



**Authors**

Megan Winzeler, undergraduate student; Seth LaGrange, undergraduate student; and Jason Hoverman, Assistant Professor in Vertebrate Ecology, Department of Forestry and Natural Resources

# Ranaviruses: Emerging Threat to Amphibians

Amphibians are integral to our ecosystem as predators, as a food resource, and as ecological indicators for water pollution and habitat quality. And currently, amphibians face increasing disease challenges.

Like any free-living species, amphibians are host to a variety of pathogens including bacteria, fungi, viruses, and parasitic worms. While the majority of pathogens are relatively benign, viral pathogens in the genus *Ranavirus* are responsible for catastrophic die-offs across the globe. In the United States, ranaviruses have been linked to die-offs in 29 different amphibian species across 25 states.

Researchers haven't tracked ranaviruses closely, so they don't know how common or widespread they are in Indiana's amphibian populations. However, because they are spreading, this publication gives an

overview of ranaviruses and their effects on amphibians. We are asking biologists and recreationists to take an active role in helping track these diseases to preserve Indiana amphibians.

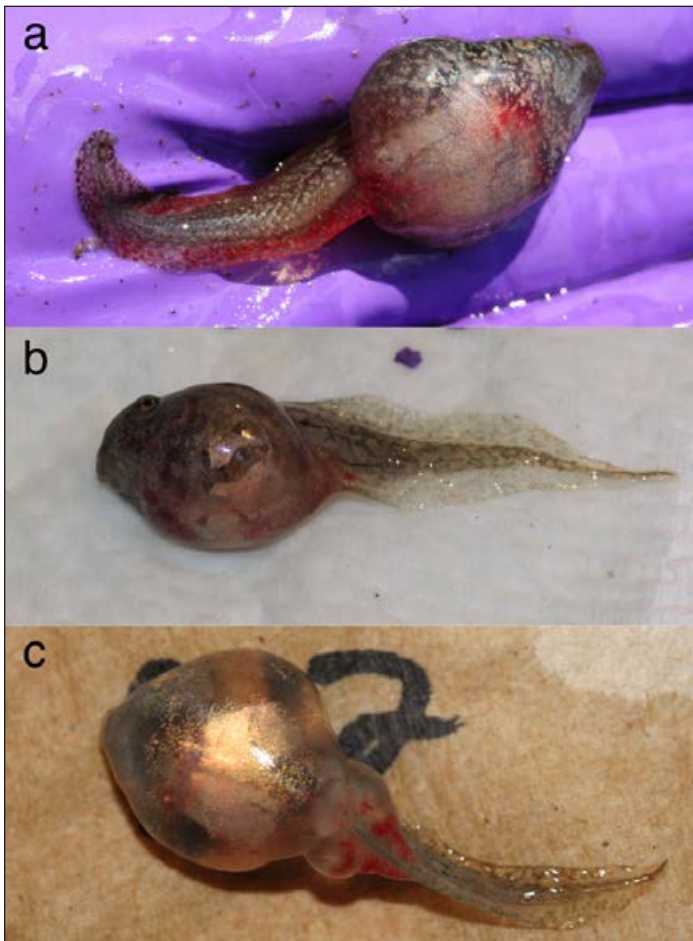
## What are ranaviruses?

Ranaviruses infect cold-blooded vertebrates, including bony fish, reptiles, and amphibians. They are double-stranded DNA viruses that multiply in host cells. Two species of ranaviruses are known to infect amphibians in North America: frog virus 3 (FV3) and *Ambystoma tigrinum* virus (ATV). While FV3 is widespread across North America, ATV has only been detected in regions west of the Mississippi River. Both FV3 and ATV can infect many different species of amphibians, but the outcome of infection varies by species.



### What are the signs of infection?

Ranaviruses can infect the liver, kidneys, and spleen. In these organs, the viruses rapidly multiply, kill cells, then spread to infect neighboring cells. Common signs of infection include lethargy, emaciation, hemorrhaging, and edema (swelling) of the legs or body (Fig. 1). The virus spreads rapidly within infected hosts. Signs of disease can be seen within days of infection, and death can occur as early as seven days after infection. Ranavirus infections appear to be more lethal in larval than adult amphibians. This may be due to the fact that in the larval stage the amphibian immune system is underdeveloped, as well as that larvae must put a great deal of their energy into metamorphosis. Although infection rates vary dramatically from species to species, research shows that, once infected with ranavirus, a large percentage of individuals die. This raises significant concern.



**Figure 1.** Gross signs of ranaviral infection include hemorrhaging and edema of the legs or body. Shown are green frog (a) and gray tree frog (b, c) tadpoles exhibiting multiple signs of ranaviral infection.

### How does it spread?

Most amphibian eggs and larvae develop in water, while juveniles and adults live on land. Ranaviruses can spread in both environments and by direct and indirect routes (Fig. 2).

*Direct transmission* occurs when an uninfected individual contacts an infected individual. Terrestrial juveniles and adults may be exposed during migration or breeding. Infected, breeding adults may introduce the virus to a breeding pond; however, researchers have not seen transmission from mother to offspring in amphibians. Larval amphibians can contact and infect each other while swimming. Amphibians may also become infected when feeding on the bodies of other live or dead infected individuals.

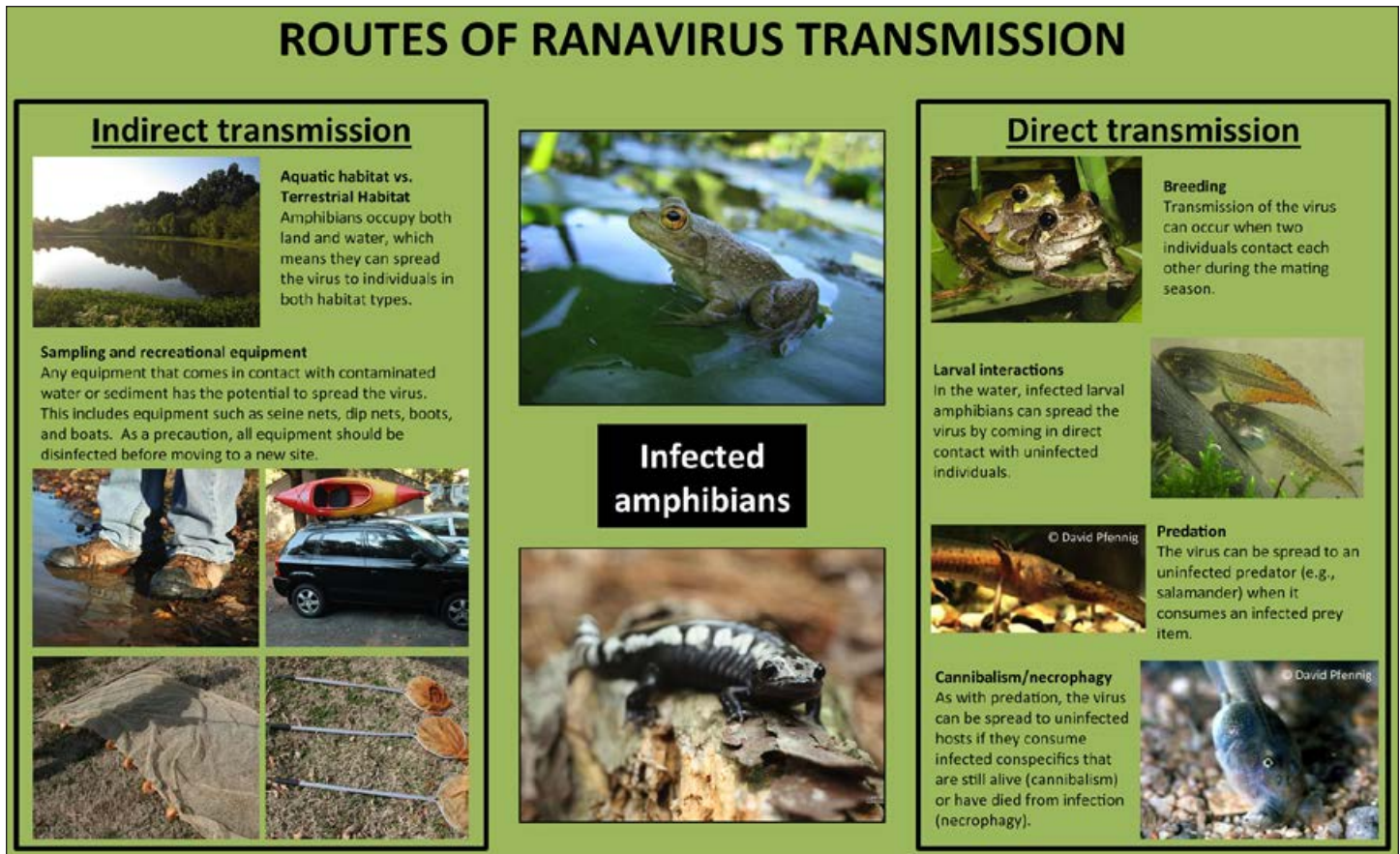
*Indirect transmission* can occur through contact with soil or water contaminated by infected individuals. Depending on conditions, ranaviruses can survive outside the host for days or weeks (especially in water), which increases their chance of spreading. However, when breeding sites dry out, the virus is no longer infectious.

We still don't understand how ranavirus spreads to new habitats and persists in populations after ponds dry out. Amphibians, especially larvae, have been known to carry low-level infections in the wild. There is evidence that these infected larvae can metamorphose and carry the pathogen into the next breeding season.

Additionally, fish and reptiles, particularly turtles, may harbor ranaviruses. Fish and reptiles are susceptible to infection; in fact, recent studies show that highly virulent strains of FV3 can cause dramatic die-offs in fish and reptiles. This suggests that ranavirus outbreaks may result from interactions among amphibian, reptilian, and fish hosts. So far, however, few studies have looked at how these different host species affect disease transmission in amphibian populations. This will take more research.

### What species are most susceptible?

The growing number of studies focused on ranavirus infection of amphibians shows substantial variation across species, but some general trends. For instance, species that are relatively rare, breed in semi-permanent wetlands, and develop quickly as larvae are more susceptible to infection. However, in laboratory experiments, certain amphibians (e.g., African clawed frog, *Xenopus laevis*) have developed immune responses to ranaviruses, which gives them increased protection against future infection.



**Figure 2.** Ranaviruses can spread through direct and indirect routes within amphibian populations.

Species within the family Ranidae (the true frogs) appear to be more susceptible than species from other families (e.g., Hylidae: tree frogs, Ambystomatidae: mole salamanders) (Fig. 3). Because Indiana has a diverse group of true frogs (eight species; Table 1), we must monitor these species for ranavirus infection. Wood frogs—highly susceptible to infection and broadly distributed in Indiana—are a particular concern. There are numerous accounts of wood frog die-offs throughout North America.

In laboratory tests, species in other amphibian families (e.g., Bufonidae: true toads, Hylidae, Ambystomatidae) appear less susceptible to ranavirus infection. However, there have been die-offs in some of these families in North America. This suggests that factors such as environmental stress could contribute to disease development. Collectively, the research demonstrates that ranaviruses can infect and cause disease in a broad range of host species. This is a significant concern, because when there are

multiple host species in a community, transmission rates are higher. Higher transmission rates mean that managers and naturalists need to watch for die-off events in all species in Indiana.

### How widespread is the disease?

To date, over 50 salamander, frog, and toad species around the world have reportedly suffered die-offs or have been found to be infected in the wild. In the United States, there are nearly 300 species of amphibians, but fewer than 10% have been examined to assess their susceptibility to ranavirus infection and disease. Moreover, the majority of the research has focused on pond-breeding amphibians; there is limited information on stream-breeding and terrestrial families (e.g., Plethodontidae: lungless salamanders). While research efforts have increased across the country, the risk that ranaviruses pose to amphibian species remains unclear.

**Table 1.** List of all native amphibians in Indiana, including their susceptibility to ranavirus infection in the lab and whether the species has been reported in mortality events or infected in the field across its range in the United States.

Species	Susceptibility in the lab <sup>1</sup>	Field data <sup>2</sup>
Cope's gray treefrog, <i>Hyla chrysoscelis</i>	High	M, I
Wood frog, <i>Lithobates sylvaticus</i>	High	M, I
Southern leopard frog, <i>Lithobates sphenoccephalus</i>	High	M, I
Green frog, <i>Lithobates clamitans</i>	High	M, I
Pickerel frog, <i>Lithobates palustris</i>	High	M, I
Eastern tiger salamander, <i>Ambystoma tigrinum</i>	High	M, I
American toad, <i>Anaxyrus americanus</i>	Low	I
American bullfrog, <i>Lithobates catesbeianus</i>	Low	M, I
Spotted salamander, <i>Ambystoma maculatum</i>	Low	M, I
Mole salamander, <i>Ambystoma talpoideum</i>	Low	ND
Marbled salamander, <i>Ambystoma opacum</i>	Medium	M
Northern leopard frog, <i>Lithobates pipiens</i>	Medium	M, I
Eastern spadefoot, <i>Scaphiopus holbrookii</i>	Medium	M, I
Western chorus frog, <i>Pseudacris triseriata</i>	Medium	ND
Northern cricket frog, <i>Acris crepitans</i>	ND	I
Eastern hellbender, <i>Cryptobranchus alleganiensis alleganiensis</i>	ND	I
Northern dusky salamander, <i>Desmognathus fuscus</i>	ND	I
Long-tailed salamander, <i>Eurycea longicauda longicauda</i>	ND	I
Southern two-lined salamander, <i>Eurycea cirrigera</i>	ND	I
Cave salamander, <i>Eurycea lucifuga</i>	ND	I
Northern slimy salamander, <i>Plethodon glutinosus</i>	ND	I
Green treefrog, <i>Hyla cinerea</i>	ND	M
Spring peeper, <i>Pseudacris crucifer</i>	ND	M
Plains leopard frog, <i>Lithobates blairi</i>	ND	M
Jefferson's salamander, <i>Ambystoma jeffersonianum</i>	ND	M
Eastern newt, <i>Notophthalmus viridescens</i>	ND	M, I
Fowler's toad, <i>Anaxyrus fowleri</i>	ND	ND
Gray treefrog, <i>Hyla versicolor</i>	ND	ND
Crawfish frog, <i>Lithobates areolatus</i>	ND	ND
Blue-spotted salamander, <i>Ambystoma laterale</i>	ND	ND
Small-mouthed salamander, <i>Ambystoma texanum</i>	ND	ND
Streamside salamander, <i>Ambystoma barbouri</i>	ND	ND
Four-toed salamander, <i>Hemidactylium scutatum</i>	ND	ND
Green salamander, <i>Aneides aeneus</i>	ND	ND
Northern zigzag salamander, <i>Plethodon dorsalis</i>	ND	ND
Eastern red-backed salamander, <i>Plethodon cinereus</i>	ND	ND
Northern ravine salamander, <i>Plethodon electromorphus</i>	ND	ND
Common mudpuppy, <i>Necturus maculosus maculosus</i>	ND	ND
Western lesser siren, <i>Siren intermedia nettingi</i>	ND	ND

<sup>1</sup> Species are categorized into high, medium, and low susceptibility based on the laboratory infection results of Hoverman et al., 2011. "ND" refers to a species with no data.

<sup>2</sup> The *Field data* column was determined from Miller et al., 2011. "M" refers to mortality due to ranaviruses (die-off event) and "I" refers to infection present in an individual. "ND" refers to a species with no data.

## What environmental stresses increase disease?

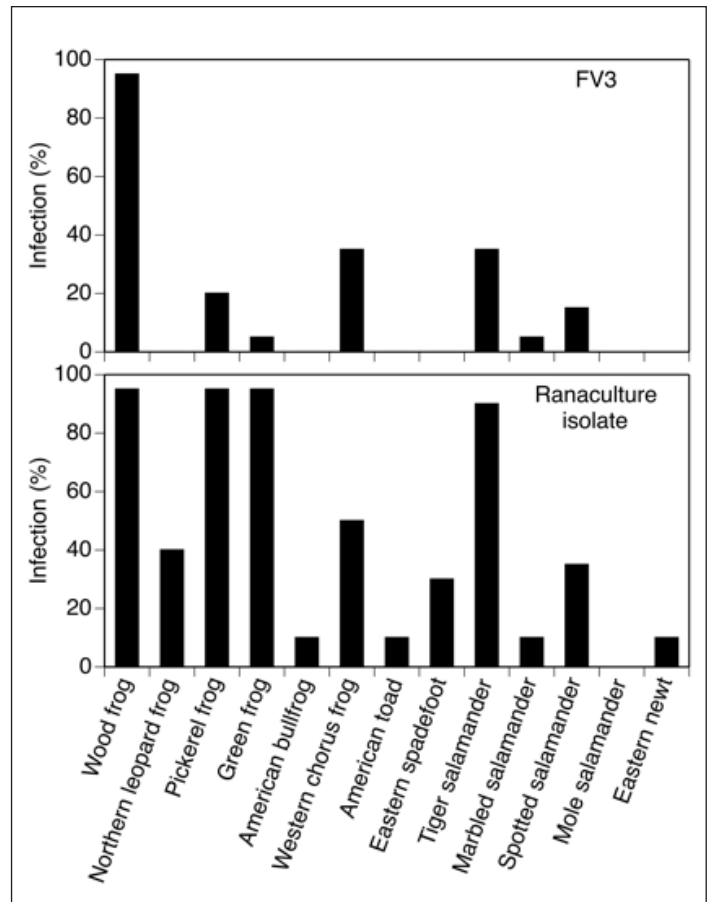
The emergence of ranaviruses in amphibian populations may be linked to environmental stressors that are natural (e.g., competition, breeding) or human-related (e.g., habitat destruction, poor water quality, climate change). These stressors may directly or indirectly make amphibians more susceptible to infection and lead to outbreaks of viral diseases in amphibian populations. For instance, experiments show that pesticide exposure can increase the susceptibility of larval tiger salamanders to ATV infection and increase larval death. Additionally, poor water quality caused by cattle activity around wetlands was linked to increased ranavirus prevalence in pond-breeding amphibians. Thus, cattle ponds and run-off retention ponds could be hotspots of ranavirus disease due to enhanced environmental stress.

Human activity is a significant cause of ranavirus spread across the landscape. For example, ATV moved long distances in the western United States when fishermen used infected tiger salamanders (*Ambystoma tigrinum*) as fishing bait. The infected bait introduced ranavirus to many formerly uninfected areas. Additionally, amphibian culture facilities and fish hatcheries can become sources of more virulent strains of the virus. These facilities house large populations of frogs (usually American bullfrogs) that can evolve resistance to infection. To counter this resistance, more virulent strains of the virus can develop. For instance, an FV3-like isolate from an American bullfrog culture facility caused 50% more mortality in several amphibian species when compared to standard FV3 in laboratory trials (Fig. 3). Thus, the movement of contaminated water and infected animals by humans could be an underlying cause of the increasing reports of ranavirus-associated die-off events.

Few studies have been done to assess long-term effects of ranaviruses on populations. A single study in North Carolina has shown recurrent ranavirus outbreaks reduced population size of several amphibian species (e.g., wood frogs, spotted salamanders) in a wetland. This suggests that recurrent outbreaks can have long-term effects on amphibian populations, especially for highly susceptible species like wood frogs. Unfortunately, Indiana currently lacks long-term surveillance projects focused on ranavirus.

## What you can do?

Biologists and recreationists can take an active role in preventing the spread of ranaviruses by disinfecting all equipment after use around bodies of water. Boots, waders, boats, buckets, and any items that come into contact with water or soil in bodies of water where amphibians breed are potential carriers of the virus.



**Figure 3.** Ranavirus infection varies greatly across species and virus isolates. Shown are the results from laboratory experiments examining the susceptibility of different amphibian species to infection by frog virus 3 (FV3) and an FV3-like virus isolate from an American bullfrog culture facility (Ranaculture isolate). Only species found in Indiana have been included. Modified from Hoverman et al. (2011).

To reduce the spread of ranaviruses:

- Use bleach (10%) or nolvasan (2%) to disinfect all gear (including vehicles in contact with water). Nolvasan is non-corrosive and safe to use on metal equipment such as boats and boat trailers.
- Do not transport soil from one site to another.
- Do not move amphibians, fish, or reptiles from one site to another.

Researchers are working to determine the distribution and presence of ranaviruses in Indiana, but until we know more, do everything you can to prevent its spread.

### What if you find dead amphibians?

There have been no reported ranavirus-associated die-off events of amphibians in Indiana. However, the virus recently was found in amphibian and reptiles populations in the state, so a die-off is possible in the future.

If ranaviruses cause a die-off in an amphibian population, you will see numerous individuals (>10) dead at a single location at a time—most likely during a breeding event or larval development. If you discover a die-off:

- Contact the Indiana Department of Natural Resources ([dfw@dnr.in.gov](mailto:dfw@dnr.in.gov), 317-234-5191) for additional guidance.
- Leave dead and moribund individuals where you find them. Moving them increases the risk of pathogen spread. You must have a permit and state approval prior to the collection of a live or dead amphibian.

If you have approval from the Indiana Department of Natural Resources to collect amphibians, we recommend one of the following approaches.

- Because clinical signs of infection vary greatly among species, take tissue samples (preferably liver or kidneys) and oral or cloacal swabs and store them in 95% ethanol for future diagnostic tests.
- Alternatively, you can preserve entire bodies of individuals separately in 95% ethanol or freeze them for testing.

Importantly, increased surveillance and testing will help researchers and managers better estimate ranaviruses' distribution in Indiana and the threat it poses to our amphibians.

### Resources

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, E. W. Davidson, and J. P. Collins. 2004. Intraspecific reservoirs: Complex life history and the persistence of a lethal ranavirus. *Ecology* 85:560-566.

Chinchar, V. G. 2000. Ecology of viruses of cold-blooded vertebrates. Pages 413-445 in C. J. Hurst, editor. *Viral ecology*. Academic Press, San Diego, California.

Currylow, A. F., A. J. Johnson, and R. N. Williams. 2014. Evidence of ranavirus infections among sympatric larval amphibians and box turtles (*Terrapene carolina carolina*). *Journal of Herpetology*

Gray, M. J., D. L. Miller, and J. T. Hoverman. 2009. Ecology and pathology of amphibian ranaviruses. *Diseases of Aquatic Organisms* 87:243-266.

Gray, M. J., D. L. Miller, A. C. Schmutzer, and C. A. Baldwin. 2007. Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. *Diseases of Aquatic Organisms* 77:97-103.

Green, D. E., K. A. Converse, and A. K. Schrader. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. *Annals of the New York Academy of Sciences* 969:323-339.

Haislip, N. A., M. J. Gray, J. T. Hoverman, and D. L. Miller. 2011. Development and disease: How susceptibility to an emerging pathogen changes through anuran development. *PLoS ONE* 6:e22307.

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, 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.

Hoverman, J. T., M. J. Gray, D. L. Miller, and N. A. Haislip. 2012a. Widespread occurrence of ranavirus in pond-breeding amphibian populations. *EcoHealth* 9:36-48.

Hoverman, J. T., J. R. Mihaljevic, K. L. D. Richgels, J. L. Kerby, and P. Johnson. 2012b. Widespread Co-occurrence of Virulent Pathogens Within California Amphibian Communities. *EcoHealth* 9:288-292.

Jancovich, J. K., E. W. Davidson, N. Parameswaran, J. Mao, V. G. Chinchar, J. P. Collins, B. L. Jacobs, and S. A. 2005. Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Molecular Ecology* 14:213-224.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451:990-994.

Kerby, J. L., A. J. Hart, and A. Storfer. 2011. Combined effects of virus, pesticide, and predator cue on the larval tiger salamander (*Ambystoma tigrinum*). *EcoHealth* **8**:46-54.

Kerby, J. L. and A. Storfer. 2009. Combined effects of atrazine and chlorpyrifos on susceptibility of the tiger salamander to *Ambystoma tigrinum* virus. *EcoHealth* **6**:91-98.

Miller, D., M. Gray, and A. Storfer. 2011. Ecopathology of ranaviruses infecting amphibians. *Viruses-Basel* **3**:2351-2373.

Nazir, J., M. Spengler, and R. E. Marschang. 2012. Environmental persistence of amphibian and reptilian ranaviruses. *Diseases of Aquatic Organisms* **98**:177-184.

Petranka, J. W., E. M. Harp, C. T. Holbrook, and J. A. Hamel. 2007. Long-term persistence of amphibian populations in a restored wetland complex. *Biological Conservation* **138**:371-380.

Robert, J., H. Morales, W. Buck, N. Cohen, S. Marr, and J. Gantress. 2005. Adaptive immunity and histopathology in frog virus 3-infected *Xenopus*. *Virology* **332**:667-675.

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.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**:1783-1786.

Wake, D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* **105**:11466-11473.

Feb 2014

It is the policy of the Purdue University Cooperative Extension Service that all persons have equal opportunity and access to its educational programs, services, activities, and facilities without regard to race, religion, color, sex, age, national origin or ancestry, marital status, parental status, sexual orientation, disability or status as a veteran. Purdue University is an Affirmative Action institution. This material may be available in alternative formats.