On the road to disease:
testing the stress-induced susceptibility hypothesis in amphibian populations adjacent to roads

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Photo: Alex Shepak

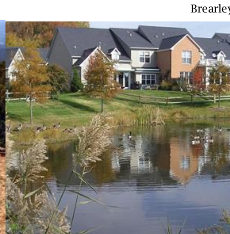
Road Map

1. Why stress can increase susceptibility to disease
2. Combining surveillance (eDNA) and dose-response experiments
3. Understanding why variance within a species across an ecological context is important

Anthropogenic effects infectious diseases in wildlife

53% (10/19) studies showing an increase in wildlife disease prevalence related to human-modified landscapes

Brearley et al. (2012) *Biol. Rev.*


Anthropogenic effects infectious diseases in wildlife

Human modified landscapes

Landscape Processes (e.g.):
- Continuity and structure
- Seasonality and timing
- Biogeochemical and physical

Transmission

+ vector/reservoir density
+ inter/intra-specific contact
+ transport from distant locations
+ patchiness/resource clumping

Host response

- Behavior and migration
- Life history trade-offs
- Physiological stress response
- Disruption of homeostasis (e.g. hormone disruptors)

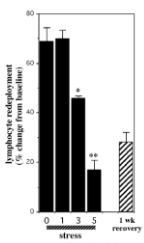
Disease impact

Modified from Brearley et al. (2012) *Biol. Rev*

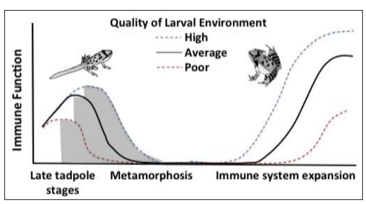
Stress-induced susceptibility hypothesis

Chronic stress is immunosuppressive, therefore environmental change that causes chronic stress will increase susceptibility to infection

(Carey et al., 2006 *Dev Comp. Immunol)*
(Rollins-Smith, 2001 *Immun Res)*



Dhabhar and McEwen 1997
Brain, Behav, Immun



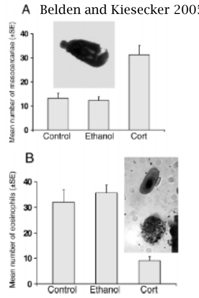
Crespi et al, *in prep.*

Examples: stress-induced susceptibility in amphibians

Stressors that suppress immune function:

- Glucocorticoids (naturally during metamorphosis)
  (e.g., Rollins-Smith 2001, *Immunol Res*; Belden and Kiesecker 2005, *J. Parasitol*; but see Searle et al 2014, *J Exp Zool*)
- Toxicity (Contaminants, agrochemicals, etc)
  (e.g., Rohr et al 2008, *Nature*; Forson and Storfer 2006, *Ecol Appl*)
- Nutritional deficit
  (Gervasi and Foufopoulos 2008, *Funct Ecol*, Venesky 2012, *Oecologia*)

Belden and Kiesecker 2005

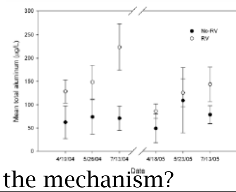


Scale up to population level?

Examples: stress-induced susceptibility in amphibians

Environmental correlates with disease:

- Agricultural fields
  (e.g., Miller et al 2009)
- Urbanization/Industry
  (e.g., Skelly et al 2006, St. Amour et al 2008)
- Catchment position
  (Gahl and Calhoun 2010)
- Roads
  (Urban 2006, *Cons Bio*; Pauza et al 2010, *DOA*)

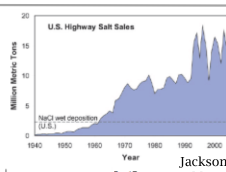


Gahl and Calhoun 2010

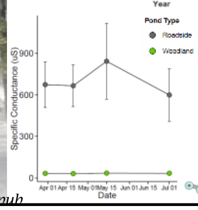
What is the mechanism?

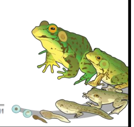
Roads are a major stressor to amphibians

Hall et al. *Unpub*

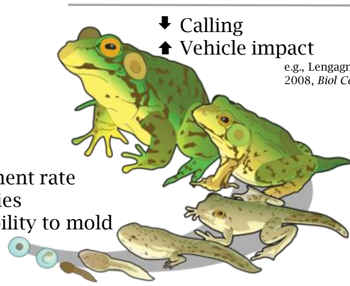


Jackson and Jobbagy, *PNAS* (2005)





Roads are a major stressor to amphibians



⬇ Calling
⬆ Vehicle impact
e.g., Lengagne 2008, *Biol Cons*

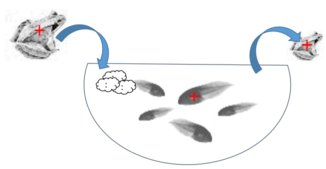
⬇ Survival
⬇ Development rate
⬆ Deformities
⬆ Susceptibility to mold
e.g., Karraker et al 2008, *Ecol Appl*

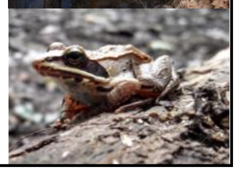
?

⬇ Growth rate
⬇ Development rate
e.g., Sanzo and Hecnar, 2006, *Environ Pollut*

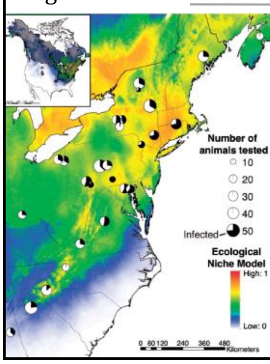
Wood frog system

- Explosive breeders
- Ephemeral fishless ponds
- Mortality from ranavirus reaches >90% of tadpoles, symptoms are observable





Wood frog system: ranavirus infection across the range



Found a higher prevalence of low-level infection in wood frog adults at center of range

- No correlation with stress hormones (GCs)
- Center of range is also highest density of human population

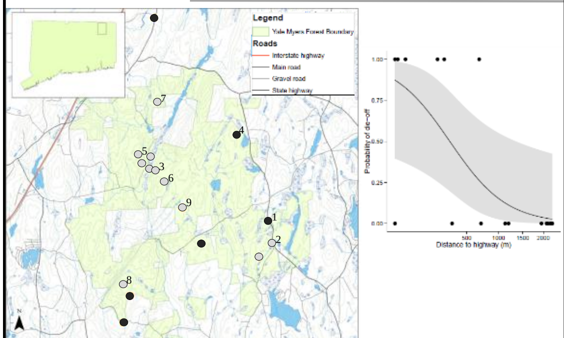
Crespi *et al* (2015), *ICB*

Testing the stress-induced susceptibility hypothesis

1. **Are roads associated with ranavirus die-offs?**
   Host density, behavior, and physiology are affected by roadside conditions, thus ranavirus dynamics will vary by proximity to roads.

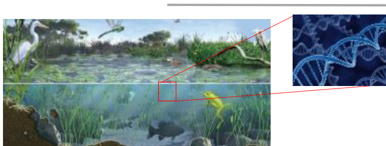
Approach:
Die-off and eDNA surveillance across a forested area dissected by roads

Die-offs were more likely to occur near highways



Hall et al., *in prep*

Wildlife disease surveillance: eDNA



eDNA is trace DNA in environmental samples. Mixture of degraded DNA from many different organisms. (Bohmann et al 2014, *Trends Ecol Evol*)

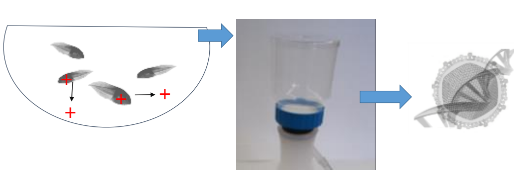
Strengths
- Detect multiple species with one sample (rare & cryptic)
- Relate pathogen and host densities
- Non-invasive, fast and easy
- High sensitivity

Weaknesses
- Not able to distinguish host species infected with pathogen
- High sensitivity prone to false positives
- Cannot distinguish between infectious/degraded pathogens

Using eDNA to detect ranaviruses in pond water

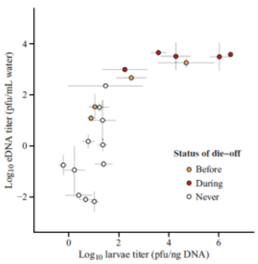
- Strengths: Not prone to false positives
- Weaknesses: Not as sensitive
- Some false negatives found -> sample more!

Sampled 20 wood frog ponds twice (before and after metamorphic climax) for eDNA (3 filters) and larvae (5 in 12 ponds)

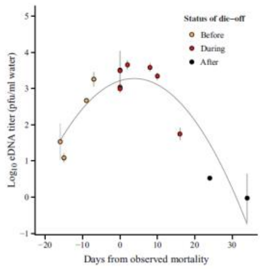


Using eDNA to detect ranaviruses in pond water

RV eDNA titers reflected tadpole titers



RV titers peaked around die-off



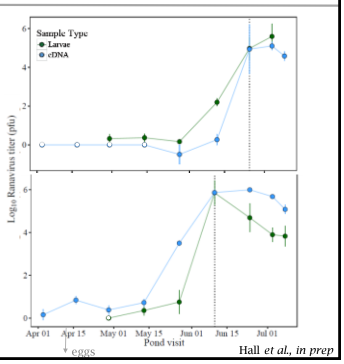
Hall *et al.*, 2015, *Molecular Ecology Resources*

Using eDNA to detect ranaviruses in pond water

2014: Sampled 8 ponds every two weeks:

- 3 eDNA samples
- 10 larval samples
- Stages, densities, water chemistry

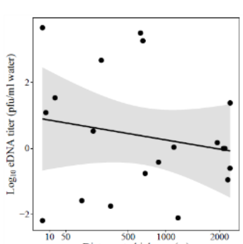
Monitored for die-offs every week (> 5 carcasses)



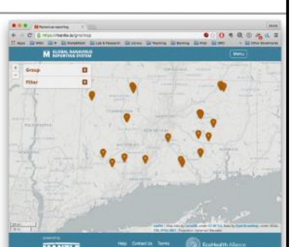
Hall *et al.*, *in prep*

What's different about where die-offs occur?

Ranaviruses are ubiquitous…



Hall *et al.*, *in prep*



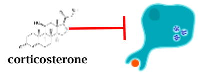
O'Connor, K.M., T.A.G. Rittenhouse, and J.L.Brunner, in prep.

…so why are die-offs more likely in some ponds but not others?

Specific hypotheses

How might road run-off increase susceptibility to infection?

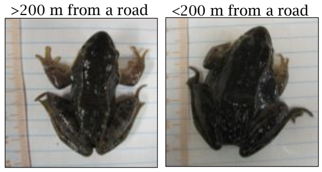
1. Glucocorticoids may directly down regulate immune function, increasing susceptibility.



Adults (Hall et al., *in prep*)
- Roadside had more bloating, and bloated adults had hormone profiles indicative of chronic stress

Tadpoles (Hall et al., *in prep*)
- Higher baseline GCs in those collected from roadside ponds (when raised in freshwater or saltwater)

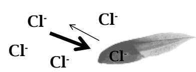
>200 m from a road | <200 m from a road



Specific hypotheses

How might road run-off increase susceptibility to infection?

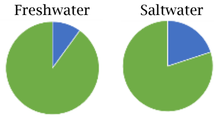
2. Osmoregulation is costly and reduces the amount of energy available for fighting infection.



Tadpoles (Hall et al., *in prep*)
- Slower growth in roadside ponds with higher salinity
- Reduced feeding behavior when raised in road salt
- Gill edema in road salt exposure in lab

Tadpole energy budget

Freshwater | Saltwater



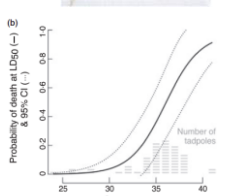
Osmoregulation
VS
Growth, storage, immune function

Specific hypotheses

How might road run-off increase susceptibility to infection?

3. Stress of poor conditions alters the timing of developmental traits associated with susceptibility.

Tadpoles (Hall et al,. *in* prep)
- Slower development rate in road salt exposure in lab
- Greater variation in development rate in roadside ponds



Warne *et al.*, 2011, *Functional Ecology*

Testing the stress-induced susceptibility hypothesis

2. Do roads increase susceptibility to ranavirus infection?
Roadside conditions influence host physiology, thus individual susceptibility to infection will be related to proximity to roads.

Approach: Dose-response exposure to FV3 ranavirus

Collect animals from ponds varying in proximity to a road
Avg. stage = 36
N=9*

→

Ranavirus (FV3) Dose Response Exposure:
Control: 0 pfu
Low: 3x10³ pfu
Med: 3x10⁴ pfu
High: 3x10⁵ pfu
N=10

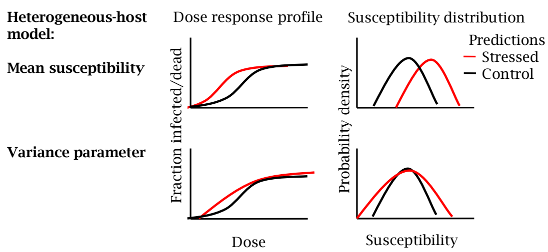
→

Measure susceptibility:
•Mortality rate
•Proportion infected

*Collected from ponds that did not have RV die-offs at that time

The power of dose-response experiments

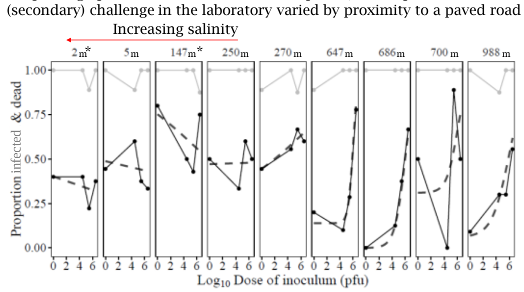
We can determine both the LD_{50} and the distribution of susceptibility using a dose-response experiment



Modified from Ben-Ami 2010 *Am Nat*

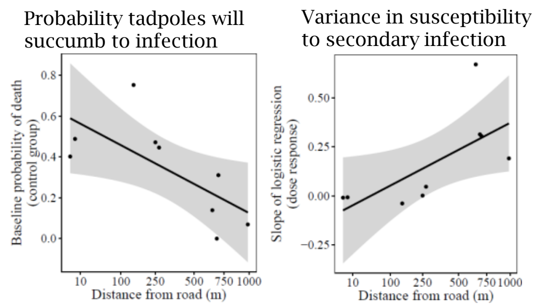
Variation across and within populations

Despite high prevalence of infection across ponds, the response to a (secondary) challenge in the laboratory varied by proximity to a paved road



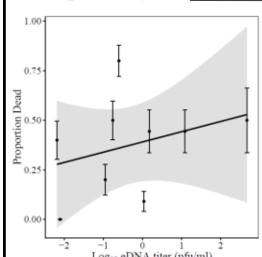
Hall et al., *in prep*

Variation across and within populations

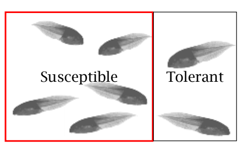


Hall et al., *in prep*

Potential explanations for differences in susceptibility

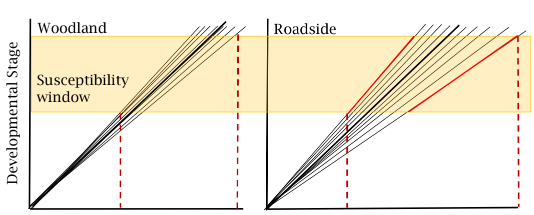


- ~~Initial exposure~~
- ~~Mortality to salinity~~
- Susceptible population already succumbed to infection



Potential explanations for differences in susceptibility

Roadside experimental enclosures had greater variance in development rate (Hall et al., *in prep*), and developmental stages vary in mortality to infection



Take home messages

1. Support for stress-induced (variance in) susceptibility (not as simple as it seems)
2. eDNA is a great tool for studying epidemiology
   - Can be related to titer in host community
   - Look at shedding rate and transmission
   - Non-invasive, minimal resources, easy to do
3. Dose-response experiments capture important factors
   - Can determine differences in lethal dose/mean susceptibility
   - Found interesting variance in susceptibility

Take home messages

1. Towards a mechanistic understanding of disease-associated declines
2. Combined lab and field experiments to strengthen associations between stressors and disease
3. Within-species differences in susceptibility across an ecological context is as important as among species

Findings and future directions

1. Does road salt stress alter transmission efficiency, immune function, or stress response to infection?
2. Does variance in development rate within a population increase the probability of a die-off?
3. Are there factors associated with the temporal pattern of when die-offs occur in a pond community?

Many Thanks

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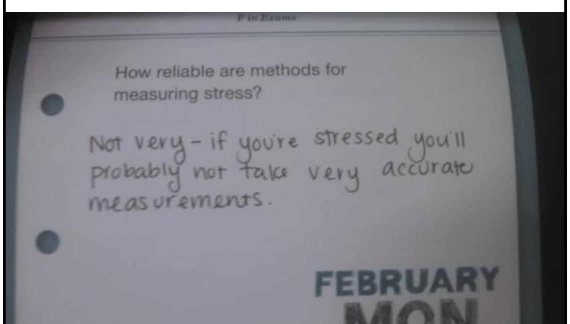
Questions?



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