Natural stressors and ranavirus susceptibility in larval Wood frogs (*Rana sylvatica*)

By Brooke C. Reeve, Erica J. Crespi, Christopher M. Whipps & Jesse L. Brunner

Chronic exposure to stressors has been shown to suppress immune function in vertebrates, making them more susceptible to pathogens. It is less clear, however, whether many natural stressors are immunosuppressive. Moreover, whether stressors make disease more likely or more severe in populations is unclear because animals respond to stressors both behaviorally and physiologically. We tested whether chronic exposure to three natural stressors of Wood frog tadpoles—high-densities, predator-cues, and low-food conditions—influence their susceptibility to a lethal ranavirus both individually in laboratory experiments, and collectively in outdoor mesocosms.

Prior to virus exposure, we observed elevated corticosterone only in low-food treatments, although other treatments altered rates of growth and development as well as tadpole behavior. None of the treatments, however, increased susceptibility to ranavirus as measured by the proportion of tadpoles that became infected or died, or the time to death compared to controls. In fact, mortality in the mesocosms was actually lower in the high-density treatment even though most individuals became infected, largely because of increased rates of metamorphosis.

Overall we find no support for the hypothesis that chronic exposure to common, ecologically relevant challenges necessarily elevates corticosterone levels in a population or leads to more severe ranaviral disease or epidemics. Conditions may, however, conspire to make ranavirus infection more common in metamorphosing amphibians.

B. C. Reeve, E. J. Crespi, C. M. Whipps, J. L. Brunner, *EcoHealth* (2013). Volume 10, Issue 2, pp 190-200 http://dx.doi.org/10.1007/s10393-013-0834-6

Cross-tolerance in amphibians: Wood frog mortality when exposed to three insecticides with a common mode of action

By Jessica Hua, Rickey D. Cothran, Aaron B. Stoler & Rick A. Relyea

The evolution of insecticide tolerance and cross-tolerance to multiple insecticides has attracted substantial attention. However, tolerance and cross-tolerance in non-target organisms, such as amphibians, is often overlooked despite its potential to

Wood frog (*Lithobates sylvaticus*) adult. Photo J. Hua.

buffer natural systems from anthropogenic contamination. To test for tolerance and cross-tolerance, we exposed 15 populations of *Lithobates sylvatica* (Wood frogs) to three common acetylcholine esterase-inhibiting insecticides (carbaryl, chlorpyrifos, and malathion). To quantify tolerance, we conducted a time to death assay and found widespread population-leval variation in wood frog sensitivity to the three insecticides. We also demonstrate that these population-level patterns of tolerance were correlated between carbaryl and both chlorpyrifos and malathion. These findings suggest that amphibian populations that are tolerant to one pesticide may also be tolerant to several other similarly acting pesticides. With amphibians worldwide experiencing unprecedented declines and some of these declines being associated with insecticide use, quantifying the existence and prevalence of cross-tolerance may contribute important insights for conservation efforts.

J. Hua, R. Cothran, A. Stoler, R. A. Relyea, *Env. Tox. Chem.* **32**, 932 (2013).

In vitro culture of skin cells from biopsies from the Critically Endangered Chinese giant salamander, *Andrias davidianus* (Blanchard, 1871) (Amphibia, Caudata, Cryptobranchidae)

By Sarah Strauß, Thomas Ziegler, Christina Allmeling, Kerstin Reimers, Natalie Frank-Klein, Robert Seuntjens & Peter M. Vogt

A primary skin cell culture of the Critically Endangered Chinese giant salamander, Andrias davidianus, was established from small biopsies using minimal invasive methodologies. Biopsies were taken from three animals simultaneously with assessment of two biopsy sampling techniques using samples from the tail tip. Cell culture was performed in a wet chamber at room temperature. Several culture media and supplementations were tested as well as culture containers and surface coatings. The handling of A. davidianus in a landing net, without transfer out of the tank, allowed easier biopsy withdrawal. Best outgrowth of cells from explants was achieved in 60% DMEM/F12 medium with supplementation. Cells started to grow out as monolayer within the first 12 hours, and after three weeks formed pigmented multilayers, then died after 10 weeks. Primary cultures of Andrias skin cells, as well as other amphibian primary cell cultures, can be used in future studies to evaluate effects of disease, pollution, regeneration, wound healing and could provide cells for use in reproduction technologies such as cryopreservation to preserve gene lines in this and other Critically Endangered species with minimal harm to the animals.

S. Strauß *et al.*, Amphib. Rep. Conserv. 5, 51 (2013).

Experimental mesocosms used to test the interactions between amphibian density, species composition and infectious disease. Photo: Catherine L. Searle.

Development and infectious disease in hosts with complex life cycles

By Catherine L. Searle, Gisselle Yang Xie & Andrew R. Blaustein

Metamorphosis is often characterized by profound changes in morphology and physiology that can affect the dynamics of species interactions. For example, the interaction between a pathogen and its host may differ depending on the life stage of the host or pathogen. One pathogen that infects hosts with complex life cycles is the emerging fungal pathogen of amphibians, *Batrachochytrium dendrobatidis* (*Bd*). We sought to determine how conditions at the larval stage can affect variation in development and patterns of *Bd* infection across amphibian life stages. We used outdoor experimental mesocosms to simulate natural pond habitats and manipulated the presence of *Bd*, the larval density, and the number of host species in larvae of two co-occurring amphibian species (*Rana cascadae* and *Pseudacris regilla*). We found that infection differed between species throughout development; *P. regilla* consistently had higher infection severity compared to *R. cascadae*. Additionally, while up to 100% of larvae were infected, only

18.2% of *R. cascadae* and 81.5% of *P. regilla* were infected after metamorphosis. This indicates that amphibians have the ability to recover from *Bd* infection as they undergo metamorphosis. Higher larval densities in *P. regilla* led to a shorter larval period, and individuals with a shorter larval period had lower infection severity. This led to a trend where *P. regilla* larvae reared at high densities tended to have lower infection prevalence after metamorphosis. We also found that exposure to *Bd* increased larval mortality and prolonged the larval period in *P. regilla*, indicating that *P. regilla* are susceptible to the negative effects of *Bd* as larvae. This study demonstrates that host density, species composition and pathogen exposure may all interact to influence development and infection in hosts with complex life cycles.

C. L. Searle, G. Y. Xie, A. R. Blaustein, *PLoS ONE* **8**, e60920 (2013). doi:10.1371/journal.pone.0060920

A Green eyed treefrog (*Litoria serrata*) fitted with a temperature-recording radio-transmitter. This species disappeared from many sites during epidemics of the disease chytridiomycosis in the late 1980's and early 1990's, but has subsequently recovered. Photo: Jodi J. L. Rowley.

Hot bodies protect amphibians against chytrid infection in nature

By Jodi J. L. Rowley & Ross A. Alford

Environmental context strongly affects many host-pathogen interactions, but the underlying causes of these effects at the individual level are usually poorly understood. The amphibian chytrid fungus has caused amphibian population declines and extinctions in many parts of the world. Many amphibian species that have declined or have been extirpated by the pathogen in some environments coexist with it in others. Here we show that in three species of rainforest frogs in nature, individuals' probability of infection by the amphibian chytrid fungus was strongly related to their thermal history. Individuals' probability of infection declined rapidly as they spent more time above the pathogen's upper optimum temperature. This relationship can explain population-level patterns of prevalence in nature, and suggests that natural or artificial selection for higher thermal preferences could reduce susceptibility to this pathogen. Similar individual-level insights could improve our understanding of environmental context-dependence in other diseases.

J. J. L. Rowley, R. A. Alford, *Sci. Rep*. **3**, 1515 (2013)

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