SECOND INTERNATIONAL SYMPOSIUM ON RANAVIRUSES

In conjunction with:

62nd International Conference of the

Wildlife Disease Association

"Utilizing Wildlife Health to Conserve Biodiversity in the Appalachians and Beyond"

July 27 – 29, 2013

Holiday Inn, World's Fair Park, Knoxville, TN, USA

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WELCOME TO THE SECOND INTERNATIONAL SYMPOSIUM ON RANAVIRUSES!

Following the success of the First International Symposium on Ranaviruses held on 8 July 2011 in conjunction with the Joint Meeting of Ichthyologists and Herpetologists in Minneapolis, Minnesota USA, scientists and veterinarians from around the globe were eager to hold a second, expanded symposium to discuss advances in ranavirus research, new diagnostic cases and methodologies, important future directions, and to build additional collaborations. The Global Ranavirus Consortium (GRC) took the lead in soliciting possible meeting locations, and the consensus was for the second symposium to be held in conjunction with the International Conference of the Wildlife Disease Association (WDA) in Knoxville, Tennessee USA, in July 2013. The WDA was receptive to the partnership, and members of the GRC have worked tirelessly to secure sponsors, solicit prominent and diverse speakers, and design a program with professional presentations, breakout discussions, social activities and field trips. We extend our deepest thanks to those professionals and students who strived to make this symposium a success. We also extend sincere appreciation to the 14 sponsors who provided >$30,000 USD in funds or donations for travel grants, coffee breaks, and the symposium social.

The Second International Symposium on Ranaviruses includes five thematic sessions over two days. Each session will begin with an overview presentation delivered by a scientist with expertise on the session theme. These overview presentations will be followed by shorter research presentations. There will be over 25 presentations delivered by researchers, veterinarians, post-doctoral research associates and students from Asia, Australia, North America, South America and Europe, totaling 11 countries. Each day will end with breakout sessions, where participants have the opportunity to discuss relevant issues or topics raised during the preceding sessions. The goal of the breakout sessions will be to identify urgent research directions, reoccurring issues with ranavirus research and diagnostic cases, and immediate outreach education needs. At the end of the symposium, a summary of each breakout session will be provided to the group for additional input. There will be an opportunity to volunteer subsequently on GRC committees to address topics or urgent tasks that are identified. We will also be hosting a poster session during the social on Saturday evening; there will be >35 poster presentations from 10 countries.

We would like to welcome you to the Second International Symposium on Ranaviruses. We look forward to interacting and hope this meeting generates ideas and new collaborations!

Sincerely,

Matthew Gray, Ph.D.
Director of the Global Ranavirus Consortium
Center for Wildlife Health, University of Tennessee

Debra Miller, D.V.M., Ph.D.
Center for Wildlife Health and Department of Biomedical and Diagnostic Services, College of Veterinary Medicine, University of Tennessee

Amanda Duffus, Ph.D.
Secretary/Treasurer for the Global Ranavirus Consortium
Department of Biology, Gordon State College

(*Organizers of the Second International Symposium on Ranaviruses*)

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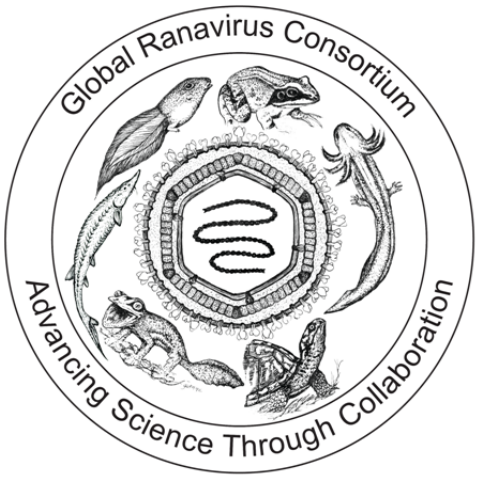
Matthew Gray – Chair
Matthew Allender
Amanda Duffus
Debra Miller

WELCOME FROM THE DIRECTOR OF THE GLOBAL RANAVIRUS CONSORTIUM

Following the First International Symposium on Ranaviruses, the Global Ranavirus Consortium (GRC) was formed with the goal to facilitate communication and collaboration among scientists and veterinarians conducting research on ranaviruses and diagnosing cases of ranaviral disease. The GRC is composed of an Executive Board (see below) and over 30 professionals that study ranaviruses. The Executive Board maintains a website (http://fwf.ag.utk.edu/mgray/ranavirus/ranavirus.htm) and LISTSERV where new publications are posted and questions can be submitted for advice and discussion. The GRC is responsible for facilitating organization of a biennial international symposium on ranaviruses with a hosting institution. Scientists of the GRC produced four publications following the first ranavirus symposium summarizing topics and important research directions. The GRC Executive Board was formed in April 2013 via election, and will hold its first meeting on Friday, 26 July, to outline future initiatives. Initial activities may include drafting a charter and bylaws for the GRC, securing non-profit (501c) status in the USA so gifts can be received, establishing continental discussion groups that annually share new research findings, and creating a new website with interactive options. Initiatives discussed during this meeting will be shared with symposium attendees on Sunday, 28 July, afternoon, which will be an opportunity to provide additional input on urgent tasks that the GRC should address. In particular, we hope to identify important research directions, outreach education needs, and tools or publications that could be developed to facilitate ranavirus research and diagnosis of ranaviral disease. We anticipate forming GRC committees to address initiatives that are identified. If you are interested in serving on a GRC committee or being a part of a continental discussion group, please sign up during one of the breakout sessions or give your contact information to a GRC Executive Board member.

Mechanisms that are causing the emergence of ranaviruses in ectothermic vertebrate communities are complex. In order to unravel the causes of ranavirus emergence and identify disease intervention strategies, it will require teams of professionals with various areas of expertise. The GRC strives to foster development of new professional relationships that lead to the advancement of knowledge on ranaviruses. Please take a part in this mission by serving on a GRC committee, participating in a continental discussion group, and attending future symposia. The GRC Executive Board looks forward to interacting with you during this symposium and future initiatives.

All the Best—
Matt Gray
Director, Global Ranavirus Consortium



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KEYNOTE SPEAKER – ABSTRACT AND BIOGRAPHY

EMERGING INFECTIOUS DISEASES AND AMPHIBIAN POPULATION DECLINES: HOW ARE WE GOING?

Richard (Rick) Speare

Emeritus Professor, Anton Breinl Centre for Public Health and Tropical Medicine, James Cook University, Townsville, Australia and Director, Tropical Health Solutions Pty Ltd, Townsville, Australia. Email: rickspeare@gmail.com

Amphibians suffer from two formidable infectious diseases, chytridiomycosis and ranaviral disease, capable of causing high mortality in wild and captive populations. Both are emerging infectious diseases and both are globally notifiable diseases with the World Organisation for Animal Health. Chytridiomycosis is due to a single species, the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), while ranaviral disease is caused by several species of viruses in the *Ranavirus* genus. If one predicted virulence from pathology, ranaviruses would easily win since the pathological changes in acute disease are severe and involve many organs, with viruses tearing cells apart. On the other hand amphibians dying from chytridiomycosis have negligible pathological changes; the fungus lives only in the superficial layer of the epidermis and there is minimal inflammation locally or abnormalities internally. Death in chytridiomycosis is subtle and elegant; the heart stops. Severe chytridiomycosis is due to low potassium and sodium in the blood causing cardiac arrest. Bd damages sodium pumps in the epidermis, probably through a selective action of proteases and other chemicals produced by the fungus. The pathophysiology of frogs dying from ranaviral disease does not appear to have been investigated.

Chytridiomycosis is responsible for a global epidemic in wild amphibians that has now been detected in all continents with amphibians. Ranaviruses cause local outbreaks with high mortality. Ranaviruses are carried between countries (as is Bd), but once detected, they are typically very elusive. Once Bd arrives in a population, it usually becomes endemic and can be detected with appropriate surveys. Ranaviruses do not appear to be causing a “pandemic”, while the evidence for Bd’s role is indisputable. The two diseases often attack different populations of amphibians: Bd hammers populations in pristine environments, often in elevated regions, while ranaviruses seem more severe in isolated populations in disturbed or contaminated areas. However, recent work hypotheses that ranaviruses may be devastating to amphibian populations already compromised by endemic chytridiomycosis. The impact of this double whammy from two formidable infectious diseases certainly needs more clarification in a range of environments and populations.

Although both pathogens are water-borne, Bd is much less resistant to unsuitable environmental conditions than ranaviruses. Bd is very susceptible to dehydration and highly sensitive to temperatures above 32°C. Ranaviruses can survive drying. Interestingly, Bd is resistant to medical sterilising ultraviolet light, while ranaviruses are sensitive. Bd is thought to largely persist in amphibian carriers, while some of the amphibian ranaviruses can utilise a range of hosts, some using three classes (amphibians, reptiles and fish). Thus, the transmission web of ranaviruses is potentially much more complex than that of Bd.

The realisation that a pathogen (Bd) could so severely impact the dynamics of wild host populations that the host can go extinct caused a major paradigm shift in not only amphibian ecology, but ecological thinking. Many ecological studies on amphibians now monitor for Bd as a routine. Ranaviruses and other amphibian pathogens have not yet been accorded this status by ecologists. As for most emerging infectious diseases, research benefits from multidisciplinary teams composed of members with specialised skills and a broad knowledge of host, pathogen and environment with team members respecting and valuing the special skills of each discipline.

Rick Speare, PhD, MBBS(hons), BVSc(hons), FAFPHM, FACTM, MACVS, has done applied research for 35 years on control of communicable diseases in humans and other animals. He is registered as a veterinary surgeon and a public health physician in Australia. In 1989 Rick discovered the Bohle Iridiovirus, the first amphibian Ranavirus found in Australia. In 1998 he was part of the team that proposed that amphibian declines in tropical Australia and in Central America were due to a strange novel pathogen, the amphibian chytrid fungus. Rick continues doing research on amphibian diseases along with projects on tuberculosis, soil transmitted helminths, head lice, veterinary infection control, and flying foxes. He is actively involved in research capacity strengthening in the Pacific, particularly Solomon Islands and Papua New Guinea.

OVERVIEW AND SPEAKER BIOGRAPHIES

Trent W. J. Garner, Ph.D., Theme Leader, Evolution and Molecular Ecology, and Senior Research Fellow, Institute of Zoology, ZSL. Dr. Garner completed his Ph.D at the Universität Zürich, his MSc. and BSc.H. at the University of Victoria. He is a confirmed academic opportunist and his research interests include amphibian infectious diseases, the ecology of host/pathogen interactions, evolutionary conflicts including parasite evolution, invasive species, population genetics, sexual selection and conservation. He freely admits that most of the better work he takes credit for has been done by the students he supervises.

Matthew J. Gray, Ph.D., Associate Professor of Wildlife Ecology, University of Tennessee Center for Wildlife Health, Knoxville, TN. Dr. Gray received his B.S. from Michigan State University, M.S. from Mississippi State University, and Ph.D. from Texas Tech University. His research focuses on ranavirus-host interactions, particularly species- and community-level attributes that affect host susceptibility and the likelihood of ranavirus emergence. He has performed over a dozen surveillance studies and over 200 laboratory experiments with ranaviruses. He is co-chair of the Disease Task Team of the Southeast Partners in Amphibian and Reptile Conservation, and has led several workshops on ranavirus ecology and designing surveillance studies to detect ranaviruses. Dr. Gray is president-elect of the Tennessee Chapter of The Wildlife Society, and is Director of the Global Ranavirus Consortium.

Debra L. Miller, D.V.M., Ph.D., Professor/Wildlife Pathologist, University of Tennessee. Dr. Miller completed her pathology residency and postdoc at the University of Miami, her Ph.D., D.V.M. and M.S. at Mississippi State University and her B.S. at the University of Wisconsin-Stevens Point. Her research interests include comparative pathology of ranaviral diseases across classes, the effects of concurrent infection on the development of disease and the interclass transmission dynamics of ranaviruses.

Allan Pessier, D.V.M., Senior Scientist, Amphibian Disease Laboratory, Institute for Conservation Research, San Diego Zoo Global. Dr. Pessier received his DVM from Washington State University and completed a residency in veterinary pathology at the Smithsonian National Zoological Park. He is a Diplomate of the American College of Veterinary Pathologists. Allan is a veterinary advisor to the IUCN Amphibian Ark and the AZA Amphibian Taxon Advisory Group. His interests include diagnostic pathology of amphibians, improving the application and interpretation of diagnostic tests for amphibian infectious diseases, development of practical biosecurity measures for amphibian conservation and reintroduction programs, and methods to control infectious and non-infectious disease in amphibian captive survival assurance populations.

Jacques Robert, Ph.D., Associate Professor of Microbiology and Immunology at the University of Rochester Medical Center, Rochester, New York. He is the Director of the *Xenopus laevis* research resource for Immunobiology, which is the world's most comprehensive facility specializing in the use of this species for immunological research. His research interests include evolutionary aspects of immune surveillance, tumor and viral immunity. He has worked extensively in the area of T cell development, immunomodulation and anti-tumor immune responses elicited by heat shock proteins, molecular evolution of immunologically relevant genes, and immunity to ranaviruses. An important part of his research interest concerns basic comparative and applied studies of viral pathogenesis and immunity in amphibians caused by ranaviruses such as Frog Virus 3.

PROGRAM OF EVENTS – JULY 26TH, 2013

15:00 – 17:00 **Global Ranavirus Consortium Executive Board Meeting**
Location: Mt. Laurel Conference Room

16:00 – 20:00 **Registration**
Location: Parlor 1

PROGRAM OF EVENTS – JULY 27TH, 2013

Unless noted otherwise, all presentations will be in the Medallion Room – Holiday Inn - Downtown, Knoxville

8:15 – 8:30 ***Welcome***

Matthew Gray
Director of the Global Ranavirus Consortium, University of Tennessee, Knoxville

8:30 – 9:30 ***Keynote Address – EMERGING INFECTIOUS DISEASES AND AMPHIBIAN POPULATION DECLINES: HOW ARE WE GOING?***

Richard Speare
Emeritus Professor, School of Public Health, James Cook University, Australia

9:30 – 10:00 ***Emergence and Conservation Overview***

CAN PATTERNS OF RANAVIRUS EMERGENCE BE USED TO ASSESS CONSERVATION THREAT?

Trenton Garner
Institute of Zoology, Zoological Society of London

10:00 – 10:15 ***Ranavirus could potentially speed up extinction for the endangered frog (Rana sevosa)***

Julia Earl
National Institute for Mathematical and Biological Synthesis, University of Tennessee

10:15 – 10:30 ***Repeated detection of frog virus 3 (FV3) during aquaculture health surveys***

Thomas Waltzek
College of Veterinary Medicine, University of Florida

10:30 – 10.45 Coffee Break

10:45 – 11:00 ***Study of highway construction mitigation leads down to an unexpected road: Concurrent die-offs of turtles, salamanders and frogs at one site in Maryland, USA***

Scott Farnsworth
School of Biological Sciences, Washington State University

11:00 – 11:15 ***Distribution of ranaviruses in Japan***

Yumi Une
Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University

11:15 – 11:30 ***Ranavirus infection in Costa Rican amphibians***

Jacob Kerby
Department of Biology, University of South Dakota

11:30 – 11:45 ***Characterization of amphibian ranavirus in the international wildlife trade***

Kristine Smith
EcoHealth Alliance

11:45 – 12:00 ***Ranaviruses: An underestimated pathogen of cool water species in Northeast China***

XiaoLong Wang
Center of Conservation Medicine & Ecological Safety, and Wildlife Resource College, Northeast Forestry University

12:00 – 13:30 Lunch

13:30 – 14:00 ***Pathology and Physiology Overview***

RANAVIRAL DISEASE PATHOLOGY AND PHYSIOLOGY

Debra Miller

College of Veterinary Medicine and Center for Wildlife Health, University of Tennessee, Knoxville

14:00 – 14:15 ***Frog virus 3 in eastern box turtles: Agents seen with coinfections***

James Wellehan
College of Veterinary Medicine, University of Florida

14:15– 14:30 ***Ranavirus associated dermatitis in lizards***

Anke Stöhr
Institute of Environmental and Animal Hygiene, Universität Hohenheim

14:30 – 14:45 ***The wood frog*, Rana sylvatica (Lithobates sylvaticus)*, as a model to study the pathogenesis and host-pathogen interactions of frog virus 3 (FV3)***

Maria Forzán
Canadian Cooperative Wildlife Health Centre and Department of Pathology and Microbiology, Atlantic Veterinary College

14:45 – 15:00 ***Ranaviruses in snakes, lizards and chelonians***

Rachel Marschang
Institute of Environmental and Animal Hygiene, Universität Hohenheim

15:00 – 15:15 Coffee Break

15:15 – 15:45 ***Virology and Immunology Overview***

THE HOST IMMUNE SYSTEM: A DOUBLE-EDGED SWORD CONTROLLING RANAVIRUS INFECTION BUT PROMOTING VIRAL PERSISTENCE

Jacques Robert
Microbiology & Immunology, University of Rochester Medical Center

15:45 – 16:00 ***The three dimensional structure and morphogenesis of Singapore grouper iridovirus***

Jinlu Wu
Mechanobiology Institute Singapore, National University of Singapore, Singapore

16:00 – 16:15 ***Experimental challenge study of ranavirus infection in previously infected eastern box turtles (*Terrapene carolina carolina*) to assess immunity***

Jennifer Hausmann
Medical Department, Maryland Zoo

16:15 – 16:30 ***Immune response in fathead minnow cells following infection with frog virus 3***

V. Gregory Chinchar
Department of Microbiology, University of Mississippi Medical Center

16:30 – 16.:45 ***Signapore grouper iridovirus (SGIV) induced parapoptosis-like death in host cells via the activation of MAPK signaling***

Qiwei Qin
Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences

17:00 – 18:00 **DISCUSSIONS ON SYMPOSIUM OVERVIEW TOPICS**

Emergence and Conservation – Led by Trent Garner – Location: Medallion Room

Pathology and Physiology – Led by Debra Miller – Location: Parlor 2

Virology and Immunology – Led by Jacques Robert – Location: Parlor 4

18:00 – 20:00 Poster Session and Social

Location – Tennessee Ballroom, Holiday Inn

PROGRAM OF EVENTS – JULY 28TH, 2013

Unless noted otherwise, all presentations will be in the Medallion Room – Holiday Inn - Downtown, Knoxville

8:30 – 9:00 ***Diagnosis, Treatment and Management Overview***

AN OVERVIEW OF RANAVIRUS DIAGNOSTICS, TREATMENT AND MANAGEMENT

Allan Pessier
Amphibian Disease Laboratory, Institute for Conservation Research, San Diego Zoo

9:00 – 9:15 ***Chelonian diagnostics, pathology, and therapy***

Matthew Allender
Department of Comparative Biosciences, , College of Veterinary Medicine, University of Illinois

9:15 – 9:30 ***Monitoring ranavirus-associated mortality in Dutch heathland in the aftermath of an outbreak***

Annemarieke Spitzen
Reptile, Amphibian & Fish Conservation Netherlands (RAVON) (Jolianne Rijks presenting)

9:30 – 9:45 ***Surveillance of ranavirus in frog farms and surrounding environments in Brazil***

Rolando Mazzoni
Centro de Pesquisa em Alimentos, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás

9:45 – 10:00 ***Risk analysis common midwife toad-like virus, the Netherlands***

Jolianne Rijks
Dutch Wildlife Health Centre, Faculty of Veterinary Medicine, Utrecht University

10:00 – 10:15 ***Ranavirus outbreak in captive eastern box turtle (Terrapene carolina carolina) population with mycoplasma and herpesvirus co-infection: Management and monitoring***

Richard Sim
Wildlife Center of Virginia

10:15– 10: 45 Coffee Break

10:45 – 11:15 ***Ecology and Epidemiology Overview***

ECOLOGY AND EPIDEMIOLOGY OF RANAVIRUSES: MECHANISMS CONTRIBUTING TO OUTBREAKS

Matthew Gray
Center for Wildlife Health, University of Tennessee, Knoxville

11:15 – 11:30 ***Mathematical Modeling of Ranavirus Ecology***

Amanda Duffus
Department of Biology, Gordon State College

11:30 – 11:45 ***Environmental dependency of ranavirus/amphibian genotypic interactions: A coevolutionary rubik's cube***

David Lesbarrères
Genetics and Ecology of Amphibians Research Group (GEARG), Department of Biology, Laurentian University

11:45 – 12:00 ***The within-pond epidemiology of an amphibian ranavirus***

Jesse Brunner
School of Biological Sciences, Washington State University

12:00 – 12:15 ***Amphibian ranavirus disease dynamics in an industrially altered landscape***

Danna Schock
Keyano College

12:15 – 13:45 Lunch

13:45 – 14:00 ***Transmission of ranavirus between ectothermic vertebrate hosts***

Roberto Brenes
Center for Wildlife Health, University of Tennessee, Knoxville

14:00 – 14:15 ***Stress effects on susceptibility and transmission of ranavirus infection in amphibians***

Robin Warne
Department of Zoology, Southern Illinois University

14:15 – 14:30 ***Temperature affects anuran susceptibility to ranavirus***

Mabre Brand
Center for Wildlife Health and College of Veterinary Medicine, University of Tennessee, Knoxville

14:30 – 14:45 Coffee Break

14:45 – 15:45 **DISCUSSIONS ON SYMPOSIUM OVERVIEW TOPICS**

Diagnosis, Treatment and Management – Led by Allan Pessier – Location: Parlor 2

Ecology and Epidemiology – Led by Matthew Gray – Location: Medallion Room

15:45 – 16:45 **SUMMARY FROM THE DISCUSSIONS ON THE OVERVIEW TOPICS**

Led by – Trent Garner, Debra Miller, Jacques Robert, Allan Pessier, and Matthew Gray

Location: Medallion Room

16:45 – 17:15 ***Synthesis of Meeting and the Role of the GRC***

Matthew Gray
Director of the GRC, Center for Wildlife Health, University of Tennessee

Location: Medallion Room

PROGRAM OF EVENTS – JULY 29TH, 2013 – OPTIONAL FIELD TRIPS

USING TURTLE DOGS FOR RANAVIRUS SURVEILLANCE AND BOX TURTLE HEALTH ASSESSMENTS

Leader: Matt Allender, Department of Comparative Biosciences, University of Illinois

Participants will join the existing field team that includes veterinarians, biologists, high school students, and of course the turtle dogs in locating, sampling, and releasing eastern box turtles. Active surveillance for ranavirus and other health parameters has taken place for the last 6 years and the field protocols will be observed during the morning. Following lunch, participants will head to the field lab station where they will assist in hematologic assessment of samples as well as protocols for processing samples for biochemical analysis, pathogen surveillance, protein electrophoresis, and acute phase protein responses.

Limit: 8
Cost: $30/person

RANAVIRUS SAMPLING IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

Leader: Matthew Gray, Center for Wildlife Health, University of Tennessee, Knoxville

Participants will join a team of disease ecologists and veterinarians that have been monitoring ranavirus prevalence in amphibian populations in the Great Smoky Mountains National Park for seven years. The Monday field trip will focus on ranavirus surveillance in lungless salamander (Plethodontidae) populations at three locations (lotic streams) that differ in elevation and human disturbance. On Wednesday, participants will sample three depressional (lentic) wetlands in Cades Cove; one location (Gourley Pond) has been the site of reoccurring die-offs from ranavirus for >15 years. In the event that the Cades Cove wetlands are dry, additional stream sites will be sampled (similar to Monday). Participants will be exposed to amphibian identification, amphibian capture and population estimation techniques, and non-lethal sampling for ranavirus and the amphibian chytrid fungus. We will discuss life history of captured amphibians, issues with amphibian pathogen surveillance, and decontamination procedures. Participants should wear field clothes (rain gear if necessary) and hiking boots. Hip boots or waders are recommended for the Wednesday field trip if Cades Cove wetlands are sampled. Lunch will be provided.

Limit: 20
Cost: $30/person

ORAL PRESENTATION ABSTRACTS

EMERGENCE AND CONSERVATION

CAN PATTERNS OF RANAVIRUS EMERGENCE BE USED TO ASSESS CONSERVATION THREAT?

Trenton W. J. Garner
Institute of Zoology, Zoological Society of London, Regents Park, London, UK.

Ranaviruses are considered to be potential threats to amphibians, fish and Testudines because they appear to be emerging more frequently and causing mass mortality when they do. However, emergence and mass mortality do not always mean disease is a conservation issue, even if the emergence is novel and mortality levels are high. Evolutionary and ecological theory both say that disease emergence associated with high levels of mortality may result in host/pathogen equilibrium that may be perturbed, but is unlikely to result in persistent decline and host extinction. But is there evidence of stable equilibria established between ranaviruses and their hosts? I'll present what little I know of this topic, and will be fully braced for criticism from the better informed.

RANAVIRUS COULD SPEED UP EXTINCTION FOR THE ENDANGERED FROG, *RANA SEVOSA*

J.E. Earl[1], M.J. Gray[2], and W.B. Sutton[2,3]
[1]National Institute for Mathematical and Biological Synthesis, University of Tennessee, Knoxville, TN, USA. [2]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA. [3]School of Agricultural, Forest and Environmental Sciences, Clemson University, Clemson, SC, USA.

There has been much debate about the ability of ranavirus to affect population dynamics in host species. To examine this possibility, we created a stage-structured population model for the dusky gopher frog (*Rana sevosa*), the most endangered anuran in North America, based on seven years of data from the largest of the four remaining populations. The matrix model included demographic stochasticity from natural variation in the population. We performed experimental challenges to ranavirus, and found that 100% of adult dusky gopher frogs that were exposed to the pathogen in water died in <2 weeks. Using these laboratory results and field data from the population, we examined the effects of ranavirus exposure during the adult stage on time to extinction. We also explored whether extinction probability interacted with hydroperiod duration, which is known to affect recruitment in this population. We found that hydroperiod duration was the most important factor driving population dynamics; however, the introduction of ranavirus resulted in the population going extinct faster. Our simulations are based on published data between 1996 – 2002. Since then, biologists have been raising tadpoles and releasing juvenile frogs to thwart the declining population trend. Nevertheless, our results demonstrate that ranaviruses could play a role in the population persistence of this endangered species. Additionally, biologists that study endangered frogs should be very conscientious about disinfecting boots and equipment to prevent accidental ranavirus introduction. Future research includes testing the susceptibility of *Rana sevosa* embryos, hatchlings, larvae and metamorphs, and incorporating these results into our model.

REPEATED DETECTION OF FROG VIRUS 3 DURING AQUACULTURE HEALTH SURVEYS

T.B. Waltzek
College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

After viral hemorrhagic septicemia virus (VHSV) was confirmed in 2003, state and federal agencies in the Great Lake Basin (GLB) responded by increasing surveillance. These efforts led to the discovery of dozens of previously unknown fish viruses including the unexpected isolations of *Frog Virus 3* (FV3), the type species for the genus *Ranavirus*. Ranavirus (RV) epizootics in amphibians are reportable to USDA APHIS and the World Organization for Animal Health due to the impact these agents have had on declining amphibian populations. In contrast, fish RV infections within the US have nearly all been attributed to Santee-Cooper ranavirus, which typically is associated with low or no mortality in largemouth bass. The recent isolations of FV3 from iconic GLB fish species are noteworthy given prior to VHSV surveillance, the host range of FV3 was believed to be primarily restricted to reptiles and amphibians. The pathogenicity of FV3 in fish was recently confirmed following the investigation of a 2009 epizootic at a Missouri hatchery that killed approximately 10,000 pallid sturgeon fingerlings. Archived FV3 isolates from significant disease episodes in hatchery-reared white, Russian, and lake sturgeon independently suggest FV3 can be pathogenic in fish. These findings suggest FV3 is among the least host specific viruses known infecting poikilothermic vertebrates across three taxonomic classes (Osteichthys, Amphibia, and Reptilia).

STUDY OF HIGHWAY CONSTRUCTION MITIGATION LEADS DOWN AN UNEXPECTED ROAD: CONCURRENT DIE-OFFS OF TURTLES, SALAMANDERS, AND FROGS AT ONE SITE IN MARYLAND, USA.

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Reports of mortalities attributed to *Ranaviruses* are becoming increasingly common, especially for amphibians. Unfortunately, information on the timing, extent, and frequency of occurrence of such outbreaks remain limited, due to the transitory nature of the disease. From 2008-2011, we studied the ecology and movement patterns of Eastern Box Turtles (*Terrapene carolina*) at North Branch Rock Creek Park in south-central Maryland. The first mortality from *Ranavirus* was seen at our study site in August 2009, when we found seven turtles dead in the field and two turtles with apparent signs of disease. Throughout the study 27 telemetered turtles and over 20 incidentally found turtles were found dead. Of the fresh carcasses sent to the USGS Wildlife Health Center for analysis, over 80% were positive for infection of a *Ranavirus.* Although no unusual amphibian mortality was seen in 2008 or 2009, during the early spring of 2010, we examined larval frogs (*Rana* [*Lithobates*] *sylvatica*) and salamanders (*Ambystoma spp.*) for signs of *Ranavirus* infection. We found multiple vernal pools where an apparent 100% of larval amphibians were infected with *Ranavirus*, later confirmed by the USGS Wildlife Lab. Mortality at these sites was an effective 100%. We found the same pattern of infection and subsequent complete mortality among larval amphibians during spring 2011 at these and additional sites. The simultaneous impacts of *Ranavirus* on amphibians and box turtles at the same site suggests a potential link, but the mechanism for this remains poorly understood.

DISTRIBUTION OF RANAVIRUS IN JAPAN

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In 2008, we reported the first outbreak of ranaviral disease in Japan in wild populations of the American Bullfrog (*Lithobates catesbeianus*). In this study, we collected and tested 1,117 Indian rice frogs (*Fejervarya kawamurai*) from 13 locations in 8 prefectures between May 2011 and October 2012 as a first attempt to learn about the distribution of ranavirus in Japan. Capture frogs were euthanized, necropsied, and tested for ranavirus infection using kidney tissue and PCR. Ranavirus was found in all 8 Japanese prefectures. Infection prevalence of ranavirus was 12.9% (152/1,177), with lower infection in 2011 (8.7%) compared to 2012 (16.5%). Infection prevalence differed among seasons in 2011 (0% in spring, 7.7% in summer, 17.8% in autumn) and in 2012 (7.7% in spring, 7.7% in summer, 36.6% in autumn), with prevalence greatest during autumn of both years. We sequenced 495, 72 and 193 base pair regions of the MCP, and identified potentially four types of ranavirus (RCV-JP, HNV, FKV, and TFV) present in Indian rice frog populations, with prevalence differing among types (30.1%, 15%, 46.9% and 9%, respectively). In conclusion, it appears ranavirus is distributed widely in Japan, with multiple variations of the virus circulating in amphibian communities. Based on these preliminary results, it is unknown if these types of ranavirus are endemic to Japan or if they are having negative impacts on populations. However, controlled studies that we have performed indicate that certain Japanese species that are rare (e.g., salamanders in Hynobiidae) are very susceptible to ranavirus. Given that the Indian rice frog is an invasive species with a rapidly expanding distribution that is a suitable host for ranavirus, their occurrence may contribute to the emergence of ranavirus in native amphibian communities in Japan.

RANAVIRUS INFECTION IN COSTA RICAN AMPHIBIANS

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Ranaviruses are a globally widespread group of iridoviruses capable of causing mass mortality events in amphibian populations, and are acknowledged to be a significant threat to amphibian populations in many parts of the world. Central America hosts a diverse, unique, and highly threatened amphibian fauna, yet there has been little effort to describe presence, systematics, host range, or impacts to hosts or populations of Ranaviruses. In one study, we examined toe clips from 104 individuals of twelve different species of amphibians collected at La Selva Biological Station in Costa Rica. Utilizing quantitative PCR methodology, we detected the presence of ranavirus in four of these samples all from a single direct developing species, *Craugastor bransfordii.* In a second study, we evaluated the prevalence of infection of both the chytrid fungus and ranavirus in a larger sampling effort. We sampled 253 amphibians from 20 species and 8 families. Of these, 54 individuals of 11 species tested positive for Bd (overall prevalence 21.3%), and 42 amphibians from 9 species tested positive for ranavirus (overall prevalence 16.6%). We found a positive but non-significant association between infection by ranavirus and infection by Bd among species overall. We found evidence for positive associations between ranavirus and Bd infection in one of our four most commonly sampled species, *Craugastor fitzingeri*. These studies represent one of the first detailed evaluations of ranavirus

prevalence in the American tropics, and to our knowledge our study is the first report detecting a positive association between Bd and ranavirus in any species.

CHARACTERIZATION OF AMPHIBIAN RANAVIRUS IN THE INTERNATIONAL WILDLIFE TRADE

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Amphibian ranaviruses have been responsible for significant amphibian die-offs worldwide since first recognized in the 1960's. Given that trade is believed to be one of the main routes of spread of this pathogen, *Ranavirus* infection (along with chytrid fungus) was recently added to the World Organization for Animal Health (OIE) list of notifiable diseases. Yet specifics regarding the role of wildlife trade in the epidemiology of ranaviruses remain unknown, and better characterization is warranted. Building upon previous work investigating the presence of infection in US markets, we evaluated the prevalence of ranaviruses in international amphibian shipments upon arrival at US ports of entry. We tested individuals from multiple shipments of amphibians originating in the Dominican Republic, Hong Kong and Madagascar. Animals were sampled immediately upon arrival directly in their shipping containers preceding exposure to other animals or holding facilities and hence avoiding the potential for iatrogenic contamination of animals post importation. Cloacal swabs were collected from live animals and organ tissues were collected from animals that were dead upon arrival. Polymerase chain reaction (PCR) testing using ranavirus-specific primers revealed a high prevalence in the majority of shipments tested. These findings suggest that *Ranavirus* infection may be extremely common in the global amphibian pet trade and repeated introduction of the pathogen into the USA likely occurs via this route.

RANAVIRUSES: AN UNDERESTIMATED PATHOGEN OF COOL WATER SPECIES IN NORTHEASTERN CHINA

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Ranaviruses are recognized for causing disease in fishes and amphibians in tropical and subtropical regions. However, little attention has been paid to the importance of this pathogen in cool water species in China. From 2006 to 2012, surveys for ranaviruses in amphibians in the northern part of China were carried out. The target area was divided into 24 regions and 72 sampling sites located more than 3 km from each other. The sample size was calculated by a 95% confidence limit for prevalence of 5%. At each sampling site, 15 samples were collected randomly. PCR-based clone and sequencing, nucleotide and amino acid sequence analysis, restriction endonuclease analysis and phylogenetic analysis of their Major capsid protein genes were used for viral identification and genomic characteristics analysis. The prevalence of ranavirus infection across the 24 sampling regions varied greatly, with prevalence of 0-5.7% and 0-90% in adults and in tadpoles. Prevalence also varied amongst species. All obtained strains were highly homologous to FV3 with different numbers of mutations/substitutions, which shortened or lengthened the position of restriction enzyme cutting sites of *Fnu4H I* in all strains, while that of *Hinc, Acc I* and *PflM I* were not disturbed by these mutations. Differences in conformation amongst FV3 and China isolates reflected their integral differences. Results indicated that ranaviruses maintained a broad distribution and presented a specific

genetic diversity throughout northeastern China. These findings may relate to the health of local species and their roles in sustaining local food webs and ecological functions.

PATHOLOGY AND PHYSIOLOGY

RANAVIRAL DISEASE PATHOLOGY AND PHYSIOLOGY

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During the two years since the last symposium, mortalities due to ranaviruses have continued, with select mass mortality events (i.e., Maryland box turtle mortality event) making headlines. Inasmuch as the genus *Ranavirus* infects amphibians, reptiles and fish and that morbidity and mortality events in each of these classes have been documented throughout the world, it behooves us to investigate the pathogenesis of ranaviral disease for identifying key factors to consider in conservation and management programs. Over the past 2 years, there has been much progress in documenting the pathological changes that occur in ranavirus-infected individuals, including how these changes might be influenced by virus isolate or infection with concurrent or secondary/opportunistic pathogens. Controlled experimental challenges have allowed us to document cross-class transmission of ranaviruses and the associated pathological changes. Hemorrhage, swelling and necrosis are common gross changes noted across classes. Microscopic lesions may vary but generally include cellular necrosis (e.g., hematopoietic tissue, vascular endothelium, and epithelial cells) and intracytoplasmic inclusion bodies. Current research directives are focused on 1) characterizing the pathogenesis of ranaviral disease among virus isolates and hosts, 2) perfecting techniques such as *in situ* hybridization and immunohistochemistry that will allow us to visualize the presence of the virus within the tissues, and 3) characterizing the effects of concurrent pathogens on disease progression.

FROG VIRUS 3 IN EASTERN BOX TURTLES: AGENTS SEEN WITH COINECTIONS

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Chelonians have low fecundity, low juvenile survival rate, and a long adult lifespan; a life history strategy where loss of adult animals (such as loss by disease) has a significant impact on populations. Frog virus 3, a *Ranavirus*, is strongly associated with mass mortality events in eastern box turtles (*Terrapene carolina*). A currently unnamed *Mycoplasma* sp, distinct from other known species, has been associated with upper respiratory tract disease in *T. carolina*. More recently, a novel adenovirus has been identified in association with enterohepatic disease in eastern box turtles, and a herpesvirus has been identified in animals with concurrent ranaviral disease. The diversity and significance of infectious diseases beyond this are just beginning to be understood. Frog virus 3, Box turtle adenovirus 1, Terrapene herpesvirus 1, and an unnamed *Mycoplasma* sp. were identified in a group of confiscated eastern box turtles. Coinfection was common in this group, and may have played a significant role in the expression of disease. An overview of these agents, along with Terrapene herpesvirus 2 and the intranuclear coccidiosis agent of tortoises will be presented.

RANAVIRUS ASSOCIATED DERMATITIS IN LIZARDS

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Several cases of ranaviral infection in lizards were detected in our laboratory in the years 2011-2012. Affected species were: green striped tree dragons (*Japalura splendida*), Asian glass lizards *(Dopasia gracilis),* green anoles *(Anolis carolinensis),* green iguanas *(Iguana iguana),* and a central bearded dragon *(Pogona vitticeps).* The lizards were from a variety of owners, but all animals had skin lesions (purulent to ulcerative-necrotizing dermatitis and hyperkeratosis). In some of these cases, large groups of lizards developed disease and low to high mortality occurred, in others only single animals were affected. In several cases, coinfections with additional viruses (including adenoviruses, invertebrate iridovirus) were also detected. Ranaviruses were identified by PCR and (in three cases) virus was isolated in cell culture. Sequencing of a portion of the MCP genes of the detected viruses demonstrated that all viruses were distinct from one another. The nt sequences were 98.4 -100% identical to the corresponding portion of the frog virus 3 (FV3) genome. This is the first detection of ranaviruses in all described species. The similarity in pathological lesions observed in these different cases indicates that ranaviral infection may be an important differential diagnosis for skin lesions in lizards.

THE WOOD FROG*, RANA SYLVATICA* (*LITHOBATES SYLVATICUS*), AS A MODEL TO STUDY THE PATHOGENESIS AND HOST-PATHOGEN INTERACTIONS OF FROG VIRUS 3 (FV3)

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Experimental infection of a native species with a ranavirus, such as Frog Virus 3 (FV3), is an indispensable counterpart to field research. The wood frog, *Rana sylvatica*, serves as a good model for laboratory experimentation based on its wide distribution in North America and known susceptibility to ranavirus infection. A protocol for housing and reproduction of ranavirus-free wood frog populations in captive settings is necessary to firmly establish the species as an experimental model. The present work includes recommendations on housing and feeding of pre- and post-metamorphic frogs in the laboratory, oral dosing of post-metamorphic frogs for experimental infection with pathogens in liquid suspension, and collection and evaluation of blood pre and post-infection to determine cellular and humoral responses to FV3. Based on these recommendations, an experimental infection was conducted in 40 wild-caught adult wood frogs to determine the pathogenesis of an FV3-infection based on histological examination of lesions and PCR testing. Frogs were acclimated to captivity for 6 months prior to infection. Oral dosing of 0.25ml of virus suspension equivalent to a $TCID_{50}$ $10^{3.03}$ was administered to individually-housed frogs (day 0), which were subsequently euthanized at days 0.25, 0.5, 1, 2, 4, 9 and 14 post-infection (n=5 frogs at each time point, except n=3 at day 14). A necropsy examination was performed; samples of skin, liver, kidney and spleen were frozen for PCR testing; all organs were fixed in 10% formalin for histological examination. The benefits, problems and future directions of this methodology will be discussed.

RANAVIRUSES IN SNAKES, LIZARDS AND CHELONIANS

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Ranavirus isolates were obtained from two Hermann's tortoises (*Testudo hermanni*), an Egyptian tortoise (*Testudo kleinmanni*), a marginated tortoise (*Testudo marginata*), a leaf-tailed gecko (*Uroplatus fimbriatus*), an Iberian mountain lizard (*Lacerta monticola*), a green striped tree dragon (*Japalura splendida*), a brown anole (*Anolis sagrei*), an Asian glass lizard (*Dopasia gracilis*), a green anole (*Anolis carolinensis*), and a red blood python (*Python brongersmai*). Virus characterization was carried out based on sequences from the MCP gene (1402 bp), the DNA polymerase gene (560 bp), the RNR-α gene (806 bp), and the RNR-β gene (646 bp). In addition, a portion of the vIF-2α gene of each isolate was amplified and sequenced. Analysis of the obtained sequences showed that an isolate from a Hermann's tortoise from Switzerland was most closely related to isolates from European frogs, while the other chelonian isolates were all most closely related to one another and were distinct from the Swiss tortoise isolate. The isolates from the anoles, the Iberian mountain lizard, and the green striped tree dragon were all closely related to one another and to FV3, while the isolate from the gecko was closely related to BIV. The isolates from the python and from the Asian glass lizard were most closely related to one another and to tiger frog virus. Lengths of the vIF-2α gene sequences were between 211 and 1000 bp. These studies underline the ability of ranaviruses to switch hosts between different classes of animals and demonstrate the role that these viruses play in reptilian hosts.

VIROLOGY AND IMMUNOLOGY

THE HOST IMMUNE SYSTEM: A DOUBLE-EDGED SWORD CONTROLLING RANAVIRUS INFECTION BUT PROMOTING VIRAL PERSISTENCE

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Infections and die-offs caused by Ranaviruses (RVs) are increasing in prevalence and range of host species infected (from amphibian to fishes and reptiles) at alarming rates. The rapid increase in prevalence of RV infections, the wide range of host species infected by RVs, the variability in host resistance among populations of the same species and among different developmental stages, as well the capacity of RVs to cross species barriers of numerous poikilotherms, all suggests that RVs possess potent immune evasion mechanisms. Indeed, while some of the 95–100 predicted RV genes encode putative evasion proteins (e.g., vIFα), roughly two-thirds of them do not share significant sequence identity with known viral or eukaryotic genes. Although amphibians, as all jawed vertebrates, possess an immune system fundamentally similar to mammals including innate (macrophages, NK cells) and adaptive (B and T cells) cell effectors, investigation of antiviral immunity, especially against RVs, is still in its infancy. This is partially due to the needs of species-specific tools (e.g., antibodies) and genetically-defined MHC-matched host systems. Therefore, the use of appropriate animal models remains critical in examining viral–host interactions.

To alleviate this problem, we have established a reliable infection model system using the frog *Xenopus* and Frog Virus 3 (FV3). We have shown that susceptible *Xenopus* tadpoles mount poor FV3-elicited type I interferon and innate immune responses compared to resistant adults, and that a greater proportions of larval than adult peritoneal leukocytes were infected by FV3. Furthermore, our findings indicate that although the adult frog immune system is efficient in controlling FV3 infection, FV3 persists in a fraction of otherwise healthy asymptomatic frogs, and this persistence involves macrophages harboring the quiescent virus. After viral clearance, peritoneal macrophages isolated from some, but not all, asymptomatic animals harbor transcriptionally inactive FV3. Preliminary evidence suggests that pro-inflammatory signals can reactivate quiescent FV3 infections. These data are consistent with the idea that FV3 targets adult macrophages to escape host immunity and persist in asymptomatic host, whereas in contrast FV3 targets larval macrophages to overcome immune defenses and rapidly disseminate.

THE THREE DIMENSIONAL STRUCTURE AND MORPHOGENESIS OF SINGAPORE GROUPER IRIDOVIRUS

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Singapore grouper iridovirus (SGIV), a major pathogen in grouper aquaculture, was first isolated in 1998 from brown-spotted grouper. In the past decade, we carried out the viral genomic, transcriptomic, proteomic and lipidomic studies, dissected its molecular compositions and revealed its gene expression profiles. Our results show that the complex virion contains a dsDNA genome of 140,131 bp, at least 44 structural proteins and 220 lipid species. How these molecules are assembled to form a viral particle is unknown. Recent advances in cryoEM/ET technology and computational power have made it possible to examine the structure and morphogenesis of large complex viruses in three dimensions. We took more than 1000 frames with an FEI Titan Krios microscope and selected about 6000 particles for 3D reconstruction. A subnano resolution map was obtained, which reveals: 1) hexamers and pentamers distributed on a T = 247 icosahedral lattice; 2) an irregular lipid bilayer between the capsid shell and viral core; 3) anchor proteins located between the capsid shell and the inner lipid bilayer. High-pressure freezing and freeze substitution were used to prepare SGIV-infected cells for electron microscopy. The viral capsid precursors first appear as closed membrane structures, then develop into headphone shape structures and capsid shells. We identified viral intermediates showing that the viral DNA is packaged into viral capsid during capsid formation. Knockdown of MCP disrupts the viral morphogenesis and diminishes the production of viral particles, while knockdown of viral DNA core protein leads to reduction of viral titer and deformities in viral particles.

EXPERIMENTAL CHALLENGE STUDY OF *RANAVIRUS* INFECTION IN PREVIOUSLY INFECTED EASTERN BOX TURTLES (*TERRAPENE CARLONIA CAROLINA*) TO ASSESS IMMUNITY

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The Maryland Zoo in Baltimore lost 13 of 27 (48%) captive Eastern box turtles (*Terrapene carolina carolina*) to an outbreak of *Ranavirus* (100% homology to 531 bp segment of FV3 MCP) during the summer of 2011. To assess survival and shedding post-infection, an experimental challenge study was performed, in which the surviving, previously infected turtles were reinfected with the outbreak strain of *Ranavirus*. Seven turtles were inoculated with a predetermined dose of infectious virus IM and four control turtles were injected with saline IM. The turtles were monitored for 8 weeks with blood and oral swabs collected for qPCR. During that time only one of the seven (14%) inoculated turtles and none of the controls (0%) died; there was no significant difference in survival. All clinical signs seen in the inoculated turtles, except for the turtle that died, were very mild when compared to the original outbreak. qPCR for *Ranavirus* was positive for all inoculated turtles on blood or oral swabs, while all controls were negative. Histopathology of the turtle that died showed intracytoplasmic inclusion bodies in multiple organs. Five of the surviving ten turtles were euthanized at the end of the study and no inclusion bodies were present. qPCR detected *Ranavirus* in the spleen of a control turtle, which may indicate persistance of the virus in tissues. In conclusion, previously infected Eastern box turtles can be reinfected with the same strain of *Ranavirus* and show mild to no clinical signs, but will shed the virus from the oral cavity.

DIFFERENTIAL TRANSCRIPTION OF FATHEAD MINNOW IMMUNE-RELATED GENES FOLLOWING INFECTION WITH FROG VIRUS 3.

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Frog virus 3 (FV3) and other ranaviruses trigger severe, systemic disease in susceptible fish, reptiles, and amphibians. However, although mortality rates may approach 100%, considerable variability is present reflecting the species infected, age of the host, and immune status. For example, FV3 infection leads to marked mortality in tadpoles and immunocompromised adults, but healthy adults generally experience transient disease and recover fully. Immunity to FV3 and similar viruses appears to depend upon acquired immunity, i.e., B cell and T cell responses, as well as various innate cellular and molecular effectors. To gain a global understanding of anti-viral immune responses following FV3 infection, we infected fathead minnow (*Pimephales promelas,* FHM) cells with wild type (wt) virus and an FV3 knock out (KO) mutant that had lost the 18 kDa immediate-early protein and whose replication in tadpoles was attenuated. Total RNA was isolated from replicate cultures at 8 hr after infection and

gene expression analysis was performed using a well-characterized custom FHM microarray. Comparison of transcripts present among mock, wt, and KO virus-infected cells indicated that viral infection resulted in the differential upregulation of numerous immune-related genes, e.g., IL-8, TRAF3, SOCS3, FTR14, PDCD6, IL-11, IL-1β, CASP9, IFN, IL-17c, IRF1, IRF7, MHC I/II, etc., affecting both the innate and acquired arms of the immune system Collectively these results indicate that FV3 infection leads to the induction of multiple immune-related molecules that may act to slow the course of infection and lead to long-term survival via pathways dependent upon both innate and acquired responses.

SINGAPORE GROUPER IRIDOVIRUS (SGIV) INDUCED PARAPTOSIS-LIKE DEATH IN HOST CELLS VIA THE ACTIVATION OF MAPK SIGNALING

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Virus induced cell death, including apoptosis and nonapoptotic cell death, plays a critical role in the pathogenesis of viral diseases. Singapore grouper iridovirus (SGIV), a novel iridovirus of genus *Ranavirus*, causes high mortality and heavy economic losses in marine aquaculture. Here, using fluorescence microscopy, electron microscopy and biochemical assays, we found that SGIV infection in host (grouper spleen, EAGS) cells evoked nonapoptotic programmed cell death (PCD), characterized by appearance of cytoplasmic vacuoles and distended endoplasmic reticulum in the absence of DNA fragmentation, apoptotic bodies, and caspase activation. In contrast, SGIV induced typical apoptosis in non-host (fathead minnow, FHM) cells, as evidenced by caspase activation and DNA fragmentation, suggesting that SGIV infection induced nonapoptotic cell death by a cell type dependent fashion. Furthermore, viral replication was essential for SGIV induced nonapoptotic cell death, but not for apoptosis. Notably, the disruption of mitochondrial transmembrane potential (ΔΨm) and externalization of phosphatidylserine (PS) were not detected in EAGS cells but in FHM cells after SGIV infection. Moreover, MAPK signals, including the extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK) were activated in SGIV induced nonapoptotic cell death. However, only ERK and JNK signals were essential for virus induced non-apoptotic cell death and viral replication. This is a first demonstration of MAPK signaling-mediated nonapoptotic cell death induced by DNA virus. These findings contribute to better understanding of the mechanisms of iridovirus pathogenesis.

DIAGNOSIS, TREATMENT AND MANAGEMENT

AN OVERVIEW OF *RANAVIRUS* DIAGNOSTICS, TREATMENT AND MANAGEMENT

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With the increasing recognition of ranaviruses as contributors to mass mortality events of amphibians and chelonians, there is a need to refine diagnostic methods in order to facilitate test interpretation and informed decision making in the management of *Ranavirus* infection in both wild and captive populations. Diagnosis of ranaviral disease can be straightforward by observation of suggestive necrotizing lesions and in some cases intracytoplasmic inclusion bodies on histopathology with confirmation by PCR (+/- DNA sequencing), virus isolation or electron microscopy. In some cases histologic evidence of ranaviral disease is obscured by secondary or concurrent infections or is missed

because lesions are subtle (e.g. hematopoietic tissue necrosis in tadpoles). Commonly used PCR methods for diagnosis (conventional and rt-PCR) of infection seem reliable from both antemortem (e.g. oropharyngeal or cloacal swab) and postmortem tissue samples of animals with ranaviral disease, but are not validated for detecting subclinical infections. This has implications for interpretation of infection presence/absence or prevalence surveys in outwardly healthy animals as well as quarantine or pre-release disease screening for captive animals and reintroduction programs. In all instances it should be recognized that positive PCR does not necessarily indicate ranaviral disease or a significant population threat. Many ranaviruses and especially FV3-like strains can appear to be very similar or even identical when diagnostic efforts are limited to PCR and DNA sequencing of portions of the MCP gene. Because these viruses can potentially have different host ranges or virulence development of practical methods to distinguish between strains are needed for reintroduction programs as well as surveys of *Ranavirus* distribution. Observations of decreased mortality in infected animals maintained at higher temperatures as well as the potential use of guanine analogue antiviral drugs may be very useful in clinical situations or even reintroduction programs for endangered species.

CHELONIAN DIAGNOSTICS, PATHOLOGY, AND THERAPY

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Ranaviruses have been proposed as a major threat to amphibian biodiversity, however the impact of these pathogens on reptiles is less well understood. A qPCR was developed that was 100% efficient in detecting the 54 bp segment of the major capsid protein of frog virus 3 (FV3) down to 5 viral copies. This assay was used to estimate the prevalence of ranavirus infections in 780 eastern box turtles (*Terrapene carolina carolina*). The overall prevalence was higher in turtles presented to rehabilitation centers and offers an opportunity for enhanced surveillance. In challenge studies at two separate environmental temperatures, red-eared sliders (*Trachemys scripta elegans*) inoculated with a FV3-like virus had higher mortality rates when maintained at 22^{o}C than at 28^{o}C, supporting the theory that this virus is less virulent at higher temperatures. Significant histopathological changes included fibrinoid vasculitis in all tissues. In a third attempted challenge study, un-inoculated turtles developed spontaneous ranaviral disease when the environmental temperature was dropped to 16^{o}C, but recovery occurred in the majority of cases when temperature was raised to 28^{o}C. Hematologic changes were evaluated in both free-ranging box turtles and experimentally inoculated red-eared sliders. Red-eared sliders showed only one significant change, a reduction in total solids over time. Box turtles were non-significantly lymphopenic. Treatment of ranavirus with anti-viral therapy has been reported to have variably poor success, but was based on anecdotal dosing recommendations. Pharmacokinetic analysis of a single oral dose of valcyclovir demonstrated measureable levels, and may prove useful against this virus.

MONITORING RANAVIRUS-ASSOCIATED MORTALITY IN A DUTCH HEATHLAND IN THE AFTERMATH OF AN OUTBREAK

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When ranavirus epizootics lead to mass mortalities of adult life stages, this is relatively easy to spot in the wild. However, in the aftermath of such an outbreak, mortality associated with ranavirus infection may be more difficult to detect. We provide an overview on the spatial distribution and temporal dynamics of ranavirus infections in a Dutch National Park in the year following a mass mortality event caused by ranavirus infection in 2010.

SURVEILLANCE OF RANAVIRUS IN FROG FARMS AND SURROUNDING ENVIRONMENTS IN BRAZIL

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American bullfrog (*Rana catesbeiana/Lithobates catesbeianus*) populations have been signaled as endemic ranavirus carriers. Ranaviruses have been detected and associated with mass mortality events in farmed tadpoles, and frog farms are very likely spreading pathogens to wild populations. The objectives of the present study were to assess ranavirus prevalence in Brazilian frog farms and pathogen spread in surrounding environments. Samples were obtained from the whole farm cycle (eggs, tadpoles, metamorphs, frogs and brood stock), and sympatric amphibians, fish and reptiles, and preserved in 95% ethanol and processed routinely for DNA extraction and further RT-PCR amplification. Primers RCVBR-f, GAGCGTCACCCTCTCATTC; RCVBR-r, GCGTCCAGGTATGCCGTG; and probe RCVBR-s, CGACATCAGCGCCCAGTC specific for ranavirus major capsid protein gene were applied. Although samples are still being processed, results thus far have yielded varied, positive prevalences for ranavirus occurrence in frogs on farms. In contrast, ranavirus has not been detected in samples obtained from surrounding areas, and neither mortalities nor outbreaks were reported in aquatic organisms near frog farms. For now, we tentatively conclude that ranavirus present in farmed American bullfrogs from Brazil is not a major threat to native species.

RISK ANALYSIS COMMON MIDWIFE TOAD-LIKE VIRUS, THE NETHERLANDS

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Common Midwife Toad-like virus (CMTV-like virus) infection is an emerging infectious disease among wild amphibians in the Netherlands. The Belgian Invasive Species Environmental Impact Assessment (ISEIA) protocol was used to assess risks associated with dispersion potential and ecological impacts.

Detected CMTV-like virus disease cases in 2011 and 2012 were clustered near the location of first record in 2010. Natural infections occurred in several common native amphibian species known to disperse >1 km per year, suggesting high dispersion potential. Amphibians in one area of high conservation value were affected, indicating medium risk for the colonization of high conservation value habitats. Susceptible species included species listed as threatened in the Netherlands; therefore the adverse impacts on native species were considered high risk. Finally, taking the view that viruses may alter ecosystem functions indirectly via the effects on host populations, the alteration on ecosystem functions was considered likely to be medium risk. Overall, the assessment revealed that CMTV-like virus should be placed on a watch list. However, without interference, the distribution of CMTV-like virus is likely to be more widespread in the future, with more adverse effects on high conservation habitats. Possible risk management opportunities focus on minimizing spread and impact.

RANAVIRUS OUTBREAK IN A CAPTIVE EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) POPULATION WITH MYCOPLASMA AND HERPESVIRUS CO-INFECTION: MANAGEMENT AND MONITORING

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In July 2011, 22 captive Eastern box turtles (*Terrapene carolina carolina*) at the Maryland Zoo in Baltimore were found to have varying degrees of fibrinonecrotic stomatitis, cloacitis, and blepharoedema. Three etiologic agents were identified within this group: *Ranavirus*, *Herpesvirus*, and *Mycoplasma* sp.. Prevalence of these etiologic agents by PCR on either ante-mortem oral swab and/or postmortem tissues was 86.4%, 54.5%, and 68.2% respectively. Treatment under quarantine included thermal support, nutritional support, fluid therapy, antibiotics, and the antiviral famciclovir. Table 1 shows the disease patterns of the three etiologic agents, prevalence of infection among the 22 treated Eastern box turtles, and survival rates among groups. No animal was solely infected with *Mycoplasma* sp., so this pathogen was excluded from statistical analysis of survival. Focusing only on the infection patterns of *Ranavirus* and *Herpesvirus*, survival rates were non-significantly different (p=0.345): no PCR detection of either pathogen (100% survival [1/1]), *Herpesvirus* alone (100% survival [2/2]), *Ranavirus* alone (44.4% survival [4/9]), and *Ranavirus* with *Herpesvirus* (60% survival [6/10]). Of the eight turtles that died during the treatment period, all were *Ranavirus*-positive and were co-infected to varying degrees, which suggests that *Ranavirus* infection was directly associated with the level of mortality. The survival rate among *Ranavirus*-positive turtles was 57.9% (11/19), which is higher than previously reported survival in similar captive *Ranavirus* outbreaks. This treatment protocol could be successful in future *Ranavirus* outbreaks regardless of occurrence of other etiologic agents.

ECOLOGY AND EPIDEMIOLOGY

ECOLOGY AND EPIDEMIOLOGY OF RANAVIRUSES: MECHANISMS CONTRIBUTING TO OUTBREAKS

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Ranavirus epizootics have been reported from most continents in captive and wild ectothermic vertebrate populations. However, the mechanisms that result in an outbreak vary and involve biotic and abiotic characteristics. Susceptibility to ranavirus differs among individuals in a population, and some individuals may be capable of superspreading events. Populations also vary in susceptibility to ranavirus, with genetic isolation as a known contributor to increased susceptibility. Common garden

experiments indicate that susceptibility to ranavirus differs among species. Highly susceptible species may function as amplification species, where their presence results in greater than expected infection likelihood of syntopic species. Reservoir hosts that are infected sublethally with ranavirus likely play a role in its persistence. In addition to active, low-grade infection in organ cells, ranaviruses cause quiescent infections in host macrophages. Reservoirs could include osteichthyean fish, reptiles, and pre- and post-metamorphic amphibians. Recent studies have shown that transmission of FV3-like ranaviruses is possible among these vertebrate groups. Environmental conditions also can affect the likelihood of an outbreak, and interact with species via complex pathways. For example, rapid drying of a water body can accelerate development of larval amphibians, causing the production of immunosuppressive hormones that increase susceptibility to ranavirus. Changes in vegetation structure or volume of an aquatic ecosystem can result in clustering of hosts and accelerated ranavirus transmission. Pollutants such as elevated nitrogenous compounds and pesticides have been linked to increased infection by ranavirus. Pathogenicity of ranavirus also is related to isolate genotype, and different isolates may result in different forms of ranaviral disease. The relatively long environmental persistence of ranavirus undoubtedly plays a role in its epidemiology, and may contribute to pathogen pollution via anthropogenic transport of virions on fomites. Indeed, the mechanisms that result in a ranavirus outbreak are complex and context-dependent. Sites with reoccurring die-offs presumably can decline. Epidemiological theory states that population extinction by a pathogen is possible if frequency dependent transmission occurs, even for brief periods. In certain cases, the ranavirus-host system likely meets these conditions. A future challenge will be identifying the most important host-pathogen mechanisms that result in elevated mortality, simulating population and community outcomes under differing scenarios, and conceiving disease intervention strategies to interrupt the host-pathogen cycle.

MATHEMATICAL MODELING OF RANAVIRUS ECOLOGY

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The transmission dynamics of the ranavirus(es) present in the common frog (*Rana temporaria*) populations in the United Kingdom provide a good starting point for understanding the more complex ecology of ranaviruses present in other regions. Initially, a simple susceptible-infected (SI) model using parameters derived from the literature is examined. This model is extended to explore the disease dynamics when two different disease syndromes, ulcerative and hemorrhagic forms, are present in the population, as well as the conditions for their persistence. An exploration of how this type of mathematical model may be useful in amphibian communities and other species will also be considered. Data necessary to make the models both representative and informative, from natural and experimental infections, will also be discussed.

ENVIRONMENTAL DEPENDENCY OF *RANAVIRUS*/AMPHIBIAN GENOTYPIC INTERACTIONS: A COEVOLUTIONARY RUBIK'S CUBE

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Despite an increasing understanding of ranaviral disease determinants, our understanding of ranavirus ecology and evolution is obscured by environmental contingencies reflecting the context-dependent nature of the disease dynamic. Additionally, interactions between host and pathogen genotypes (G_HxG_P) are modulated by environmental conditions in terms of host immune response and pathogen virulence. Therefore, investigating G_HxG_PxE interactions has the potential to explain variations in fitness related traits in host-pathogen systems with greater accuracy by accounting for both genetic and environmental influences. Using two common North American frog species (*Lithobates pipiens* and *L. sylvaticus*) and three strains of frog virus 3 (FV3) at different temperatures, our results revealed significant variations in host susceptibility and strain infectivity, suggesting the potential for frequency-dependent selection in this system. However, our results also suggest that the strength of the mutual selective pressure exerted by the host and the pathogen is temperature-dependent, revealing for the first time in a vertebrate-pathogen system the occurrence of G_HxG_PxE interactions.

THE WITHIN-POND EPIDEMIOLOGY OF AN AMPHIBIAN RANAVIRUS

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Researchers have learned a great deal about the transmission, persistence, and virulence of ranavirus from laboratory and mesocosm experiments, particularly with amphibian larvae. Yet translating these findings into epidemiological dynamics and consequences has remained ad hoc and idiosyncratic. I will present a series of epidemiological models built on and, to the extent possible, parameterized by empirical results to synthesize our understanding of ranavirus epidemiology in larval amphibian populations. In particular I show that transmission through water is at best a minor component of ranavirus epidemics, that habitat structure is likely to play an important role in the speed and size of epidemics, that host susceptibility may play a dominant role in the course of an epidemic, and that metamorphs may commonly leave ponds infected. The degree to which multi-host communities differ in their dynamics from the simple community I modeled is an important, unanswered question.

AMPHIBIAN RANAVIRUS DYNAMICS IN AN INDUSTRIALLY ALTERED LANDSCAPE

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The emergence of pathogens as major threats to wildlife is not well understood, although changes in ecological conditions and environmental stressors have been implicated. The boreal forest of northeast Alberta and the Northwest Territories, Canada, plays host to several economically important activities including oil sands and base metal mining, forestry, and in some areas, rapid urban expansion. Health assessments of amphibian populations in the region are examining the dynamics of ranaviruses in conjunction with several other parameters linked to health including population biology and contaminant levels at breeding ponds and in amphibian tissues (heavy metals, naphthenic acids, polycyclic aromatic hydrocarbons). Ranaviruses have been detected widely, but to date, only in wood frogs even at breeding sites shared with other species (boreal chorus frogs and Canadian toads).

Recurrent ranavirus-related die-offs have taken place at some sites with no apparent impact on population size or structure. At other sites there has been complete loss off all young-of-the-year for multiple years in a row, and predictably, the population structure has shifted to one of large adults only. Analyses are ongoing to tease apart interactions between ranavirus infections and environmental stressors such as contaminant levels and proximity to highways and mining operations. For the 2013 field season, protocols are coming online that will allow measurement of levels of metabolites indicative of chronic stress. It is expected that this additional layer of information about how amphibians are experiencing their rapidly changing landscape will shed much needed light on ranavirus dynamics in wild amphibian populations.

TRANSMISSION OF RANAVIRUS BETWEEN ECTOTHERMIC VERTEBRATE HOSTS

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Transmission among hosts can be an essential process to perpetuate the survival of pathogens. Ranaviruses are known to infect different classes of lower vertebrates including amphibians, fishes, and reptiles. Differences in capacity of infection among ectothermic hosts could partly explain the successful annual persistence of ranaviruses in aquatic environments. The goal of this study was to determine the capacity of transmission of a highly virulent FV3-like ranavirus isolate among three commonly sympatric ectothermic classes (Cope's gray treefrog larvae [*Hyla chrysocelys*], mosquito fish [*Gambusia affinis*], and red-eared slider [*Trachemys scripta*]). We housed naïve and infected individuals in containers divided by mesh screening to permit water flow between subjects. Our results showed that infected gray treefrog larvae were capable of transmitting the pathogen to naïve larval conspecifics, turtles, and fish (70%, 30%, and 15%, respectively). Also, infected turtles and fish transmitted the pathogen to 50% and 20% of the naïve gray treefrog larvae, respectively. Although infection of turtles and fish was observed when naïve individuals were housed with infected gray treefrog larvae, no mortality was observed. Our results demonstrate that ranaviruses can be transmitted through water among syntopic ectothermic classes. The capacity of ranavirus to infect multiple ectothermic vertebrate hosts, added to the differences in susceptibility of the tested species, demonstrates that both fish and reptiles could serve as reservoirs for ranavirus. Persistence of ranaviruses in permanent residents of the aquatic environment might sustain its survival when highly susceptible hosts like amphibians are absent as a result of breeding phenology.

STRESS EFFECTS ON SUSCEPTIBILITY AND TRANSMISSION OF RANAVIRUS INFECTION IN AMPHIBIANS

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Individual variation in exposure, susceptibility and recovery to infection are fundamental drivers of disease dynamics in structured populations. Yet, how varying stressors interact with individual differences in physiological and behavioral traits to influence disease dynamics at the population level are not well understood. In addition, while environmentally induced stress is thought to be a key driver of disease outbreaks in wildlife populations, the mechanistic links between differing stressors, immune function and epidemic outbreaks are not well tested. Our work examines how chronic environmental stressors, that include pollutants and population structure, interact with individual traits (e.g. growth,

behavior and glucocorticoid hormone expression) to influence *Ranavirus* susceptibility and transmission dynamics. Here we use a *Ranavirus* and wood frog (*Lithobates sylvaticus*) study system and experimental manipulation of physiological stress to explore how individual responses to stressors and population structure influence disease outbreaks. This system is well suited for such research because wood frog larvae naturally form size structured populations in which growth, development, stress physiology and possibly immune function vary consistently with size and developmental stage. Through these efforts our research provides insight into the potential effects of environmental stress on disease outbreaks in amphibians, as well as a broader understanding of how individual traits may influence the epidemiology of contact dependent diseases in heterogeneous populations.

TEMPERATURE AFFECTS ANURAN SUSCEPTIBILITY TO RANAVIRUS

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Ranaviruses are emerging pathogens that cause morbidity in fish, reptiles and amphibians. Surveillance studies suggest that winter is a season with high infection prevalence, yet die-offs are often reported during summer or fall. Several factors vary seasonally (e.g., temperature) that could affect susceptibility to ranavirus. There are two competing hypotheses that might drive relationships between host susceptibility to ranavirus and temperature. Ranavirus virulence could be positively correlated with water temperature because virus replication *in vitro* increases with temperature; thus, warmer temperature could lead to more outbreaks. Alternatively, water temperature may function as a stressor; thus, larvae of summer breeding species may be more susceptible to ranavirus at colder temperatures, while spring breeding species may be more susceptible to ranavirus at warm temperatures. We tested the relative susceptibility of three amphibian species (*Lithobates sylvaticus, L. clamitans, Ambystoma maculatum*) whose larvae typically develop during winter and spring in North America compared to one species whose larvae develop during summer only (*Hyla chrysoscelis*). Larvae were challenged with ranavirus in a water bath under controlled conditions at two temperatures (10 and 25 C). After 28 days, susceptibility to ranavirus was lower at 10 C compared to 25 C for *L. sylvaticus, L. clamitans,* and *A. maculatum*, whereas susceptibility was greater at 10 C compared to 25 C for *H. chrysoscelis*. Additional species will need to be tested in the near future to determine if these trends are consistent. However, preliminary results suggest that temperature may act as a stressor; thus, abnormal temperatures during larval development may increase susceptibility to ranavirus and result in an outbreak.

POSTER PRESENTATION ABSTRACTS

COMMON MIDWIFE TOAD VIRUS: OUTBREAKS IN EUROPE, PATHOLOGY AND GENOMIC ANALYSES

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In Europe, the first known outbreak of common midwife toad virus (CMTV) occurred in northern Spain in 2007. Since then, three more outbreaks have occurred in Spain and in The Netherlands, affecting both wild and captive amphibians, and indicating that the host range and geographic distribution of CMTV are much wider than previously suspected. CMTV is known to infect wild tadpoles of the common midwife toad (*Alytes obstetricians*) and juvenile alpine newts (*Triturus alpestris*) in Spain; and adult wild water frogs (*Pelophylax* spp.) and common newts (*Lissotriton vulgaris)* in The Netherlands. Recently it has been reported in captive frogs in the former country. Common gross lesions include systemic hemorrhagic disease. Histological findings revealed the presence of intracytoplasmic inclusion bodies and the necrosis of epithelial and endothelial cells, which result in destruction of several organs, including skin, liver, pancreas, spleen, kidney and intestine. Phylogenetic and gene content analyses of CMTV showed that this virus is an amphibian-like ranavirus (ALRV). However, the CMTV genome structure was novel and represented an intermediate evolutionary stage between the two previously described ALRV groups, shedding light on the phylogenetic relationships within this complex group of emerging, disease-causing viruses. It will be important to determine the mechanisms of emergence of CMTV-caused disease and whether it may be linked to environmental factors, virus spread, or both. Better knowledge of the ranavirus species and strain structure will be crucial to set up detection methods and surveillance strategies in order to minimize their impact on amphibian wildlife and cultivated species.

IMMUNOHISTOCHEMICAL STAINING FOR RANAVIRUSES

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The ability to detect and confirm ranaviruses in tissue sections is necessary to link pathological changes to the virus. We have begun a study to investigate the feasibility of using immunohistochemistry to identify cell populations targetted by ranaviruses and the cell damage caused by the viruses. Rabbit antisera was prepared against common midwife toad virus (CMTV) and frog virus 3 (FV3), respectively. In brief, two New Zealand white rabbits were immunized with purified virions emulsified with complete Freund´s adjuvant at days 1, 14, 21 and 28. A final booster was administered intravenously at day 35 and serum collected on day 38. Pre and post-immunization sera were used for immunohistochemical studies. Initial staining was performed on tissues from 3 species of North American anurans and one species of North American turtle that were challenged with 4 different FV3-like ranavirus isolates. Positive staining was not achieved using the CMTV antibody; however, positive staining was achieved using the FV3 antibody. Positive staining was observed in various cell types including hepatocytes, renal tubular epithelium and intestinal epithelium; cytoplasmic inclusions also displayed positive staining. Initial evidence suggests that immunohistochemistry may a be useful tool to aid diagnostic investigations of morbidity and mortality events that are suspected to be caused by ranaviruses; however, their success may be ranavirus specific.

PHYLOGENETIC AND LIFE HISTORY TRAITS CORRELATED WITH SUSCEPTIBILITY TO RANAVIRUS: AN EXPANDED ANALYSIS WITH 35 AMPHIBIAN SPECIES

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Ranaviruses have been linked to amphibian die-off events all over the world; susceptibility to the pathogen has been reported to be extremely variable among species and age classes. It has been suggested that differences in susceptibility may be a consequence of species-specific natural history and ancestral phylogenetic relationships. In 2011, we tested the susceptibility of 19 amphibian species from the eastern United States to two ranavirus isolates and related susceptibility (infection prevalence) to life history traits and phylogenetic lineages. We expanded this dataset to 35 species from 9 families, including representatives from the Pacific Northwest, Europe, and the pet trade. Experimental procedures and statistical and phylogenetic comparative methods followed previous experiments, with the addition of a canonical correspondent analysis to determine the influence of natural history traits on species susceptibility. Phylogenetic comparative methods demonstrated high variability within family susceptibility. Variation in infection within the Ranidae, Hylidae, Ambystomatidae, and Scaphiopodidae families ranged from >90% to ≥ 5%, while Bufonidae and Salamandridae showed susceptibilities ≤ 40%. Geographically distant species like the common frog and threatened species like the gopher frog exhibit mortalities ≥ 95%. This expanded database constitutes the most comprehensive collection of records on amphibian susceptibility to ranavirus infection currently available, and could be invaluable in the design of amphibian conservations strategies. The risk factor of an area to suffer population outbreaks could be predicted based on the levels of susceptibility of its amphibian community; this information could be directed to guide conservation efforts to areas of high risk, maximizing available resources.

RANAVIRUS IS LETHAL TO BOREAL TOAD (*BUFO BOREAS*) TADPOLES

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Ranaviruses are known to infect and cause disease in common amphibian species such as the wood frog (*Lithobates sylvaticus*). However, there is increasing evidence that rare amphibians also may be affected negatively by this emerging pathogen. For example, Mississippi gopher frogs (*L. sevosus*) and Chinese giant salamanders (*Andrias davidianus*) are highly susceptible to ranavirus. Wild populations of the boreal toad (*Bufo boreas*) have been declining for over 20 years, and pathogens are believed to be playing a role. The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) has been shown to cause mortality in post-metamorphic boreal toads, and affect adult survival rates in wild populations. No studies have been performed to explore the susceptibility of boreal toad tadpoles to ranavirus. Thus, we exposed boreal toad tadpoles to an environmentally relevant concentration (10^3 PFU/mL) of two *Frog Virus 3* (FV3)-like isolates (*n* = 20 tadpoles per isolate) in water and monitored survival for 21 days. Tadpoles began dying at five days post-exposure and 100% mortality was documented after eight days for both isolates. This amount and rate of mortality indicates that boreal toad tadpoles are highly susceptible to ranavirus. Future pathogen surveillance and conservation planning should consider ranavirus as a threat to this species.

GENOME ANNOTATION OF *A* RANAVIRUS (ADRV) ASSOCIATED WITH HIGH MORTALITY IN CHINESE GIANT SALAMANDER AND CROSS-SPECIES TRANSMISSION.

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Ranaviruses caused serious fatal outbreaks of disease in various aquatic animals, such as systemic hemorrhage with high mortality in wild and farmed amphibians. As a rarely endangered species and the largest amphibian in the world, the Chinese giant salamander *Andrias davidianus* has undergone an epidemic outbreak disease of with a high proportion of deaths since 2010. Here w*e* identified *Andrias davidianus* ranavirus (ADRV) as a lethal virus pathogen that causes a highly lethal hemorrhagic syndrome in giant salamanders, and genomic sequencing was carried out. Its complete genome was sequenced and gene annotation of 101 ORFs was compared with other sequenced amphibian ranaviruses. The ADRV genome contains 26 iridovirus core genes, 24 ranavirus-specific genes, 11 amphibian subgroup-specific genes and 40 other genes. *A* phylogenetic tree was constructed based on concatenated sequences of 26 core-gene-encoded proteins from known genome sequence iridoviruses. ADRV belongs to the amphibian subgroup in the genus *Ranavirus*. Significantly, the ADRV genome was discovered to contain 99%, 97%, 93%, 93% and 85% homologous genes in RGV, FV3, CMTV, TFV, and ATV genomes respectively. One inversion, one inserted and one deletion fragment were detected in the ADRV genome in comparison with RGV. And several variable major genes, such as highly diversified duplicate *US22* family-like genes, variable vIF2αs, and a novel 75L gene with both motifs of NLS and NES, were identified to contribute to pathogen virulence, host susceptibility, and cross-species transmission. These findings broaden our understanding of evolutionary emergence and cross-species transmission of ranaviruses.

IDENTIFICATION OF A BOHLE IRIDOVIRUS-LIKE AGENT IN CAPTIVE NORTH AMERICAN BOREAL TOADS

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A captive survival assurance population of 55 endangered boreal toads (*Anaxyrus boreas boreas*) experienced high mortality (93%) between April and July of 2010. Toads were housed in a cosmopolitan collection with other amphibians including some originating from Southeast Asia. Histologic examination of tissues demonstrated lesions consistent with severe ranaviral disease including multicentric necrosis (especially skin, kidney, liver, spleen and hematopoietic tissue), vasculitis, and myriad basophilic intracytoplasmic inclusion bodies. Initial confirmation of *Ranavirus* infection in affected toads was made with real-time PCR from a portion of the MCP gene and by demonstration of iridovirus virions in tissues by transmission electron microscopy. Initial conventional PCR and DNA sequencing for MCP, DNA pol and NF-H1 genes demonstrated 86.3, 99.2 and 99% identity respectively with Bohle iridovirus (BIV). The virus was isolated in fathead minnow cells and viral protein profiles, RFLP analysis and next generation DNA sequencing using the Ion Torrent PGM™ system (Life Technologies) were performed. Comparisons of several characteristic *Ranavirus* genes

(MCP, 18K and 46K) to FV3, BIV, ATV, RGV and SGIV and representatives of other iridovirus genera supported the view that the toad virus was most similar to BIV. This is the first report of a BIV-like agent in North America. It remains unclear if the virus is a novel North American variant of BIV or if it was acquired by exposure to amphibians originating from a different geographic location. The few surviving toads were euthanized 10 weeks after the conclusion of the outbreak and remained PCR-positive for the same *Ranavirus*. Observations from this outbreak have implications for the captive management of amphibians destined for use in reintroduction programs.

SUSCEPTIBILITY TO THE TADPOLE EDEMA VIRUS AMONG EARLY LIFE STAGES OF THE FOWLER'S TOAD (*ANAXYRUS FOWLERI*)

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Tadpole edema virus (TEV) is a ranavirus closely related to Frog virus-3 and pathogenic to amphibians. Here, we investigate the comparative virulence of TEV among life stages of the Fowler's toad, a common anuran species, from hatch to metamorphosis. A single mass of fertilized Fowler's toad eggs was field collected, and the hatched tadpoles reared under laboratory conditions. Replicate groups of untreated tadpoles and groups challenged with TEV (10^5 $TCID_{50}$/ml for 24h) were maintained for 14 day trials for the following approximated life stages: hatchlings (Gosner 20), mouth parts developed (Gosner 25), rear limbs developing (Gosner 36), and metamorphosis (Gosner 42). Mortality was recorded daily, and dead tadpoles and representative survivors were evaluated for presence and titer of virus using a quantal viral infectivity assay to determine 50% endpoint in cell culture. Mortality levels were negligible for all control groups, and no virus was detected among any control groups or the pre-screened eggs and embryos. Among the TEV-challenged groups, there was variability in mortality and associated detection of virus among the 4 life stages. High mortality associated with TEV (89% or greater) was observed among the earliest and latest life stages (Gosner 20 and Gosner 42) while lower mortality levels (32% and below) were observed from the intermediate aquatic life stages examined. Viral titers among exposed survivors and tadpoles dying with TEV ranged from 10^3 to 10^6 $TCID_{50}$/g. Susceptibility appeared greatest during the periods of heightened physiological stress near hatch and metamorphosis.

IRIDOVIRUS PHYLOGENETICS: IS THE MAJOR CAPSID PROTIEN ENOUGH?

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Iridoviruses are emerging infections in both invertebrate and lower vertebrate hosts. They have a global distribution and low host specificity, which makes understanding their ecology and evolution a challenge. Many attempts to understand their phylogenetic relationships have relied solely on the sequence of a portion of the major capsid protein (MCP), which encodes for the major structural protein of the viral capsid and is highly conserved across all Iridoviruses. In this study, we compare the phylogenetic trees made by both Neighbor Joining and Maximum Likelihood methods for both the truncated and complete MCP sequences. The phylogenetic trees produced by the Neighbor Joining method were less well defined (i.e. had fewer clades) than those produced by Maximum Likelihood. This trend was also observed when the trees produced by the use of the partial MCP sequence were compared to those built with the complete MCP sequences. This demonstrates that the trees produced using more information are more accurate, and that using the more complex algorithm of the Maximum Likelihood construction method produces better trees (i.e. more defined). This information

is extremely important as many of the published phylogenies for Iridoviruses use only the partial MCP sequences, which are not as informative as they could be, thus requiring a different perspective when they are interpreted.

EFFECTS OF HERBICIDES AND RANAVIRUS ON SURVIVAL AND HEALTH OF JUVENILE FRESHWATER TURTLES

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Emerging pathogens within the genus Ranavirus (family Iridoviridae) are carried by and can be lethal to ectotherms including amphibians, reptiles, and fish. Impacts of this virus in reptiles are under-studied, and factors contributing to pathogen virulence and host susceptibility are not well understood. We conducted a controlled laboratory experiment to examine the interactive effects of ranavirus and herbicides on turtle health and survival. Twenty juvenile Red Eared Sliders (*Trachemys scripta elegans*) were assigned to each of eight groups containing all combinations of treatments for ranavirus (exposure, control) and three herbicides (ShoreKlear®, 2,4-D, Atrazine, control). We found no difference in either survival or swelling of the neck and axillary region (a common symptom of ranavirus) due to either ranavirus or chemical exposures. Similarly, neither treatment impacted growth in mass or shell length during the experiment. There was little to no growth in turtles over this period. Among turtles exposed to ranavirus, chemical treatments did not impact whether turtles tested positive or negative for ranavirus at the conclusion of the experiment. These results suggest that low concentrations of the herbicides used in this experiment may not impact growth or survival of juvenile turtles exposed to ranavirus. However, we hesitate to make broader conclusions and recommendations based on this study, because of the lack of growth in our study and the death of some turtles in our control treatments. We suspect that cool rearing temperatures may have impacted the general health of turtles across treatments.

PRESENCE OF AMPLIFICATION HOSTS INCREASES MORTALITY OF SYNTOPIC AMPHIBIANS BY RANAVIRAL DISEASE

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Declines in amphibian populations from disease outbreaks could be mediated by host susceptibility. Our objective was to determine if the outcome of a ranaviral disease outbreak in an amphibian community was dependent on which species was initially exposed to the virus, and if trophic shifts in an experimental aquatic ecosystem occurred as mortality progressed. We created two amphibian communities: (1) an Appalachian community composed of wood frog (*Lithobates sylvaticus*), upland chorus (*Pseudacris feriarum*), and spotted salamander (*Ambystoma maculatum*) larvae, and (2) a coastal plain community composed of gopher frog (*Lithobates capito*), upland chorus frog (*P. feriarum*), and southern toad (*Anaxyrus terrestris*) larvae. The experiment was conducted outdoors in 320-L mesocosms, and treatments consisted of one, all, or none of the species initially exposed to *frog virus 3*. Initial exposure occurred under controlled laboratory conditions in 1-L water baths (10^3 PFU/mL) for 3 days prior to distribution of the larvae to the mesocosms. Mortality rates after 60 days depended on which species was initially exposed to the pathogen. In the Appalachian community, exposed wood frog tadpoles caused an outbreak of ranaviral disease in unexposed chorus frogs (40% mortality) and

amplified mortality of spotted salamander larvae 2X greater than when this species was directly exposed to the virus. In the coastal plains community, all species were able to cause outbreaks of ranaviral disease (>40% mortality) in co-inhabitant unexposed species. Our results demonstrate that amphibian community composition can affect ranaviral disease outcomes.

INSIGHT INTO THE PATHOGENIC DETERMINANTS OF AMBYSTOMA TIGRINUM VIRUS

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Ranaviruses (RVs) (family *Iridoviridae*, genus *Ranavirus*) are large, double-stranded DNA viruses that infect economically and ecologically important freshwater cold-blooded vertebrates including fish, amphibians and reptiles. RVs are a unique group of viruses for two reasons: (i) No other group of viruses infect such a wide diversity of hosts (i.e. amphibians, fish and reptiles) except perhaps for the orthomyxoviruses (e.g. influenza viruses); (ii) Members of the genus *Ranavirus* have undergone multiple evolutionarily recent hosts species shifts, jumping from fish to amphibians, amphibian species to amphibians species, amphibians to reptiles and perhaps from amphibians and/or reptiles to fish. However, in spite of the global impact RVs have on economically and ecologically important hosts of freshwater ecosystems, our current understanding of the mechanisms of RV pathogenesis, including the genes that contribute to RV pathogenesis and host range, is extremely limited. Therefore, using *Ambystoma tigrinum virus* (ATV) and its salamander host (*Ambystoma tigrinum*) as our model host-pathogen system, we are testing the hypothesis that 7 conserved ATV genes are involved in viral pathogenesis. To test this hypothesis, we are generating: (i) plasmid vectors to express ATV proteins in cells in culture; (ii) recombinant ATV viruses where we delete one of these potential pathogenesis genes. We are then using these tools to characterize these putative pathogenesis genes in cells in culture and in its natural host, tiger salamanders, and we will present an update on our progress of characterizing these ATV proteins.

GENOMIC SEQUENCE OF LARGEMOUTH BASS VIRUS

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Largemouth Bass Virus (LMBV) is associated with lethal disease of North American bass species (*Micropterus salmoides*; *M. floridanus*). LMBV was first observed in Lake Weir, FL in 1991, and since that time outbreaks of LMBV have been observed throughout the midwestern and southern United States. A major symptom of LMBV disease is the over-inflation of swim bladders, which alter equilibrium and prevent submergence of infected hosts. Other characteristics of disease are lesions on swim bladders with a yellow exudate, thickening of swim bladder walls, and secondary bacterial and fungal infections. The level of susceptibility and the degree of infection differ among outbreaks as some show disease symptoms, while others appear unaffected by exposure to LMBV. It is unclear if this variability is due to dissimilarities of immune responses between host populations, or due to the pathogenic diversity among LMBV strains from different geographic regions. Therefore, genomic sequencing of LMBV will allow us to gain a better understanding of this important pathogen of largemouth bass. In addition, having complete genomic sequence information for LMBV will provide insight into the evolutionary relationship among fish iridoviruses and increase our understanding of

how ranaviruses infect such a wide variety of hosts. To that end, we have begun sequencing LMBV and we will present our results of this sequencing effort.

PREVALENCE OF RANAVIRUS AND BD IN HELLBENDER POPULATIONS IN TENNESSEE AND ARKANSAS

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The Hellbender, *Cryptobranchis alleganiensis*, is a large aquatic salamander containing two subspecies (Eastern Hellbender, *C. a. alleganiensis* and Ozark Hellbender, *C. a. bishopi*) from the eastern and Ozark mountain regions, respectively. Both subspecies have seen population declines over the past 25 years, especially in *C. a. bishopi* which is federally endangered. Habitat degradation and possible low genetic diversity may lead to secondary infections, with amphibian pathogens such as *Ranavirus* and *Batrachochytrium dendrobatidis* (Bd) playing a potential role. The objective of this study was to determine prevalence of these pathogens in both subspecies as a first step in understanding the role of emerging amphibian pathogens in *C. alleganiensis* declines. We collected tail tissue and skin swabs from *C. a. bishopi* and *C. a. alleganiensis* individuals from Arkansas and Tennessee respectively over the summers of 2011 and 2012. We used qPCR analysis to determine presence of *Ranavirus* and Bd from tail samples and skin swabs, respectively. Overall, for *C. a. bishopi*, we detected 20% prevalence of Bd and no cases of ranaviral infections; for *C. a. alleganiensis* 7% Bd and 5% positive for *Ranavirus*. Additionally, we observed leeches parasitizing on many individuals, and identified one *C.a. allenganiensis* leech positive for *Ranavirus*. These short-term data reveal that Bd is prevalent in these populations and, as Bd is known to favor keratinized tissue, may play a role in causing physical deformities (e.g., missing and fused toes) seen in *C. a. bishop*. Furthermore, early analyses of *Ranavirus* suggest a link to watershed and ectoparasitism.

CONSTRUCTION AND ANALYSIS OF A RECOMBINANT FROG RANAVIRUS (RGV) CONTAINING THE LAC REPRESSOR-OPERATOR SYSTEM

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Rana grylio virus (RGV), a member of ranavirus belonging to the family *Iridoviridae*, is a pathogenic ranavirus that has resulted in high mortality in cultured and wild frogs, and it induced cytopathic effect and plaque formation in fish cells. 53R gene, a core gene of *Iridoviridae*, encodes an envelope protein of RGV, and the gene involved in DNA replication, gene transcription, viral infection and assembly. Here, the plasmid pRFP-lacO-53R, which contains a chimeric gene p50-RFP and hybrid promoter p50-lacO flanked by RGV 53R gene sequences, was used to insert the hybrid promoter p50-lacO in front of 53R gene by homologous recombination. A recombinant RGV (Δ53R-RGV-lacIO) contains the *lac* repressor/operator system that was a thoroughly studied and best understood system that regulates gene transcription by protein-nucleic acid interaction, and dual (green-red) fluorescent labeling for regulating the expression of protein 53R was generated. Δ53R-RGV-lacIO shared similar characteristics with the wild-type RGV in the presence of isopropyl β-D-thiogalactoside (IPTG). However, the

expression of 53R levels, the ability of formed plaques and the virus titer of Δ53R-RGV-lacIO was strongly reduced in the absence of IPTG. Meanwhile, over-expression of 53R *in vitro* could rescue virus titer in the absence of IPTG. Electron microscopy showed that Δ53R-RGV-lacIO produces many defective viral particles in the absence of IPTG. Therefore, the current data confirmed that the *lac* repressor/operator system is able to regulate the gene expression in recombinant ranavirus during infection in fish cells and was thought to be the first report of this system in aquatic virus.

INVOLVEMENT OF MAPK SIGNALING PATHWAY IN SOFT-SHELLED TURTLE IRIDOVIRUS (STIV) INDUCED APOPTOSIS

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Iridoviruses are large DNA viruses that infect invertebrates and poikilothermic vertebrates, and result in significant economic losses in aquaculture production, and drastic declines in amphibian populations. Soft-shelled turtle iridovirus (STIV) is the causative agent of severe systemic diseases in farm-raised soft-shelled turtles (*Trionyx sinensis*). In the present study, the mechanisms of STIV-induced cell death and the roles of the mitogen-activated protein kinase (MAPK) signaling pathway were investigated. STIV infection evoked typical apoptosis in fish cells, as demonstrated by the formation of apoptotic bodies, positive terminal deoxynucleotidyl transferase-mediated nicked-end labeling, and caspase-3 activation. The translocation of cytochrome c from mitochondria to cytoplasm, and caspase-9 activation suggested that a mitochondria-mediated pathway was involved in STIV induced apoptosis. Moreover, MAPK pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK signaling were activated during STIV infection. Using specific inhibitors, we found that MAPK signaling molecules, including ERK, JNK and p38 MAPK, were important for virus release, whereas only ERK and p38 MAPK were involved in STIV-induced apoptosis by modulating caspase-3 activity. Taken together, our findings shed light on the roles of the MAPK signaling pathway in iridovirus-induced apoptosis and virus replication, which provides new insights into understanding iridovirus–host interaction.

ISOLATION AND PARTIAL SEQUENCING OF A FV3-LIKE RANAVIRUS FROM THE CARCASS OF A JUVENILE EASTERN PAINTED TURTLE (*CHYSEMYS PICTA PICTA*)

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In Spring, 2006, a painted turtle carcass recovered in Kearneysville, WV was presented to the Leetown Science Center. Due to moderate decomposition, a limited necropsy was performed with no remarkable gross findings. Cell-free extract of an internal organ homogenate was inoculated on to BF-2 and EPC cell culture monolayers, producing cytopathic effect that remained evident following serial passage. Supernatant from infected cells was pelleted for electron microscopy, and virions were morphologically consistent with that of Ranaviruses. DNA was extracted from infected BF-2 monolayers and amplified with a number of primer sets that amplify Ranaviruses. Specific genes targeted were the major capsid protein (MCP), DNA polymerase, and neurofilament triplet H1-like protein (NF-H1). Two other regions of the genome that include genes of unknown function were amplified as well. The isolate was 100% identical to FV3 and a number of other related Ranaviruses across 475 bp of the MCP. No differences were noted in the sequences region of the DNA polymerase gene. Differences were noted between the isolate and the NF-H1 of FV3. The sequence was 97%

identical over 839 bp. The sequencing of 5 loci and over 3,200 bps revealed 99.1% identity between the turtle isolate and FV3. Further sequencing is in queue to further distinguish this isolate from other Ranaviruses. Whether this represents an interclass host shift followed by subsequent genomic adapation of the virus is unknown. Pathogenicity studies are required to fulfill Koch's postulates and evaluate the comparative pathogenicity of this isolate to FV3 in an anuran host.

IDENTIFICATION OF LARGEMOUTH BASS VIRUS IN THE INTRODUCED NORTHERN SNAKEHEAD (*CHANNA ARGUS*) INHABITING THE CHESAPEAKE BAY WATERSHED

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The Northern snakehead (*Channa argus*) is an introduced species that now inhabits the Chesapeake Bay. During a preliminary survey for introduced pathogens possibly harbored by these fish in Virginia waters, a filterable agent was isolated from five specimens that produced cytopathic effects in BF-2 cells. Based on PCR amplification and partial sequencing of the major capsid protein (MCP), DNA polymerase (DNApol) and DNA methyltransferase (Mtase) genes, the isolates were identified as largemouth bass virus (LMBV). Nucleotide sequences of the MCP (492 bp) and DNApol (419 pb) genes were 100% identical to those of LMBV. The nucleotide sequence of the Mtase (206 bp) gene was 99.5% identical with that of LMBV and the single nucleotide substitution did not lead to a predicted amino acid coding change. This is the first report of LMBV from the Northern snakehead, and evidence that non-Centrarchids may be susceptible to this virus.

THE EFFECT OF COMMUNITY INTERACTIONS ON AMPHIBIAN RANAVIRUS PERSISTENCE IN AQUATIC ECOSYSTEMS

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Ranaviruses are transmitted between their cold-blooded vertebrate hosts by direct contact as well as through the environment, particularly in pond water, sediment, and on fomites. It is unclear how long ranaviruses remain infectious in the aquatic environment. Previous research has suggested persistence times from days to years, depending on conditions. Viral interactions with the biotic community (e.g., microbes, plankton) may explain some of these discrepancies as studies in which virions were held in sterile conditions had much longer persistence times than those where virions were unprotected. To address the role of the biotic community and particulate matter on ranavirus persistence we experimentally inoculated filter-sterilized, UV-sterilized, and unmanipulated pond water with 10^5 pfu/mL of an FV3-like virus. We then took samples over 78 days, quantifying viral titers with real-time quantitative PCR and plaque assays. We also measured viral titers through time (0, 5, and 24 hours) in spring water with 0, 1, 2, 5, or 10 *Daphnia pulex* at two different algal food concentrations. Water and *D. pulex* samples were taken after inoculation to determine whether *D. pulex* filter virus out of water and if filtered virus remains infectious. Viral counts dropped quickly in all pond water treatments, losing an order of magnitude between 1 and 6 days, even in filter-sterilized pond water. The microbial community had a much greater influence in reducing viral titer in water samples than *D. pulex*. We conclude that ranaviruses, at least when in the form of free-floating virions, do not persist for long in ponds.

CONCURRENT RANAVIRUS AND *BATRACHOCHYTRIUM DENDROBATIDIS* INFECTION IN CAPTIVE FROGS (PHYLLOBATES AND DENDROBATES SPECIES), THE NETHERLANDS, 2012. A FIRST REPORT.

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In the Netherlands, ranavirus infection has recently been discovered in wild water frogs (*Pelophylax* spp.) and common newts (*Lissotriton vulgaris*). The sequence obtained showed a 100% identity with the one deposited for common midwife toad virus (CMTV). Now we report on a ranavirus infection with concurrent *Batrachochytrium dendrobatidis* infection and mortality in captive *Phyllobates* and *Dendrobates* species. Young as well as adult animals were involved. Clinical signs in the frogs were dry, greyish skin with the animals spending 90 % of their time in the water, anorexia and death. Young metamorphosed tadpoles that just emerged onto the land lay suddenly dead, others did not grow well. Some lay dead in the water. Some of the adult frogs still ate but were emaciated, and died after two to three weeks. Greyish skin with hepato- and renomegaly was evident. Microscopically, *Batrachochytrium dendrobatidis* was present in the stratum corneum of the hyperkeratotic skins. Intracytoplasmic inclusion bodies were present in erythrocytes and multiple organs. All samples examined tested positive using PCR for the major capsid protein (MCP) gene of ranavirus and the ITS-1–5.8S region of *B. dendrobatidis*. The sequence obtained showed a 99 % identity with the deposited sequence of the MCP gene of the CMTV. These findings highlight the importance of monitoring ranaviral and *B. dendrobatidis* infections in captive as well as wild amphibians, especially with the discovery of ranaviral infection in *Dendrobatidae* in Japan that is thought to originate from imported poison dart frogs from the Netherlands.

SPATIAL EPIDEMIOLOGY OF AMPHIBIAN EMERGING INFECTIOUS DISEASES IN ONTARIO, CANADA

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Emerging infectious diseases have significant effects on biological communities. In some cases, pathogens have caused host extinctions. The majority of research has focused on a 'one host – one pathogen framework'. However, individual hosts encounter multiple pathogens simultaneously, which may lead to additive, antagonistic or synergistic effects on hosts. The dynamic interaction between pathogens is an important issue in conservation biology, as it can increase infection prevalence and severity. While establishing the cause of extinction is difficult and candidate model species are few, amphibians appear to be an ideal specimen as increasing evidence suggests that we are facing a global population decline. Ranavirus (family *Iridoviridae*) and the Chytrid fungus (*Batrachochytrium dendrobatidis*) are the primary pathogens associated with amphibian mortalities. While there have been several reports of ranavirus and chytrid infection within Europe, both pathogens have become prominent in North America and particularly in Canada. We aim to investigate the distribution of both pathogens throughout Ontario, Canada, by testing 3,000 *adult* Northern Leopard frogs *(*Lithobates (formerly *Rana*) *pipiens)* for presence and intensity of disease*.* Utilizing these results, we hope to model the dynamics of both pathogens simultaneously. We are interested in evaluating the dynamical impact of seclusion and disease-induced mortality on the pathogen community. This provides us with a mechanism in which to study competitive dynamics on the scale of individuals, and their large-scale consequences.

A RANAVIRUS-RELATED MORTALITY EVENT AND THE FIRST REPORT OF RANAVIRUS IN NEW JERSEY

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Although Ranavirus has been reported in many states throughout the Northeast, it has until now not been documented in New Jersey. We conducted a side-by-side comparison of PCR and RT-PCR to screen 114 animals from a site in southern New Jersey that experienced a mass mortality event involving *Lithobates clamitans* and *Anaxyrus fowleri* tadpoles. Twenty-four of 114 animals tested positive for Ranavirus with PCR and 32/114 tested positive with RT-PCR, suggesting RT-PCR may be a more effective detection method. Three species were infected at this site: *L. clamitans* (tadpole), *A. fowleri* (tadpole), and *L. sphenocephala* (adult). All animals positive for Ranavirus were symptomatic or dead. We have since documented the presence of Ranavirus, including another mass mortality event, in two other counties in southern New Jersey.

USE OF THE IMMUNOELECTRON MICROSCOPY TECHNIQUE FOR THE DIAGNOSIS OF RANAVIRUS IN AMERICAN BULLFROGS (*LITHOBATES CATESBEIANUS*)

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Iridoviruses from genus Ranavirus are responsible for amphibian population declines worldwide. This study aimed to identify ranavirus particles in bullfrogs (*Lithobates catesbeianus*) from three commercial frog farms in South-eastern Brazil, by immunoelectron microscopy techniques. During this surveillance, 120 liver samples from tadpoles and frogs were collected at three different places. Three Transmission Electron Microscopy (TEM) methods were used: negative staining (rapid preparation) for detection of viral particles; immunoelectron microscopy and immunocytochemistry (immunolabelling with colloidal gold particles), to confirm the presence of iridovirus. For negative staining, suspensions were obtained and placed on metal grids, previously covered with colloidal film and stabilized with carbon and negatively contrasted with 2% ammonium molybdate. For immunoelectron microscopy, copper grids were sensitized with Anti-Iridovirus capsid protein antibody (Abcam®), obtained from iridovirus Major Capsid Protein. Immunoelectron microscopy positive samples were submitted to immunolabelling with colloidal gold (incubated for 30 minutes with secondary antibody - protein A conjugated with colloidal gold particles - Electron Microscopy Sciences®). Negative staining TEM showed Iridovirus-like particles, further confirmed by immunoelectron microscopy and immunocytochemistry in all sampled farms (90%, 65% and 15% respectively). TEM methods were a suitable tool for Iridovirus detection and further research is recommended to confirm viral involvement in disease outbreaks in frog farms and pathogen pollution into the environment.

RANAVIRUS PREVALENCE IN WOOD FROG TADPOLES THROUGHOUT CONNECTICUT

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Between 40 and 60 percent of amphibian mass mortality events in the United States are currently attributed to ranavirus. Despite the potential impact of these mortality events on regional amphibian population dynamics, little effort has been made to quantify the spatial distribution or frequency of

these events. The first mortality event in Connecticut attributed to ranavirus was in summer of 2010. In spring of 2012, we sampled wood frog (*Lithobates sylvatica*) tadpoles from 4 wetlands in the watershed with the known mortality event and 4 wetlands in a watershed where historic mortality events were reported but cause of mortality was unknown. Quantitative real-time polymerase chain reaction (qPCR) was used to amplify and quantify viral DNA extracted from liver tissue. Each sample was assayed in triplicate. Out of the 122 tadpoles collected, 113 tadpoles tested positive for ranavirus. All wetlands contained tadpoles that tested positive for ranavirus. Plaque forming units (PFU) varied greatly among wetlands, but not among tadpoles within a wetland. We detected one mortality event with weekly visits to all wetlands. Our results indicate that ranavirus prevalence is high within wetlands in Connecticut, and yet high prevalence did not always result in mortality events. We are expanding surveillance sampling to 12 randomly selected state-owned properties stratified by ecophysiological region. This sampling effort will allow us to determine the spatial distribution of ranavirus throughout Connecticut. This project contributes to our understanding of the spatial distribution of ranavirus within Connecticut and will serve as baseline information for future ecological research.

THE GLOBAL RANAVIRUS REPORTING SYSTEM

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The Global Ranavirus Reporting System is an informational web portal focused on ranaviruses and other iridoviruses (e.g. *Lymphocystivirus*), featuring an interactive online occurrence database and mapping program, for world data uploading and viewing. We developed the system in consultation with Global Ranavirus Consortium scientists, to allow ranavirus surveillance and information sharing, and as a networking tool to foster communication and collaborations among scientists and managers. The reporting system data structure and capabilities are designed to include data importing and exporting functions for scientists and managers. Compilation of existing ranavirus data is in progress and we are hoping to launch the website with historical records already uploaded. We also are seeking additional input from interested parties for additional ideas on what content to include and how the system can be further refined for efficient data input.

INTRODUCTIONS OF DIVERSE RANAVIRUSES TO NORTHERN SPAIN: LIKELY CAUSE OF POPULATION DECLINES IN MULTIPLE HOSTS

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Ranaviruses have been present in the UK for 25 years and there have been sporadic reports of infections in other European countries, including Spain, in the past decade. Whilst we understand something of the pathogen distribution, the impact on host populations and possible adaptation by hosts in the UK, to date there is little published data on the distribution, ecology and diversity of ranaviruses in Spain. We show that there are distinct viruses circulating in northern Spain, showing no evidence of host adaptation or specificity, with infections in multiple hosts from diverse taxa, and often accompanied by pathology and mortality. The timing of emergence of infections and signs of disease

are correlated with local population declines in a number of host species. The diverse viruses and lack of divergence among viruses from different host species suggests multiple recent introductions. When combined with the virulence and broad host range exhibited by these viruses, the short-term future appears bleak for Iberian amphibians.

NEGATIVE EFFECTS OF BELOW-TOXIC LEVELS OF ATRAZINE ON IMMUNITY TO RANAVIRUS USING XENOPUS AS A NOVEL MODEL SYSTEM

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Evidence suggests ranaviruses, such as frog virus 3 (FV3), may contribute to a worldwide decline in amphibians, bringing attention to host antiviral immunity. Common environmental pollutants, such as the herbicide atrazine, are believed to induce lasting negative effects on host immune system function, even at below-government levels. The extent to which such pollutants can modulate immune function, however, remains unclear due to the lack of appropriate, cost-effective models. We have established the frog Xenopus laevis as an experimental model with which to study, in particular, waterborne pollutants and their potential to modulate host antiviral defense. Given ample genetic resources, the extensive characterization of the Xenopus immune system, and its notable genetic and functional similarities to mammalian systems, this model has the potential to extend to other aquatic vertebrates. We hypothesized that while waterborne atrazine may induce deleterious effects on antiviral immunity at higher doses, sub-toxic and legal doses may yield similar effects by negatively impacting the proper development of Xenopus antiviral immunity. Our findings thus far indicate low levels of atrazine markedly reduce expression of 5 immune genes, (TNF-α, IL-1β, IFN-□, Il-10, CSF-1) in pre-metamorphic animals infected with FV3. Assessment of larval exposure to atrazine on the development of effective viral immunity after metamorphosis is underway. Our preliminary results suggest that atrazine durably impairs amphibian host antiviral immunity possibly leading to increased susceptibility to pathogenic infections.

DOES GEOGRAPHIC DISTANCE BETWEEN HOST POPULATION AND ISOLATE LOCATION EQUATE TO RANAVIRUS PATHOGENICITY?

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Hosts that coevolve with pathogens presumably have a greater immune response when infected. Ranaviruses are an emerging pathogen and have been associated with die-offs in wood frog (*Lithobates sylvaticus*) populations from Georgia to Alaska, USA. We hypothesized that pathogenicity of a ranavirus would increase as distance between isolate and host population locations increased. We are testing pathogenicity of two FV3-like ranaviruses isolated from morbid amphibians in Minnesota and Tennessee, USA, among four populations of wood frog tadpoles collected from Tennessee, Michigan, Manitoba Canada, and Alaska. Inasmuch as temperature affects amphibian immune response and viral replication, we are performing our experiments in environmental chambers at 15 and 25 C. If our predictions hold true, pathogenicity of the Tennessee isolate should decrease in the following order: Alaska, Manitoba, Michigan and Tennessee populations. Similarly, pathogenicity of the Minnesota isolate should decrease in the following order: Alaska, Tennessee, Manitoba and Michigan populations. We anticipate that pathogenicity will be greater in the 25°C treatment, because

ranavirus replication and tadpole development increase with temperature. By the time of the symposium, we will be completed with the Tennessee, Michigan and Manitoba experiments. Our results have potential implications in host-pathogen evolutionary theory and conservation relevance regarding the transport of amphibians sub-lethally infected with ranavirus across large geographic distances. Additionally, our temperature results may provide insight into possible effects of global climate change on ranavirus emergence.

A BULLFROG RANAVIRUS STRAIN ISOLATED FROM A FARM IS MORE VIRULENT THAN A WILD-CAUGHT STRAIN

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Ranaviruses are considered emerging pathogens due to the increases in both geographic range and number of reported epizootics over the last 20 years. Most recently, they are listed as reportable pathogens by the World Organization for Animal Health because they are spread in international trade. Bullfrogs (*Rana catesbiana*) are relatively resistant carriers of ranaviruses and are farmed and consequently traded as a food source in large numbers throughout the globe. An increasing concern is whether artificially high densities of bullfrogs in farms can select for high virulence of ranavirus strains, which can then be introduced into native amphibian populations via bullfrog escape. We tested the relative virulence of two ranavirus strains isolated from either a captive or wild bullfrog population. Using a 3x2 fully-factorial cross-infection design, we infected larvae of southern leopard frogs (*Rana sphenocephala*) and *R. catesbiana* to either of the two ranavirus strains or no virus (control). Overall, we found that the ranavirus strain isolated from the captive bullfrog population grew significantly faster and was significantly more virulent than the strain isolated from the wild population. These data provide support for the virulence-transmission tradeoff, insomuch as high bullfrog densities in farms artificially select for high virulence because transmission is virtually guaranteed. In addition, because bullfrogs are a highly successful invasive species, management should include widespread testing of imported bullfrogs, as well as stringent measures to prevent escape from captive populations.

TRENDS IN RANAVIRUS PREVALENCE AMONG PLETHODONTID SALAMANDERS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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Emerging infectious diseases are a potential contributor to worldwide amphibian declines. Ranaviruses, which infect ectothermic vertebrates and are common in aquatic environments, have been implicated in mass mortality of at least 72 amphibian species worldwide. As a majority of these surveillance studies have focused on pool-breeding amphibians, the primary objective of our study was to evaluate trends in ranavirus infection within a salamander assemblage (Family Plethodontidae) in the Great Smoky Mountains National Park, USA. We sampled a total of 691 plethodontid salamanders representing 16 species at a total of eight sites over a six-year period (2007 – 2012). We identified ranavirus positive individuals in 11 of the 16 (69%) sampled species, with salamanders in the genus *Desmognathus* having greatest infection prevalence. We observed species-specific effects of elevation

on infection prevalence with individuals sampled at lower elevation sites having greater infection prevalence compared to high-elevation sites. Infection prevalence was significantly different by year and was greatest in 2007, with 82.5% of sampled individuals testing positive for ranavirus. We hypothesize that extremely low rainfall amounts during 2007 was responsible for high infection prevalence. Body condition was not a significant predictor of ranavirus infection; however, average body condition for the five most commonly captured individuals was greatest during the year (2010) with lowest infection prevalence. Overall, our results indicate that natural history differences among streamside salamander species along with elevation and environmental stressors may play a profound role in the way that ranavirus infections occur in streamside salamanders.

HIGH SUSCEPTIBILITY OF THE MOST ENDANGERED FROG IN NORTH AMERICA (*RANA SEVOSA*) TO RANAVIRUS

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The Dusky Gopher Frog (*Rana sevosa*) is the most endangered anuran in North America, with only two natural populations remaining in southern Mississippi composed of <200 individuals. Various factors likely led to the reduction of *R. sevosa* populations, including reduction in habitat quantity and quality. Ranaviruses have caused significant die-offs in at least seven amphibian species in the southeastern United States, and this emerging pathogen has the potential to negatively impact *R. sevosa* in the wild. Controlled studies at the University of Tennessee demonstrated that the Carolina gopher frog (*R. capito*) was very susceptible to ranavirus. Thus, our objective was to test the relative susceptibility of adult *R. sevosa* to ranavirus via three routes of exposure: intra-peritoneal (IP) injection, oral (OR) inoculation, and transdermal (TD) exposure in a water bath over a 28 day period. These are standard exposure routes tested in pathogen challenge experiments, and provide evidence of host suitability (IP), transmission by consuming infected conspecifics or contaminated water (OR), and transmission by exposure in water (TD). We observed 100% mortality of adult *R. sevosa* in the IP and TD treatments after 10 and 19 days, respectively. Ninety-five percent mortality occurred in the OR treatment after 19 days. No mortality was observed in the control treatment after 28 days. Challenge studies with other species have demonstrated that adult anurans typically clear ranavirus within a week of infection. Thus, our results indicate that *R. sevosa* is highly susceptible to ranavirus. Considering the high mortality observed, ranavirus could result in extinction of *R. sevosa* if this pathogen is introduced into the remaining populations.

SUSCEPTIBILITY OF COMMON FISH AND CHELONIANS TO RANAVIRUS

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Ranaviruses have been associated with mortality of lower vertebrates around the world. FV3-like ranaviruses have been isolated from different ectothermic vertebrate classes; however, few studies have demonstrated whether this pathogen can be transmitted among classes. In this study, we tested for the occurrence of ranavirus transmission (infection prevalence) and relative susceptibility (percent mortality) for five fish and four turtle species exposed to FV3-like ranaviruses isolated from three different ectothermic vertebrate classes. Fish hosts were exposed to a ranavirus isolated from an

amphibian (*Lithobates catesbeianus*) and turtle (*Terrapene carolina carolina*) species, and turtle hosts were exposed to a ranavirus isolated from an amphibian and fish (*Scaphirhynchus albus*) species. Exposure was administered via water bath (10^3 PFU/mL) for three days and survival was monitored for 28 days. Greatest mortality (35%) occurred in the red-eared slider (*Trachemys scripta elegans*) exposed to the fish isolate. Soft-shelled turtles (*Apalone ferox*) experienced no mortality but 10% and 20% of individuals were infected by the reptile and fish isolates, respectively. Similarly, 5% of Mississippi map turtles (*Graptemys pseudogeographica kohni*) were sublethally infected with the turtle isolate at the end of the experiment. Channel catfish (*Ictalurus punctatus*) experienced 5% mortality when exposed to the turtle isolate, while mosquitofish (*Gambusia holbrooki*) experienced 10% mortality when exposed to the turtle and amphibian isolates. Our results demonstrate that FV3-like ranaviruses are capable of infecting hosts from different ectothermic classes. Although substantial mortality did not occur in our experiments, the occurrence of low mortality and sublethal infections suggests that fish and chelonians may function as reservoirs for FV3-like ranaviruses. Additionally, our study is the first to report that a chelonian species can be infected by a ranavirus originating from a fish.

OUTBREAK OF RANAVIRAL DISEASE IN AMPHIBIAN COLONIES IN JAPAN

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In this presentation, we will introduce some outbreaks of ranaviral disease in Japan in 2011-2012. Case 1: Three *Tylototriton kweichowensis* that were imported from China and two *T. kweichowensis* that had been kept previously were housed together. All five animals died within 4 days after cohabitation. Further, two *T. kweichowensis* and four *T. taliangensis* died within three days after purchase. FV-3 was detected in all animals. In addition, these animals were sold in the same pet shop. Case2: Five *Hynobius naevius* that were wild-caught died within 10 days after purchase. After water plants from the aquarium of *H. naevius* were put into the aquarium of larvae of *Hynobius hidamontanus*, 52 larvae died within one week. RCV-JP closely related viruses have been detected in all animals tested. Case3: In February, 2012, an importer (Trader A) imported 12 poison dart frogs from the Netherlands via Canada. These frogs showed onset of disease two or three days after arrival, and all died within a month. All other frogs that had come into contact with the dead frogs died within the month of April. The total number of dead frogs was forty-eight adults and about 30 juveniles. Trader B imported frogs of three genera from the Netherlands in March, 2012. Onset of disease and death began about the 10th day after importation. Fifty-three adults of 5 species died in about one month. Conclusion: These are the first reports of ranavirus in imported animals of the Salamandridae and Dendrobatidae in Japan.

PREVALENCE OF RANAVIRUS ACROSS INDIANA

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Emerging infectious diseases are responsible for significant declines in amphibian populations around the world. Ranavirus is considered a primary pathogen currently threatening amphibians, attacking the immune system of larval amphibians and causing mass die-off events across six continents. This disease can persist in populations through sub-lethal infection of adults, spreading from one site to the next through dispersal or anthropogenic disturbance. It is important for the conservation and proper management of amphibian species to determine the prevalence of this disease throughout a variety of ecosystems. Indiana has a highly fragmented landscape which can lead to higher degrees of

anthropogenic disturbance and possibly a greater likelihood of ranavirus spreading throughout the state. Minnow trap arrays will be used to collect green frog (*Lithobates clamitans*) tadpoles at six sites covering Indiana. Tadpole livers will be used to extract DNA and PCR analysis will be performed on each individual sample. Each sample will be extracted and amplified under sterile conditions to prevent the cross-contamination of any one individual to another. Positive samples will be identified in triplicate on agarose gels run with multiple positive and negative controls. The virus has been found in Indiana but the prevalence of this disease in the state is undocumented. This study will determine the prevalence of ranavirus in green frog tadpoles at six sites across the state of Indiana.

EFFECT OF LIPOSOME-ENCAPSULATED CIDOFOVIR ON FROG VIRUS 3 IN CELL CULTURE

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To date, there are no known effective pharmaceutical treatments for ranaviruses. Cidofovir is an acyclic nucleoside phosphonate with antiviral activity against a wide variety of large DNA viruses including herpesviruses, adenoviruses, and poxviruses. We present data on the effect of cidofovir on Frog virus 3, a Ranavirus, in cell culture.

IDENTIFICATION OF A NOVEL MARINE FISH VIRUS, SINGAPORE GROUPER IRIDOVIRUS-ENCODED MICRORNAS EXPRESSED IN GROUPER CELLS BY SOLEXA SEQUENCING

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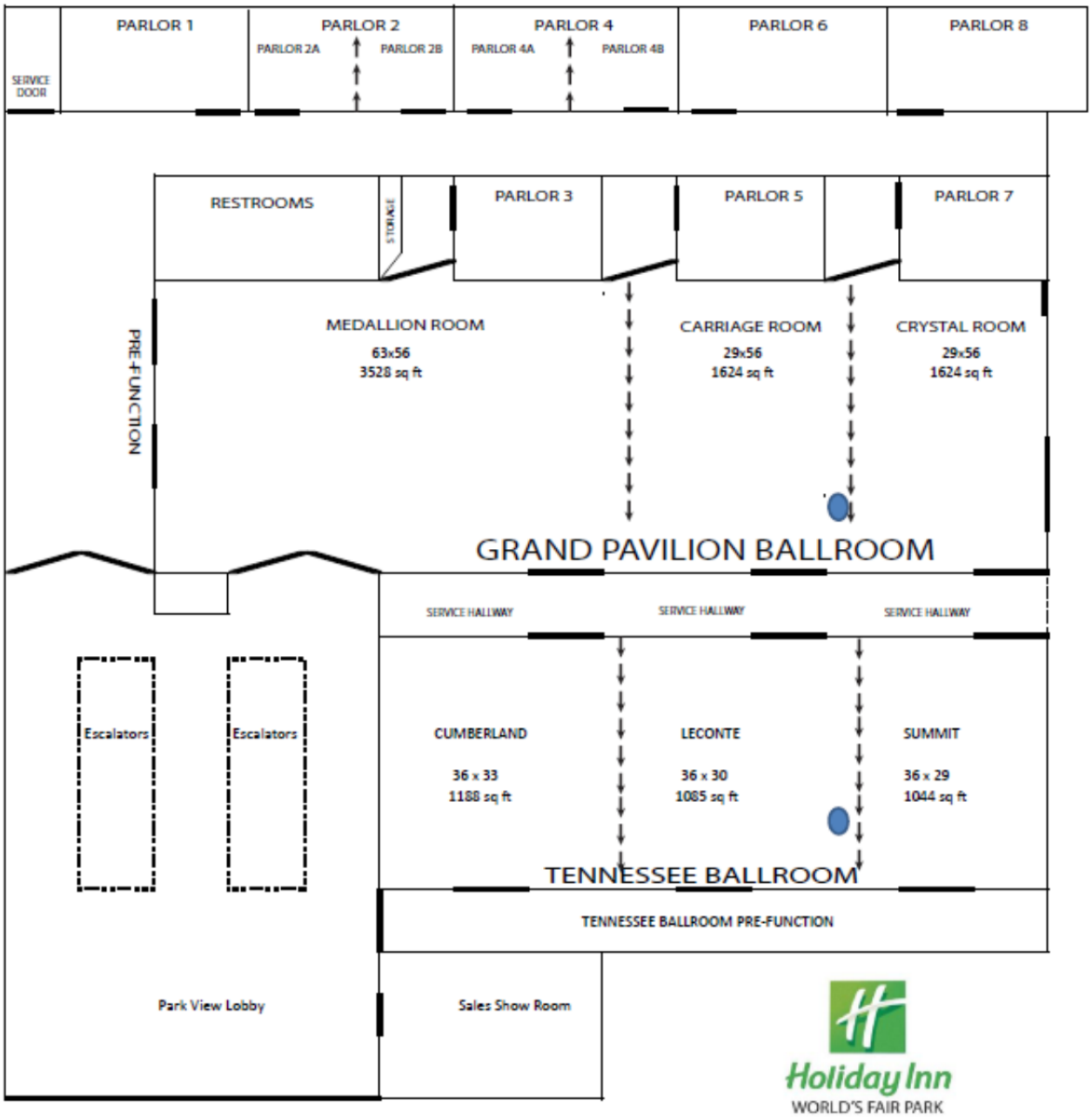
MicroRNAs (miRNAs) are ubiquitous non-coding RNAs that regulate gene expression at the post-transcriptional level. An increasing number of studies have revealed that viruses can also encode miRNAs, which are proposed to be involved in viral replication, persistence, and angiogenesis. Singapore grouper iridovirus (SGIV) is a pathogenic iridovirus that has severely affected grouper aquaculture in China and Southeast Asia. To determine whether SGIV encoded miRNAs during infection, a small RNA library derived from SGIV-infected grouper (GP) cells was constructed and sequenced by Illumina/Solexa deep-sequencing technology. We recovered 6,802,977 usable reads, of which 34,400 represented small RNA sequences encoded by SGIV. Sixteen novel SGIV-encoded miRNAs were identified by a computational pipeline, including a miRNA that shared a similar sequence to herpesvirus miRNA HSV2-miR-H4-5p, which suggests miRNAs are conserved in far related viruses. Generally, these 16 miRNAs are dispersed throughout the SGIV genome, whereas three are located within the ORF057L region. Some SGIV-encoded miRNAs showed marked sequence and length heterogeneity at their 3' and/or 5' end. Expression levels and potential biological activities of these viral miRNAs were examined by stem-loop quantitative RT-PCR and luciferase reporter assay, respectively, and 11 of these viral miRNAs were present and functional in SGIV-infected GP cells. Our study provided a genome-wide view of miRNA production for iridoviruses and identified 16 novel viral miRNAs. To the best of our knowledge, this is the first experimental demonstration of miRNAs encoded by aquatic animal viruses. The results provide a useful resource for further in-depth studies on SGIV infection and iridovirus pathogenesis.

FLOOR PLAN OF THE HOLIDAY INN



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