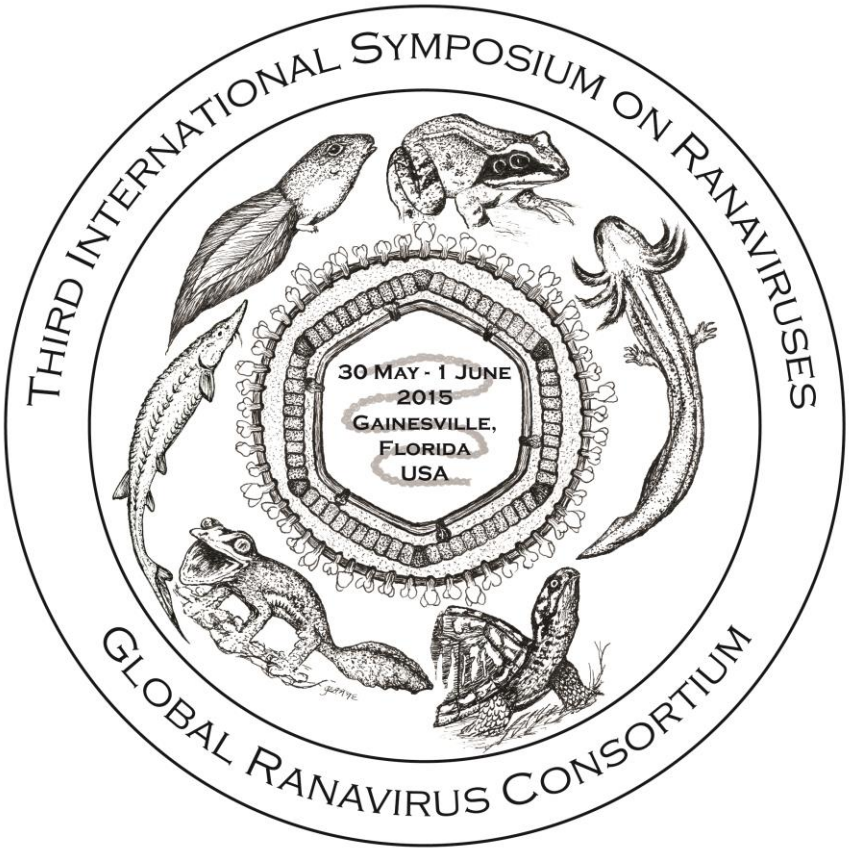
Book of Abstracts



Third International Symposium on Ranaviruses

May 30 - June 1, 2015
Gainesville, Florida USA

UF UNIVERSITY of FLORIDA College of Veterinary Medicine

AQUATIC ANIMAL HEALTH

http://conference.ifas.ufl.edu/aeh/ranavirus/index.html

Use #RV2015 to tweet!

Thank You to our Sponsors!

Diamond Level (>$10,000)

UF University of Florida College of Veterinary Medicine

Aquatic Animal Health

Florida Fish and Wildlife Conservation Commission

Platinum Level ($5,001-10,000)

Visit Gainesville where nature and culture meet

USDA United States Department of Agriculture National Institute of Food and Agriculture

Gold Level ($2,501-5,000)

Clearwater Marine Aquarium

UF Emerging Pathogens Institute University of Florida

Silver Level ($1,001-2,500)

Gordon

ARAV

Georgia Aquarium at Pemberton Place

UT

The Center for Wildlife Health

WDA Wildlife Disease Association Founded in 1951

Bronze Level (< 1,000)

Tennessee Wildlife Resources Agency

SFRC School of Forest Resources & Conservation

Swamp Head Brewery

Table of Contents

Welcome From the Organizers

With the success of the First and Second International Symposia on Ranaviruses (ISR), the Global Ranavirus Consortium (GRC) was eager to start planning for the Third ISR. In 2013, the GRC solicited proposals from potential hosts and the membership chose to partner with the Aquatic Animal Health Program, College of Veterinary Medicine at the University of Florida. The Third ISR is part of the 2015 Aquatic Animal Health Conference, which includes the Fifth Florida Marine Mammal Health Conference to be held at the Hilton University of Florida, Gainesville, Florida USA.

The Third ISR includes seven thematic sessions over two days, thematic group discussions where participants will have the opportunity to discuss relevant issues, workshops and fieldtrips, the Regional GRC meetings and the Business Meeting of the GRC. There are 34 oral presentations and more than 20 posters from researchers, veterinarians, post-doctoral researchers, and students from around the globe (representing 9 different countries).

We are very pleased to welcome Dr. Richard Whittington as our Keynote Speaker. We would especially like to thank Drs. Gray, Chinchar and Brunner for their advice and help in both the organization and solicitation of funding for the Symposium. Also, thanks to Dr. Lesbarreres for his help with the organization of the presentations. We would sincerely like to thank the Executive of the GRC, and the professionals and students who have worked hard to make the symposium a success.

We would like to welcome you to the Third ISR. We look forward to interacting with you and hope that you enjoy the meeting!

Sincerely,

Thomas Waltzek MS, DVM, PhD
Conference Co-Chair
Third International Symposium on Ranaviruses
North America Representative, Global Ranavirus Consortium
Co-Director, Aquatic Animal Health Program, University of Florida

Amanda Duffus PhD
Conference Co-Chair
Third International Symposium on Ranaviruses
Secretary-Treasurer, Global Ranavirus Consortium
Department of Biology, Gordon State College

Patrick Thompson
Conference Coordinator
Third International Symposium on Ranaviruses
Aquatic Animal Health Program, University of Florida

Welcome From the Director of the GRC

The mission of the Global Ranavirus Consortium (GRC) is to facilitate communication and collaboration among scientists, veterinarians, and others interested in ranaviruses. Specifically, the GRC is dedicated to: (1) advancing knowledge in all areas of ranavirus biology and disease, (2) facilitating multi-disciplinary, scientific collaborations, (3) disseminating information on ranaviruses, and (4) providing expert guidance and training opportunities. The first GRC Board was elected and formed in April 2013, and consists of a Director (Dr. Matthew Gray), Associate Director (Dr. Jesse Brunner), Secretary/Treasurer (Dr. Amanda Duffus), and five regional representatives (Drs. Ellen Ariel, Rachel Marschang, Rolando Mazzoni, Yumi Une, and Thomas Waltzek). In addition, Dr. Greg Chinchar serves as an honorary advisor to the Board. Since formation, the Board drafted and approved bylaws for the GRC, created a new website (http://www.ranavirus.org/) with various resources, helped lead organization of the Second and Third International Symposia on Ranaviruses, and was instrumental in securing funds to publish the first eBook on ranaviruses as Open Access. Members of the Board also helped organize a workshop on ranaviruses in Harbin, China in 2014. The GRC became incorporated as a non-profit organization in the USA in 2014, and opened charter membership in 2015. We encourage symposium attendees to become members of the GRC. Membership fees help maintain the GRC website, generate funds for travel grants and outreach activities, and defer costs of the biennial symposia. The GRC also is in the process of creating the Global Ranavirus Reporting System (GRRS), which will be an online geospatial database for reporting cases of ranavirus infection and disease. Membership fees will help operate the GRRS. Future activities of the GRC could include securing 501c tax-exempt status in the USA, establishing continental discussion groups that annually share new research findings, offering an online course on ranaviruses using virtual classroom technology, and organizing regional workshops or training activities on ranaviruses. The Board encourages attendees to take advantage of the workshops offered on Monday, 1 June, which will provide guidance on how to design ranavirus surveillance studies, aseptically collect samples in the field, techniques used to test for ranavirus infection and diagnose disease, and analyze data from ranavirus studies. The workshops will be a great opportunity to discuss current shortcomings in sampling, diagnostic and analytical techniques, and identify possible solutions. Structured discussions on Sunday, 31 May, also will be an opportunity to identify urgent research directions and outreach education needs. As needed, *ad hoc* committees may be formed to address tasks that are identified.

Factors responsible for 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 becoming a member of the GRC, serving on a GRC *ad hoc* committee, participating in a GRC continental discussion group, and attending future symposia. The GRC Board looks forward to interacting with you during this symposium and future endeavors.

All the Best—
Matthew Gray
Director, Global Ranavirus Consortium
http://www.ranavirus.org/

About Aquatic Ecosystem Health 2015

The University of Florida's Aquatic Animal Health Program is proud to host **Aquatic Ecosystem Health 2015**, consisting of two back-to-back conferences: the **Third International Symposium on Ranaviruses** and the **Fifth Florida Marine Mammal Health Conference**.

Florida is the perfect location to host a joint discussion addressing global aquatic ecosystem health issues because its ecosystems, economy, and heritage are inextricably linked to its diverse water resources. The Florida ecosystem faces many of the global challenges affecting aquatic ecosystems worldwide, such as population growth, rising temperatures, ocean acidification, chemical contamination of surface waters, an unprecedented number of unexplained marine mammal mortality events. Furthermore, significant disease epizootics in freshwater habitats, including ranavirus infections in amphibians, have been increasingly documented. These conferences provide a forum for Marine Mammal and Ranavirus experts to address the complexity of global aquatic ecosystem health in a broad perspective.

About the Third International Symposium on Ranaviruses

The scientific community is increasingly aware that emerging infectious diseases pose a significant threat to global biodiversity. A group of viruses in the genus Ranavirus (Family *Iridoviridae*) cause disease in ectothermic vertebrates (e.g. amphibians, reptiles and fish) and appear to be emerging in many populations. Ranavirus-associated die-offs in larval and adult amphibians have been documented in the Americas, Europe, and Asia, with mortality often exceeding 90% during an outbreak. Ranavirus infections also have been reported in wild and cultured fish populations worldwide. While research on reptiles has been slower to accumulate, recent evidence suggests that ranaviruses are capable of causing morbidity and mortality in free-ranging populations.

Morbidity and mortality events in both wild and captive populations of ectothermic vertebrates have sparked a diversity of research programs in all areas of Ranavirus biology. In an attempt to bring scientists together from across the globe to learn and share information about Ranaviruses, the Third International Symposium on Ranaviruses includes five thematic sessions over two-and-a-half days. Symposium sessions will begin with an overview presentation delivered by a topical expert on the session theme, followed by shorter research presentations on the relevant themes.

Conference Organizers

Thomas Waltzek
Conference Co-Chair
Aquatic Animal Health Program Co-Director
University of Florida
College of Veterinary Medicine

Patrick Thompson
Conference Coordinator
Aquatic Animal Health Program
University of Florida
College of Veterinary Medicine

Amanda Duffus
Conference Co-Chair
Gordon State College

Detailed Program Agenda

Friday May 29, 2015		
8:00am - 5:00pm	Optional Research Group Meetings (See Group Meeting Schedule For Details)	Cypress & Live Oak
1:00pm - 3:00pm	GRC Executive Board Meeting	Live Oak
4:00pm - 8:00pm	**Registration**	Registration Desk
4:00pm - 8:00pm	**Poster Set-Up**	Century Ballroom B/C

Saturday May 30, 2015			
7:00am - 5:00pm	**Registration & Poster Set-Up**		Registration Desk/ Century Ballroom B/C
7:00am - 8:15am	**Morning Refreshments**		Century Ballroom B/C
8:15am - 8:30am	Welcome From The GRC President & Symposium Host	Matthew Gray & Thomas Waltzek	Century Ballroom A
8:30am - 9:30am	Keynote Address: *Ranavirus* – The Australian Story	Richard Whittington	Century Ballroom A
Diagnostics Moderator: Debra Miller			
9:30am - 9:45am	Lessons Learned From An Aquatic Pathogen PCR Ring-Test In Indonesia	Paul Hick	Century Ballroom A
9:45am - 10:00am	The Positives And Negatives Of *Ranavirus* Detection With eDNA: It's Useful	Jesse Brunner	Century Ballroom A
10:00am - 10:15am	Repeated Detections Of Ranaviruses In Aquaculture And The Development Of Improved Molecular Tools	Natalie Steckler	Century Ballroom A
10:15am - 10:30am	Revealing Ranaviruses Within The Tissues: An Immunohistochemical Study	Debra Miller	Century Ballroom A
10:30am - 10:45am	**Break**		Century Ballroom B/C
Ecology Moderator: Jesse Brunner			
10:45am - 11:00am	Grim Reaper: Role Of Wood Frogs In *Ranavirus* Outbreaks	Matthew Gray	Century Ballroom A
11:00am - 11:15am	The Impact Of *Ranavirus* On UK Amphibian Population Genetics And Demographics	Lewis Campbell	Century Ballroom A
11:15am - 11:30am	Prior Exposure To The Trematode, *Echinostoma trivolvis,* Decreases Ranavirus Infection Prevalence In Gray Tree Frogs (*Hyla versicolor*)	Vanessa Wuerthner	Century Ballroom A
11:30am - 11:45am	Too Late Or Too Soon: How Does Host Phenology Mediate The Impact Of Ranavirus?	Goncalo Rosa	Century Ballroom A
11:45am - 1:30pm	**Lunch**		Albert's Restaurant

Saturday May 30, 2015 (cont'd)			
Stressors, Physiology and Immunology Moderator: Gregory Chinchar			
1:30pm - 1:45pm	Life History Trade-Offs Induced By Copper And Temperature In The *Lithobates pipens* - *Ranavirus* System	David Lesbarrères	Century Ballroom A
1:45pm - 2:00pm	On The Road To Disease: Susceptibility To Ranavirus Infection Of Wood Frog Populations Near Roads	Emily Hall	Century Ballroom A
2:00pm - 2:15pm	Complex Interactions Between Host Macrophages And Ranaviruses	Jacques Robert	Century Ballroom A
2:15pm - 2:30pm	*Xenopus laevis* Tadpole Type III Interferon Responses To *Frog Virus 3*	Leon Grayfer	Century Ballroom A
2:30pm - 2:45pm	Hematological Reference Intervals For *Rana sylvatica* (*Lithobates sylvaticus*) And Alterations Due To Infection With Frog Virus 3 (*Ranavirus* Sp, Iridoviridae)	María Forzán	Century Ballroom A
2:45pm - 3:00pm	**Break**		Century Ballroom B/C
Taxonomy and Evolution Moderator: Amanda Duffus			
3:00pm - 3:15pm	Ranavirus Taxonomy And Phylodynamics	Thomas Waltzek	Century Ballroom A
3:15pm - 3:30pm	The Genomic Sequence And Taxonomic Classification Of Largemouth Bass Virus	James Jancovich	Century Ballroom A
3:30pm - 3:45pm	Comparative Genomics Of An Emerging *Ranavirus*	Andrew Storfer	Century Ballroom A
4:00pm - 5:00pm	North & South America Regional Meeting –Thomas Waltzek and Rolando Mazzoni		Century Ballroom A
4:00pm - 5:00pm	Europe Regional Meeting – Rachel Marschang		Live Oak
4:00pm - 5:00pm	Australia & Asia Regional Meeting – Ellen Ariel		Cypress
5:30pm - 7:30pm	**Poster Session With Hors D'Oeuvres**		Century Ballroom B/C

Sunday May 31, 2015			
7:00am - 5:00pm	**Registration**		Registration Desk
7:00am - 8:30am	**Morning Refreshments**		Century Ballroom B/C
Virology Moderator: Jacques Robert			
8:30am - 8:45am	The Next Chapter In The Ranavirus Story: Elucidation Of Viral Genes Mediating Host Range, Virulence, And Immune Evasion	V. Greg Chinchar	Century Ballroom A
8:45am - 9:00am	Entry Of Singapore Grouper Iridovirus (SGIV) Into Host Cells Via Clathrin-Mediated Endocytosis And Macropinocytosis In A pH-Dependent Manner	Qiwei Qin	Century Ballroom A
9:00am - 9:15am	Rapid Assembly Of Recombination Cassettes And Generation Of Knock-Out Ranaviruses	Mariah Aron	Century Ballroom A
9:15am - 9:30am	Ranavirus And Turtle Tactics	Ellen Ariel	Century Ballroom A
9:30am - 9:45am	Molecular Characterization Of Three Ranaviruses Detected In Reptiles In Europe	Rachel Marschang	Century Ballroom A
9:45am - 10:00am	Characterizing the Epidemiology of Ranaviral Disease in Chelonians: Natural Outbreaks, Challenge Studies, And Continued Surveillance	Matt Allender	Century Ballroom A
10:00am - 10:15am	**Break**		Century Ballroom B/C
Surveillance Moderator: Matthew Gray			
10:15am - 10:30am	*Ranavirus* And The Chinese Giant Salamander Farming Industry	Andrew Cunningham	Century Ballroom A
10:30am - 10:45am	Investigation Of The International Amphibian Trade As A Pathway Of Global *Ranavirus* Dispersal	Jonathon Kolby	Century Ballroom A
10:45am - 11:00am	Isolation Of *Ranavirus* From Ornamental Amphibians In The International Pet Trade And Their Pathogenicity To Selected Amphibians And Fish	Amanda Bayley	Century Ballroom A
11:00am - 11:15am	Molecular Epidemiology of CMTV-Like *Ranavirus* In The Netherlands	Steven van Beurden	Century Ballroom A
11:15am - 11:30am	Patterns of Prevalence of Two Amphibian Pathogens, *Ranavirus* and *Batrachochytrium dendrobatidis*, Across Wetlands of the Savannah River Site	Stacy Lance	Century Ballroom A
11:30am - 11:45am	Reconstructing UK Ranavirus Emergence Supports Key Role For Translocations By Humans	Stephen Price	Century Ballroom A
11:45am - 12:00pm	A 5-State Surveillance Of Wood Frog (*Lithobates sylvaticus*) Breeding Ponds for Ranavirus	Scott Smith	Century Ballroom A
12:00pm - 1:30pm	**Lunch**		Albert's Restaurant

Sunday May 31, 2015 (cont'd)			
Clinical Case Reports And Pathology Moderator: Matthew Allender			
1:30pm - 1:45pm	Ranavirus Infection In A Group Of African Spur-Thigh Tortoises (*Geochelone sulcata*)	Lisa Farina	Century Ballroom A
1:45pm - 2:00pm	Pathologic Findings From A Mortality Event Associated With Frog Virus 3 In Lee County, Florida In Florida Box Turtles (*Terrapene carolina bauri*) In 2014	Heather Fenton	Century Ballroom A
2:00pm - 2:15pm	Ranavirus Infection In The Invasive *Xenopus laevis* And Endemic *Calyptocephallela gayi* In Chile	Claudio Soto	Century Ballroom A
2:15pm - 2:30pm	Vestibular Syndrome Associated To *Ranavirus* In Farmed Frogs (*Rana catesbeiana* (Shaw, 1802)/*Lithobates catesbeianus*)	Rolando Mazzoni	Century Ballroom A
2:30pm - 2:45pm	**Break**		Century Ballroom B/C
2:45pm - 3:45pm	Topical Discussion: Ecology, Stressors And Surveillance	Jesse Brunner	Century Ballroom A
2:45pm - 3:45pm	Topical Discussion: Pathology And Diagnostics	Deb Miller	Cypress
2:45pm - 3:45pm	Topical Discussion: Virology And Immunology	Jacques Robert	Live Oak
2:45pm - 3:45pm	Topical Discussion: Evolution, Phylogenetics And Taxonomy	Greg Chinchar	Dogwood
3:45pm - 4:30pm	Discussion Summaries		Century Ballroom A
4:30pm - 5:30pm	State Of The GRC	Matthew Gray	Century Ballroom A
5:00pm - 7:00pm	Poster Removal		Century Ballroom B/C
7:00pm - 9:00pm	**Reception / Social**		Florida Museum of Natural History

Keynote Speaker Biography & Abstract

Professor Richard Whittington

Richard is the Chair of Farm Animal Health in the veterinary school at the University of Sydney where he leads research in diverse areas with emphasis on infectious diseases in aquatic animals and ruminants. He translates basic findings from the disciplines of microbiology, virology, immunology, pathology and epidemiology for application at population level. He is a veterinary graduate with a PhD in microbiology and immunology, a Fellow of the Australian Society for Microbiology, a registered specialist in Veterinary Pathobiology and an OIE nominated technical expert on ranavirus. Richard has been engaged in research on ranaviruses since 1989, in both field work and in the laboratory, supported by intermittent research grants from fisheries research and conservation agencies.

RANAVIRUS – THE AUSTRALIAN STORY

Richard Whittington
Faculty of Veterinary Science, The University of Sydney, Sydney, Australia

Australia has the dubious distinction of hosting the first recognized natural disease outbreak caused by a ranavirus in fish. That was 29 years ago, in 1986. The agent was named *Epizootic haematopoietic necrosis virus* and the host was an introduced species, the redfin perch *Perca fluviatilis.* Prior to this, ranaviruses, represented by *Frog virus 3*, were considered to be interesting DNA viruses with fascinating molecular replicative mechanisms, and were still looking for an epizootic. In this address I will touch on this history, the international regulatory aspects of ranavirus, the role of the OIE reference laboratory, and some recent research in Australia.

The World Organisation for Animal Health (OIE) recognized the virulence of EHNV, its restricted geographic range, and the availability of tests to identify it, and listed the pathogen. This created a requirement for international disease notification and certification by national veterinary authorities worldwide, to facilitate trade in finfish products. It also created a need for more accurate identification and differentiation of viral isolates, and a need for a reference laboratory to develop standardized reagents and validated test protocols.

This regulatory activity ran parallel to the global emergence of ranaviruses in disease contexts in lower vertebrates, including in Australia where *Bohle iridovirus* and Mahaffey Road virus (MHRV) appeared in frogs in northern Australia, and Wamena virus was detected in illegally imported pythons. Ranavirus disease events in native frogs have been rare and sporadic and have been confined to northern Australia in captive colonies. Concurrently, there was serological evidence of ranavirus in the introduced cane toad (*Rhinella marina*) in northern Australia; efforts to isolate and identify the virus are ongoing.

Based on the results of a structured survey completed in 2011 using serology, and virus isolation, EHNV does not appear to have spread widely in finfish beyond the locations in the south east where it was first identified. This is despite rapid initial spread in redfin perch populations, recurrent seasonal outbreaks in redfin perch, the resistance of the virus, and experimental confirmation that other finfish species are susceptible.

Important questions remain about the origin, epidemiology and ecological significance of ranaviruses in Australia and elsewhere. Although they lost prominence as the putative agent of an amphibian doomsday when chytrid fungus (*Batrachochytrium dendrobatidis*) was discovered, they must be regarded with ongoing respect and recognized for their role in disease events in many countries.

Contact Information: Prof. Richard Whittington, Faculty of Veterinary Science, The University of Sydney, 425 Werombi Road Camden NSW 2570 Australia, Email: richard.whittington@sydney.edu.au

Oral Presentation Abstracts

CHARACTERIZING THE EPIDEMIOLOGY OF RANAVIRAL DISEASE IN CHELONIANS: NATURAL OUTBREAKS, CHALLENGE STUDIES, AND CONTINUED SURVEILLANCE

Matthew C. Allender[1]*, Ashley Barthel*[1]*, Elena Dzhaman*[1]*, and John Byrd*[2]

[1]Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA
[2]Clinch River Environmental Studies Organization, Oak Ridge, TN, USA

Ranaviral disease has caused mass mortality events in wildlife populations worldwide, but the conservation threat to reptiles is widely unknown. Experimental and observational studies were performed to better characterize the epidemiology of this virus in turtles. Two natural outbreaks in eastern box turtles (*Terrapene carolina carolina*) were observed in east Central Illinois in 2013 and 2014. Outbreaks were observed over the three week duration with no sex predilection, but all individuals were adults. Surveillance efforts following the outbreaks demonstrated zero prevalence in box turtles from the surviving population three weeks after completion of the outbreak. A challenge study was then performed in hatchlings of four species of terrapins at two environmental temperatures. Median survival time was between 6 and 12 days for all species at both temperatures demonstrating extreme sensitivity to FV3-like virus. Quantitative polymerase chain reaction of post-mortem tissues showed positive infection in all exposed subjects (n=8 per species per temperature) and negative infection in all control subjects (n=4 per species per temperature); all subjects in the 22°C environment had higher virus copies detected in the tissues. Finally, surveillance of 1200 free-ranging box turtles demonstrated a prevalence of less than 1% in populations not experiencing an outbreak. This data was then used to construct models of population viability following an outbreak. Model estimates can then be used to influence management decisions that aim to minimize the impact of this disease on biodiversity.

Contact Information: Matt Allender, Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, 2001 S. Lincoln Ave, Urbana, IL 61802 USA, Phone: 217-265-0320, Email: mattallender@vetmed.illinoi.edu

RANAVIRUS AND TURTLE TACTICS

***Ellen Ariel**, Wytamma Wirth, Helene Brien, Tiffany Grandjean, Leigh Owens, and Suzy Munns*
James Cook University, Townsville, Queensland, Australia

The outcome of an infection in any animal is a combination of the virulence of the infectious agent, host susceptibility/immune competence and environmental factors influencing the balance of power between the host and the pathogen.

Ranavirus have only been reported to infect poikilothermic animals. The apparent inability of ranaviruses to infect mammals has been attributed to the temperature preference of ranaviruses replication below 32°C and possible inability to survive higher temperatures. The body temperature of the host therefore seems to be a critical factor in the outcome of infection and several studies report the influence of temperature on outcome of an infection in fish, amphibians and reptiles.

Poikilotherms are able to alter body temperatures by selecting different thermal niches within their environments. Behaviourally elevating body temperatures (behavioural fever) may enable poikilotherms to overcome a viral infection by directly killing the virus. Elevated body temperatures also up-regulate the immune system in poikilotherms and this may further aid in combating an infection. The potential for behavioural fever to control/eradicated a ranavirus infection in Krefft's river tortoises (*Emydure krefftii*) was tested *in vivo* and *in vitro*.

The temperature sensitivity of ranavirus was tested in cell culture to determine the temperature and time needed to inactivate virus outside the host. The challenge with culture systems is that they are multi-factorial and it is difficult to separate the ability of the virus to replicate from the ability of the propagation system (e.g. cell lines) to support the viral replication at temperatures outside the cells preferred range. While the interactions between the virus and the host cells or host animal are important from the point of view of virulence, pathogenicity, laboratory diagnosis and transmission trials, it is also important to separate the two systems in order to understand aspects of the epidemiology of the virus outside a host system. We re-defined the functional temperature range ranavirus can tolerate outside the host by testing its ability to replicate in cell culture at normal culture conditions after exposure to various temperatures and exposure times.

The basking behaviour of wild tortoises was determined and correlated to laboratory measures of thermal equilibration times across a range of temperature gradients. Laboratory modelling of thermal equilibration times was used to predict the core body temperatures of basking tortoises during different seasons. The combination of in vitro and in vivo data was used to predict the potential for behavioral fever in tortoises to be an effective mechanism to combat a possible ranaviral infection.

Contact Information: Ellen Ariel, James Cook University, Townesville, Queensland 4814 Australia. Phone: +61 7 478 14123, Email: ellen.ariel@jcu.edu.au

RAPID ASSEMBLY OF RECOMBINATION CASSETTES AND GENERATION OF KNOCK-OUT RANAVIRUSES.

***Mariah Aron**, Jennifer H. Hill, Alexander G. Allen, Mathew Kromer, and James K. Jancovich*
California State University, San Marcos, CA, USA

Ambystoma tigrinum virus (ATV) was first isolated in the mid 1990's from the Sonoran tiger salamander, *Ambystoma tigrinum stebbinsi*. Since that time, similar ranaviruses have been associated with salamander epizootics throughout western North America. Salamanders infected with ATV become lethargic and show extreme epidermal sloughing and hemorrhaging. ATV can be spread by direct contact or through water. However, we know very little about how the molecular mechanisms of pathogenesis of ATV in its salamander host work. Genomic sequencing of ATV and similar ranavirus isolates reveal a number of open reading frames (ORFs) that may be associated with viral pathogenesis and many other ORFs with unknown function. Therefore, in order to better understand the molecular mechanism(s) of ATV pathogenesis, we have initiated studies to characterize a handful of ATV genes that we predict enhance ATV pathogenesis using knock-out techniques to delete these genes from the virus. However, current technology to delete putative pathogenesis genes from ATV requires a timely process of assembling recombination cassettes – chimeric DNA molecules that contain expression elements (EE) driving the expression of effective screenable and selectable markers, flanked by homologous sequences targeting a specific viral gene – and then generating and purifying recombinant viruses. Therefore, in order to quickly and efficiently generate recombinant ATV, we have generated recombination cassettes that use different EE to express a green fluorescent protein (GFP), a screenable marker, fused to a neomycin resistance gene (neoR), a selectable marker. This GFP-neoR (GNR) cassette is then assembled in a semi-high-throughput (HT) manner to construct recombinant cassettes that target and knock-out specific genes in ATV. Using this technique to synthesize recombination cassettes has been highly successful in comparison to current technology, as it is a faster and more cost effective technique. We will present our data on the HT technique to assemble recombination cassettes, the EE's we have tested for enhanced selection of recombinant ATV and our current procedure to quickly and efficiently generate knock-out ATV.

Contact Information: James Jancovich, Department of Biological Sciences, California State University, San Marcos, CA 92096 USA. Phone: 760-750-8525. Email: jjancovich@csusm.edu

ISOLATION OF RANAVIRUS FROM ORNAMENTAL AMPHIBIANS IN THE INTERNATIONAL PET TRADE AND THEIR PATHOGENICITY TO SELECTED AMPHIBIANS AND FISH

***Amanda E. Bayley**, D. Stone, and S.W. Feist*
Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, UK

The European Union imports large numbers of live ornamental amphibians from all over the world and this trade is a potential route for the introduction or spread of ranaviruses to aquatic ecosystems. A survey of imports into the UK of consignments of live amphibians resulted in the isolation of ranaviruses on seven occasions from amphibians originating from three different continents – the Americas (USA and Central America), Africa (Ghana) and Asia (Indonesia). The isolates were characterized by sequence analysis of a 544 nucleotide region of the major capsid protein (MCP) gene and displayed between one and seven nucleotide differences to the same region of the *Frog virus 3* (FV3) genome.

Infection trials were carried out to assess the pathogenicity of the isolates in some UK amphibian and fish species. Common frog *Rana temporaria* larvae were susceptible to two of the isolates from the Americas; whereas common toad *Bufo* and smooth newt *Lissotritun vulgaris* larvae were susceptible to all isolates. Although the fish species tested (common carp *Cyprinus carpio*, tench *Tinca tinca*, goldfish *Carassius auratus*, stickleback *Gasterosteus aculeatus* and chub *Squalius cephalus*) appeared not to be susceptible to disease, virus was recovered from a number of chub sacrificed during the course of the 28 day experiment. These findings provide evidence for repeated introduction of ranaviruses into the EU and corroborate reports elsewhere of ranavirus isolations from international amphibian shipments arriving at US ports. Susceptibility results indicate that these viruses could pose a threat to UK amphibians.

Contact Information: Amanda Bayley, Aquatic Health and Hygiene Division, Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, Weymouth, Dorset DT4 8UB, UK. Phone: +44(0)1305206665, Email: amanda.bayley@cefas.co.uk

THE POSITIVES AND NEGATIVES OF RANAVIRUS DETECTION WITH EDNA: IT'S USEFUL

***Jesse L. Brunner**, Caren Goldberg, Erica J. Crespi, and Emily Hall*
Washington State University, Pullman, WA, USA

Ranaviruses, like most wildlife pathogens, can be difficult to detect and monitor in captive and wild populations. The most sensitive diagnostic methods use internal tissues (e.g., liver, kidneys, spleen), which generally require lethal sampling. In addition obtaining sufficient sample sizes can be expensive and time consuming, particularly for elusive and rare species, and when the pathogen prevalence is low. Environmental DNA (eDNA)-based approaches are rapidly being adopted as convenient and at least theoretically powerful alternatives to traditional individual-based detection. At a minimum they offer the promise of streamlining sampling and minimizing the need to collect and handle large numbers of individual animals. One or a handful of eDNA samples can be used to detect multiple pathogens from an entire population (e.g., a pond or aquarium), thus reducing the costs and making routine surveillance more feasible. These methods, however, have not been validated for detecting ranaviruses. We present the results of several studies in the laboratory and field aimed at determining the utility and diagnostic performance of eDNA-based ranavirus detection in amphibians. In particular we focus on what a researcher can and cannot determine with eDNA results. We end with general guidelines for the application of eDNA-based methods for various surveillance programs and research.

Contact Information: Jesse Brunner, School of Biological Science, Washington State University, PO Box 644236, Pullman, WA 99164 USA. Phone: 509-335-3702, Email: jesse.brunner@wsu.edu

UNDERSTANDING THE IMPACT OF *RANAVIRUS* ON UK AMPHIBIAN POPULATION GENETICS AND DEMOGRAPHICS

Lewis J. Campbell[1+2]*, Trenton W.J. Garner*[2]*, Xavier A. Harrison*[2]*, Alexandra C. North*[1]*, Amber G.F. Griffiths*[1]*.*

[1]University of Exeter, Environment and Sustainability Institute, Cornwall, UK
[2]Institute of Zoology, London Zoo, London, UK

In the UK the *Ranavirus* strain Frog Virus 3 (FV3) is responsible for sharp declines in the numbers of common frog (*Rana temporaria*) in several areas of England. Site infection often results in a mass mortality event with pond owners reporting relatively large numbers of frogs dead in a short space of time during the summer months. When temperatures are high enough the host shows symptoms such as ulcerated skin lesions and haemorrhaging, however there is usually little sign of infection to an individual during the spring mating season. Nevertheless previous work has shown that frog populations infected by *Ranavirus* present a population genetic structure indicative of selection and disease-induced assortative mating.

The current aims of our research are (in no particular order):
1) To find explicit evidence for assortative mating via MHC genotyping using wild populations as a study system.
2) To evaluate which particular genes such selection acts upon in wild populations of varying infection status using RNA-sequencing.
3) Assess the impact of potential parental mate-choice on offspring survivability using laboratory experiments.

Field sites were chosen based on known previous exposure to *Ranavirus*, both known infected and uninfected sites were used. Tissue samples were taken from adult pairs caught during amplexus that were known to have produced fertile spawn. Spawn was harvested and raised to Gosner stage 25 before inclusion in a large scale infection trial.

Based upon observations on body size collected during the 2014 field season, planned research for spring 2015 also includes projects studying the impacts of the disease on the body size and age demographics of wild populations.

We will present the project aims as well as experimental data from the ongoing research projects.

Contact Information: Lewis Campbell, Environment and Sustainability Institute, Penryn Campus, Penryn, Cornwall, TR10 9FE UK. Phone: +44(0)7787439639, Email: LC461@exeter.ac.uk

THE NEXT CHAPTER IN THE RANAVIRUS STORY: ELUCIDATION OF VIRAL GENES MEDIATING HOST RANGE, VIRULENCE, AND IMMUNE EVASION

V. Gregory Chinchar
University of Mississippi Medical Center, Jackson, MS, USA

Ranavirus virology has followed the same story arc as other viruses: discovery, description of virion morphology, identification of nucleic acid type, characterization of major structural and catalytic proteins, and determination of genomic sequence. The functions of about a third of the approximately 100 ranavirus open reading frames (ORFs) have been inferred by homology to other known cellular or viral genes. What remains is the hard part, ascertaining the role of the remaining ORFs that share no, or limited, homology to known genes, but which likely play critical roles in virus replication. Identification of these genes and determination of their various functions is critical because it may permit the construction of attenuated viruses suitable as vaccines. Furthermore, identifying those viral genes that impair key elements of anti-viral immunity will not only enhance our understanding of host immune responses critical for combatting ranavirus infections, but provide insights into the evolutionary origins of the vertebrate immune response. Here I summarize past and current efforts employed by my laboratory and others to determine viral gene function using classical (e.g., metabolic inhibitors, temperature-sensitive and drug-resistant mutants) and contemporary (e.g., antisense morpholino oligonucleotides, siRNA, knock out and conditionally-lethal mutants, and recombinant viral proteins) approaches. Collectively, these studies will allow us to identify those viral gene products absolutely required for replication in every cell type (i.e., essential genes), those that expand host range and cell tropism or increase viral yields (i.e., host range and efficiency genes) and those that facilitate escape from, or evasion of, host immune responses (i.e., immune evasion genes).

Contact Information: V. Gregory Chinchar, Department of Microbiology, University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216 USA. Phone: 601-984-1743, Email: vchinchar@umc.edu

RANAVIRUS AND THE CHINESE GIANT SALAMANDER FARMING INDUSTRY

Andrew A. Cunningham[1]*, Feng Zhou*[2]*, Stephen J. Price*[3]*, and Minyao Wu*[2]

[1]Institute of Zoology, Zoological Society of London, London, U.K.
[2]Shaanxi Normal University, Xi'an, Shaanxi Province, P.R. China
[3]University College London, London, U.K.

The Chinese giant salamander (*Andrias davidianus*; CGS), endemic to China and Critically Endangered, has been identified by the ZSL's Evolutionarily Distinct and Globally Threatened (EDGE) project (http://www.edgeofexistence.org/amphibians) as the amphibian species most in need of conservation action. Over recent years a rapidly growing industry to farm CGS for food has developed throughout much of China, centred on the Qinling Mountain region of Shaanxi Province. The first farm was licensed to breed CGS in Shaanxi Province in 2004. By mid-2012, 141 CGS farms had been licensed in the province, but there are many more unlicensed farms. In 2011, 2.6 M farmed CGS were recorded in Shaanxi Province, but wild-caught CGS continue to be in demand for breeding farms even though their capture in the wild is illegal. This is partly due to problems in getting > F1 animals to breed and partly due to huge (up to 100%) losses of farm stock from disease epidemics. We visited 14 CGS farms during epidemic disease outbreaks. Clinical signs included swelling and bleeding of the head (locally termed "big head" disease) or feet ("big foot"), necrosis and bleeding of the oral mucosa ("bad mouth") or tail ("bad tail"). Swabs (n = 60) were taken from skin and oral lesions and one CGS *in extremis* with a combination of "big head" and "bad mouth" was euthanased. DNA extracted from the liver, kidney and spleen of this animal and from the skin and mouth swabs was analysed for the presence of *Ranavirus* DNA using PCR with primers that amplify a 466 base-pair region of the ranavirus genome coding for the C-terminal 163 amino acids of the *Ranavirus* major capsid protein (MCP). Amplicons of the expected size were obtained from all tissues of the euthanased CGS and from 39 of 60 swabs (representing 11 of the 14 farms sampled), were sequenced and found to be identical with each other and with sequences reported for *Ranavirus* MCP from farmed CGS and farmed frogs (*Rana grylio*) elsewhere in China. Thirty-nine of 43 farms surveyed reported that they had suffered disease outbreaks consistent with ranaviral disease. Three of the four farms that did not report disease held stock of ≤ 3,000 animals, lower than the mean number of 8,354 CGS per surveyed farm. The industrial-scale farming, high stocking densities, and trade in animals across China in the absence of biosecurity measures has led to a system that has fostered the propagation and spread of ranaviral disease. This virus threatens wild CGS (and possibly other wild fauna) through the discharge of contaminated farm wastewater or the release of infected individuals to the wild. Farmed CGS are released annually as a government-led conservation action, but there is no pre-release assessment of their health, nor is any post-release monitoring conducted. The current structure and management of the CGS farming industry presents conservation threats to extant wild CGS through the introduction and spread of ranaviral disease. We recommend separation of farmed and wild CGS populations and improved CGS farm management, including the quarantining of new stock and the disinfection of waste water, to reduce disease risks to both farmed and wild animals.

Contact Information: Andrew A. Cunningham, Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, U.K., Phone: +44(0)20 7449 6674, Email: a.cunningham@ioz.ac.uk

RANAVIRUS INFECTION IN A GROUP OF AFRICAN SPUR-THIGH TORTOISES (*GEOCHELONE SULCATA*)

***Lisa L. Farina*[1], *Natalie Steckler*[1], *Greg Krane*[1], *Scott P. Terrell*[2], *Heather Grodi*[1], *Thomas Waltzek*[1], *and James Wellehan*[3]**

[1]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[2]Disney's Animal Programs, Lake Buena Vista, FL, USA
[3]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Tortoises from a disease outbreak at a privately-owned breeding facility were examined by the University of Florida Anatomic Pathology Service and Wildlife and Aquatic Veterinary Disease Laboratory. Tortoise species at the facility included the African spur-thigh tortoise (Geochelone sulcata), Indian star tortoise (Geochelone elegans), Galapagos tortoise (Chelonoidis nigra), Aldabra tortoise (Aldabrachelys gigantea), red-footed tortoise (Chelonoidis carbonaria), leopard tortoise (Geochelone pardalis), and Burmese mountain tortoise (Manouria emys). Only the African spur-thigh tortoises were affected, and the diseased animals were limited to a single pen.

On 19 Dec 2014, three tortoises were found dead. An adult male African spur-thigh tortoise was submitted to the UF Anatomic Pathology Service for necropsy. The animal was in poor post-mortem condition. Large portions of the mucosa of the tongue, palate and proximal esophagus were covered by adherent, yellowish-tan to slightly green material. Histologically, there was a fibrinonecrotic stomatitis, glossitis and pharyngitis with intracytoplasmic viral inclusions and necrotizing orchitis with fibrin thrombi. Remaining parenchymal organs were too autolyzed to identify lesions.

Over the next several days, several more tortoises in the same pen developed upper respiratory symptoms. Oral swabs were obtained from two tortoises. The referring veterinarian performed bacterial culture and susceptibility testing on one swab, which yielded multi-drug-resistant Staphylococcus xylosus and S. sciuri; both organisms were susceptible to enrofloxacin. Three tortoises were treated with danofloxicin SC every 48 hours.

Ranavirus quantitative real-time PCR was performed at UF WAVDL on the oral swabs from the same two tortoises. Both were positive for ranavirus. Conventional PCR targeting the MCP region was performed on both samples, and the virus in both samples was 100% identical at the nucleotide level to Frog virus 3 (FV3), the type species for ranavirus.

On 07 Jan 2015, a 35 kg adult female, reproductively active African spur-thigh tortoise in good post-mortem condition was submitted for necropsy. Tan to grey to yellow friable plaques, shallow depressed lesions, and/or red foci were identified in the mucosa of the oral cavity, tongue, glottis, pharynx, esophagus and pylorus. Throughout the myocardium of the ventricle, there was patchy tan discoloration. Histologically, there was necrosis and heterophilic inflammation in the spleen, kidney, lung, and submucosa and/or mucosa of the nasal cavity, pharynx, glottis, tongue, esophagus, stomach and small intestine. In many of these organs, as well as the heart, there was vasculitis, fibrinoid vascular necrosis and/or thrombosis.

Contact Information: Lisa L. Farina, Department of Infectious Diseases and Pathology, University of Florida College of Veterinary Medicine, PO Box 110880, Gainesville, FL 32611-0880 USA. Phone 352-294-4114, Email:farinal@ufl.edu

PATHOLGOIC FINDINGS FROM A MORTALITY EVENT ASSOCIATED WITH FROG VIRUS 3 IN LEE COUNTY, FLORIDA IN FLORIDA BOX TURTLES (*TERRAPENE CAROLINA BAURI*) IN 2014

Heather Fenton[1], *Heather Barron*[2], *Michael Yabsley*[1,3], *Sonia Hernandez*[1,3], *Whitney Kistler*[4], *Jennifer Ballard*[1], *Dan Wolf*[5], *Mark Cunningham*[5], *and Debora Miller*[6]

[1]Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA, USA
[2]Clinic for the Rehabilitation of Wildlife, Sanibel Island, FL USA
[3]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, USA
[4]The Institute for Environmental and Human Health, Texas Tech University, Lubbock, TX, USA
[5]Florida Fish and Wildlife Commission, Gainesville, FL, USA
[6]Center for Wildlife Health, University of Tennessee. Knoxville, TN, USA

Infections with *Frog Virus 3* (FV3) in box turtles can be fatal, but the epidemiology of such infections in free-ranging wildlife is poorly understood. In December 2013, a mortality event was recognized on Captiva Island in Lee County, Florida, and mortalities were reported through March 2014. During this time, seven Florida box turtles (Terrapene carolina bauri) from Captiva Island and one turtle from Sanibel Island presented to the Clinic for the Rehabilitation of Wildlife (CROW). Clinical signs included facial swelling, stomatitis, open-mouth breathing, mucopurulent nasal discharge, bilateral chemosis, dehydration, emaciation, and skin sloughing over the head and neck. The animals were either dead or euthanized upon presentation to the clinic. Four intact carcasses and samples from four additional turtles were shipped to the Southeastern Cooperative Wildlife Disease Study in Athens, Georgia for necropsy and ancillary testing. Post mortem findings in all carcasses examined included buphthalmos, oronasal discharge, fibrinonecrotizing stomatitis, glossitis, and red-tinged fluid and blood within the coelomic cavity. Histologic findings included fibrinonecrotizing and heterophilic stomatitis and glossitis as well as marked skeletal muscle degeneration.

Ranavirus specific polymerase chain reaction (PCR) amplifying a conserved region of the coding region of the major capsid protein was done on blood samples and oral swabs when available, from all turtles (n=8) examined. Sequencing and phylogenetic analysis confirmed FV3 in 4/7 oral swabs and 3/8 blood samples. Virus isolation was successful in only one individual despite characteristic lesions being present in all four carcasses. The single turtle from Sanibel Island had pneumonia associated with *Pasteurella testudinis* and FV3 was not detected by PCR or virus isolation. This mortality investigation details the pathologic findings of an epizootic in southern Florida and emphasizes that diagnosing infection in free-ranging box turtles can be challenging in chronic stages of disease. Further investigations of similar outbreaks may help to alleviate these diagnostic challenges in order to better understand the epidemiology of ranaviruses in free-ranging box turtles.

Contact Information: Heather Fenton, Southeastern Cooperative Wildlife Disease Study, Population Health, University of Georgia, 589 D. W. Brooks Drive, Athens, GA 30602 USA. Phone: 706-340-3452, Email: hfenton@uga.edu

SYLVATICUS) AND ALTERATIONS DUE TO INFECTION WITH FROG VIRUS 3 (*RANAVIRUS* SP, IRIDOVIRIDAE)

María J. Forzán[1,2], *Raphaël V. Vanderstichel*[2], *and Cornelia V. Gilroy*[2]
[1]Canadian Wildlife Health Cooperative, Charlottetown, PE, Canada
[2]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

Hematological profiles, routinely used in human and domestic animal medicine, could prove a valuable tool in assessing amphibian health and provide an insight into the way their immune system responds to a specific infectious agent. Little is known, however, of the hematologic profiles of even the most abundant and frequently studied frog species. The wood frog, *Rana sylvatica* (*Lithobates sylvaticus*), is a member of the large *Ranidae* family and a species widely distributed in North America. Woods frogs have been used in research on ranavirus infection for several years, but reference intervals (RI) for routine hematological parameters have not been established. Neither has the effect of infection with *Ranavirus* sp on their hematological profile been studied. Our objectives were to: 1) Establish hematologic RI for adult wood frogs maintained in the laboratory (PCV, total RBC, WBC and thrombocyte counts, and absolute numbers of neutrophils, lymphocytes, monocytes, eosinophils and basophils) following guidelines from the American Society for Veterinary Clinical Pathology, 2) Determine whether an automated particle counter could be reliably used as a partial substitute to the hemocytometer counting technique, and 3) Determine whether oral infection with Frog Virus 3 (FV3) would result in significant alterations in the hematic profile 4, 9 and 14 days post-infection (dpi).

Forty adult *Rana sylvatica* were caught and maintained in captivity for 6 months prior to sampling. Blood was collected with heparinized capillary tubes via puncture of the facial vein and diluted in Natt-Herrick solution. Complete blood cell counts (hemocytometry), differential WBC counts (Wright-Giemsa-stained smears), PCV and automated total cell counts (Sysmex particle counting) were calculated. Thirteen of those frogs were infected with $10^{4.43}$ plaque forming units of FV3; a hematological profile of infected frogs was established through a sample collected immediately before euthanasia 4 (n=5), 9 (n=5), and 14 (n=3) days post-infection. Leukocyte morphology was similar to other amphibians and mammals. Lymphocytes were the most numerous WBC. PCV was similar to other frogs. High agreement was found between hemocytometry and automated total cell counts (concordance correlation coefficient = 0.879). Infection with FV3 had no significant effect on the overall hematological profile when infected frogs were compared to the reference intervals but resulted in several trends when individual frogs were analyzed pre and post infection. Additionally, beginning 9 dpi cytoplasmic inclusion bodies were evident in lymphocytes, monocytes, neutrophils and eosinophils; the inclusions were much more prevalent 13-14 dpi.

We provide hematological reference intervals for research and clinical practice of *Rana sylvatica*, and report the hematological alterations resulting from a lethal FV3 infection.

Contact Information: María J. Forzán, Canadian Wildlife Health Cooperative, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE C1A4P3 Canada. Phone: 902-566-0545, Fax: 902-566-0871, Email: mforzan@upei.ca

GRIM REAPER: ROLE OF WOOD FROGS IN RANAVIRUS OUTBREAKS

Matthew J. Gray[1]*, Patrick N. Reilly*[1,2]*, Roberto Brenes*[1]*, Jordan C. Chaney*[1]*, Rebecca P. Wilkes*[2]*, Rachel D. Hill*[1]*, and Debra L. Miller*[1,2]

[1]Center for Wildlife Health, University of Tennessee, Knoxville TN;
[2]College of Veterinary Medicine, University of Tennessee, Knoxville, TN.

Transmission of pathogens among hosts is a fundamental process in epidemiology that can affect the probability of disease outbreaks. In multi-species communities, the susceptibility of a host species is integral in affecting transmission, because pathogen shedding and contact rates with uninfected hosts may be elevated. We tested the importance of host susceptibility, pathogen shedding, and contact rates on ranavirus outbreak dynamics for larvae of two common amphibian species (wood frog, *Lithobates sylvaticus*; Cope's gray treefrog, *Hyla chrysoscelis*) in the southeastern United States. Our experiments consisted of a combination of experimental challenges with the pathogen for each host species, estimating viral shedding via eDNA sampling, and quantifying activity and number of contacts of infected individuals. We found that wood frog and gray treefrog tadpoles differ in their ability to transmit ranavirus and cause disease outbreaks. Infected wood frog tadpoles transmitted ranavirus to >85% of uninfected conspecifics and caused widespread mortality, whereas gray treefrog tadpoles transmitted the pathogen to only 15% of conspecifics. Interestingly, infected wood frog tadpoles transmitted ranavirus to only 35% of cohabitant gray treefrog tadpoles, and none of those individuals died. The primary mechanism resulting in different rates of transmission between species was viral shedding not contact rates. Our results indicate that wood frog tadpoles can rapidly transmit ranavirus to conspecifics and cause within-species outbreaks. The effect of wood frog tadpoles infected with ranavirus on cohabitant species needs further investigation.

Contact Information: Matthew J. Gray, Center for Wildlife Health, University of Tennessee, 274 Ellington Plant Sciences Building, Knoxville, TN 37996 USA, Phone: 865-974-2740, Fax: 965-974-4714, Email: mgray11@utk.edu

XENOPUS LAEVIS TADPOLE TYPE III INTERFERON RESPONSES TO FROG VIRUS 3

Jacques Robert, Francisco De Jesús Andino, and ***Leon Grayfer***
Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA

Ranaviruses (Iridoviridae) are posing an increasing threat to amphibian populations, with anuran tadpoles being particularly susceptible to these emerging viral infections. From a phylogenetic perspective, amphibians are the most basal class of vertebrates known to possess both type I and type III interferon cytokines, which are integral to mammalian antiviral immunity. Despite this, the respective roles of these immune mediators during amphibian antiviral defenses to ranaviruses remain poorly understood. Accordingly, we transcriptionally and functionally compared the amphibian Xenopus laevis type I (IFN) and type III (IFNλ) IFNs in the context of the ranavirus Frog Virus 3 (FV3) infections. X. laevis IFN and IFNλ displayed distinct tissue expression profiles in tadpoles, metamorphic froglets and adult frogs, suggesting possibly unique physiological roles for these two molecules. Notably, in comparison to virally challenged adult frogs, FV3-infected X. laevis tadpoles possessed delayed and modest type I IFN responses to infection. By contrast, tadpoles mounted timely and robust type III IFN gene responses, suggesting that this cytokine may be more important to tadpole antiviral defenses. To functionally compare these immune mediators further, we produced both IFN cytokines in recombinant form (rXlIFN, rXlIFNλ) using an insect protein expression system. Interestingly, the rXlIFN and rXlIFNλ induced distinct antiviral gene expression profiles in the kidney-derived A6 cell line as well as in tadpole leukocytes and tissues. However, in comparison to rXlIFN, rXlIFNλ was less effective in preventing FV3 replication in A6 cells and tadpoles, and inferior at extending infected tadpole survival. Intriguingly, FV3 impaired the A6 cell and tadpole kidney type III IFN receptor gene expression. Moreover, in comparison to rXlIFN, rXlIFNλ conferred respectively equal or greater protection of A6 cultures against recombinant FV3 deficient for the putative immune evasion genes encoding either a viral caspase recruitment and activation domain (vCARD) or a truncated viral translation initiation factor-2-alpha (vIF-2α. Together, our findings indicate that in contrast to previous beliefs, tadpoles possess intact antiviral defenses reliant on type III IFNs, which are effectively overcome by FV3 through virulence determinants such as vCARD and vIF-2α.

Contact Information: Leon Grayfer, Department of Microbiology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642 USA. Phone: 585-275-5359, E-mail: Leon_Grayfer@urmc.rochester.edu

ON THE ROAD TO DISEASE: SUSCEPTIBILITY TO RANAVIRUS INFECTION IN WOOD FROG POPULATIONS NEAR ROADS

***Emily Hall**, Jesse Brunner, and Erica Crespi*
School of Biological Sciences, Washington State University, Pullman, WA, USA

Roads are a major anthropogenic disturbance covering around 1% of the area of the US and affecting nearly one fifth. Northern states routinely apply de-icing salts in winter which is associated with chronic salinitization of wetlands. We hypothesized increased salinity (an osmoregulatory stressor) in roadside ponds affects energy available for growth and immune function in amphibian larvae thus decrease performance and susceptibility to disease. Roads can affect disease susceptibility of amphibians in two ways, by increasing transmission of pathogens or by decreasing host resistance to infection. We examined the effects living adjacent to roads on susceptibility to ranavirus (FV3) infection in a dose response exposure experiment with larval and juvenile wood frogs. We found high prevalence of ranavirus infection in wood frog larvae in Yale Myers Forest, CT and ranavirus-associated die-offs were more likely to occur near roads. Infection intensity (liver titer) was higher in larvae from roadside ponds in the control group (sham exposure) and at a low dose animals from roadside ponds had similar infection intensities but lower survival than larvae from woodland ponds, suggesting roadside animals are more susceptible to a secondary ranavirus infection. Overall, roads may contribute to population declines by decreasing size and performance of individuals and as a source of ranavirus propagation in this matrix of ponds.

Contact Information: Emily Hall, School of Biological Science, Washington State University, PO Box 644236, Pullman, WA 99164 USA. Email: emily.m.hall@wsu.edu

LESSONS LEARNED FROM AN AQUATIC PATHOGEN PCR RING-TEST IN INDONESIA

Paul Hick[1]*, Alison Tweedie*[1]*, Murwantoko*[2]*, Toha Tosihadi*[3]*, Mike Rimmer*[1]*, and Richard Whittington*[1]

[1]Faculty of Veterinary Science, University of Sydney, Australia
[2]Gadjah Mada University, Daerah Istimewa Yogyakarta, Indonesia
[3]LP2IL – Centre for Fish Disease and Environment Investigation, Serang, Indonesia

A ring-test that included the ranavirus *Epizootic haematopoietic necrosis virus* (EHNV) provided fish health laboratories in Indonesia with the opportunity to evaluate their capacity to use PCR assays accurately. The ring-test was conducted in conjunction with a PCR proficiency training workshop hosted by the Centre for Fish Disease and Environment Investigation (LP2IL), Serang. This training initiative recognised the growing importance of laboratory accreditation when testing for OIE listed pathogens to facilitate trade of ornamental fish.

A panel of samples was prepared using heat inactivated, cultured virus spiked into a matrix of fish tissue. Aliquots of each sample were distributed in freeze dried form to 20 eligible laboratories overseen by the Directorate General of Aquaculture (DGA), Indonesia. The ring-test was designed to be challenging, with a limited quantity of virus added to each sample. A combination of two viruses was chosen to ensure that most laboratories needed to implement at least one unfamiliar assay: Epizootic Haematopoietic Necrosis Virus (EHNV) and Nervous necrosis virus (NNV). There was no restriction on the choice of assays or the method of testing. The ring-test was both less formal and more challenging than a proficiency test. Thus, participants could critically evaluate PCR methods in an anonymous setting, but also engage in collegial discussion with the organisers. This format was well received by the participants.

The program was successful due to participation of 18/20 laboratories. These participants correctly identified 73.1% (n=238) of positive and negative samples under blind testing conditions. These aggregated results include laboratories which failed to correctly implement a new assay, as well as 11 panels that were tested with 100% specificity and 100% sensitivity. The sensitivity and specificity could only be interpreted in the context of this ring-test, and did not reflect the diagnostic capacity of the laboratory network. The key observation was that expertise with a specific test protocol was a better predictor of a successful result compared to assay choice or the type of equipment available. Conventional PCR assays performed equally well compared to real-time methods. Identification of factors that impacted the accuracy of PCR tests provided evidence to guide future training initiatives and to support the establishment of a reference laboratory system amongst the DGA network.

Contact Information: Paul M. Hick, Faculty of Veterinary Science, University of Sydney, 425 Werombi Road, Camden, NSW 2570 Australia. Phone: +61293511608, Email: paul.hick@sydney.edu.au

THE GENOMIC SEQUENCE AND TAXONOMIC CLASSIFICAITON OF LARGEMOUTH BASS VIRUS.

*Joei Quevedo[1], Kristina S. Hamel[1], Maxine J. Bravo[1], Jeanine Abrams McLean[2], Thomas Waltzek[3], **James K. Jancovich**[1]*

[1]Department of Biological Sciences, California State University, San Marcos, CA, USA
[2]School of Biological Sciences, University of Texas at Austin, TX, USA
[3]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Largemouth bass virus (LMBV) (family *Iridoviridae*, genus *Ranavirus*) 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. Major symptoms of LMBV disease include lesions and over-inflation of swim bladders, which alter equilibrium and prevent submergence of infected hosts. The level of susceptibility and the degree of infection differ among outbreaks as some show detrimental 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. We have sequenced the genome of LMBV using next generation sequencing technology and the assembled LMBV genome has a unique organization. Dot plot comparisons between LMBV and all of the completely sequenced ranavirus genomes show that LMBV is not completely co-linear with any known ranavirus genome. In addition, phylogenetic analysis using the 26 core iridovirus genes shows that LMBV is a unique taxonomic group within the genus *Ranavirus*. Together, these data support the hypothesis that last common ancestor for the amphibian-like ranaviruses was a fish virus and that jumps from fish to other cold-blooded vertebrates have occurred during ranavirus evolution. In addition, these data show that the Santee-Cooper ranavirus group forms a unique clade within the genus *Ranavirus*. As a result, there appears to be a need for taxonomic reorganization within the family *Iridoviridae* and genus *Ranavirus*. Therefore, possible changes in ranavirus taxonomy will be discussed.

Contact Information: James Jancovich, Department of Biological Sciences, California State University, San Marcos, CA 92096 USA. Phone: 760-750-8525, Email: jjancovich@csusm.edu

INVESTIGATON OF THE INTERNATIONAL AMPHIBIAN TRADE AS A PATHWAY OF GLOBAL RANAVIRUS DISPERSAL

Jonathan E. Kolby *[1,2], Lee F. Skerratt[1], and Lee Berger[1]*

[1]College of Public Health, Medical, and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia
[2]EcoHealth Alliance, New York, NY, USA

The contemporary global dispersal of amphibian pathogens appears to be closely associated with the international trade in live amphibians. Millions of amphibians are traded annually, primarily for the purpose of human consumption of frog legs or as exotic pets, providing a potential avenue for the spread of ranavirus and amphibian chytrid fungus. Despite the listing of both emerging pathogens as globally notifiable by the World Organization for Animal Health (OIE) in 2009, most countries still have not developed regulatory measures to mitigate their spread, and global dispersal continues largely unabated.

The practice of disease surveillance within the international wildlife trade can provide timely information for both policy-makers and the scientific research community. Sampling commercial amphibian shipments directly upon import to the USA is a relatively low-cost and rapid technique that can: 1) Identify the prevalence of ranavirus in traded amphibians and evaluate the risk of introduction and spillover through trade activities and 2) Detect pathogen presence in any given country of interest that exports amphibians to the USA, particularly from regions where field surveillance resources are limited.

To demonstrate these concepts, we tested amphibians for ranavirus that were imported to the USA from Madagascar, Hong Kong, Taiwan, and the Dominican Republic. Ranavirus was detected in the majority of amphibians sampled and in nearly all shipments tested, showing that this trade activity does play a considerable role in the international spread of this pathogen. We further interpreted these data to suggest the presence of infected free-ranging amphibians at locations where these exported amphibians may have been collected, both in Madagascar and Hong Kong. Targeted field surveillance was then performed in these regions to identify pathogen distribution and prevalence in potential source populations.

Contact Information: Jonathan E. Kolby, One Health Research Group, College of Public Health, Medical, and Veterinary Sciences, James Cook University, Townsville, Queensland 07083 Australia. Phone: 908-875-6165 (USA), Email: jonathan.kolby@my.jcu.edu.au

PATTERNS OF PREVALENCE OF TWO AMPHIBIAN PATHOGENS, RANAVIRUS AND *BATRACHOCHYTRIUM DENDROBATIDIS*, ACROSS WETLANDS OF THE SAVANNAH RIVER SITE

Stacey L. Lance[1]*, Cara N. Love*[1]*, Megan E. Winzeler*[1]*, Rochelle Beasley*[1]*, Austin Coleman*[1]*, Schyler O. Nunziata*[2]*, and David E. Scott*[1]

[1]Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA
[2]Department of Biology, University of Kentucky, Lexington, KY, USA

A variety of natural and anthropogenic stressors have been hypothesized to increase the emergence of wildlife diseases in amphibians via increased host susceptibility. In South Carolina, a high incidence of *Batrachochytrium dendrobatidis* (Bd), the fungus that causes chytridiomycosis, was observed in bullfrog larvae in constructed wetlands on the Savannah River Site (SRS), where there are also elevated levels of copper, zinc, and mercury. However, no studies to date have explicitly examined the linkages between metal contaminants and the disease ecology of chytridiocmycosis or ranavirus in amphibians, and this knowledge gap impedes our understanding of disease transmission. We sampled adult and larval amphibians from four contaminated and eight reference wetlands on the SRS. We examined 1004 individual amphibians representing 14 frog and 7 salamander species sampled from 2012 to 2013. Overall, for Bd, *Pseudacris ornata* had the highest prevalence (46.4%), followed by *Lithobates catesbeianna* (27.4%) and *L. sphenocephalus* (24.4%). Three species of salamanders tested positive including *Ambystoma tigrinum*, *A. opacum*, and *A. talpoideum*, though all at low zoospore loads. For ranavirus, *P. ornata* again had the highest prevalence (100%), followed by *A. tigrinum* (85.7%), *A. talpoideum* (52.5%), and *A. opacum* (45.9%). Significantly higher proportions of animals with Bd were observed in contaminated sites (19%) versus uncontaminated sites (6.4%), with the odds of infection 71% higher in contaminated wetlands. Slightly lower proportions of individuals at contaminated sites were ranavirus positive than at reference sites (32.1% vs. 39.1%, respectively). In addition to wetland type, both life stage and season had significant impacts with adults 2.6 times more likely to test positive for ranavirus than larvae and the highest incidence occurring in autumn and then winter. The presence of Bd was highest in the winter and it was not detected in summer or fall. Few studies have examined coinfection of these diseases but in this study 4.9% of individuals tested positive for both Bd and ranavirus and individuals positive for ranavirus had a significantly higher Bd infection frequency than negative individuals. Based on these data we have now established a monitoring program on the SRS that includes 24 wetlands and six common species. Initial follow-up studies on Ambystomatids from Spring 2014 suggest large decreases in ranavirus and Bd prevalence. In addition, prevalence of Bd and ranavirus in the metal contaminated H-02 constructed wetlands decreased to zero from 27.3% and 35.7% respectively. We discuss these results as well as ongoing monitoring efforts on the SRS and experimental challenge studies directly examining the impacts of copper, hydroperiod, and temperature on the susceptibility of *A. opacum* to FV3-like ranaviruses.

Contact Information: Stacey L. Lance, Savannah River Ecology Laboratory, University of Georgia, PO Drawer E, Aiken, SC 29802 USA. Phone: 803-725-0988, Email: lance@srel.uga.edu

LIFE HISTORY TRADE-OFFS INDUCED BY COPPER AND TEMPERATURE IN THE *LITHOBATES PIPIENS -RANAVIRUS* SYSTEM.

Joël Leduc[1], Darryl Edwards[2], Pierre Echaubard[1], Vance Trudeau[3], and ***David Lesbarrères***[1]

[1]Genetics and Ecology of Amphibians Research Group (GEARG), Department of Biology, Laurentian University, Sudbury, ON, Canada

[2]Department of Biology, Laurentian University, Sudbury, ON, Canada

[3]Centre for Advanced Research in Environmental Genomics, Department of Biology, University of Ottawa, ON, Canada

Emerging infectious diseases, pollution and climate change are directly or indirectly associated with amphibian population declines worldwide. However, identifying causation is often obscured by the interplay of environmental and anthropogenic stressors. In this context, understanding interactions between evolutionary, ecological and epidemiological determinants of disease is critical to improve our knowledge of host-pathogen co-evolution. Due to the increased effects of climate change and anthropogenic disturbances in northern landscapes, pathogens, whose replication rate is often enhanced in warmer conditions, are increasing their range of occurrence, migrating northward and threatening many aquatic vertebrate communities. We designed a factorial experiment where Northern Leopard Frog tadpoles were subjected to a combination of three experimental conditions: (1) copper-spiked medium; (2) sublethal exposure to ranavirus; and (3) two temperature regimes (14°C and 20°C). Both host and pathogen responses were temperature-dependent suggesting differential trade-offs between host and pathogen fitness with regards to future global warming. We also observed antagonistic effect of the stressors whereby tadpoles exposed to the combination of pathogen and metal grew and developed at a rate similar to the control tadpoles. The arms-race between host and pathogen may be difficult to forecast because of environmental factors hiding the phenotypic and physiological effects of the pathogen on its host and leading infected hosts to become silent propagators of the pathogen.

Contact Information: David Lesbarrères, Biology Department, Laurentian University, Ramsey Lake Road, Sudbury, ON P3E2C6 Canada. Phone 705-675-1151, Email: dlesbarreres@laurentian.ca

MOLECULAR CHARACTERIZATION OF THREE RANAVIRUSES DETECTED IN REPTILES IN EUROPE

Anke C. Stöhr[1], Alberto López-Bueno[2], Silvia Blahak[3], Alí Alejo Herberg[4], ***Rachel E. Marschang***[1,5]

[1]Fachgebiet für Umwelt- und Tierhygiene, Universität Hohenheim, Stuttgart, Germany

[2]Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Madrid, Spain

[3]Chemisches und Veterinäruntersuchungsamt OWL, Detmold, Germany

[4]Centro de Investigación en Sanidad Animal, Instituto Nacional de Investigación y Technología Agraria y Alimentaria, Valdeomos, Spain

[5]Laboklin GmbH & Co. KG, Laboratory for Clinical Diagnostics, Bad Kissingen, Germany

In recent years, reports of ranaviral disease outbreaks in reptiles have been increasing. However, our understanding of the role of this animal class in the epidemiology of ranaviruses is still limited. The aim of this study was to fully sequence the genomes of ranavirus isolates from different reptile species obtained in different years and to use the obtained sequences to better understand the relationships between the individual isolates and their relationship to previously described ranaviruses. The isolates used originated from a diseased Hermanns's tortoise (*Testudo hermanni*) from Switzerland, an Egyptian tortoise (*Testudo kleinmanni*) from Germany and a leaf-tailed gecko (*Uroplatus fimbriatus*) from Germany. The results demonstrated that all isolates are different from one another and belong to the amphibian-like ranaviruses (ALRV). Based on the phylogenetic analysis and the genomic structure, we determined that our reptilian isolates clustered more closely to ranaviruses detected in amphibians than to each other. We also found that the isolates belong to different ALRV groups: the ranaviruses from the tortoises both cluster to the CMTV-group, whereas the gecko ranavirus has FV3-like characteristics. This is the first analysis of full-lenght ranavirus genomes detected in European reptiles. Our findings underline the wide host range of ranaviruses, support the host-switch theory and stress the emergence of ranaviral disease in reptiles.

Contact Information: Rachel E. Marschang, Laboklin GmbH & Co. KG, Steubenstr. 4, 97688 Bad Kissingen, Germany, Phone: +4997172020, Email: rachel.marschang@gmail.com

VESTIBULAR SYNDROME ASSOCIATED TO RANAVIRUS IN FARMED FROGS (*RANA CATESBEIANA* (SHAW, 1802)/*LITHOBATES CATESBEIANUS*).

Rolando Mazzoni[1], *Adriano Q. Mesquita*[2], *Luiz Fernando F. Fleury*[3], *Marcio Hipolito*[4], *Cláudia Maris Ferreira*[5], *Ana M. Cristina R.P.F. Martins*[4], *Márcia H.B. Catroxo*[4], *and Luara L. Cassiano*[4]

[1]Universidade Federal de Goiás, Goiânia, GO, Brazil
[2]Empresa Brasileira de Pesquisa Agropecuária, Rio Branco, AC, Brazil
[3]PUC, Goiânia, GO, Brazil
[4]Instituto Biológico, São Paulo, SP, Brazil
[5]Instituto de Pesca, São Paulo, SP, Brazil

Frog farming is a consolidated aquaculture activity in Brazil. American bullfrogs (Rana catesbeiana, Shaw 1802/Lithobates catesbeianus). Intensive culture conditions trigger disease occurrence and diseases with variable morbidity and mortality rates have been reported in frog farms. However, most of them are still not enough described, mostly concerning their etiology. A common disease with high prevalence in frogs is a nervous syndrome characterized by diverse degree of postural and locomotive alterations resembling a vestibular disease in other species. Frogs with nervous syndrome are characterized by lack of coordination, abnormal posture with lordosis, varying degrees of scoliosis and head tilt to the left or right. All frog categories are affected without any distinction between sex and age, including frogs reaching the market size. The disease has a chronic course and its prevalence and incidence varies in different farms and periods from 10 to 50% population. Most frogs remain sick without being able to feed; becoming emaciated, but some of them will eventually recover.

To study this syndrome, ninety-one sick American bullfrogs (Rana catesbeiana/Lithobates catesbeianus) were obtained from two different farms located at Goiás State in Central-western Brazil. Frogs were first clinically assessed and further necropsied to identify lesions. Samples from the whole head, liver, kidney, spleen, stomach, intestine, lungs, gonads, skin and blood were obtained for histopathology, immunohistochemistry (IHQ), bacteriology, transmission electron microscopy (TEM) and real-time PCR (RT-PCR).

We found macroscopic lesions affecting the inner ear region. Histopathology confirmed inflammatory lesions with necrosis and lymphocytic infiltrate in the choroid plexus and labyrinthine endorgans, with necrotic foci, abundant macrophages and cellular debris associated with inflammatory infiltrates and acidophil inclusion bodies. No bacteria were isolated or identified in these lesions. IHQ showed the presence of positive staining for Iridoviridae and TEM confirmed viral particles in these lesions. RT-PCR also detected a virus from Ranavirus genus. These findings indicate that Ranavirus cause frog vestibular syndrome.

As far as we know this is the first report of Ranavirus producing nervous lesions. Although Ranavirus diseases in bullfrogs may only happen in stressing conditions, frog farms are very likely spreading pathogens to wild populations and this will be a potential threat to susceptible wild species.

Contact Information: Rolando Mazzoni, CPA-EVZ-UFG, Campus II, Goiânia, GO 74000-900 Brazil, Phone: +556281786489, Fax: +556232051222, Email: rolo1001@gmail.com

REVEALING RANAVIRUSES WITHIN THE TISSUES: AN IMMUNOHISTOCHEMICAL STUDY

***Ana Balseiro*[1], *Debra L. Miller*[2], *Thomas B. Waltzek*[3], *and Matthew J. Gray*[2]**

[1]Centro de Biotecnologia Animal-Serida, Gijon, Asturias, Spain

[2]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA

[3]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Ranavirus-associated mortality events that occur in the wild often involve species from multiple classes, including amphibians, reptiles (particularly chelonians), and fishes. Unfortunately, it can be difficult to determine whether a particular lesion might be caused by a ranavirus. Further, the occurrence and severity of lesions varies depending upon host susceptibility and ranavirus isolate. Thus, we used experimentally challenged species to compare and characterize lesions associated with ranaviral disease. Our challenges included multiple development stages and multiple ranavirus isolates. We used immunohistochemical staining to identify ranavirus within the tissues. Specifically, we compared immunohistochemical staining using antibodies obtained from OIE, antibodies developed against the Common Midwife Toad Virus, and antibodies developed against Frog Virus 3. Staining was similar among the three antibodies and highlighted inclusion bodies as well as tissues with minimal gross or histopathological lesions. Identifying the target tissues of ranavirus and factors related to virulence allows us to explore treatment options and develop management plans.

Contact Information: Debra L. Miller, Center for Wildlife Health, University of Tennessee, 274 Ellington PSB, Knoxville, TN 37996 USA. Phone: 865-974-7948, Email: dmille42@utk.edu

RECONSTRUCTING UK RANAVIRUS EMERGENCE SUPPORTS KEY ROLE FOR TRANSLOCATIONS BY HUMANS

Stephen J. Price[1,2,3]*, Trenton W.J. Garner*[2]*, and Richard A. Nichols*[3]

[1]UCL Genetics Institute, Gower Street, London, UK
[2]Institute of Zoology, Zoological Society of London, Regents Park, London, UK
[3]School of Biological and Chemical Sciences, Queen Mary University of London, London, UK

Emerging infectious diseases (EIDs) have received much attention due to the massive public health and economic burdens they incur, though interest in wildlife diseases has often been indirect - a consequence of their roles in human and livestock disease. Phylodynamic techniques have proved exciting and highly effective in reconstructing emergence of well sampled and fast evolving pathogens where research is supported by significant funds but the majority of emerging wildlife pathogens do not fit these criteria even though they may be of conservation concern. We have combined genetic information about the pathogen and a database of citizen science surveillance effort to reconstruct a national ranavirus outbreak.

We used “twinstim”, a function in the R package Surveillance to analyse the UK spread of ranavirus-consistent mortality events. Models have two components – the endemic and epidemic – which represent “imported” cases and “self-exciting” spread from pond to pond. We used different covariates to explore the evidence for two alternative hypotheses for spread of UK ranavirosis: human translocation of virus and climate change effects on host or virus whilst robustly controlling for reporting effort. We also used a concatenated multiple-sequence alignment (7 loci, 2267 base pairs in length) to visualize virus relationships within the UK and in a global context.

The phylogenetic and spatial epidemiological approaches are complementary in reconstructing the pattern of ranavirus emergence. Genetic data suggest international translocations of virus involving the UK and the spatio-temporal models link disease events to human populations. We show that ranavirosis is an emerging infectious disease among UK amphibians, which has spread through a combination of transmission between ponds leading to ‘secondary infections’ and imported events which are well correlated with human population density and are likely to result from a combination of local and international movement of animals and/or other infectious materials.

Contact Information: Stephen J. Price, UCL Genetics Institute, Gower Street, London, WC1E 6BT, UK Phone: +447800896114, Email: s.price@ucl.ac.uk

ENTRY OF SINGAPORE GROUPER IRIDOVIRUS (SGIV) INTO HOST CELLS VIA CLATHRIN-MEDIATED ENDOCYTOSIS AND MACROPINOCYTOSIS IN A PH-DEPENDENT MANNER

*Shaowen Wang[1], Xiaohong Huang[1], Youhua Huang[2], Xian Hao[2], Haijiao Xu[2], Mingjie Cai[2], Hongda Wang[2], and **Qiwei Qin**[1]*

[1]Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China

[2]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China

Virus entry into host cells is critically important for initiating infections and is usually recognized as an ideal target for the design of antiviral strategies. Iridoviruses are large DNA viruses which cause serious threats to ecological diversity and the aquaculture industry worldwide. However, the current understanding of iridovirus entry is limited and controversial. Singapore grouper iridovirus (SGIV) is a novel marine ranavirus isolated from diseased grouper, *Epinephelus tauvina*. Using single-virus tracking technology in combination with biochemical assays, we investigated the crucial events during virus entry based on the established SGIV virus-cell infection model.

SGIV infection in host cells was strongly inhibited when cells were pretreated with drugs blocking clathrin-mediated endocytosis, including sucrose and chlorpromazine. Inhibition of key regulators of macropinocytosis, including Na+/H+ exchanger, Rac1 GTPase, p21-activated kinase 1 (PAK1), protein kinase C (PKC), and myosin II, significantly reduced SGIV uptake. Cy5-labeled SGIV particles were observed to colocalize with clathrin and macropinosomes. In contrast, disruption of cellular cholesterol by methyl-β-cyclodextrin and nystatin had no effect on virus infection, suggesting that SGIV entered grouper cells via the clathrin-mediated endocytic pathway and macropinocytosis but not via caveola-dependent endocytosis. Furthermore, inhibitors of endosome acidification such as chloroquine and bafilomycin A1 blocked virus infection, indicating that SGIV entered cells in a pH-dependent manner. In addition, SGIV particles were observed to be transported along both microtubules and actin filaments, and intracellular SGIV motility was remarkably impaired by depolymerization of microtubules or actin filaments. The results of this study for the first time demonstrate that not only the clathrin-dependent pathway but also macropinocytosis are involved in fish DNA enveloped virus entry, thus providing a convenient tactic for exploring the life cycle of DNA viruses.

Contact Information: Qiwei Qin, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, China. Phone & Fax: +862089023638, Email: qinqw@scsio.ac.cn

COMPLEX INTERACTIONS BETWEEN HOST MACROPHAGES AND RANAVIRUSES

***Jacques Robert**, Francisco De Jesús Andino, Eva-Stina Edholm, and Leon Grayfer*
Microbiology & Immunology, University of Rochester Medical Center, Rochester, NY, USA

Growing evidence suggests that macrophages are central in host/ranavirus pathogen interactions. We have established a reliable infection model system using FV3 infections of the anuran *Xenopus laevis* for investigating the complex roles of different macrophage populations of adult frogs and tadpoles in orchestrating host immune defenses as well as in permitting ranaviruses to escape immunity and either disseminate or persist in the host.

In tadpoles, we have investigated the respective role of two key macrophage mediators CSF-1 and IL-34 that both interact with the same receptor, CSF-1R. In *Xenopus laevis*, CSF-1 and IL-34 genes displayed striking non-overlapping developmental and tissue-specific expression profiles. Notably, using recombinant forms of these cytokines to derive and elicit macrophage responses, we found that IL-34 promotes more potent anti-FV3 macrophages, whereas CSF-1 stimulates more phagocytic macrophages that are more susceptible to FV3 infection. Moreover, tadpole survival upon FV3 infection was increased by pretreatment with recombinant IL-34 but decreased by recombinant CSF-1. As another intriguing evidence of macrophage involvement in ranavirus dissemination, our observation indicate that in tadpoles but not adults FV3 infection compromises the blood brain barrier leading to macrophage infiltration and FV3 spreading into the brain.

In *X. laevis* adults, macrophages critical involvement in the residual quiescence of FV3 occurring in asymptomatic animals long past viral clearance was investigated by triggering their recruitment and activation. We showed that inflammation induced by peritoneal injection of heat-killed bacteria in asymptomatic frogs one month after infection with FV3 resulted in viral reactivation including detectable viral DNA and viral gene expression in otherwise asymptomatic frogs. FV3 reactivation was most prominently detected in kidneys and in peritoneal macrophages. Notably, unlike adult frogs that typically clear primary FV3 infections, a proportion of the animals succumbed to the reactivated FV3 infections, indicating that previous exposure does not provide protection against subsequent reactivation in these animals.

Finally, in both tadpoles and adults, we identified the critical role of an unconventional or innate T cell subset interacting with the nonclassical MHC class I molecules (XNC10) expressed by macrophages and possibly other leukocytes. Transgenic tadpoles and adult *X. laevis* deficient for XNC10 expression and lacking XNC10-iT cells were markedly more susceptible to FV3 infections and elicited delayed anti-FV3 immune response.

Collectively, these findings implicate macrophage-lineage cells as central players of FV3 infection in both tadpoles and adult of *X. laevis* and presumably other host species, serving as likely cellular targets for persistence, quiescence and dissemination.

Contact Information: Jacques Robert, Department of Microbiology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642 USA. Phone: 585-275-1722, E-mail: Jacques_robert@urmc.rochester.edu

TOO LATE OR TOO SOON: HOW DOES HOST PHENOLOGY MEDIATE THE IMPACT OF RANAVIRUS?

Gonçalo M. Rosa[1,2,3], *Joana Sabino-Pinto*[3], *Telma G. Laurentino*[3], *Isabela Berbert*[3], *An Martel*[4], *Frank Pasmans*[4], *Jaime Bosch*[5], *Rui Rebelo*[3], *Trenton W. J. Garner*[2], *and Richard A. Griffiths*[1]

[1]Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, Kent, UK

[2]Institute of Zoology, Zoological Society of London, London, UK

[3]Centre for Ecology, Evolution and Environmental Changes (CE3C), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[4]Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[5]Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Ranavirus is one of five genera of viruses within the family Iridoviridae, and have been implicated as a contributing factor in extinctions and global decline of amphibian populations. Its impact has been compared to the chytrid fungus. Amphibians of different species and life stages may vary in their susceptibility to *Ranavirus* infections and the impact of this pathogen can even be conditioned by other ecological factors.

A population of Bosca's newts (*Lissotriton boscai*) was monitored for 4 years during and after an outbreak of ranaviruses (CMTV-like) in Serra da Estrela (Portugal). We aimed to understand how the host phenology mediated the impact of the pathogen, using a control population to allow demographical comparisons through time.

Between 2011 (when outbreak was first recorded) and 2012 the adult population of *L. boscai* declined 45.5%, and 68.8% when compared the numbers of 2011 to 2013. Due to the phenology of the species, the mass mortality affected mostly the females that stayed in the water after breeding. This lead to a complete reversal of the sex ratio of the population shifting from 25% in late Spring 2011 to over 60% in the two subsequent years. A relation between age and infection will help to better understand the dynamics of the disease.

Contact Information: Gonçalo M. Rosa, Institute of Zoology, Zoological Society of London, Regents Park, London, NW1 4RY UK. Email: goncalo.m.rosa@gmail.com

A 5-STATE SURVEILLANCE OF WOOD FROG (*LITHOBATES SYLVATICUS*) BREEDING PONDS FOR RANAVIRUS

Scott A. Smith[1], *Kirsten Monsen-Collar*[2], *Holly S. Niederriter*[3], *Mackenzie L. Hall*[4], *Kathy Gipe*[5], *Chris Urban*[5], *Kimberly Terrell*[6], *Craig A. Patterson*[7], *and D. Earl Green*[8]

[1]Maryland DNR-Wildlife & Heritage Service, Wye Mills, MD, USA
[2]Department of Biology & Molecular Biology, Montclair St. University, Montclair, NJ, USA
[3]Delaware Division of Fish & Wildlife, Smyrna, DE, USA
[4]Conserve Wildlife Foundation, Frenchtown, NJ, USA
[5]Pennsylvania Fish & Boat Commission, Bellefonte, PA, USA
[6]Smithsonian Conservation Biology Institute, Washington, DC, USA
[7]Maryland DNR-Wildlife & Heritage Service, Wye Mills, MD, USA
[8]USGS National Wildlife Health Center, Madison, WI, USA

Ranavirus and other emerging infectious diseases are increasingly becoming a concern for natural resource managers. In order to begin to better understand the extent to which Ranavirus is impacting amphibian and reptile populations in the Northeast U.S. and to develop and test a sampling protocol that could be used throughout the region, we conducted a survey of amphibian larvae at wood frog (*Lithobates sylvaticus*) breeding ponds in Delaware (DE), Maryland (MD), New Jersey (NJ), Pennsylvania (PA), and Virginia (VA). Wood frogs have the highest mortality and infection rates of northeast amphibians and their breeding ponds (primarily vernal pools) may be the main source of the disease for other affected species.

A random sample of 30 wood frog breeding ponds were chosen in each state with a minimum distance between all study ponds of >3 km. Only ponds with ≥5 wood frog egg masses were included in the study, and were monitored through metamorphosis, die-off or pond dry-out. Standard Samples of 30 wood frog larvae per study pond at Gosner stage ≥27 were collected and analyzed at Montclair State University (NJ) by PCR for presence of Ranavirus. Die-off Samples were collected whenever a die-off of any species was observed and analyzed at USGS-National Wildlife Health Center by PCR and virus culture. In 2013 sampling was conducted in DE, MD and NJ. In 2014 PA and VA ponds were also sampled, while ponds that had confirmed Ranavirus in 2013 were resampled. In 2013, 24 of 60 (40%) study ponds where a Standard Sample was collected tested positive for Ranavirus (DE:12/21; MD:1/19; NJ:11/20). Additionally, 8 of 11 Die-Off Sampled ponds tested positive (DE:2/2; MD:6/6; NJ:0/3). In total, Ranavirus was detected at 28 of 64 (43.8%) study ponds. All isolated viruses were further identified as FV-3. Species (all larvae) with Ranavirus included wood frog, spring peeper (*Pseudacris crucifer*), spotted salamander (*Ambystoma maculatum*), and eastern spadefoot (*Scaphiopus holbrookii*). The 2014 samples, which included 30 PA ponds and 26 VA ponds plus all 28 ponds that tested positive in 2013, were still being analyzed but results will be available and discussed. This study is the largest geographic data set ever assembled for Ranavirus testing. It represents the first cases of lab-confirmed Ranavirus in Delaware (all 3 counties) and the first in eastern spadefoots (in MD).

Contact Information: Scott Smith, Maryland DNR-Wildlife & Heritage Service, P.O. Box 68, Wye Mills, MD 21679 USA. Phone: 401-827-8612 x103, Email: scott.smith@maryland.gov

RANAVIRUS INFECTION IN THE INVASIVE *XENOPUS LAEVIS* AND ENDEMIC *CALYPTOCEPHALLELA GAYI* IN CHILE

Claudio Soto-Azat[1]*, Nicole Sallaberry-Pincheira*[1]*, Sebastian Sarmiento*[1]*, and Andrew A. Cunningham*[2]

[1]Universidad Andres Bello, Santiago, Chile
[2]Institute of Zoology, Zoological Society of London, London, UK

Currently, emerging infectious diseases (EIDs) are recognized as a threat to biodiversity. The class Amphibia faces a global extinction crisis with no precedent, caused by varied factors including EIDs. Ranaviral disease caused by viruses within the genus *Ranavirus* (Iridoviridae) has been linked with high mortality events in many regions of the world and with amphibian population declines in Europe. Since the 1970s the African clawed frog (*Xenopus laevis*) has established feral populations in central Chile and recently the species has been proposed as a vector for ranaviruses based on laboratory trials. Chilean amphibians comprise 60 species of native anurans, which are characterized by a high degree of endemism (70%) and with more than half threatened with extinction. Dead amphibians found in the wild between 2010 to 2013 in central and south Chile, including single individuals to mass mortality events were obtained, DNA extracted from kidney, spleen and liver and amplified using conventional PCR with *Ranavirus*-specific primers following published protocols and using a positive control obtained from previously isolated *Ranavirus*. Overall, eight of 187 individuals (4.3%) produced amplicons of the expected size for *Ranavirus.* Two of four sampled species were positive: *X. laevis* (7/175) and the Chilean frog (*Calyptocephallela gayi*; 1/9). Neither the two four-eyed toads (*Pleurodema thaul*) nor the Bullock's toad (*Telmatobufo bullocki*) sampled gave positive results. All positive cases were from the Metropolitan Region, the original area of *X. laevis* invasion and where the highest densities of this frog are found in Chile. The positive *C. gayi* was a 2 kg female, which presented abundant serosanguinous subcutaneous and intracelomic fluids. Once abundant and widely used as food resource, *C. gayi* is currently classified as Vulnerable by the IUCN and populations have markedly declined over the last two decades. Most cases of mortality could not be associated with ranaviral disease and none of the PCR-positive frogs using histopathology of key tissue had lesions typical of *Ranavirus* infection. Although these results are consistent with a possible reservoir role of *X. laevis*, this needs to be further investigated. Also, whether *Ranavirus* could be considered a threat or is having negative impacts on native Chilean amphibians should be matter of future research. Isolation and genetic characterization of ranaviruses in Chile is required.

Contact Information: Claudio Soto-Azat, Facultad de Ecologia y Recursos Naturales, Universidad Andres Bello, Republica 440, Santiago Chile. Phone: +56226615790, Email: csoto@unab.cl

REPEATED DETECTIONS OF RANAVIRUSES IN AQUACULTURE AND THE DEVELOPMENT OF IMPROVED MOLECULAR TOOLS

Natalie Steckler[1], *James Wellehan*[2], *Salvatore Frasca Jr.*[3], *Richard Whittington*[4], *Paul Hick*[4], *Joy Becker*[4], *Diana Jaramillo*[4], *Ellen Ariel*[5], *Anna Toffan*[6], *Riikka Holopainen*[7], *James Jancovich*[8], *Niels Jorgen Olesen*[9], *Debra Miller*[10], *Jaree Polchana*[11], *Somkiat Kanchanakhan*[11], *and Thomas Waltzek*[1]

[1]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[2]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[3]Connecticut Veterinary Medical Diagnostic Laboratory, University of Connecticut, Storrs, CT, USA
[4]Faculty of Veterinary Science, University of Sydney, Camden, New South Wales, Australia
[5]Discipline of Tropical Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia
[6]Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy
[7]Elintarviketurvallisuusvirastovira Evira, Helsinki, Finland
[8]Department of Biological Sciences, California State University, San Marcos, CA, USA
[9]Technical University of Denmark, Copenhagen, Denmark
[10]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[11]Department of Fisheries, Kasetsart University, Bangkok, Thailand

Ranaviruses have been detected with increasing frequency among aquacultured and wild fishes including endangered species such as pallid sturgeon. To examine the impact and spread of ranaviruses with a focus on aquaculture, we conducted phylogenomic analyses to elucidate significant biologic and epidemiologic trends. Our comparative genomic analyses facilitated the design and validation of improved molecular diagnostic assays for the rapid detection of ranaviruses. We designed a quantitative real-time Taqman PCR assay capable of detecting all known ranaviruses while excluding other iridoviral genera affecting fish. Additionally, we have developed an *in situ* hybridization assay for the detection of ranaviral DNA in tissue sections. These newly validated molecular tools will be used in future challenge studies examining whether stocking density and water temperature influence survival among aquacultured species. Improved diagnostics and a better understanding of ranaviral ecology and epidemiology in aquaculture will be imperative in the design of future mitigation strategies.

Contact Information: Natalie Steckler, Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA. Phone: 352-727-0721, Fax: 352-392-4707, Email: steckler@ufl.edu

COMPARATIVE GENOMICS OF AN EMERGING *RANAVIRUS*

Andrew Storfer *and Brendan Epstein*
School of Biological Sciences, Washington State University, Pullman, WA, USA

Ranaviruses have caused severe epizootics in commercial frog and fish populations and are currently classified as notifiable pathogens in international trade. Previous work demonstrates that a ranavirus that infects tiger salamanders throughout Western North America (*Ambystoma tigrinum* virus, or ATV) is in high prevalence among salamanders in the fishing bait trade. These ATV strains have been shown to have elevated virulence and are transported long distance by humans, providing widespread opportunities for pathogen pollution. We sequenced the genomes of 15 strains of ATV collected from tiger salamanders across western North America and performed phylogenetic and population genomic analyses and tests for recombination. We find that ATV forms a monophyletic clade within the rest of the Ranaviruses and that it likely emerged within the last several thousand years. Bayesian analyses suggest effective population sizes of ATV have remained nearly stable for several hundred years, although Tajima's D was slightly negative, suggesting recent range expansion, consistent with human bait salamander movement. We also identify several genes under strong positive selection, some of which are involved in viral virulence and/or host immune evasion. We provide evidence for recombination among ATV strains, and potential recombination of strains isolated from salamanders used as fishing bait and those isolated from natural tiger salamander populations raises particular concern.

Contact Information: Andrew Storfer, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236 USA. Phone: 509-335-7922, Email astorfer@wsu.edu

MOLECULAR EPIDEMIOLOGY OF CMTV-LIKE RANAVIRUS IN THE NETHERLANDS

Jolianne Rijks[1], Bernardo Saucedo[2], Annemarieke Spitzen-van der Sluijs[3], Joseph Hughes[4], Gavin S. Wilkie[4], Fons J.A.M. van Asten[1,2], An Martel[5], Frank Pasmans[5], Andrea Gröne[1,2], ***Steven J. van Beurden***[2] *and Marja Kik[1,2]*

[1]Dutch Wildlife Health Centre (DWHC), Utrecht, The Netherlands
[2]Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[3]Reptile, Amphibian and Fish Conservation the Netherlands (RAVON), Nijmegen, The Netherlands
[4]MRC - University of Glasgow Centre for Virus Research, UK
[5]Faculty of Veterinary Medicine, Ghent University, Belgium

Several recent amphibian die-offs in Europe were found to be associated with amphibian-like ranaviruses of the family *Iridoviridae*. The ranavirus emerging in Spain since 2007 was tentatively named common midwife toad virus (CMTV-E), after one of its host species. In 2010, a CMTV-like virus (CMTV-NL) was detected during a mass-mortality event affecting wild water frogs (*Pelophylax* spp.) and other amphibians in the Netherlands. In order to define the relationship of CMTV-NL virus with other ranaviruses, its complete genome sequence was determined. Amphibian mortality events during the following years were monitored to assess the significance of the CMTV-NL outbreak, and to detect, characterize and map ranavirus disease among different host species in the Netherlands over time.

CMTV-NL was isolated from the internal organs of a typically affected fresh dead wild edible frog (*Pelophylax* kl. *esculentus*), using the BF-2 cell line. The animal was collected during an outbreak in a small semi-artificial pond near the index site. Next, the virus was purified, viral DNA extracted, and the complete viral genome determined by deep sequencing. The double-stranded DNA genome was 107,772 bp in length, encoding 104 predicted protein-coding open reading frames. The genome demonstrated a high degree of co-linearity and nucleotide sequence similarity with CMTV-E (96.5%), as well as with *Andrias davidianus* ranavirus isolate 1201 (ADRV-1201) (94.1%). Phylogenetic analysis based on concatenated alignments of the 26 iridovirus core proteins confirmed the grouping of CMTV-NL with CMTV-E and ADRV-1201.

Since the outbreak in the Netherlands in 2010, almost 50 marked amphibian mortality events were investigated, involving multiple amphibian species. In more than a dozen of these events, ranavirus infection was demonstrated by histopathology, immunohistochemistry, and PCR. Using phylogeny based on partial gene sequences from seven different regions along the ranavirus genome, two distinct groups of isolates could be differentiated, which were also separated geographically. Additional viral genomic sequence data is required to provide further insights in the spread and evolution of ranaviruses in the Netherlands.

Contact Information: Steven J. van Beurden, Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, P.O. box 80165, 3508 TD Utrecht, The Netherlands, Phone: +31 30 253 42 96, Email: s.j.vanbeurden@uu.nl

RANAVIRUS TAXONOMY AND PHYLODYNAMICS

Thomas Waltzek[1]*, Natalie Steckler*[1]*, Kuttichantran Subramaniam*[1]*, Patrick Thompson*[1]*, James Wellehan*[2]*, Sieara C. Claytor*[3]*, Richard Whittington*[4]*, Paul Hick*[4]*, Ellen Ariel*[5]*, Anna Toffan*[6]*, Riikka Holopainen*[7]*, James Jancovich*[8]*, Niels Jorgen Olesen*[9]*, Matthew Gray*[10]*, Debra Miller*[10]*, Jaree Polchana*[11]*, Somkiat Kanchanakhan*[11]*,Jesse Brunner*[12]*, Taj Azarian*[13]*, and Marco Salemi*[13]

[1]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[2]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[3]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, USA
[4]Faculty of Veterinary Science, University of Sydney, Camden, New South Wales, Australia
[5]Discipline of Tropical Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia
[6]Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy
[7]Elintarviketurvallisuusvirastovira Evira, Helsinki, Finland
[8]Department of Biological Sciences, California State University, San Marcos, CA, USA
[9]Technical University of Denmark, Copenhagen, Denmark
[10]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[11]Department of Fisheries, Kasetsart University, Bangkok, Thailand
[12]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, USA
[13]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

As globally emerging pathogens impacting poikilothermic vertebrates across three taxonomic classes, ranaviruses (RV) have captured the attention of scientists as well as conservation and regulatory agencies. This recent renewed interest in ranavirology has underscored deficiencies in our understanding of these pathogens, including the lack of an updated taxonomic scheme to capture the diversity of an increasing number of discovered RV. Additionally, there is a pressing need to understand the dispersion process(es) by which RV have become globally distributed and whether naïve populations exist that could be protected.

Viral taxonomy and epidemiology are now heavily based upon molecular evidence, and recent advances in sequencing technology and improved cost effectiveness have facilitated the gathering of sequence data for RV. In this presentation, Next Generation Sequencing and phylodynamic approaches useful for resolving these issues will be discussed. Increasing our knowledge of RV taxonomy and epidemiology is fundamentally important not only to basic virology but also to more applied agencies interested in conservation and policy.

Contact Information: Thomas Waltzek, Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA. Phone: 352-273-5202, Fax: 352-392-4707, Email: tbwaltzek@ufl.edu

PRIOR EXPOSURE TO THE TREMATODE *ECHINOSTOMA TRIVOLVIS* DECREASES RANAVIRUS INFECTION PREVALENCE IN GRAY TREE FROGS (*HYLA VERSICOLOR*)

***Vanessa P. Wuerthner**, Jessica Hua, and Jason T. Hoverman*
Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA

Epidemiological research has traditionally focused on interactions between a single host and pathogen, yet growing evidence suggests that coinfections can alter disease patterns. A central question concerns how interactions between co-occurring pathogens affect disease severity and pathogen transmission. We explored whether the addition of ranavirus (frog virus 3, FV3) at different time points will have an effect on co-infection prevalence of the trematode *Echinostoma trivolvis* and ranavirus within larval gray tree frogs (*Hyla versicolor*). We conducted a 2 x 2 x 3 factorial experiment with two trematode exposure treatments (0 or 50 cercariae), two virus treatments (0 or 10^6 plaque forming units [PFUs]), and three time points of virus addition (simultaneous with *Echinostoma* treatments, 5 days after exposure to *Echinostoma*, or 10 days after exposure to *Echinostoma*) for a total of 240 experiment units. Tadpoles were euthanized two weeks after virus addition and trematode load and viral infection were quantified. Trematode infection rate was similar across all treatments, with an average infection rate of 40%. Interestingly, ranavirus infection prevalence was influenced by prior exposure to trematodes. In the presence of trematodes, we typically saw lower ranavirus infection rates. In addition, for virus treatments 10 days post echinostome exposure, mortality is higher for virus treatments when compared to co-infected treatments. These results may suggest prior exposure to trematodes can decrease susceptibility to ranavirus infection and mortality. This effect may be mediated by immune system trade-offs. Collectively, our results underscore the need to explore the implications of coinfection for amphibians.

Contact Information: Vanessa Wuerthner, Department of Forestry and Natural Resources, Purdue University, 715 W State St, West Lafayette, IN 47907 USA. Phone: 219-204-0983, Email: vwuerthn@purdue.edu

Poster Presentation Abstracts

CHARACTERIZATION OF THE AMBYSTOMA TIGRINUM VIRUS (ATV) RNASE III-LIKE GENE (ORF 25R)

Alexander G. Allen *and James K. Jancovich*
Department of Biological Sciences, California State University San Marcos, CA, USA

Iridoviruses are large, double-stranded DNA viruses that infect economically and ecologically important host species. The family *Iridoviridae* is currently made up of five genera: two genera that infect invertebrate host species (*Chloriridovirus*, *Iridovirus*) and three genera that infect cold-blooded vertebrate hosts (*Ranavirus*, *Megalocytivirus*, *Lymphocystivirus*). All known members of the family *Iridoviridae* encode 26 conserved core proteins. One of those core proteins is a ribonuclease (RNase) III-like gene. It has been suggested that this viral RNase III-like protein works by binding to and degrading viral double stranded (ds) RNA, an activator of host immune responses, particularly the interferon (IFN) response, or by processing micro RNAs, molecules that are essential for post transcriptional modifications of messenger RNA. Viral processing of host and virus derived RNA is an essential mechanism for controlling immune surveillance and detection. Therefore, to test these hypotheses and to determine the function of the *Ambystoma tigrinum virus* (ATV; genus *Ranavirus*) RNase III-like gene (ORF 25R) we first constructed a plasmid expression vector encoding the ATV 25R protein. Ectopic expression of 25R in polyI:C, a synthetic form of dsRNA that activates IFN pathways, treated fathead minnow (FHM) cells lead to rapid degradation of 25R and decreased production of IFN-like activity. Therefore, our preliminary data suggest that the ATV RNase III works at helping evade the host's immune system, and more specifically the IFN system, by masking the dsRNA created by the virus. We then generated a recombinant ATV deleted of the RNase III gene (ATVΔ25R) by homologous recombination to further characterize the function of this ATV protein. ATVΔ25R displays a small plaque phenotype in FHM cells as compared to wtATV and reduced growth under multi-cycle conditions. In addition, preliminary experiments suggest that ATVΔ25R is more sensitive to IFN treatment as compared to wtATV. Taken together, our preliminary data suggest that the ATV 25R protein is non-essential for virus growth in cells in culture and functions to help evade the host's immune response by masking activators of the IFN pathway. In addition, the ATV 25R protein may work in concert with the 57R gene (i.e. vIF2αH), in a manner similar to poxvirus E3L and K3L proteins, to control the cellular innate immune response. We are continuing to characterize the molecular action(s) of this highly conserved iridovirus protein in cells in culture and examine ATVΔ25R pathogenesis in tiger salamanders.

Contact Information: James Jancovich, Department of Biological Sciences, California State University, San Marcos, CA 92096 USA. Phone: 760-750-8525. Email: jjancovich@csusm.edu

VISUALIZING THE RANAVIRUS SCIENTIFIC LANDSCAPE

*Wytamma Wirth and **Ellen Ariel***
James Cook University, Townsville, Queensland, Australia

This poster shows several visualizations of bibliometric networks of scientific papers published in the field of ranavirus research. Bibliometrics is a set of tools used for the statistical analyses of publication data. To obtain an overview of the published works in this field, 310 references relating to the topic of “ranavirus” were downloaded from the Web of Science™ core collection database. The combined bibliographies of these papers included 6002 cited references. These 6002 references were analyzed and visualized using VOSviewer 1.6.1 to show relatedness of key words, the location of publication and co-authors of papers based on citation data. The resulting 'maps’ include the cumulative temporal changes in common key words used in the last 23 years of ranavirus research, the landscape and popularity of journals for publication, and a visualization of collaboration of organizations based on the co-authorship of papers.

Contact Information: Ellen Ariel, James Cook University, Townesville, Queensland 4814 Australia. Phone: +61 7 478 14123, Email: ellen.ariel@jcu.edu.au

RANAVIRUS TRANSMISSION STUDIES IN COMMON FROG *RANA TEMPORARIA*

Amanda E. Bayley *and S.W. Feist*

Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, UK.

In order to determine the level of risk a particular virus represents once it enters the environment and to make epidemiological inferences to provide management and control strategies, the transmission dynamics of that virus must be understood. We carried out transmission studies in common frog *Rana temporaria* using a ranavirus isolate from a 2007 disease event in adult common frogs in Sussex, UK. Challenge variables included pathogen dose, exposure method and stocking density. In our studies a bath challenge dose of 10-100 $TCID_{50}$ ml^{-1} was required to induce mortality in larvae; in post metamorphs a higher dose of 100-1000 $TCID_{50}$ ml^{-1} was necessary. Virus was transmitted in both larvae and post metamorphs by (transfer of) water from tanks of infected animals. Finally, transmission was demonstrated between common frog and common toad *Bufo bufo* post metamorphs following cohabitation with infected individuals. Results indicate that infected animals release high levels of ranavirus into their environment, sufficient to cause infection in naïve animals; and that released virus can survive in the environment for enough time to initiate infection.

Contact Information: Amanda Bayley, Aquatic Health and Hygiene Division, Centre for Environment Fisheries and Aquaculture Science, Barrack Road, Weymouth, Dorset DT4 8UB UK. Phone: +44(0)1305 206665, Email: amanda.bayley@cefas.co.uk

SURVIVAL OF *FROG VIRUS 3* IN FRESHWATER AND SEDIMENT FROM AN ENGLISH LAKE

*J. Munro[1,2,3], **Amanda E. Bayley**[1], and S.W. Feist[1]*

[1]Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, UK

[2]Current address: School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, Australia

[3]Current address: Australian Research and Development Institute, and Marine Innovation Southern Australia, 2 Hamra Avenue, West Beach, South Australia, Australia

Ranaviruses can be transmitted by infected water and sediment but must retain infectivity for a sufficient period to reach and infect a susceptible host. In order to determine the level of risk a particular virus represents once it enters the environment, its persistence in that environment must be determined. In this study we evaluated the survival of frog virus 3 (FV3) in water and sediment over time at temperatures of 4°C, 15°C, 20°C and 30°C. In sediment, FV3 viability ranged from one day when maintained at 30°C to 25 days at 4°C. Likewise, in freshwater, FV3 viability extended from 21 days when maintained at 30°C to 70 days when maintained at 4°C. These results can be used to estimate the persistence of FV3 in the environment and indicate that virus could remain infectious in temperate locations for extended periods during winter.

Contact Information: Amanda Bayley, Aquatic Health and Hygiene Division, Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, Weymouth, Dorset DT4 8UB UK. Phone: +44(0)1305206665, Email: amanda.bayley@cefas.co.uk

PHYLOGEOGRAPHY OF *FROG VIRUS 3* IN NORTH AMERICA

Sieara C. Claytor[1], *Jesse Brunner*[2], *Matthew Gray*[3], *V. Gregory Chinchar*[4], *Gavin Glenney*[5], *April Johnson*[6,7], *James Wellehan*[7], *Taj Azarian*[8], *Marco Salemi*[8], *Natalie Steckler*[9], *Kuttichantran Subramaniam*[9], *and Thomas Waltzek*[9]

[1]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, USA
[2]School of Biological Sciences, Washington State University, Pullman, WA, USA
[3]Center for Wildlife Health, University of Tennessee, Knoxville TN, USA
[4]Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA
[5]US Fish and Wildlife Service, Lamar Fish Health Center, Lamar, PA, USA
[6]Signature Science LLC, Baku, Azerbaijan
[7]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[8]Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, Gainesville, University of Florida, Gainesville, FL, USA
[9]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Ranaviruses are emerging aquatic animal pathogens that have contributed to the global decline of amphibians. The type species for the genus, *Frog Virus 3* (FV3), has resulted in amphibian, reptile, and fish epizootics around the world. To our knowledge, phylogeographic analyses have not been performed on FV3 isolates. Thus, we investigated the phylogeographic structure of FV3 across its range, to (1) determine patterns among hosts and geography and (2) determine the origin of introductions. Currently, we have gathered genomic sequences from 26 FV3 isolates from fish, amphibian, and reptilian hosts occurring throughout North America. Although data collection is ongoing, we have begun preliminary phylogeographic analyses utilizing non-coding and coding regions of the FV3 genomes. These results will provide insight into ranavirus-host evolution and a better understanding of the temporospatial patterns of FV3 in North America.

Contact Information: Sieara Claytor, Department of Wildlife Ecology and Conservation, University of Florida, Building #0116, 2322 Mowry Road, Gainesville, FL 32611 USA. Phone: 785-341-5357, Email: sclayto@ufl.edu

EFFECTS OF TEMPERATURE ON SUSCEPTIBILITY OF MARBLED SALAMANDERS (*AMBYSTOMA OPACUM*) TO RANAVIRUS

***Austin Coleman**, Megan E. Winzeler, and Stacey L. Lance*
Savannah River Ecology Lab, University of Georgia, Aiken, SC, USA

Increasingly, a major conservation concern for amphibians and other ectothermic vertebrates is the emergence of the Ranavirus (RV) pathogen. Ranavirus (RV) infection can be influenced by various biotic and abiotic factors, however, little is known about the influence temperature has on the dynamics of and susceptibility to this disease. In a previous study we found the highest prevalence of RV on the Department of Energy's Savannah River Site (SRS) during autumn and winter. The goals of this study are to 1) determine the effects temperature has on marbled salamander (*Ambystoma opacum*) larvae susceptibility to RV and 2) to examine RV viability outside of host-pathogen interactions. In the fall of 2014 we collected adult marbled salamanders during their breeding migration to Ginger's Bay, a small Carolina bay located on the SRS, using partial drift fences with pit-fall traps. We housed several males and females together in outdoor mesocosms to allow for breeding and oviposition. In February of 2015 we collected four clutches of eggs from the mesocosms, hatched them, and assigned individuals to treatment groups. We used a factorial design (n=120) consisting of four clutches assigned to two RV treatments (RV+/RV-) and two temperature treatments (5°C/25°C) with six replicates each. Our temperatures were chosen based on historical records of low and high water temperature during February in Carolina Bays on the SRS. We also included a set of replicates without larvae in order to examine viability of RV in water alone. We are rearing larvae in 500 mL plastic containers with soft water in environmental chambers. After allowing larvae to acclimate to their temperature treatment for 48 hours we inoculated the RV+ treatments with 500 µL (PFUs being calculated now) of an FV3-like ranavirus cultured from a local marbled salamander. The RV- treatments were sham inoculated with 500 µL of the cell culture media the virus is stored in. After 24 hours of exposure, we rinsed the larvae gently with soft water and moved them fresh containers with water already adjusted to the appropriate temperature. Ten days post exposure we will sacrifice the larvae and test them for RV using qPCR. We will then repeat the experiment and swap temperature treatments between the two environmental chambers. Our results will provide insight into the role temperature plays on susceptibility to an important amphibian disease.

Contact Information: Austin Coleman, Savannah River Ecology Laboratory, University of Georgia, PO Drawer E, Aiken, SC 29802 USA. Phone: 803-725-0988, Email: acolem@uga.edu

ANALYSIS OF MAJOR CAPSID PROTEIN, ORF 52R GENES AND CONCONATED SEQUENCES FROM 24 *RANAVIRUS* ISOLATES FROM THE UNITED KINGDOM

Amanda L.J. Duffus[1], Trent W.J. Garner[2], Ashley W. Dean[1], ***Amanda R. Davis***[1]*, and Richard. A. Nichols*[3]

[1]Department of Biology, Gordon State College, Barnesville, GA, USA
[2]School of Biological and Chemical Sciences, Queen Mary, University of London, London, UK
[3]Institute of Zoology, Zoological Society of London, London, UK

Ranaviruses are emerging infections in lower vertebrates and have been associated with population declines in common frogs (*Rana temporaria*) in the UK. Ranaviruses began to emerge in the UK over 25 years ago, yet to date only preliminary attempts have been made to determine the number of amphibian species that are affected or to isolate and characterize the virus(es) present. In this study, different species of amphibians were opportunistically sampled and screened for the presence of the major capsid protein (MCP) gene of frog virus 3 (FV3). FV3 – like ranavirus(es) were isolated from infected tissues and grown in cell culture. This culture was then used to provide materials to obtain partial sequences of the MCP and whole sequences of open reading frame (ORF) 57r, an eIF-2α homologue. These gene sequences were then used to make inferences about the relationships between the different isolates. Some geographic clustering of related sequences was observed. High homology between all ranavirus isolates, regardless of species of origin, suggests that transmission between species is the result of pathogen spill over. This pattern is consistent with previous work establishing that these viruses can be multihost pathogens.

Contact Information: Amanda L. J. Duffus, Department of Biology, Gordon State College, Barnesville, GA 30204 USA. Phone: 678-359-5464, Fax: 678-359-5850, Email: aduffus@gordonstate.edu

RANAVIRUS PHYLOGENETICS USING FULL MAJOR CAPSID PROTEIN SEQUENCES: A COMPARISON OF METHODS

Ashley W. Dean *and Amanda L. J. Duffus*
Department of Biology, Gordon State College, Barnesville GA USA

Ranaviruses are globally emerging infections in ectothermic vertebrates. Much of our knowledge of the relationships between different strains and isolates of ranaviruses is based upon the phylogenetic analysis of partial or full sequences of the major capsid protein (MCP), which is highly conserved throughout the genus *Ranavirus*. Using 28 complete MCP sequences obtained from GenBank, we compare three tree building methods. Maximum likelihood (ML), Neighbor Joining (NJ) and Minimum Evolution (ME), as well as, all of the different models of nucleotide substitutions that are available in the software package Molecular Evolution and Genetic Analysis (MEGA) version 6. Regardless of the model of nucleotide substitution used, ML trees had two major clades and similar branching patterns. The trees produced using NJ methods in some cases had two or three deep multifurcating branches before further branching occurs, similar to trees build using ME methods. The NJ and ME trees build using the same nucleotide substitution models have similar, but not necessarily identical, branching patterns. In the future, it would be beneficial to use multiple isolates (minimum of two per strain) to increase the confidence that the branching pattern seen is as accurate as possible.

Contact Information: Amanda L. J. Duffus, Department of Biology, Gordon State College, Barnesville, GA 30204 USA. Phone: 678-359-5464, Fax: 678-359-5850, Email: aduffus@gordonstate.edu

THE GLOBAL RANAVIRUS REPORTING SYSTEM

Amanda L. J. Duffus[1], *Deanna H. Olson*[2], *Debra L. Miller*[3,4], *Jesse L. Brunner*[5], *Kristine M. Smith*[6], *Andrew G. Huff*[6], *and Matthew J. Gray*[3,7]

[1]Department of Biology, Gordon State College, Barnesville, GA, USA
[2]Pacific Northwest Research Station, US Forest Service, Corvallis, OR, USA
[3]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[4]Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA
[5]School of Biological Sciences, Washington State University, Pullman, WA, USA
[6]EcoHealth Alliance, New York, NY, USA
[7]Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, TN, USA

The Global Ranavirus Reporting System (GRRS) is an open-access internet-based data repository that is focused on ranaviruses. The web-based system features an online spatial database for global disease occurrence. The GRRS was developed in conjunction with scientists from the Global Ranavirus Consortium, U.S. Forest Service, and EcoHealth Alliance to enable sharing of ranavirus surveillance and pathology data. Reports will be screened by a board of experts, and ranked according to data integrity. The reporting system and spatial database include data import and export functions. Compilation of existing ranavirus data is in progress and the website is expected to launch in mid-2015.

Contact Information: Amanda L. J. Duffus, Department of Biology, Gordon State College, Barnesville, GA 30204 USA. Phone: 678-359-5464, Fax: 678-359-5850, Email: aduffus@gordonstate.edu

WHOLE GENOME RANAVIRUS PHYLOGENETICS: A COMPARISON OF TREE BUILDING METHODS AND NUCLEOTIDE SUBSTITUTION MODELS

Amanda L. J. Duffus *and Ashley W. Dean*
Department of Biology, Gordon State College, Barnesville, GA USA

Ranaviruses are emerging pathogens of ectothermic vertebrates on a global scale. Most of our understanding of the evolutionary relationships between different species, strains and isolates of ranaviruses comes from analyses of one or more partial or complete genes from genomes that are over 100kbp. Rarely, have full genomes been used, perhaps because of the difficulties associated with obtaining sequence data from full genomes in labs that are not equipped for it. Using 10 complete *Ranavirus* genomes obtained from GenBank, we compare and contrast not only three different types of tree building methods (Maximum likelihood (ML), Neighbor Joining (NJ) and Minimum Evolution (ME)), but also all of the models of nucleotide substation available in the freely available software Molecular Evolution and Genetic Analysis (MEGA) version 6. In general, the ML trees, had the same branching pattern, and the branches were highly supported (Bootstrap values greater than 95), regardless of the model of nucleotide substitution that was used. The similar branching patterns were observed in the NJ and ME trees, regardless of the model of nucleotide substitution used. However, in the ME tree built using maximum composite likelihood had several branches that were not well supported (Bootstrap values of less than 50). For both NJ and ME tree building methods, the Tamura 3 Parameter and Tamura-Nei models failed to compute phylogenetic trees. While it appears that robust trees can be obtained using most tree building and models of nucleotide substitutions with *Ranaviruses*, it is important to note that only one isolate per species/strain was used. If multiple isolates of strains/species are available, then a minimum of two should be used to ensure that the best tree is made, ideally with isolates of the same strain/species grouping together.

Contact Information: Amanda L. J. Duffus, Department of Biology, Gordon State College, Barnesville, GA 30204 USA. Phone: 678-359-5464, Fax: 678-359-5850, Email: aduffus@gordonstate.edu

RANAVIRUS COULD FACILITATE EXTINCTION IN RARE AMPHIBIAN SPECIES

Julia E. Earl[1], Jordan C. Chaney[2], William B. Sutton[3], Carson E. Lillard[2], Andrew J. Kouba[5], Cecilia Langhorne[6], Jessi Krebs[7], Rebecca P. Wilkes[4], Rachel D. Hill[2], Debra L. Miller[2,4], and ***Matthew J. Gray[2]***

[1]Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK, USA
[2]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[3]Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN, USA
[4]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA
[5]Conservation and Research Department, Memphis Zoological Society, Memphis, TN, USA
[6]Department of Biochemistry and Molecular Biology, Mississippi State University, Starkville, MS, USA
[7]Omaha's Henry Doorly Zoo and Aquarium, Omaha, NE, USA

There is growing evidence that pathogens play a role in population declines and species extinctions. We estimated the relative susceptibility of two amphibian species of conservation concern (the dusky gopher frog [*Lithobates sevosus*] and the boreal toad [*Anaxyrus boreas boreas*]) to an emerging pathogen (ranavirus) using laboratory challenge trials, and combined these data with published demographic parameter estimates to simulate the potential effects of ranavirus exposure on extinction probability in natural populations. We also examined the effects of life stage when pathogen exposure occurred, pathogen exposure interval, hydroperiod of the breeding habitat (gopher frogs only), population carrying capacity (boreal toads only), and immigration (boreal toads only) in the simulations. We found that both species were highly susceptible to ranavirus when exposed to ranavirus in water at an environmentally relevant concentration. Dusky gopher frogs experienced 100% mortality in four out of six life stages tested, while boreal toads experienced 100% mortality when exposed to ranavirus as larvae or metamorphs. Simulations generally showed population declines, greater extinction probability, and faster times to extinction when exposure to ranavirus occurred. Immigration did very little to mitigate the effects of ranavirus exposure in boreal toads unless immigration occurred every two years. Our results demonstrate that disease-induced extinction by emerging pathogens, such as ranavirus, is possible, and that threat may be high for rare species. For the species in this study, conservation organizations should incorporate ranavirus surveillance into annual monitoring programs, and devise intervention strategies in the event ranavirus exposure occurs.

Contact Information: Matthew J. Gray, Center for Wildlife Health, University of Tennessee, 274 Ellington Plant Sciences Building, Knoxville, TN 37996 USA. Phone: 865-974-2740, Fax: 965-974-4714, Email: mgray11@utk.edu

DURATION OF INFECTIOUS RANAVIRUS UNDER ENVIRONMENTAL CONDITIONS

Yi Geng[1,2]*, Matthew J. Gray*[2]*, Carson E. Lillard*[2]*, Reilly T. Jackson*[2]*, Benjamin S. Wilson*[2]*, Rebecca P. Wilkes*[3]*, Rachel D. Hill*[2]*, and Debra L. Miller*[2,3]

[1]College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, China
[2]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[3]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Environmental persistence of pathogens is a fundamental process in epidemiology that can affect the probability of disease outbreaks. Ranaviruses are a group of emerging pathogens that infect fish, reptiles and amphibians. Previous eDNA and virus culture research suggests that persistence of infectious ranavirus virions in water may be <24 hours. We explored the environmental persistence of a *Frog virus 3* (FV3)-like ranavirus by inoculating aged tap water and pond water with the pathogen (10^3 PFU/mL), and adding green frog (*Lithobates clamitans*) tadpoles at different post-inoculation (PI) durations (0, 1, 3, 6, 12, 24, and 48 hours). In both water types, infection and mortality occurred in all PI durations, with slower and lower mortality generally occurring for 24 and 48-hour durations. Also, slower and lower mortality occurred in pond water compared to aged tap water, suggesting microbes in pond water might reduce persistence or interfere with infection. Our results indicate that persistence of infectious FV3-like virions that are shed by hosts is at least 48 hours in water. Environmental persistence of pathogens outside of hosts increases the probability of density-independent transmission, which can lead to disease-induced population extinctions.

Contact Information: Yi Geng, Department of Veterinary Pathology, College of Veterinary Medicine, Sichuan Agricultural University, Xinkang Road 46, Ya'an, Sichuan, 625014 PR China. Phone (USA): 865-974-0465, Fax (USA): 965-974-4714, Email: gengyisicau@126.com

PRELIMINARY EVIDENCE FOR INCREASED VIRULENCE OF RANAVIRUSES IN CAPTIVE POPULATIONS

Yi Geng[1,2], *Matthew J. Gray*[2], *Carson E. Lillard*[2], *Reilly T. Jackson*[2], *Benjamin S. Wilson*[2], *Rebecca P. Wilkes*[3], *Rachel D. Hill*[2], *Thomas Waltzek*[4], *Yumi Une*[5], *Jesse L. Brunner*[6], *and Debra L. Miller*[2,3]

[1]College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, China
[2]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA
[3]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA
[4]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[5]School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan
[6]School of Biological Sciences, Washington State University, Pullman, WA, USA

The virulence trade-off hypothesis predicts that pathogen virulence will increase if transmission is guaranteed and there are abundant hosts. These conditions often exist in captive aquaculture facilities where fish and amphibian hosts are raised for human consumption or conservation. Several studies have shown that ranavirus isolates from captive facilities are more pathogenic than wild isolates; however, these studies compared one captive and one wild isolate (i.e., no replication). We tested for differences in virulence (as indexed by percent mortality and infection) among three ranavirus isolates from captive populations and three isolates from wild populations by performing controlled experimental challenges with tadpoles of two common amphibian host species (green frog, *Lithobates clamitans*; American bullfrog, *L. catesbeianus*). Mortality and infection were on average 30% greater for captive isolates compared to wild isolates for green frog tadpoles. Infection and mortality of bullfrog tadpoles were less than green frog tadpoles, and only one of the captive isolates was more pathogenic than the wild isolates. Our results provide preliminary evidence for the evolution of increased virulence of ranaviruses in captive populations; however, the effects on host species are not uniform. Aquaculture facilities should consider routinely monitoring captive animals for ranavirus infection, and implementing strategies to thwart the evolution of pathogen virulence, such as reducing animal densities and quarantining or euthanizing infected individuals.

Contact Information: Yi Geng, Department of Veterinary Pathology, College of Veterinary Medicine, Sichuan Agricultural University, Xinkang Road 46, Ya'an, Sichuan, 625014 PR China. Phone (USA): 865-974-0465, Fax (USA): 965-974-4714, Email: gengyisicau@126.com

BENCH TO CLASSROOM: EXPLORATION OF *RANAVIRUS* AS A TEACHING LABORATORY MODEL SYSTEM

Thomas B. Lentz *and Robert M. Kelly*
Biotechnology Program, North Carolina State University, Raleigh, NC, USA

Science education is significantly augmented by application of the subject in laboratory exercise. In developing a virus biotechnology course for the Biotechnology Program at North Carolina State University (www.ncsu.edu/biotechnology), we have sought a model system that will introduce students to challenging and relevant molecular virology techniques. Here, we outline a classroom laboratory curriculum based on Ranavirus systems. Many aspects of Ranavirus biology are well suited to the teaching laboratory, while at the same time circumventing concerns presented by many other viral systems. Ranaviruses require nominal (BSL1) biosafety precautions. The culture substrate, ectothermic animal cells, has relatively low maintenance requirements and is readily adaptable to the classroom laboratory environment. And, perhaps most importantly, Ranaviruses are complex biological systems, with tractable infection of cell cultures. In the proposed course, students will use this system to: 1) learn ectothermic animal cell culture, 2) study cytopathic effect of viral infection, 3) quantify viral genome replication, and 4) detect viral gene expression. An experimental timeline for the proposed course, which is designed for upper level undergraduate and graduate students, is provided in detail. Specific learning objectives are described for each experimental protocol. Highlighted throughout are points of consideration likely to benefit from the input of this expert audience. Through this presentation, we hope to gain insights and critique from the Ranavirus scientific community that will help improve the design and execution of this new course.

Contact Information: Thomas B. Lentz, Biotechnology Program, North Carolina State University, 2800 Faucette Drive, Raleigh, NC 27695-7512 USA. Phone: 919-513-7685, Email: tblentz@ncsu.edu

SUSCEPTIBILITY OF EASTERN HELLBENDER LARVAE TO RANAVIRUS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Carson E. Lillard[1], *Debra L. Miller*[1,2], *Reilly T. Jackson*[1], *Benjamin S. Wilson*[1], *Rebecca P. Wilkes*[2], *Rachel D. Hill*[2], *Yi Geng*[1,3], *and Matthew J. Gray*[1]

[1]Center for Wildlife Health, University of Tennessee, Knoxville TN, USA
[2]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA
[3]College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, China

The eastern hellbender (Cryptobranchus alleganiensis alleganiensis) of North America is declining in several watersheds in its geographic range, and pathogens may be playing a role. Stressors such as water temperature, decreased dissolved oxygen, and increased nitrogenous compounds may increase the virulence of pathogens. Ranaviruses are known to infect eastern hellbenders; however, reports of morbidity due to this pathogen are rare. Further, preliminary ranavirus challenge experiments that we performed at 15 °C with larval hellbenders resulted in no morbidity. Hellbenders in Tennessee, USA, can be exposed to water temperatures between 20 – 25 °C, which is within the optimal temperature range for replication of FV3-like ranaviruses. Additionally, water flow can be reduced in lotic hellbender habitats due to hydroelectric activities, which could affect concentration of dissolved oxygen and nitrogenous compounds. We performed a series of ranavirus challenges in flow-through aquaria with larval hellbenders at 22 °C and two water velocities. Hellbender survival and water quality were monitored for 28 days, and viral load in tissues quantified using qPCR. Data collection is ongoing and results from this study will be presented. These results will be useful in understanding the risk of ranaviruses to hellbenders, especially during warmer months and in rivers where water flow is reduced.

Contact Information: Carson Lillard, Center for Wildlife Health, Department of Forestry, Wildlife and Fisheries, University of Tennessee, 274 Ellington Plant Sciences Building, Knoxville, TN 37996 USA. Phone: 865-974-2740, Fax: 965-974-4714, email: clillar1@vols.utk.edu

THE IMPACT OF CO-INFECTION ON PATHOGEN FITNESS AND VIRULENCE: EXPERIMENTAL TESTS IN THE *XENOPUS LAEVIS*-RANAVIRUS MODEL SYSTEM

Jesse Brunner and ***Sarah Meiners***
School of Biological Sciences, Washington State University, Pullman, Washington, USA

The question of why some pathogens are highly virulent while others are benign is fascinating and important. Several decades of empirical and theoretical studies have shown that optimal pathogen virulence is a consequence of trade-offs between competing fitness traits. Aggressive host exploitation, for instance, may facilitate rapid transmission, but at the cost of a shorter infectious period and thus fewer transmission opportunities. While this trade-off between virulence in a host and transmission between hosts has received widespread attention, according to theoretical predictions, the outcome of competition between co-infecting pathogens within a single host is potentially more important for virulence evolution and pathogen fitness. Although studies indicate that co-infection with related pathogens is common across host taxa, there are few empirical tests of these theoretical predictions in vertebrate-pathogen systems.

We experimentally test how co-infection influences pathogen fitness and virulence in a model vertebrate system: FV3 ranaviruses infecting the African clawed frog, *Xenopus laevis.* We measure potential transmission (with various quantifications of virus replication and shedding) and virulence (with case mortality—proportion of infected tadpoles that die—divided by median time to death) of two FV3 ranaviruses in co-infections relative to single infections.

We use simple models of the basic reproductive rate, R_0, modified to include all measured routes of transmission. This integrates separate measures into a single metric of viral fitness to compare treatments of co-infected individuals to treatments of individuals infected with only one of the FV3 strains. A decrease of viral fitness in co-infections relative to single infections suggests strong competition between the strains, whereas no change or an increase in fitness in each of the strains may indicate that the closely related strains do not robustly compete and can be co- transmitted—a phenomenon that could lead to the persistence of virulent pathogens in a population.

Contact Information: Sarah Meiners, School of Biological Sciences, Washington State University, PO Box 644236, Pullman, WA 99164 USA. Phone: (636)-399-3043, Email: sarah.meiners@email.wsu.edu

PREVALENCE OF RANAVIRUS IN TERRESTRIAL AND AQUATIC TURTLES IN SOUTH GEORGIA

Brittany A. Mixon*, J.M. Lockhart, D.L. Bechler, and J.W. Hall*
Department of Biology, Valdosta State University, Valdosta, GA, USA.

Ranavirus, a virus thought to occur through ingestion or direct contact with infected organisms, has been observed as the leading cause of numerous recent amphibian and reptilian disease outbreaks and mortality events. Clinical signs associated with ranavirus infection include: palpebral edema, ocular discharge, fluid drainage, respiratory distress, nasal discharge, cutaneous abscesses, anorexia, and lethargy. Turtles were collected and sampled for ranavirus from January-December 2014 at Lake Louise Field Station in Lowndes County, GA and a private residence in Lowndes County, GA which contained a captive turtle enclosure. Upon capture, turtles were measured, weighed, and sexed. Oral swabs were collected using cotton-tipped applicators and stored at -20 °C until further analysis. 70 oral swabs were collected from four turtle species including, Eastern Box turtles (*Terrapene carolina*), Loggerhead Musk turtles (*Sternotherus minor*), Common Musk Turtles (*Sternotherus odoratus*), and Yellow-bellied Sliders (*Trachemys scripta*). Ranavirus testing will include an ELISA test to detect antibodies reactive to ranavirus. We hypothesize that the terrestrial and aquatic turtles will not test positive for ranavirus due to the fact that this virus has not been found within wild or captive populations of amphibians and reptiles in South Georgia. However, we think that the Eastern box turtle population within the enclosure may experience higher levels of stress, thus allowing for potential infections to occur.

Contact Information: Brittany Mixon, Biology Department, Valdosta State University, 1500 N. Patterson Street, Valdosta, GA 31698 USA. Phone: (912)337-4455, Email: bamixon@valdosta.edu

INVESTIGATING THE ROLE OF INVASIVE SPECIES AS RESERVOIRS OF *RANAVIRUS* FOR CHILEAN NATIVE ANURANS

Claudio Soto-Azat, ***D. Alexandra Peñafiel-Ricaurte****, and Nicole Sallaberry-Pincheira*
Facultad de Ecologia y Recursos Naturales, Universidad Andres Bello, Santiago, Chile.

Invasive species may play an important role in the spread of emerging infectious diseases (EIDs). In Chile, the African clawed frog (*Xenopus laevis*) has established feral populations since the 1970s and recently, this species has been proposed as a vector for ranaviruses. Moreover, ranaviruses are known for their low specificity, being able to infect not only amphibians, but also fish and reptiles. This low specificity could allow different species of invasive fish to participate in the epidemiology of ranaviruses, possibly acting as reservoirs or vectors to native amphibian species. In order to test this hypothesis, 69 native frogs (eight different species), 200 invasive frogs (*X. laevis*), 68 native fish, and 97 invasive fish were collected from different sites across central and southern Chile. DNA was extracted from liver, kidney and spleen of the invasive species, and from oral swabs of the native species; and then amplified using conventional PCR with *Ranavirus*-specific primers following published protocols and using a positive control obtained from previously isolated *Ranavirus*. Preliminary results showed a low prevalence of *Ranavirus* infection in invasive and native frogs. Currently, efforts to isolate the local ranaviruses for further genomic characterization are being undertaken.

Contact Information: Alexandra Peñafiel-Ricaurte, Facultad de Ecologia y Recursos Naturales, Universidad Andres Bello, Republica 440, Santiago Chile. Phone: +56226615790, Email: alexa_444@hotmail.com

GLOBAL SURVEILLANCE OF AMPHIBIAN RANAVIRUSES: A SYSTEMATIC REVIEW

Yvonne Black[1] *and* ***Stephen Price***[2]
[1]University of Edinburgh, Edinburgh, UK
[2]UCL Genetics Institute, London, UK

Amphibian species make an important and complex contribution to ecosystem services, yet 31% of them are at risk of extinction. Disease may be an important contributing factor to global amphibian declines with both the fungal pathogen Bachochytridium dendrobatidis, and species of the genus Ranavirus implicated as possible causes. Since 2009 these diseases have been notifiable to the World Organisation for Animal Health (OIE), which aims to enhance and disseminate knowledge of animal health throughout the world.

In this study a systematic review of published literature is carried out alongside questionnaires delivered to laboratories testing amphibian samples, and individuals involved in citizen science projects by an online survey engine. We find that OIE guidelines for diagnostic testing as set out in the Aquatic Manual are largely ignored and are considered irrelevant by some scientists involved in testing for amphibian ranaviruses. Reporting of the results of testing to the OIE appears inadequate, as the official summary of international disease surveillance does not reflect published data on disease distribution. Potentially useful data collected by conservation groups harnessing the enthusiasm of members of the public is available but not yet globally disseminated.

We conclude that in order to maximise the value of available surveillance resources, diagnostic testing should be internationally harmonised, reporting of results improved, and the recording and dissemination of population data collected by citizen science projects focused upon.

Contact Information: Stephen Price, UCL Genetics Institute, University College London, Gower Street, London, WC1E 6BT, UK.
Email: s.price@ucl.ac.uk

RANAVIRUS OUTBREAK IN TRANSLOCATED ENDANGERED COMMON SPADEFOOT TOADS (*PELOBATES FUSCUS*) AND SYMPATRIC WATER FROGS (*PELOPHYLAX ESCULENTUS*) AT A CONSERVATION SITE IN THE SOUTH OF THE NETHERLANDS

Bernardo Saucedo Garnica[1], *Andrea Gröne*[1], *Jolianne Rijks*[1], *Stephen van Beurden*[2], *Marja Kik*[1], *and Jouke van der Zee*[3]

[1]Dutch Wildlife Health Centre, Utrecht, Netherlands
[2]Utrecht University, Pathobiology Division, Utrecht, Netherlands
[3]Bureau Natururbalans-Limes Divergens, Utrecht, Netherlands

The translocation of amphibians conducted as part of conservation programs for endangered species pose a risk for the dispersal of pathogens. The spadefoot toad (*Pelobates fuscus*) is considered endangered in the Netherlands. The largest populations are present in the North of the country, and as part of a reintroduction project, egg masses were taken from these for the reintroduction of the species at certain sites in the South of the country, and initially raised in basins. To enhance their adaption to the environment of release, the developing amphibians were transferred as larvae into nets in outdoor ponds located in Limburg to continue their growth.

However, in 2014, soon after the transfer, both these larvae and several of the local wild water frogs (*Pelophylax esculentus*) showed mortality, and the specimens were submitted to the Dutch Wildlife Health Centre at Utrecht University for *post mortem* examination. All animals presented with necrosis in multiple organs and intracytoplasmic inclusion bodies. Conventional PCR analysis confirmed the diagnosis of Ranavirus infection.

Ranavirus infection had occurred in the spadefoot toad populations in the North in previous years, and the possibility that the egg masses could have been infected with ranavirus prior to transportation could not be discarded. Yet reliable evidence of vertical transmission is lacking in the literature. Additionally, sequencing of the major capsid protein (MCP) of the ranavirus isolate from the affected specimens revealed nucleotide differences in comparison to the strain from Common Midwife Toad Virus present in the North of the Netherlands, which may suggest the presence of a previously undetected divergent ranavirus in Limburg. Efforts to further determine the exact origin of the virus that caused the outbreak are currently being conducted.

Contact Information: Bernardo Saucedo Garnica, Department of Pathobiology, Dutch Wildlife Health Centre, Yalelaan 1, Utrecht University, Utrecht, 3584CL Netherlands. Phone: +310622339445, Email: B.SaucedoGarnica@uu.nl

PREVALENCE OF RANAVIRUS AND *BATRACHOCHYTRIUM DENDROBATIDIS* CO-INFECTIONS IN A DAKOTA COUNTY, MINNESOTA AMPHIBIAN COMMUNITY

*Dominic Travis[1], **Peter Sebastian**[1], Katie Talbott[2], Tiffany Wolf[2], Irene Bueno[1], Sara Sokolik[1], Matt McLaughlin[2], and Allan Pessier[3]*

[1]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA
[2]Minnesota Zoo, Apple Valley, MN, USA
[3]San Diego Zoo Institute for Conservation Research, San Diego, CA, USA

Major declines in amphibian populations worldwide have led to concern of a global biodiversity crisis. One of the drivers of amphibian decline is infectious disease, which includes major die-offs due to ranaviruses (family iridoviridae) and chytrid fungus (*Batrachochytrium dendrobatidis*). These pathogens are widespread and have previously been found in Minnesota, a state known for its abundance of wetlands. While our understanding of ranavirus infections continues to grow, studies have rarely investigated populations co-infected with both pathogens. Our objective was to further investigate the threat that these pathogens pose to the sustainability of wetlands and the risk factors involved with infections in a Minnesota amphibian population.

We investigated the prevalence of ranavirus and chytrid fungus as well as risk factors such as wetland type, season, and species in amphibian populations in the Minnesota Zoo's undeveloped lands (Minnesota Zoo, Apple Valley, Minnesota, USA). From May to September 2014, we sampled 271 amphibians of four species (*Anaxyrus americanus*, *Hyla spp., Lithobates sylvaticus, and Pseudacris maculata*) at three different ponds over three seasons. We swabbed each amphibian for ranavirus and chytrid fungus and submitted all samples to the Amphibian Disease Lab at San Diego Zoo for qPCR analysis. Across all samples (n=271), the prevalence of ranavirus was 46.1% (n=125) and the prevalence of chytrid fungus was 30.6% (n=83). The prevalence of co-infection was 17.3% (n=47). Despite the high prevalence of ranavirus, there was no evidence of mass die-offs. Our data also suggest that species differ in their susceptibility to both ranavirus and chytrid fungus and that ranavirus and chytrid fungus have different seasonal windows of highest susceptibility. This data represents the first step in understanding ranavirus infections in Minnesota within the framework of multiple risk factors including concurrent chytrid fungus infection.

Contact Information: Peter Sebastian, Veterinary Population Medicine, University of Minnesota, 225 Veterinary Medical Center, 1365 Gortner Ave, St. Paul, MN 55108 USA. Phone: 763-614-4296, Email: seba0028@umn.edu

PHYLOGENOMIC CHARACTERIZATION OF SQUAMATE ERYTHROCYTIC IRIDOVIRUSES

Kuttichantran Subramaniam[1], *Thomas Waltzek*[1], *Nicole I. Stacy*[2], *Claire Grosset*[3], *and James Wellehan*[4]

[1]Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[2]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA
[4]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Erythrocytic iridoviruses (EIV) have been documented in squamates within the families *Gekkonidae*, *Phyllodactylidae*, *Scincidae*, *Cordylidae*, *Lacertidae*, *Pythonidae*, *Colubridae*, *Viperidae*, *Varanidae*, *Iguanidae*, *Phrynosomatidae*, *Agamidae*, and *Chamaeleonidae*. Interestingly, similar viral agents have also been reported in more than 20 species of anadromous and marine fishes throughout the Atlantic and Pacific Oceans, as well as amphibians. However, the phylogenetic relationship of these viruses to other iridoviruses remains unclear to date. In this study, we compared the light microscopic abnormalities of infected cells, the ultrastructural morphology and phylogenetic relationship of EIVs to other iridoviruses. Recently, EIVs were partially characterized in a wild Peninsula ribbon snake (*Thamnophis sauritus sackenii*) and captive-bred inland bearded dragons (*Pogona vitticeps*). The Peninsula ribbon snake displayed two types of cytoplasmic inclusions in erythrocytes, polychromasia, anisocytosis, and hypochromasia, while the erythrocytes of the bearded dragon exhibited prominent blue-staining inclusions within normal appearing erythrocytes. Cytoplasmic inclusion bodies within erythrocytes examined by transmission electron microscopy (TEM) revealed enveloped icosahedral particles morphologically consistent with iridoviruses. The complete genome of the EIV from Peninsula ribbon snake (*Thamnophis sauritus sackenii*; TsEIV) comprises 111,413 bp nucleotides which encodes 115 potential open reading frames (ORFs). Phylogenetic analysis based on 19 conserved genes shows that the squamate EIVs form a well-supported clade distinct from other established iridoviral genera, and likely represent a novel genus. We propose the genus Hemocytivirus for this new clade of iridoviruses to reflect their predilection for red blood cells.

Contact Information: Kuttichantran Subramaniam, Department of Infectious Diseases and Pathology, University of Florida, Building #1379, Mowry Road, Gainesville, FL 32611, USA, Phone: (352) 215-7278, Email: kuttichantran@ufl.edu

EFFECTS OF MULTIPLE STRESSORS ON SUSCEPTIBILITY OF MARBLED SALAMANDERS (*AMBYSTOMA OPACUM*) TO RANAVIRUS

***Megan E. Winzeler**, David E. Scott, and Stacey L. Lance*
Savannah River Ecology Lab, University of Georgia, Aiken, SC, USA

Amphibians experience multiple natural and anthropogenic stressors during their development. The effects of these stressors on larval amphibians include decreased survival, lengthened larval periods, and other physiologic changes. How the combination of stressors can influence an amphibian's susceptibility to disease is not well understood. In a previous study we found a correlation between presence of metal contaminants, including copper (Cu), and increased prevalence of amphibian pathogens. However, that study was confounded by multiple factors, including that all contaminated wetlands also had a long hydroperiod. To begin addressing the direct relationship between exposure to Cu and/or a shortened hydroperiod we designed a laboratory study incorporating experimental challenges of FV3-like ranavirus. Our study examines the effects of shortened hydroperiod and exposure to Cu alone and in combination on the susceptibility of larval marbled salamanders (*Ambystoma opacum*) to FV3. We collected 8 clutches of eggs, mixed them, and hatched them at the same time. We are rearing individual larvae in four treatments: Cu (30 μg/ml), shortened hydroperiod (periodic removal of water from the container), a combined Cu (30 μg/ml) and shortened hydroperiod treatment and a control. Each treatment is replicated 60 times. When the larvae are 30 days old, the stress treatments will stop and for half of the replicates in each treatment (N=30) individuals will be inoculated with an FV3-like ranavirus cultured from a local marbled salamander. Exposure will occur for 24 hours and then after an additional 30 days each larvae will be euthanized. We will measure growth rates, weight, survival, and Harrison stage at the end of the experiment, as well as viral load of the FV3-like ranavirus using qPCR. The experiment is scheduled to end on 5 April 2015 and we will have the results before the symposium. This study will add to the growing body of literature on how natural and anthropogenic stressors can influence larval amphibian susceptibility to diseases like ranaviruses.

Contact Information: Megan E. Winzeler, Savannah River Ecology Laboratory, University of Georgia, PO Box Drawer E, Aiken, SC 29802 USA. Phone: 803-725-0467, Email: mewinzeler@srel.uga.edu

SINGAPORE GROUPER IRIDOVIRUS-ENCODED SEMAPHORIN HOMOLOGUE (SGIV-SEMA) CONTRIBUTES TO VIRAL REPLICATION, CYTOSKELETON REORGANIZATION AND INHIBITION OF CELLULAR IMMUNE RESPONSES

Yang Yan[1]*, Huachun Cui*[2]*, Chuanyu Guo*[1]*, Jingguang Wei*[1]*, Youhua Huang*[1]*, and Qiwei Qin*[1]

[1]Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong, China

[2]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Semaphorins are a large, phylogenetically conserved family of proteins that are involved in a wide range of biological processes including axonal steering, organogenesis, neoplastic transformation, as well as immune responses. In this study, a novel semaphorin homologue gene belonging to the Singapore grouper iridovirus (SGIV), ORF155R (termed SGIV-sema), was cloned and characterized. The coding region of SGIV-sema is 1728 bp in length, encoding a predicted protein with 575 aa. SGIV-sema contains a ~370 aa N-terminal Sema domain, a conserved plexin-semaphorin-integrin (PSI) domain, and an immunoglobulin (Ig)-like domain near the C terminus. SGIV-sema is an early gene product during viral infection and predominantly distributed in the cytoplasm with a speckled and clubbed pattern of appearance. Functionally, SGIV-sema could promote viral replication during SGIV infection in vitro, with no effect on the proliferation of host cells. Intriguingly, ectopically expressed SGIV-sema could alter the cytoskeletal structure of fish cells, characterized by a circumferential ring of microtubules near the nucleus and a disrupted microfilament organization. Furthermore, SGIV-sema was able to attenuate the cellular immune response, as demonstrated by decreased expression of inflammation/immune-related genes such as IL-8, IL-15, TNF-a and mediator of IRF3 activation (MITA), in SGIV-sema-expressing cells before and after SGIV infection. Ultimately, our study identified a novel, functional SGIV gene that could regulate cytoskeletal structure, immune responses and facilitate viral replication.

Contact Information: Yang Yan, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301 PR China. Phone: +862089023638, Email: yanyang@scsio.ac.cn

ENVIRONMENTAL FACTORS AS PREDICTORS OF RANAVIRUS EPIDEMICS AMONG AQUATIC-BREEDING AMPHIBIANS

Tess Youker-Smith[1]*, James Gibbs*[1]*, and Sadie Ryan*[2]

[1]Department of Environmental and Forest Biology, State University of New York College of Environmental Science and Forestry, Syracuse, NY, USA

[2]University of Florida, Gainesville, FL, USA

Amphibian ranavirus outbreaks are difficult to monitor in a natural setting due to their rapid and often unpredictable epidemic course, thus such studies are currently lacking. As part of a long-term wetland restoration monitoring study in a forested vernal pool system in Upstate New York, USA, we are recording ranavirus prevalence in larval populations of wood frogs (*Lithobates sylvaticus*) and green frogs (*Lithobates clamitans*) to detect epidemic trends. Our study is unique in that we are continuing surveillance both during and *between* observed outbreaks. Preliminary monitoring and testing suggests ranavirus is endemic in the area; therefore our goals are to identify both environmental and host factors that may serve as predictors of future outbreaks.

In 2010, The Upper Susquehanna Coalition in collaboration with SUNY College of Environmental Science and Forestry created a network of hydrologically isolated pools, incorporating several pre-existing pools, at Heiberg Memorial Forest in Tully, New York. Several population die-offs of larval wood frogs were observed within the first two years of restoration, and preliminary testing of liver tissue with conventional PCR assay using a 500bp segment of the FV3 major capsid protein for amplification has detected ranavirus in all die-off sites. We have since collected tissue samples of larval amphibians in each pool site where they occur, monthly from May-July over a four-year period. Samples include the two species mentioned above, ranging from Gosner stages 27-43. For each tadpole we also recorded clinical signs in the field (when applicable), total length and weight. Environmental factors including tadpole density, pool location, water temperature, depth, and pH were recorded for each sampling site.

Preliminary results from years 2011-2013 using conventional PCR analysis suggest ranavirus-positive sites have lower average water temperatures and higher average tadpole densities than negative sites. With respect to individuals, ranavirus-positive tadpoles were on average at least two Gosner stages further in development than negative tadpoles. Surprisingly, no association has thus far been detected between length of hydroperiod and likelihood of detection. We are currently verifying results and proceeding with testing of new samples using qPCR assay, to incorporate severity of infection and prevalence estimates into our analyses. We will use regression analysis to identify which suite of biotic and/or abiotic variables are most influential in ranavirus outbreaks and develop a predictive model. Since the Heiberg system incorporates both natural and constructed ponds, this is a unique opportunity to comparatively analyze disease epidemics (and continuing population responses) in each.

Contact information: Tess Youker-Smith, Department of Environmental and Forest Biology, State University of New York College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210 USA. Phone: 315-868-8719, Email: teyouker@syr.edu

Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates

Edited by Dr. Matthew Gray and Dr. V. Gregory Chinchar

Frontispiece caption: Ranaviruses are a group of emerging pathogens responsible for mass die-offs of amphibians, fish and reptiles in captive and wild populations across the globe. Global commerce of ectothermic vertebrate species and stressors may be contributing to its emergence. Photo credits (*clockwise from top left*): Matthew Allender, Nathaniel Wheelwright, Matthew Neimiller, Jonathan Kolby, Yi Geng, Yi Geng, Jonathan Kolby, and Rolando Mazzoni. Artwork by Jeanne Jones.

Available Now from Springer! Open Access!

http://link.springer.com/book/10.1007/978-3-319-13755-1