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THIRD INTERNATIONAL SYMPOSIUM ON RANAVIRUSES: ADVANCING THE UNDERSTANDING OF THE THREAT OF RANAVIRUSES TO NORTH AMERICAN HERPETOFAUNA

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Members of the genus *Ranavirus*, one of five genera within the family *Iridoviridae*, encompass a group of large, double-stranded DNA viruses that infect all three classes of ectothermic vertebrates: fish, amphibians, and reptiles. Ranaviruses are globally emerging pathogens that cause considerable morbidity and mortality among diverse populations. In North America, ranavirus epizootics are regularly reported in wild and cultured fish, amphibian, and reptile populations.

In May 2015, the Third International Symposium on Ranaviruses (ISR) was held in Gainesville, Florida in conjunction with the University of Florida's Aquatic Ecosystem Health Conference. Seventy participants from 9 countries—including scientists, managers, wildlife biologists, veterinarians, policy makers, and students—participated and shared their experiences related to ranaviruses. Events over the two-day conference included: lectures, topical discussions, poster sessions, wet labs, fieldtrips, and a social evening at the Florida Museum of Natural History.

The Symposium began with a welcome by Matthew Gray, Director of the Global Ranavirus Consortium (University of Tennessee, Knoxville) and Thomas Waltzek (University of Florida, Gainesville), host of the 2015 ISR. This was followed by the keynote address by Richard Whittington, Chair of Farm Animal Health, Faculty of Veterinary Science, University of Sydney, Australia. Dr. Whittington discussed Australian ranaviruses in both their historical and modern context. Following the keynote address, presentations covered various topics including: diagnostics, ecology, stressors, physiology, immunology, taxonomy, evolution, viral replication, surveillance, clinical case reports, and pathology. Here, we present a summary of the presentations related to the impact of ranaviruses on native North American herpetofauna.

Two oral presentations examined ranavirus infections and their effects in North American chelonians. Matthew Allender (University of Illinois) shared findings from two ranavirus outbreaks in Eastern Box Turtles (*Terrapene carolina carolina*) in central Illinois. These outbreaks appeared to only affect adult turtles, and caused significant mortality. Each outbreak lasted only three weeks, illustrating the rate at which ranaviruses can decimate populations, and the difficulty in detecting cases of clinical disease. Dr. Allender also surveyed populations that were not in the midst of ranavirus outbreaks and found that infection prevalence was less than 1% in the 1200 animals that were screened; thus, ranaviruses can remain at low prevalence then increase to nearly 100% during rapidly occurring outbreaks. Heather Fenton (Southeast Cooperative Wildlife Disease Study, University of Georgia) presented findings from a pathological investigation into a mortality event that occurred in a wild population of Florida Box Turtles (*T. c. bauri*) in Lee County, Florida, USA. The afflicted animals showed gross clinical signs of ranaviral disease. Molecular diagnostic methods revealed that some of the animals that died were infected with the ranavirus species, *Frog virus 3* (FV3); however, the pathogen was not detected in all animals involved in the outbreak despite the presence of characteristic lesions. These findings underscore the importance of full pathological investigations into cases of ranaviral disease and illustrate that detection of the virus in chelonians may be challenging in later stages of disease.

There were several presentations on North American amphibians. Matthew Gray presented findings on how Wood Frogs (*Rana sylvatica*/*Lithobates sylvaticus*) may play a crucial role in ranavirus epidemics in amphibian communities. In a series of experiments, Dr. Gray and his team examined viral shedding, tadpole activity, and the number of contacts infected individuals had with uninfected individuals. Wood frog and Cope's Gray Treefrog (*Hyla chrysoscelis*) tadpoles were found to differ in their ability to transmit infections, affecting their capacity to cause outbreaks. Infected Wood Frog tadpoles transmitted the virus at a much higher rate than Cope's Gray Treefrog tadpoles to their conspecifics. Another important difference was the way in which infection developed into disease between the two species. In wood frog tadpoles, the infection readily developed into disease, whereas when naïve gray treefrog tadpoles were exposed, virus

transmission was much lower and disease did not develop. Because contact rates were similar between the species yet Wood Frog tadpoles shed more virus than Cope's Gray Treefrog tadpoles, the team concluded that viral shedding might play a significant role in ranavirus epizootics, especially if the lethal concentration of the virus for a host species is exceeded in the environment.

Emily Hall from Washington State University shared findings regarding the proximity of roads to outbreaks of FV3 in Wood Frog populations. She examined the hypothesis that roads act as stressors, primarily through exposure to de-icing salts, that increase the likelihood of FV3 outbreaks in Wood Frog tadpoles. Through field observations in the Yale Myers Forest, Connecticut, FV3-associated disease events were more likely to occur in ponds associated with roadsides than those that were not. It was concluded that proximity to roads may play a role in the FV3 dynamics in the northern USA.

Scott Smith from the Maryland Department of Natural Resources presented findings from the largest FV3 surveillance study performed in the USA. Wood Frog tadpoles from five northeastern states were collected and screened for the presence of FV3. Samples came from populations that did not appear to be experiencing mortality, as well as, those amid FV3 epizootics. In addition to screening samples for FV3 by conventional PCR, samples were subjected to diagnostic virology. This study is the first to identify FV3 infections in Delaware, and the first study to demonstrate susceptibility of Eastern Spadefoots (*Scaphiopus holbrooki*) in the wild. It was suggested that the presence of very susceptible Wood Frog tadpoles might be an important factor determining the occurrence of ranavirus outbreaks in the northeastern USA.

David Lesbarrères from Laurentian University presented work that examined the life-history trade-offs caused by exposure to copper, different temperatures, and sub-lethal doses of FV3 during larval development in Northern Leopard Frogs (*Lithobates/Rana pipiens*). Tadpoles that were exposed to both copper and FV3 exhibited growth and developmental patterns that were similar to the unexposed control tadpoles. This finding indicates that there are potential antagonistic effects between these stressors. The interactions between different stressors will make the outcome of environmental changes harder to predict as they impact the host in unexpected ways.

Stacey Lance from the Savannah River Ecology Laboratory, University of Georgia presented a study that examined the prevalence of both *Batrachochytrium dendrobatids* (*Bd*) and FV3 in amphibian species that occur in the Savannah River Site in South Carolina. The prevalence of these two pathogens was compared between heavy metal contaminated and reference sites. *Bd* prevalence was lower (9.8%) than FV3 (37.4%) at all sites and only *Bd* was found in high prevalence in heavy metal contaminated sites. It is possible that the heavy metal contamination affects the host immune response to *Bd*, however at this point there are several confounding variables that need to be addressed.

María Forzán from the Canadian Wildlife Health Cooperative presented research which sought to establish the normal hematological values (i.e. reference intervals) for adult Wood Frogs. Dr. Forzán also studied frogs that had been experimentally infected with FV3. Infected frogs developed neutrophilia and an initial lymphopenia during the subclinical (incubation) period. This was followed by a lymphocytosis that coincided with the appearance of severe clinical signs, gross lesions and intracytoplasmic viral inclusions in white and red blood cells. Results suggest that adult Wood Frogs are capable of mounting an adaptive immune response to FV3.

Andrew Storfer from Washington State University presented the results from a genomic sequencing study of 15 isolates of *Ambystoma tigrinum* virus (ATV) from the western USA. This virus commonly infects wild tiger salamanders (*Ambystoma tigrinum*), especially those sold as part of the fishing bait trade. Bayesian analysis showed that ATV had likely evolved sometime in the past several thousand years and the fishing bait trade likely played a role its range expansion and emergence in the western USA. He also presented evidence that virulence of ATV strains might be affected by genomic recombination.

There were many excellent international presentations on ranaviruses in both amphibians and reptiles. Importantly, Jonathan Kolby (James Cook University) presented the results of work that examined the international trade of amphibians as a vector of the spread of ranaviruses. Dr. Kolby examined the prevalence of ranavirus infection in shipments of amphibians being imported into the USA from Madagascar, Hong Kong, Taiwan, and the Dominican Republic. Ranavirus infections were found at a high prevalence in nearly all shipments from all four points of origin. These findings highlight the potential involvement of the international amphibian trade in the global spread of ranaviruses, and underscore the importance of understanding the possible role of pathogen spillover from captive animals to wild populations.

Matthew Gray closed the conference with the general business meeting of the Global Ranavirus Consortium (GRC). At the general business meeting, many of the GRC's accomplishments since the last symposium were highlighted, including the publication of the first comprehensive book on ranaviruses that was edited by Matthew Gray and Gregory Chinchar. This book is available through Springer as Open Access (http://link.springer.com/book/10.1007/978-3-319-13755-1); there have been >24,000 chapter downloads since publication. Additionally, the GRC went live with their own website www.ranavirus.org, which contains various resources including a regularly updated list of ranavirus publications. Professionals and students can join the GRC for a nominal fee, which helps support activities, such as providing travel grants to the biennial international symposium. The GRC also launched the Global Ranavirus Reporting System (GRRS, https://mantle.io/grrs), which is an online spatial database for reporting cases of ranavirus infection and disease. Researchers are encouraged to contribute to this useful epidemiological database by uploading published and new cases of ranavirus. Finally, it was announced that the GRC would organize an online course on ranaviruses. The course was offered from February – April 2016 and included 46 students from 7 countries with 26 instructors contributing.

In addition to the presentations, there were opportunities for professional development activities and field trips. On the third day of the symposium, Matthew Gray and Jesse Brunner led a half-day workshop on the design of ranavirus surveillance studies and data analysis. That afternoon, Paul Hick and Debra Miller presented a half-day workshop on sterile sample collection from ranavirus hosts and molecular diagnostic techniques. These workshops were held at the University of Florida's Emerging Pathogens Institute. Symposium participants also had the option to explore Payne's Prairie or travel to Homosassa Springs near Gainesville to view the diverse wildlife of Florida.

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The Fourth International Symposium on Ranaviruses (ISR) will be held June 7 – 10, 2017 in Budapest, Hungary in conjunction with the 10th International Symposium on Viruses of Lower Vertebrates. Information about the 2017 ISR will be posted on the symposium's website (http://www.rana-2017.com/) and through the GRC's listserv, which can be joined at http://listserv.utk.edu/cgi-bin/wa?A0=GRC.

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