STRESSORS

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CORE QUESTIONS

- What is stress?
- What are the common stressors?
- What are measures of a stress response?
- How do these influence disease risk?
- How might factors interact?

WHAT IS STRESS?

- Difficult to define-
  - Mechanical stress is tension applied to a material object
  - Emotional stress is tension resulting from demanding circumstances
  - Physiological stress is chemical response to an demanding stimulus
    - Heat shock proteins
    - Long Term Stress hormones (cortisol in humans, corticosterone in amphibians and reptiles)
    - Short Term Stress Hormones (epinephrine, norepinephrine)
  - Most of the work related to RV has been on corticosterone (CORT)

NATURAL STRESSORS

- Competition- density, food availability, habitat, mates
- Predation-
- Parasitism- Fungi, Trematodes, Bacteria, Virus
- Abiotic- Temperature, pH, salinity, etc.

- Philosophical question- If you are used to living in a stressful environment, are you stressed?

COMMUNITY ECOLOGY

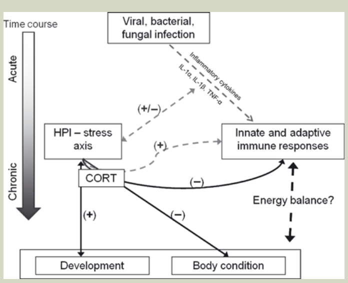
- Density vs. Trait Mediated Interactions (Pressier et al. 2005)
  - The presence of a predator alters an individual in two ways
    - Can die
    - Can be scared to die
  - Both impact resource

- Can apply to disease- how many of your behaviors are modulated via fear of disease?

- Does being afraid or stressed contribute to falling ill and vice versa?

ESCAPE FROM THE POND: STRESS AND DEVELOPMENTAL RESPONSES TO RANAVIRUS INFECTION IN WOOD FROG TADPOLES

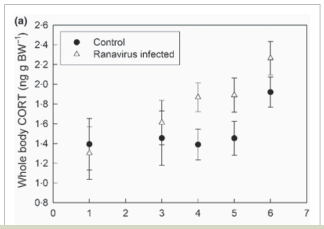
- Brunner et al. 2010



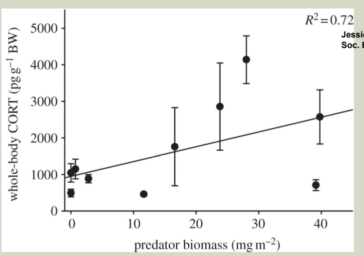
Functional Ecology
Volume 25, Issue 1, pages 139-146, 8 OCT 2010 DOI: 10.1111/j.1365-2435.2010.01793.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2435.2010.01793.x/full#f4

CORT RESPONSE TO RV

- Being sick is stressful (Warne et al. 2010)



CORT RESPONSE TO PREDATORS

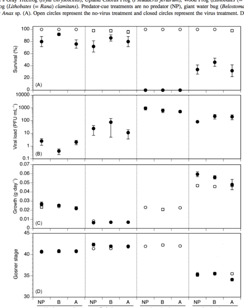


Jessica Middlemis Maher et al. Proc. R. Soc. B 2013;280:20123075

Whole-body corticosterone (CORT) content in field-collected tadpoles was positively correlated with predator biomass per square metre sampled in the same ponds (slope = 44.1, p = 0.032).

PREDATOR EFFECTS?

- Haislip et al. 2012
  - Predator cue does not increase RV susceptibility



CORTICOSTERONE

- Brunner et al. 2010

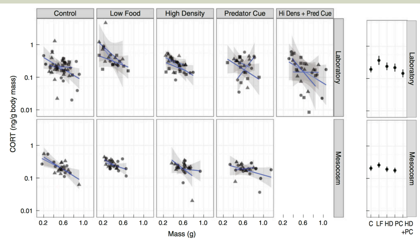


Figure 1. Relationship between log-transformed whole-body corticosterone (CORT) concentration (ng g^{-1} body mass) and body mass after 21 days (lab) or 14 days (mesocosm) of exposure to experimental treatments. Each point is an individual. *Shapes* represent replicates within the treatment. *Lines* are linear regressions fit to each replicate and the *shaded areas* are their associated 95% confidence intervals. The marginal figures show the mean corticosterone concentration for each treatment adjusted for mass with 95% confidence intervals.

MORTALITY RESULTS

- Brunner et al. 2010

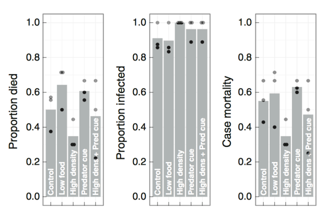


Figure 2. Proportion of tadpoles that died following ranavirus exposure in the laboratory experiment, the proportion that became infected, and the proportion of the infected animals that died (case mortality). *Bars* show the mean values and the points show the values for individual replicates.

NATURAL STRESSORS

- Strong evidence that all of these natural stressors impact survival
- No clear understanding of how they impact RV infection or recovery from infection
- Disease is another natural stressor.
  - Species have persisted with these natural stressors
  - Is it worth considering?

ANTHROPOGENIC STRESSORS

Various human impacts:

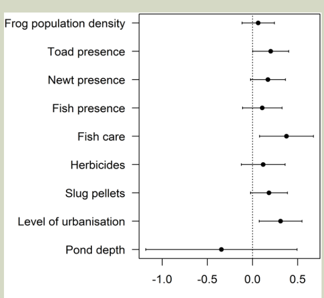
- Habitat destruction
- Global climate change
- Invasive species
- Emerging disease
- Emerging contaminants

ANTHROPOGENIC STRESSORS

Various human impacts:

- Habitat destruction
- Global climate change (Temp- Gray)
- Invasive species (Trade- Kolby)
- Emerging disease (Bd mostly)
- Emerging contaminants

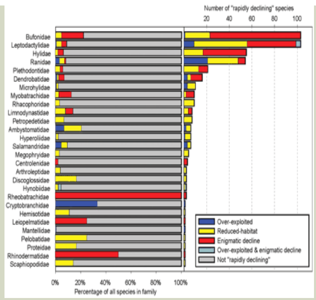
Fig 2. Abiotic and Biotic Variables Influencing Ranavirosis Occurrence.



North AC, Hodgson DJ, Price SJ, Griffiths AGF (2015) Anthropogenic and Ecological Drivers of Amphibian Disease (Ranavirosis). PLoS ONE 10(6): e0127037. doi:10.1371/journal.pone.0127037
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0127037

AMPHIBIAN DECLINES

- 33% of amphibian species threatened with extinction (Stuart et al. 2004)
- More than any other vertebrate taxon
- Enigmatic declines are disease and pollution (Collins and Storfer 2003)



Stuart et al., 2004

CONTAMINANTS

Contaminants are a important to consider because:

- Known to impact amphibians
- Impact surrounding taxa
- Ubiquitous- found even in “pristine” areas
- Variety of contaminants impact immune system in different ways.
- Lots of information available in comparable format.

CONTAMINANTS

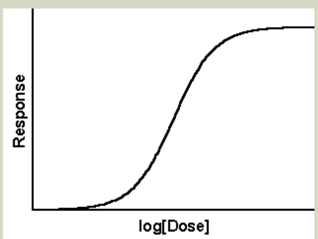
- Major groups
  - Pesticides
  - Metals
  - Pharmaceuticals
  - Organics
  - Inorganics

BASIC CONCEPTS IN TOXICOLOGY

- Dose response
- Hormesis
- Biomarkers
- Measuring risk
- Species tolerance

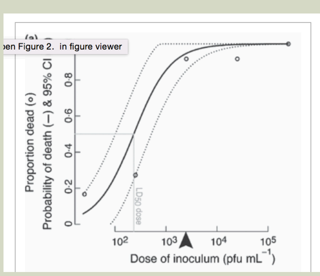
DOSE RESPONSE CURVE

- LC50- Lethal concentration where 50% of organisms die

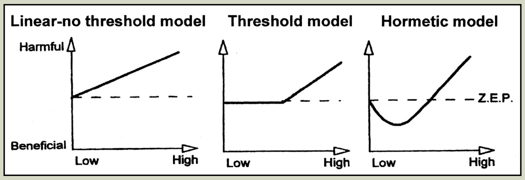


DOSE RESPONSE

- Dosage matters (Warne et al. 2010)



MODELS OF TOXICITY



BIOMARKERS

- Indicate previous exposure to a chemical
  - General or specific
- Examples
  - Heat Shock Proteins
  - Acetylcholinesterase (Neurotoxins)
  - Highly specific breakdown products

RISK

- Calculation of danger since ALL chemicals can kill at high enough concentrations
- Are specific taxa more susceptible?
- Calculations are complex but boil down to:
  - Exposure
  - Uptake
  - Elimination
- Toxicity tests help estimate effects of exposure, field data and models estimate actual exposure levels

AMPHIBIAN SENSITIVITY

- Commonly stated that amphibians are particularly sensitive:
  Science, Ecology, Conservation Biology, Popular Press
- Why are amphibians particularly sensitive?
  Reasoning: semi-permeable skin

AMPHIBIANS AS CANARIES?

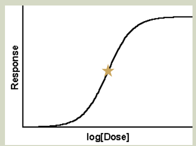
- Has this sensitivity been rigorously tested?
  - NO!
- Are amphibians the most sensitive taxon in aquatic systems?
  - Contaminants

DATABASE ANALYSIS

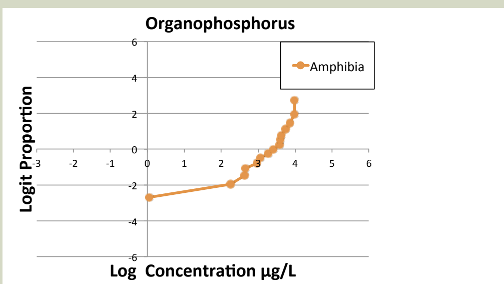
- EPA AQUIRE contains over 200,000 studies, incorporating over 9000 species and over 8000 chemicals
- 28,000 studies including 1279 species and 107 chemical agents
- Can compare across taxa and contaminant type using standard acute (48-96hr) lethality tests.

DATABASE ANALYSIS

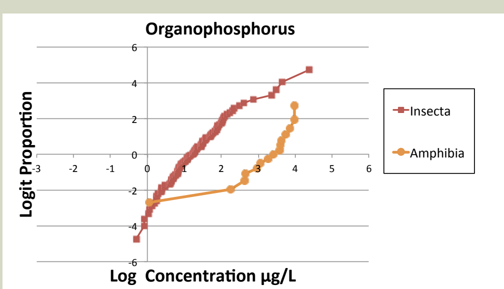
- Devised alternative method based on Ecotoxicology-
- Species Sensitivity Distribution
  - Used minimum of 7 species per taxon.
- HC50 of taxon- at what concentration are half the species impacted?



LETHALITY ESTIMATES



LETHALITY ESTIMATES



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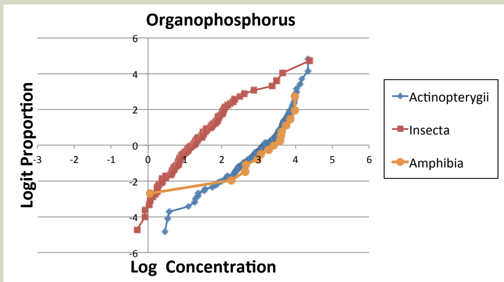
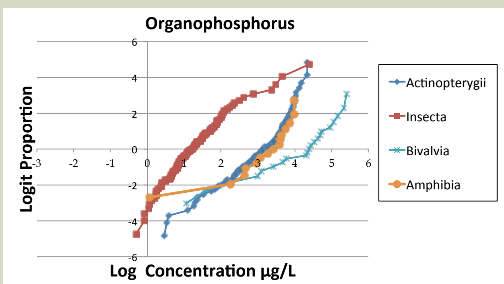
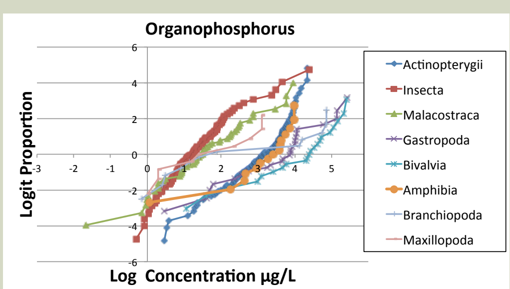
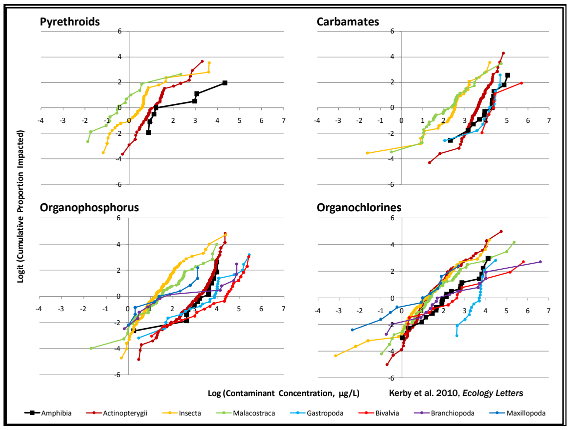


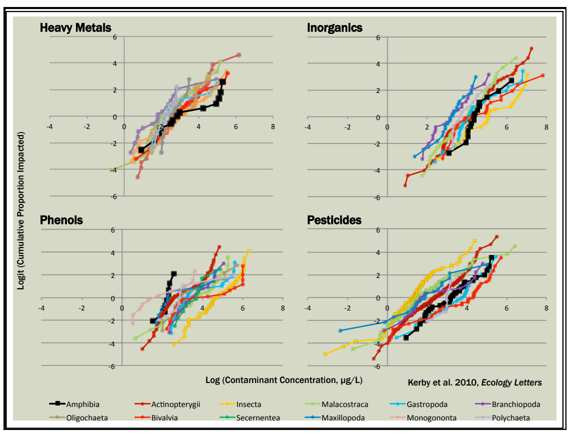
LETHALITY ESTIMATES



LETHALITY ESTIMATES

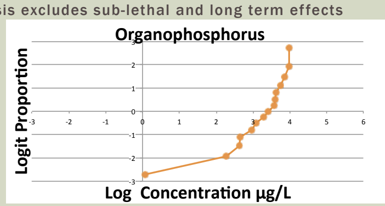






SUMMARY

- Amphibians overall are not particularly sensitive to contaminants.
- Range of sensitivity is important- particular species very sensitive to contaminants.
- This analysis excludes sub-lethal and long term effects



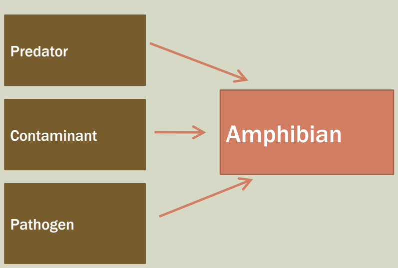
REPRISE

- The impact of natural stressors depend on the species.
- The impact of anthropogenic stressors depend on the species.
- Despite this, there are clear impacts!

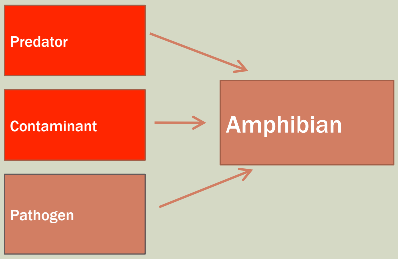
EXPERIMENTS

- Data on interaction between RV and contaminants are sparse.
- Complicated to pick out in field (enigmatic)
- I will highlight the few contaminant studies in the literature

STRESSORS

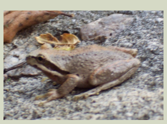


MULTIPLE STRESSORS



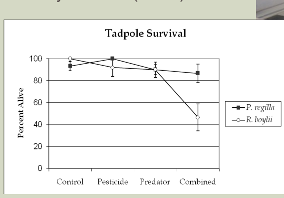
PREDATION STUDY

- Tested two species of frogs (*Pseudacris regilla, Rana boylii*)

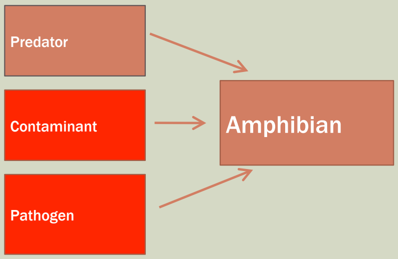


PREDATION STUDY

- 50 µg/L carbaryl for 7 days
- One crayfish predator with 5 tadpoles of each species in 10L of water.
- Kerby and Sih (2015)



MULTIPLE STRESSORS



ATRAZINE IMPACTS

- Increased concentrations impact infection rate
- Hormesis
- Forson and Storfer 2006

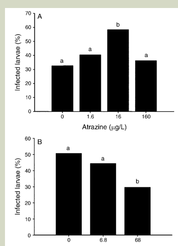
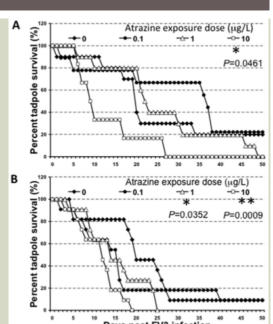


FIG. 1. Effect of atrazine ($G^2 = 52.35$, df = 36, $P = 0.038$) and sodium nitrate ($G^2 = 45.77$, df = 32, $P = 0.054$) on viral infection rate. (A) The number of ATV-infected larvae is higher at 16 μg/L atrazine than at the other atrazine treatments levels. (B) The number of ATV-infected larvae is lower at the highest treatment level of 68 mg/L compared to the other treatments. Different letters represent significantly different comparisons ($P < 0.05$).

ATRAZINE IMPACTS

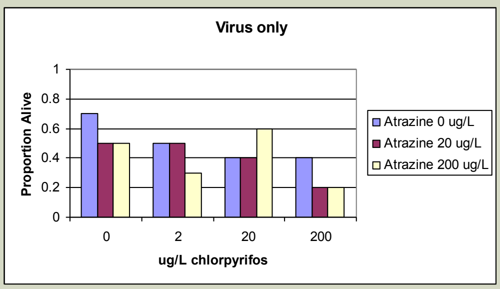
- Increased concentrations impact mortality rate
- Sifkarovski et al. 2014



MULTIPLE CONTAMINANTS

- Virus/ no virus
- Atrazine – 3 levels: 0, 20, 200 µg/L
- Chlorpyrifos – 4 levels: 0, 2, 20, 200 µg/L
- Tox model- will the weak die or will the effect be compounded?

MORTALITY

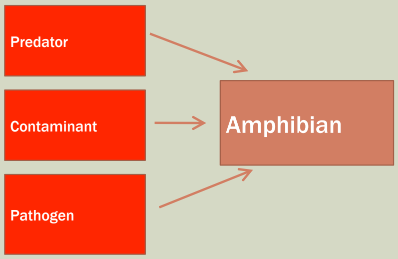


Kerby and Storfer 2009, *Ecohealth*

SUMMARY

- Sub-lethal pesticide concentrations magnify number killed by disease
- Combined chlorpyrifos, atrazine, and virus treatments show lowest survival
- Emerging pathogens – need to consider pesticides as cofactors

MULTIPLE STRESSORS

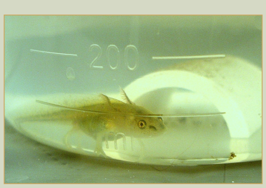


QUESTION

- Does the chemical cue of a predator alone potentiate effects of pesticides and/or virus?

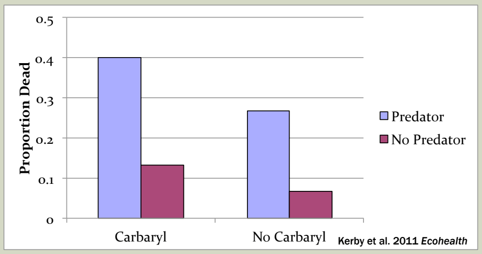
DESIGN

- Virus / No virus
- Carbaryl (500 µg/L) / No Carbaryl
- Predator Cue (Dragonfly nymph)/ No Cue



MULTIPLE STRESSORS

- Virus exposed larvae suffer higher mortality when combined with predator cue and insecticide

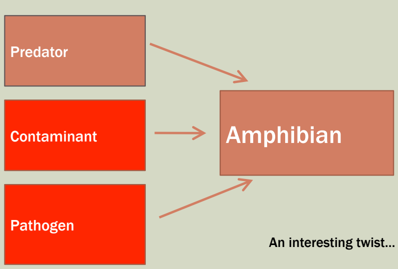


Kerby et al. 2011 *Ecohealth*

CONCLUSIONS

- Predator cue increases mortality in virus exposed larvae
- Carbaryl presence appears to increase mortality in virus exposed larvae
- Combined predator cue and pesticide exhibit least survival

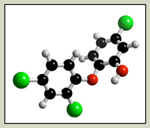
MULTIPLE STRESSORS



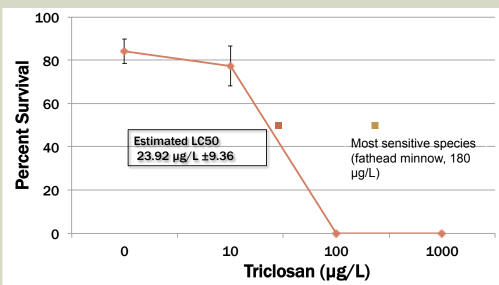
An interesting twist…

CONTAMINANT- TRICLOSAN

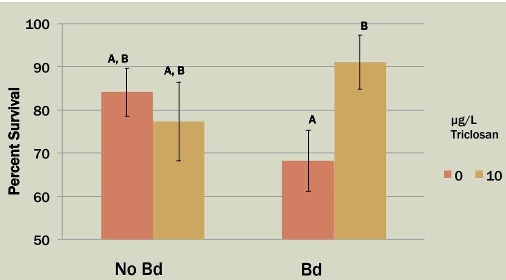
- Phenol and antimicrobial
- Commonly found in household items
  - 57.6% of US streams in 1999-2000 (Koplin et al. 2002)
  - 0.14 µg/L average (Koplin et al. 2002)
- Amphibians are sensitive to phenolic compounds (Kerby et al. 2010)



TRICLOSAN TOXICITY

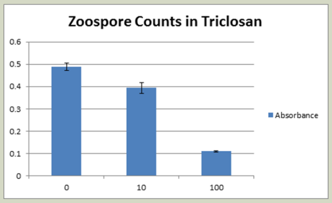


TRICLOSAN BY CHYTRID



TRICLOSAN TOXIC TO BD

- Undergraduate experiment demonstrated that Bd growth is inhibited by concentrations as low as 10μg/L
- Other work show pesticide toxicity to Bd as well



RV CASE

- Unlikely for contaminants to kill DNA virus, so less likely for this antagonism
- Other factors though might interact (Predators/Competition and RV)
  - What does FV3 do to fish predators?

ENVIRONMENTAL DEGRADATION

Open questions:

- Habitat destruction
- Global climate change
- Invasive species
- Other diseases

LARGER SCALE?

- With so many permutations, how can we know what is linked to a die off?
- What factors might a land manager mitigate?
- Is it even possible to answer?

ACADIA NATIONAL PARK

- Larger scale (Gahl and Calhoun 2010)

Table 3. Highest-ranked logistic regression models correlated with mortality events of amphibian larvae caused by ranavirus in Acadia National Park (ANP) breeding pools.

Candidate model	Log (L)*	$K^{\dagger}$	$QAIC_c$	$\Delta QAIC_c^{\ddagger}$	$w_i^{\S}$	Evidence ratio‖
Surrogate variable models						
Conductivity (–)	–13.046	3	12.95	0.00	0.12	1.00
Canopy cover (–)	–14.496	3	13.59	0.64	0.08	1.38
LICL (+)	–14.708	3	13.68	0.73	0.08	1.44
Invertebrate predators	–15.078	3	13.85	0.90	0.07	1.57
Maximum color	–15.422	3	14.00	1.05	0.07	1.69
SD temperature	–15.492	3	14.03	1.08	0.07	1.71
Larval density	–15.672	3	14.11	1.16	0.07	1.78
SD temperature, conductivity	–11.404	4	15.13	2.18	0.04	2.98
Global model	0.00	9	30.86	17.91	<0.01	7 729.75
Principal components models						
CHEM2	–12.634	3	15.97	0.00	0.15	1.00
BIO1	–14.451	3	17.23	1.26	0.08	1.88
BIO2	–14.673	3	17.39	1.42	0.07	2.03
PHYS2	–15.123	3	17.70	1.73	0.06	2.37
CHEM2, BIO2	–11.189	4	17.87	1.90	0.06	2.59
CHEM1	–15.652	3	18.07	2.10	0.05	2.85
CHEM2, BIO3	–11.49	4	18.08	2.11	0.05	2.87
PHYS1	–15.699	3	18.10	2.13	0.05	2.90
BIO3	–15.77	3	18.15	2.18	0.05	2.97
Global model	–5.796	9	34.88	18.91	<0.01	12 768.51

SUMMARY

- Causes of amphibian declines are simple in some cases and quite complicated in others
- Examining multiple stressors can provide key insights into anthropogenic impacts on wildlife.

QUESTIONS?