SEPARC DTT
Information Sheet #1R3

Ranavirus Infections in Amphibians in the Southeast USA

Amanda L. J. Duffus, Jason T. Hoverman, Debra L. Miller, Natalie K. Stilwell, Patricia L. Bartlett, and Matthew J. Gray

Amphibian Mortality Events:

In the southeastern USA, viruses from the genus Ranavirus have been responsible for many amphibian disease and mortality events (Duffus et al. 2015). When compared to the fungal pathogen, *Batrachochytrium dendrobatidis*, the number of ranavirus-associated mortality events in the USA is three to four times greater (Muths et al. 2006). Amphibian mortality events associated with ranavirus infections have been documented in many species in the southeastern USA, and an even greater number of species are known to be affected by ranavirus infections (Table 1). These infection and die-off events have occurred in both wild and captive populations (Duffus et al. 2015).

The amphibian family that appears to be the most susceptible to ranavirus infection is the Ranidae, which experiences high mortality both in the lab and in the field (Duffus et al. 2015, Hoverman et al. 2011, Miller et al. 2011). There is no doubt that ranavirus associated disease and mortality events have gone undocumented because they were not discovered, or because proper pathological investigations were not performed. Therefore, when a die-off or disease event is discovered, it is important that full pathological investigations are performed to ensure that the cause of death/disease is properly identified (See Duffus et al. 2018).

Since the majority of ranavirus mortality events occur in relatively common species (see Duffus et al. 2015, Green et al. 2002, Miller et al. 2011, Table 1), the significance of this group of pathogens is often dismissed. In the southeast, ranavirus infection has been documented in the eastern hellbender, a species of conservation concern (Souza et al. 2012). It is also very likely that ranavirus-associated disease and die-off events have happened in other species present in the southeast, even in some that are of conservation concern, and these have gone undocumented.

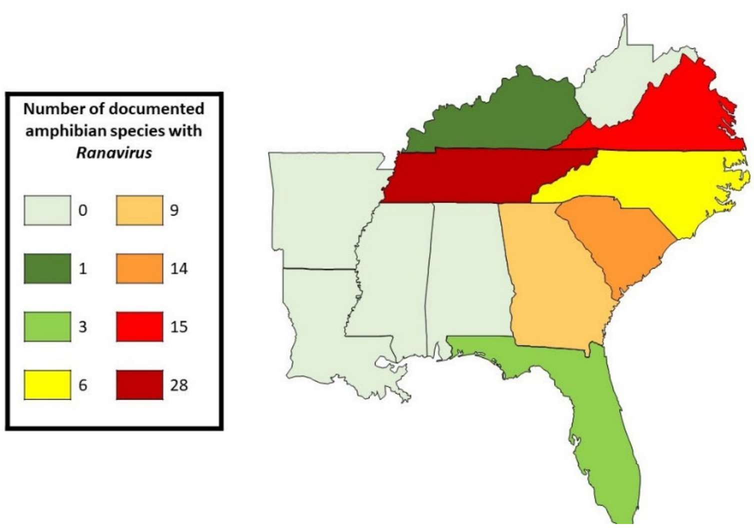

Figure 1. Number of documented amphibian species with ranavirus infections in the southeast USA.

Pathogen Characteristics:

Ranaviruses are members of the viral family *Iridoviridae* (subfamily *Alphairidovirinae*; Chinchar et al. 2020). Of the three species of ranavirus known to affect amphibians, *Frog Virus 3* (FV3), is assumed to be the most prevalent in the southeast USA (Duffus et al. 2015). However, a chimeric ranavirus made up of FV3 and *Common Midwife Toad Virus* (CMTV) was found in the southeast and described by Claytor et al. (2017). It is predicted that if this chimeric ranavirus were to invade naïve populations of amphibians, they would be decimated (Peace et al. 2019). Therefore, proper biosecurity within and between sites is paramount to avoid the introduction of new infectious agents to naïve populations.

The susceptibility of amphibians to ranaviruses actually changes of their development, with animals that are closer to metamorphosis or those that have recently metamorphosed being most sensitive (Haislip et al. 2011, Warne et al. 2011). In most cases, die-off and disease events occur in tadpoles or salamander larvae (1) and these infections are wide spread in the southeast (Hoverman et al. 2012, Figure 1, Table 1). However, infections are known to occur across all developmental and life history stages (Duffus et al. 2015).

Ranaviruses may be transmitted between amphibians in several different ways: direct contact; scavenging on infected animals or eating infected tissues; and exposure to contaminated water or sediments (Brunner et al. 2011, Brunner et al. 2015, Converse and Green 2015). Ranavirus infections and disease can also cause very high mortality (95-99%) in tadpole/larval populations (Duffus et al. 2015, Green et al. 2002, Greer et al. 2005).

Signs of Disease:

Amphibians suffering from ranaviral disease often have swollen limbs and reddening of the skin. Typically, affected animals will have redness that is concentrated on lower abdomen and on the upper hind limbs. However, it is important to note that there are no ranavirus specific signs of disease and that the signs described here can be associated with other pathogens, such as bacteria (e.g. *Aeromonas hydrophila*) or alveolates. In terms of behavioral changes, amphibians with ranaviral disease tend to be lethargic, stop eating, loose coordination and their typical avoidance behaviors (Miller et al. 2015).

Ranaviruses can infect many tissues, including the liver and kidneys. At necropsy, these organs may be pale and friable or hemorrhagic (Convers and Green 2005, Miller et al. 2008, Miller et al. 2015). Mortality due to ranavirus infection can happen as soon as a few days, or in some cases sub-lethal (also called subclinical) infections, these individuals may become carriers/reservoirs of the virus (Brunner et al. 2004, Brunner et al. 2015).

The standard ways to confirm ranavirus infection in animals involves one or more specific diagnostic tests (Miller et al. 2015). These test may include histological examination of specific tissues for pathological changes, electron microscopic examination for the presence of virions in tissue, isolation of the virus in cell culture, and polymerase chain reaction (PCR; both traditional and qPCR; Miller et al. 2015). PCR is the most commonly used diagnostic tool to determine if ranavirus is present in a specific tissue, however, it only detects the presence/absence of viral DNA in the sample and does not determine if that DNA is from viable virions (i.e. as would be detected with virus isolation; Miller et al. 2015). These techniques are available at most diagnostic laboratories. A list of laboratories that are able to identify ranavirus infections are available on the Global Ranavirus Consortium's website, www.ranavirus.org.

Factors Contributing to Emergence:

The first large scale mortality events associated with ranavirus infections in amphibians in North America occurred in the early 1990s (Duffus et al. 2015). Now, disease and mortality events have occurred in amphibians present in six southeastern states (Figure 1; Table 1). Ranavirus has been found in at least 48 amphibian species from 11 families (Table 1).

One of the major factors that has been associated with ranavirus emergence is the introduction of novel strains into naïve populations by humans (Picco et al. 2008, Storfer et al. 2007). Humans can unknowingly transport the virus between areas on footwear, clothing, recreational equipment, and field equipment. Importantly, results from recent studies suggest that ranaviruses from captive amphibian facilities (e.g. bait shops, ranaculture facilities, biological supply companies) may be more virulent than those that are found in the wild (Hoverman et al. 2011, Majji et al. 2006, Storfer et al. 2007). Therefore, novel and potentially highly virulent strains of ranavirus may be introduced to naïve populations through anthropogenic means.

Preventative Measures:

To reduce the likelihood of spreading ranaviruses to naïve populations or between populations, the soles of boots, recreation equipment, and field gear should be disinfected prior to entering a new area. Effective methods of disinfection can be found on the in SEPARC Information Sheet #10R. These methods include a 3% bleach solution or a 1% Novalsan solution with a minimum contact of one minute (Bryan et al. 2009).

Release of captive amphibians or reptiles (including chelonians) is discouraged, except in cases of repatriation, head start programs, or similar conservation efforts, and even then, care must be taken to ensure that novel pathogens are not inadvertently included with these releases. If captive animals from other situations (e.g., rehabilitation programs) are to be released, they should be returned to the location of origin, and only if there is no evidence (i.e., via diagnostic testing) that they are infected with a pathogen of concern (e.g., ranavirus, Bd, Bsal, mycoplasma, herpesvirus).

In 2008, the OIE (World Organization on Animal Health) declared that ranaviruses were Reportable Infections in amphibians (but not reptiles), recognizing the negative effects that this group of viruses has on these animals. The listing of ranaviruses as reportable infections in amphibians may eventually affect legislation that governs the movement of amphibians between states and countries, and may eventually lead to the requirement that animals be certified ranavirus-free before they are moved.

Table 1: Amphibian species known to be affected by *Ranavirus* infection in the southeastern USA.

Family	**Species**	**State**	**First Reference(s)**
Ambystomatidae	*Ambystoma maculatum*	TN	Todd – Thompson 2010
	Ambystoma opacum	TN, SC	Todd –Thompson 2010. Love et al. 2016
	Ambystoma talpoideum	TN, SC	O'Bryan et al. 2012, Love et al. 2016
	Ambystoma tigrinum	TN, SC	Hoverman et al. 2011; Love et al. 2016
Bufonidae	*Anaxyrus americanus*	TN	Hoverman et al. 2011
	Anaxyrus terrestris	SC	Love et al. 2016
Cryptobranchidae	*Cryptobranchus alleganiensis*	TN	Souza et al. 2012
Eleutherodactylidae	*Eleutherodactylus planirostris*	GA	Rivera et al. 2019
Hylidae	*Acris crepitans*	TN	Hoverman et al. 2011
	Hyla chryoscleris	TN, SC	Todd –Thompson 2010, Love et al. 2016
	Hyla chryoscleris and *H. versicolor complex*	TN	O'Bryan et al. 2012
	Hyla cinerea	GA,SC	Rivera et al. 2019, Love et al. 2016
	Hyla squirella	GA	Rivera et al. 2019
	Pseudacris crucifer	TN, SC	Todd – Thompson 2010, Love et al. 2016
	Pseudacris feriarum	TN	Todd – Thompson 2010
	Pseudacris ornate	SC	Love et al. 2016
Microhylidae	*Gastrophryne carolinensis*	SC	Love et al. 2016
Plethodontidae	*Aneides aeneus*	VA	Blackburn et al. 2015
	Desmognathus conanti	TN	Gray et al. 2009
	Desmognathus crestes	VA	Hamed et al. 2013
	Desmognathus folkesti	GA, NC	Rothermel et al. 2013
	Desmognathus fuscus	VA	Hamed et al. 2013
	Desmognathus imitator	TN	Gray et al. 2009

	Desmognathus marmoratus	GA, NC	Rothermel et al. 2013
	Desmognathus monticola	GA, NC, TN,VA	Gray et al, 2009, Davidson and Chambers 2011, Hamed et al. 2013, Rothermel et al 2013,
	Desmognathus ocoee	GA, NC, TN	Gray et al. 2009, Rothermel et al. 2013
	Desmognathus organi	VA	Hamed et al. 2013
	Desmognathus quadramaculatus	GA, NC,TN, VA	Gray et al, 2009, Davidson and Chambers 2011, Hamed et al. 2013, Rothermel et al 2013,
	Desmognathus santeetlah	TN	Gray et al, 2009
	Desmognathus wrighti	TN	Gray et al, 2009
	Eurycea cirrigera	VA	Davidson and Chambers 2011
	Eurycea longicauda	VA	Davidson and Chambers 2011
	Eurycea lucifuga	VA	Davidson and Chambers 2011
	Eurycea wilderae	GA, NC, TN	Davidson and Chambers 2011, Rothermel et al. 2013
	Gyrinophilus porphyriticus	TN	Davidson and Chambers 2011
	Plethodon glutinosus complex	VA	Davidson and Chambers 2011
	Plethodon jordani	TN	Gray et al. 2009
	Plethodon montaneus	VA	Hamed et al. 2013
	Plethodon welleri	VA	Hamed et al. 2013
Ranidae	*Lithobates calmitans*	TN	Todd-Thompson 2010
	Lithobates capito	FL	Landsberg et al. 2013
	Lithobates catesbeianus	FL, TN, VA, SC	Todd- Thompson 2010, Davidson and Chambers 2011, Hoverman et al. 2011, Landsberg et al. 2013, Love et al. 2016
	Lithobates clamitans	TN, SC	Hoverman et al. 2011, Love et al. 2016
	Lithobates paulstris	TN, VA	Todd-Thompson 2010, Davidson and Chambers 2011, Hoverman et al. 2011
	Lithobates sphenocephalus	FL,TN, SC	Hoverman et al. 2011, O'Bryan et al. 2012, Landsberg et al 2013, Love et al. 2016
	Lithobates sylvaticus	TN	Todd-Thompson 2010
Salamandridae	*Notophthalmus viridescens*	KY, TN, SC, VA	Todd-Thompson 2010,Davidson and Chambers 2011, Hoverman et al. 2011, Richter et al. 2013, Love et al. 2016
Scaphiopodidae	*Scaphiopus holbrookii*	SC	Love et al. 2016

Author's Affiliations and Contact Information:
MJG and DLM:
Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries
University of Tennessee-Knoxville
mgray11@utk.edu; dmille42@utk.edu

ALJD and PLB:
Department of Natural Sciences
Gordon State College
Barnesville, GA 30204
aduffus@gordonstate.edu

JTH:
Forestry and Natural Resources
Purdue University
jhoverm@purdue.edu

NKS
Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
University of Georgia
Athens, GA 30602
Natalie.Stilwell@uga.edu

Recommended Citation:
Duffus, A.L.J., J.T. Hoverman, D.L. Miller, N.K. Stilwell, P.L. Bartlett and M.J. Gray. 2021. Ranavirus Infections in North American Amphibians. Southeastern Partners in Amphibian and Reptile Conservation, Disease Task Team, Information Sheet #1R3

Updated Version of:
Duffus, A.L.J., J. T. Hoverman, D. L. Miller, A.W. Dean, J.M, Klaus, and M.J. Gray. 2013. Ranavirus Infections in North American Amphibians. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet # 1R2

References:

Blackburn, M., J. Wayland, W.H. Smith, J.H. McKenna, M. Harry, M. K. Hamed, M.J. Gray, and D.L. Miller (2015). First report of ranavirus and *Batrachochytrium dendrobatitidis* in green salamanders (*Aneides aeneus*) from Virginia, USA. Herpetological Review. 46:357-361.

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.

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., Storfer, A., Gray, M.J., and J.T. Hoverman. 2015. Ranavirus ecology and evolution: from epidemiology to extinction. In Gray, M.J. and V.G. Chinchar Eds, "Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates" Springer Online

Bryan, L., C. A. Baldwin, M. J. Gray, and D. L. Miller. 2009. Efficacy of select disinfectants at inactivating *Ranavirus*. Diseases of Aquatic Organisms 84:89-94.

Claytor SC, Subramaniam K, Landrau-Giovannetti N, Chinchar VG, Gray MJ, Miller DL, Mavian C, Salemi M, Wisely S, Waltzek TB. Ranavirus phylogenomics: signatures of recombination and inversions among bullfrog ranaculture isolates. Virology. 2017 Nov 1;511:330-43.

Converse, K. A., and D. E. Green. 2005. Diseases of tadpoles. Pages 72-88 *in* S. K. Majumdar, J. E. Huffman, F. J. Brenner, and A. I. Panah, editors. Wildlife diseases: landscape epidemiology, spatial distribution and utilization of remote sensing technology. Pennsylvania Academy of Science, Easton, Pennsylvania, USA.

Davidson SRA, Chambers DL (2011) Ranavirus prevalence in amphibian populations of Wise County, Virginia, USA. Herpetol Rev 42:214-215

Duffus, A.L.J., Fenton,H.M.A., Gray, M.J., and D.L. Miller. 2017. Investigating amphibian and reptile moralities: A practical guide for wildlife professionals. Herpetological Review. 48:550-557.

Duffus, A.L.J., Waltzek, T.B., Stohr, A.C., et al. 2015. Distribution and host range of ranaviruses. In Gray, M.J. and V.G. Chinchar Eds, "Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates" Springer Online

Gray MJ, Miller DL, Hoverman JT (2009) First report of ranavirus infecting lungless salamanders. Herpetol Rev 40:316-319

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 Science, 969:323-339.

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.

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.

Hamed MK, Gray MJ, Miller DL (2013) First report of ranavirus in plethodontid salamanders from the Mount Rogers National Recreation Area, Virginia, USA. Herpetol Rev 44:455-457

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 JT, Gray MJ, Haislip NA, et al. (2011) Widespread occurrence of ranavirus in pond breeding amphibian populations. EcoHealth 9:36-48

Hoverman, J. T., M. J. Gray, D. L. Miller and N. A Haislip. 2012. Widespread occurrence of ranavirus in pond-breeding amphibian populations. DOI: 10.1007/s10393-011-0731-9

Landsberg JH, Kiryu Y, Tabuchi M, et al. (2013) Co-infection by alveolate parasites and frog virus 3-like ranavirus during and amphibian larval mortality event in Florida, USA. Dis Aquat Organ 105:89-99

Love CN, Winzeler ME, Beasley R, Scott DE, Nunziata SO, Lance SL (2016) Patterns of amphibian infection prevalence across wetlands on the Savannah River Site, South Carolina, USA. Dis Aquat Org 121:1-14. https://doi.org/10.3354/dao03039

Majji, S., S. LaPatra, S. M. Long, R. Sample, L. Bryan, A. Sinning, and V. G. Chinchar. 2006. *Rana catesbeiana* virus Z (RCV-Z): a novel pathogenic ranavirus. Diseases of Aquatic Organisms 73:1-11.

Miller, D.L., Rajeev, S., Brookins, M., et al. 2008 Concurrent infection with ranavirus, Batrachochytrium dendrobatidis, and Aeromonas in a captive anuran colony. Journal of Zoo and Wildlife Medicine, 39:445-449.

Miller, D., M. Gray, and A. Storfer. 2011. Ecopathology of Ranaviruses Infecting Amphibians. Viruses, 3:2351-2373.

Miller, D.L., Pessier, Allan P., Hick, P., and R. J. Whittington. 2015. Comparative pathology of ranaviruses and diagnostic techniques. In Gray, M.J. and V.G. Chinchar Eds, "Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates" Springer Online

Muths, E., A. L. Gallant, E. H. C. Campbell, et al. 2006. The Amphibian Research and Monitoring Initiative (ARMI): 5-year report: U.S. Geological Survey Scientific Investigations Report 2006-5224.

O'Bryan CJ, Gray MJ, Brooks CS (2012) Further presence of ranavirus infection in amphibian populations of Tennessee, USA. Herpetol Rev 43:293-295

Picco, A.M., and J.P. Collins. 2008 Amphibian Commerce as a Likely Source of Pathogen Pollution. Conservation Biology, 22-1582-1589.

Richter SC, Drayer AN, Strong JR, et al. (2013) High prevalence of ranavirus infection in permanent constructed wetlands in eastern Kentucky, USA. Herpetol Rev 44:464-46

Rivera B, Cook K, Andrews K, Atkinson MS, Savage AE. Pathogen Dynamics in an Invasive Frog Compared to Native Species. EcoHealth. 2019 Jun 15;16(2):222-34.

Rothermel BR, Travis ER, Miller DL, et al. (2013) High occupancy of stream salamanders despite high *Ranavirus* prevalence in a southern Appalachians watershed. EcoHeath 10:184-189

Souza, M. J., M. J. Gray, P. Colclough, and D. L. Miller. 2012. Prevalence of infection by *Batrachochytrium dendrobatidis* and *Ranavirus* in eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in eastern Tennessee. Journal of Wildlife Diseases, 48:560-566.

Storfer, A., M.E. Alfaro, B.J. Ridenhour, J.K. Jancovich, S.G. Mech, M.J. Parris, and J.P. Collins. 2007. Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. Ecology Letters 10:1075-1083.

Todd-Thompson M (2010) Seasonality, variation in species prevalence and localized disease for ranavirus in Cades Cove (Great Smoky Mountains National Park) amphibians. MSc Thesis, University of Tennessee, Knoxville, Tennessee, USA.

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