**Question III: How do amphibian community dynamics influence ranavirus transmission and survival?**

**Objective III: I aim to determine a) how different species interactions influence virus transmission and b) how host density influences the ability of viruses to persist outside of their host.**

**Hypotheses and Predictions**:

* I hypothesize that in addition to environmental virus exposure from water and soil, the **frequency and routes of interaction between species are influencing virus transmission** in amphibian communities of Vermont. I predict ponds where species are coming into contact with each other more, such as predation, necrophagy, and mating interactions, will have increased virus transmission.
* I hypothesize that **pathogens are constrained by a survival-reproduction tradeoff** and maximize their fitness by employing different life history strategies under different environmental circumstances. I predict that increasing the length of time a pathogen must spend in the environment until it encounters a host will decrease pathogen reproductive fitness because it will invest more energy into surviving outside of the host.

**Background**

- How is it transmitted between individuals? (more detail; ingestion has bigger dose! Thus species network is warranted)

- How do humans influence transmission (other ways to go between sites)?

- Previous eDNA research

- Host-switching behavior (general/ranavirus) ## persistence outside amphibians

- Experimental evolution: viruses dominate our planet and their evolution is a broad and applied field that can be studied in the real time

-life history trade off: survival-reproduction

- SIR models (equations); previous ranavirus SIR models (Duffus 2009)

- previous studies of Wasik and De Paepe

- conflicts with curse of the pharaoh (Bonhoeffer et al. 1996)

- phages/coliphages: variety in tails, lytic vs lysogenic, T-even vs T odd

SIR models are not used frequently in the ranavirus system, and no published studies exist on ranavirus transmission rates or dynamics in wild populations (REF). Therefore, to address my first question, I will model species interactions that I believe are important in transmission dynamics and variable between ponds. From there, I will apply the different predicted contact rates to alternate SIR models, use species and environmental viral load data, and determine how closely the SIR predictions of timing and prevalence match with field observations. More generally, I would like to investigate how host density influences viruses’ ability to persist outside their host. To address this, I will also conduct an experiment using viruses that infect bacteria (bacteriophages, or phages), to a) determine if a survival-reproduction trade-off still remains in multiple phage strains after altering timing-of-transmission and b) to infer the phenotype-genotype associations.

**Approach: SIR modeling**

To begin, I will model the number of susceptible, infected, and recovered individuals for one species and incorporate its interactions with other species and the environmental virions into the model.

*Parameters*

Using my species richness and abundance data from summer sampling, as well as a matrix of potential interactions (including predation, mating, necrophagy), I will use packages in R (R Core Team 2017), such as enaR (REF), foodweb (REF), and/or bipartite (REF), to perform ecological network analyses. I can use model output, such as edge weights, to inform the transmission and contact rate parameters of alternate SIR models. In addition, I can use average viral loads of each species and environmental DNA measurements of viral load in the water and substrate at each site, as well as species-specific mortality, infection, and recovery rates (using my data and data from the literature) as parameters of the model.

*Model construction*

The creation and implementation of the SIR models will also be conducted in R. I will start with the EpiModel package (REF); and with increasing complexity, I will create ordinary differential equations and SIR models outside of the package, which is the more flexible option. After obtaining predictions of prevalence and timing from the SIR models, I can compare them to field observations of prevalence and timing. In addition, by changing parameters, I can observe which factors are strongly influencing ranavirus transmission and the amount of infected individuals.

**Approach: Experimental evolution experiment**

I would like to test how increasing timing-of-transmission influences the reproduction-survival trade off and if the effects differ between coliphages. The following experimental design was informed by the previous studies of De Paepe and Taddei (2006) and Wasik et al. (2014).

*Phages*

I selected 5 well-characterized lytic DNA coliphages that were shown by De Paepe and Taddei (2006) to have differential survival and reproduction: **φ**X174 (ssDNA; family Microviridae), T5 (dsDNA; family Siphoviridae), T4 (dsDNA; family Myoviridae), PRD1 (dsDNA; family Tectiviridae), and T7 (dsDNA; family Podoviridae) will be used in this study. As they are all coliphages, I can keep the host constant by using *E. coli* for all phages. In addition, phage stability is very dependent on salt concentration and osmotic pressure, so I will use the standard high-salt LB broth instead of medium that could potentially favor one phage over another (De Paepe and Taddei 2006).

*Experimental Design*

A single ancestral genotype of each phage will originate 8 lineages evolved in either ordinary cell culture passage (n = 4 lineages) or in delayed cell culture passage (n = 4 lineages), totaling 40 experimental units. To determine how long ‘ordinary’ and ‘delayed’ passages will be for each phage, I will conduct a pilot study to observe at what time populations reach a stationary density (ordinary), and then double that time (delayed). The lineages will be observed for 30 passages and frozen every passage to obtain an ‘evolutionary record.’ Fecundity and extracellular survival will be measured for each lineage every 10 passages.

*Sequencing*

At the end of the experiment, each lineage will have its whole genome sequenced every 3 passages (using PCR and Sanger sequencing of viral RNA), and the number of non-synonymous and synonymous substitutions will be compared between the ordinary or delayed passage treatments.

*Fecundity and survival measurements*

Fecundity will be used as a proxy for reproductive fitness and will be measured as delta-log10 titer. First, I will conduct 6-h growth productivity assays of phage populations. Approximately 104 viruses of each experimental lineage (3 replicates) will be allowed to infect replicate 25 cm2 flasks. The phages will be counted using plaque assays, and these data will be log10 transformed and divided by the population titer (104 plaque forming units) to obtain delta-log10 titer. Extracellular survival will be measured by placing 106 pfu/mL of virus in 10 mL of cell-free DMEM and calculating the remaining titer with plaque assays at 5 time points (6, 12, 24, 36, and 48 h post incubation).

*Statistical analyses*

To determine whether there is a strong relationship between survival and reproduction, I will perform a linear regression model. I can conduct an ANCOVA to determine whether the slopes (i.e. tradeoffs) are different between delayed and ordinary treatments and between the different phages. If slopes are different between treatments, I can perform a piecewise regression to estimate the ‘breakpoint’ (or break in the tradeoff). This can be done with the segmented package in R (Muggeo 2008).

**Expected Results and Implications**

Based on how similar the predictions and observations are, we can determine how much influence species interaction has on transmission and hence prevalence of ranavirus.

-First transmission network/SIR model in system

-No published studies on ranavirus transmission rates or dynamics in wild populations

-Will either see a trade-off or a break in trade-off at a certain point

-If all slopes are the same across phage species, indicates there is indeed a trade off; if slopes are different, perhaps it is more complex than that

-Expected results experimental evolution: more non-synonymous substitutions in delayed transmission lineages because signature of selection

- if no significant differences: one substitution could be enough to increase performance (pleitropy)