	2	15	0	0.6	0.39

Brunner et al. *In prep.*, see also Greer et al. 2008

Transmission summary

<u>Routes of transmission</u>

1) Most transmission occurs by "close contact"

2) Build up of virus in the environment, particularly substrate, may increase transmission

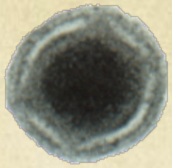
3) Cannibalism & Necrophagy/ Scavenging are probably very important

<u>Form of transmission</u>

1) Frequency-dependent (over most host densities)

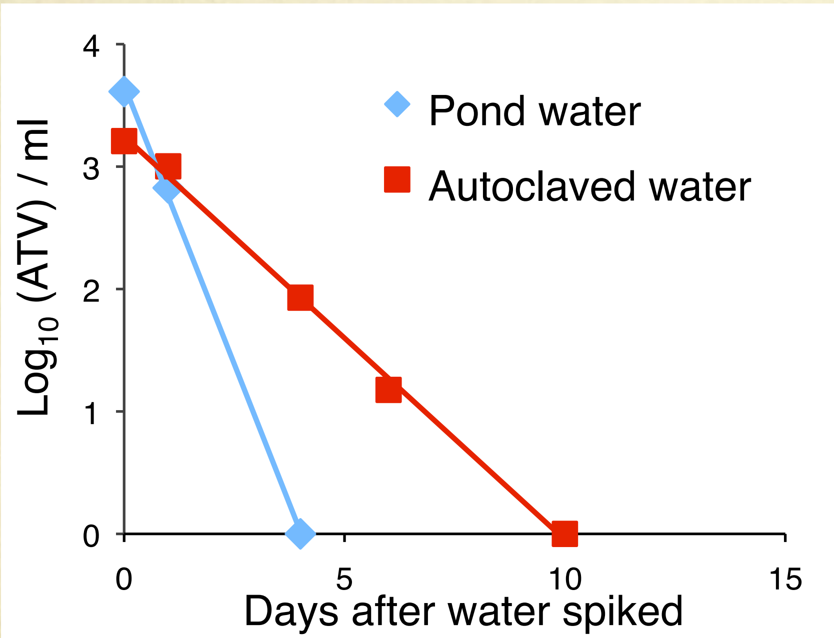
2) Dose-dependent transmission from the environment is like density-dependent transmission

3) Transmission via scavenging is an added term (keep track of carcasses) and should lead to accelerating epidemics

Persistence in the environment 

EHNV can survive ≥ 97d in distilled water & ≥113 d at 15°C in dried culture media (Langdon 1989, reviewed in Whittington et al. 2010)

Water



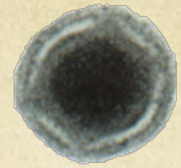
Brunner, unpublished data

Sediment

Sediment collected during an epidemic infected wood frog tadpoles in 9/12 pools (Harp & Petranka 2006)

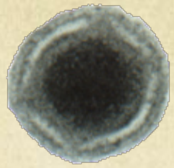
But… when ATV-spiked pond sediment was dried & rehydrated it was not infectious to salamander larvae (Brunner et al. 2007)

Persistence in frozen carcasses



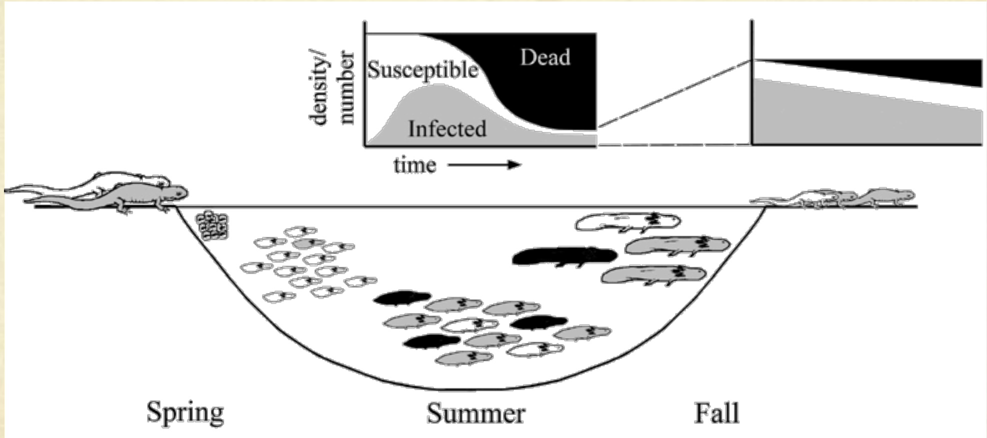
EHNV can persist in frozen fish for ≥ 2 years (Langdon 1989)

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

Persistence in carrier state 

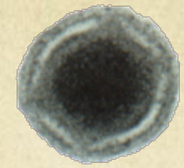
- *Notophthalmus viridescens* developed persistent (≥81 days) infections with T6-T20 (FV3-like) (Clark et al. 1969)
  - FV3 persists and replicates in peritoneal leukocytes for ≥12 days in *Xenopus laevis* (Robert et al. 2007)

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



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

Persistence in alternate hosts / biotic reservoirs



No shortage of potential hosts

- ATV infects a range of salamanders (Jancovich et al. 2001) as well as frogs (Schock et al. 2008)
- FV3 infects anurans and caudates, (Duffus et a. 2008, Schock et al. 2008), and apparently fish (Mao et al. 1999) and chelonians, too (Johnson et al. 2008)
- BIV infect frogs and fish (Moody & Owens 1994)

Interspecific transmission not assured or well understood

E.g., FV3 and ATV viruses co-circulating in wood frogs and tiger salamanders, respectively (Schock et al. 2008)

Persistence summary

1) Need to think about persistence at different time scales, with varying relevance for:
   - transmission within epidemics
   - persistence between epidemics (re-current epidemics)
   - movement between populations or regions

2) Multiple means of persistence between epidemics:
   - reservoirs
   - Carriers
   - frozen carcasses

3) Environmental persistence of ranavirus least well documented or understood

Brunner, J. L., and J. P. Collins. 2009. Testing assumptions of the trade-off theory of the evolution of parasite virulence. Evolutionary ecology research **11:1169-1188.**

Brunner, J. L., K. Richards, and J. P. Collins. 2005. Dose and host characteristics influence virulence of ranavirus infections. Oecologia **144:399-406.**

Brunner, J. L., D. M. Schock, and J. P. Collins. 2007. Transmission dynamics of the amphibian ranavirus *Ambystoma tigrinum virus. Diseases of aquatic organisms **77:87-95.***

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.**

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.*

Cullen, B. R., and L. Owens. 2002. Experimental challenge and clinical cases of bohle Iridovirus (BIV) in native Australian anurans. Diseases of aquatic organisms **49:83-92.**

Cullen, C. R., L. Owens, and R. J. Whittington. 1995. Experimental infection of Australian anurans (*limnodynastes terraereginae and litoria latopalmata) with bohle Iridovirus. Diseases of aquatic organisms **23:83-92.***

Cunningham, A. A., A. D. Hyatt, P. Russell, and P. M. Bennett. 2007. Experimental transmission of a ranavirus disease of common toads (Bufo Bufo) to common frogs (Rana temporaria). Epidemiology and Infection **135:1213-1216.**

Duffus, A. L. J., B. D. Pauli, K. Wozney, C. R. Brunetti, and M. Berrill. 2008. Frog Virus 3-like infections in aquatic amphibian communities. J wildlife dis **44:109-120.**

Grant, E. C., D. P. Philipp, K. R. Inendino, and T. L. Goldberg. 2003. Effects of temperature on the susceptibility of largemouth bass to largemouth bass virus. Journal of Aquatic Animal Health **15:215-220.**

Greer, A. L., M. Berrill, and P. J. Wilson. 2005. Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. Diseases of aquatic organisms **67:9-14.**

Greer, A. L., C. J. Briggs, and J. P. Collins. 2008. Testing a key assumption of host-pathogen theory: Density and disease transmission. Oikos **117:1667-1673.**

Harp, E. M., and J. W. Petranka. 2006. Ranavirus in wood frogs (*Rana sylvatica): Potential sources of transmission within and between ponds. Journal of wildlife diseases **42:307-318.***

Hoverman, J. T., M. J. Gray, and D. L. Miller. 2010. Anuran susceptibilities to ranaviruses: Role of species identity, exposure route, and a novel virus isolate. Dis aquat org **89:97-107.**

Jancovich, J. K., E. W. Davidson, A. Seiler, B. L. Jacobs, and J. P. Collins. 2001. Transmission of the *Ambystoma tigrinum virus to alternative hosts. Diseases of aquatic organisms **46:159-163.***

Johnson, A. J., A. P. Pessier, J. F. X. Wellehan, A. Childress, T. M. Norton, N. L. Stedman, D. C. Bloom, W. Belzer, V. R. Titus, R. Wagner, J. W. Brooks, J. Spratt, and E. R. Jacobson. 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. Journal of wildlife diseases **44:851-863.**

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.***

Mao, J., D. E. Green, G. Fellers, and V. G. Chinchar. 1999. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. Virus Research **63:45-52.**

Moody, N. J. G., and L. Owens. 1994. Experimental demonstration of the pathogenicity of a frog virus, bohle Iridovirus, for a fish species, barramundi *lates calcarifer. Diseases of aquatic organisms **18:95-102.***

Pearman, P. B., T. W. J. Garner, M. Straub, and U. F. Greber. 2004. Response of the italian agile frog (*Rana latastei) to a ranavirus, Frog Virus 3: A model for viral emergence in naive populations. Journal of wildlife diseases **40:660-669.***

Schock, D. M., T. K. Bollinger, V. G. Chinchar, J. K. Jancovich, and J. P. Collins. 2008. Experimental evidence that amphibian ranaviruses are multi-host pathogens. Copeia **1:133-143.**

Warne, R. W., E. J. Crespi, and J. L. Brunner. 2011. Escape from the pond: Stress and developmental responses to ranavirus infection in wood frog tadpoles. Functional ecology **25:139-146.**

Whittington, R. J., J. A. Becker, and M. M. Dennis. 2010. Iridovirus infections in finfish - critical review with emphasis on ranaviruses. J fish dis **33:95-122.**

Future directions / research needs

1) Establish the *ecologically relevant* environmental limits of ranavirus
   - In pond water (with algae, bacteria, etc.) & substrates
   - On potential fomites & in or on potential carriers (e.g., birds)

2) Study persistent/chronic infections
   - Commonness, duration, and cause(s) of

3) Determine the relative importance of different routes of transmission
   - From environmental sources, carcasses, and live hosts
   - Between alternate host species in the community