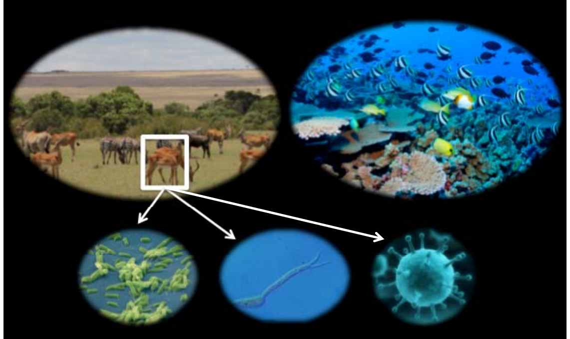
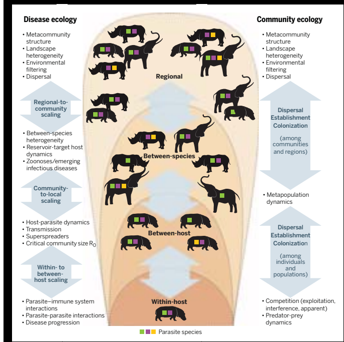

## Lecture overview

1. Community ecology and disease
2. Within host food webs
   - Theory
3. Parasite communities of amphibians
4. Field patterns
5. Experiments

## Community ecology



## Community ecology of disease




Many parallels between community ecology and disease ecology

- Variation across scales
- Relative importance of interactions
- Feedbacks

Increasing focus on integrating these two fields to understand disease dynamics in nature

Johnson et al. 2015; Science

## Parasites within hosts

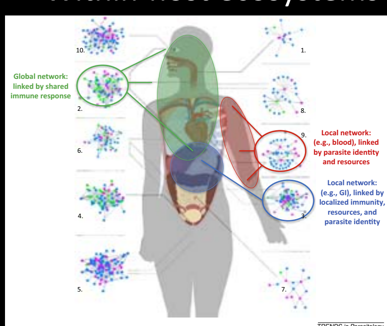
Hosts can be infected with multiple parasites – concurrently or consecutively

Observed in humans, wildlife, and plants

Coinfections can be approached from a community ecology perspective

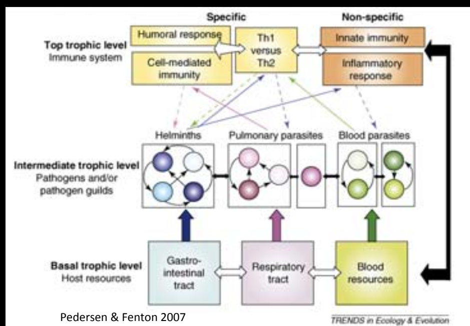
- Direct interactions between parasites
  - Competition for resources and space
  - Predation
- Indirect interactions mediated by the host's immune system
  - Positive and negative effects


## Within-host ecosystems




Rynkiewicz et al 2015. Trends in Parasitology

## Parallels to free-living food webs

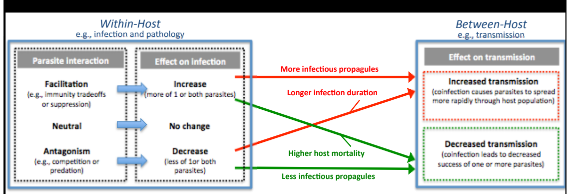



Pedersen & Fenton 2007

## Implications for disease dynamics

Within-host interactions influence host mortality, duration of infection, and production of infectious particles

The magnitude of these effects will determine transmission between host species

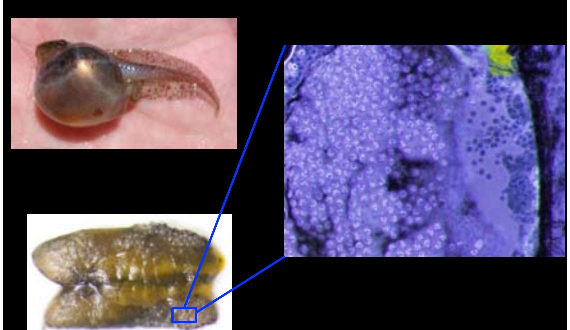



## Amphibian parasites

## Ranaviruses

## *Ribeiroia ondatrae*

## Echinostomes



## *Batrachochytrium dendrobatidis*

## Considerations

Some basic things to consider with these parasites:

- Generalists
- Mixture of direct and indirect transmission
- Transmission most likely during larval stage
- Generally target different areas
    - Interactions likely mediated by immune system

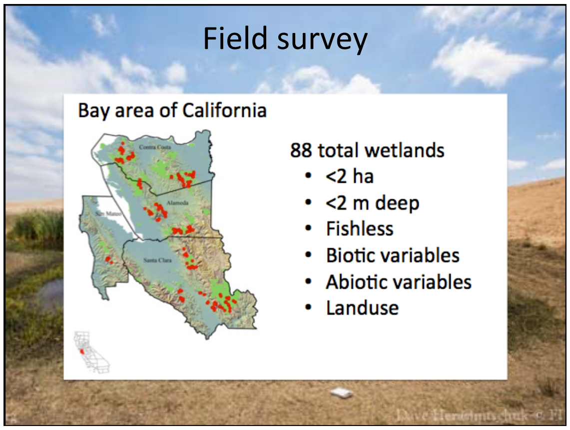
## Field survey objectives

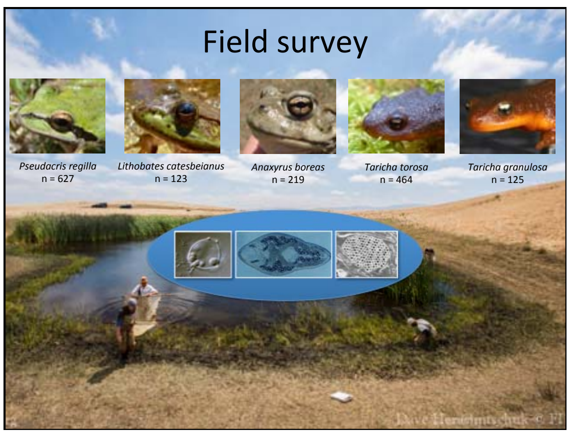
Site-level patterns of co-occurrence

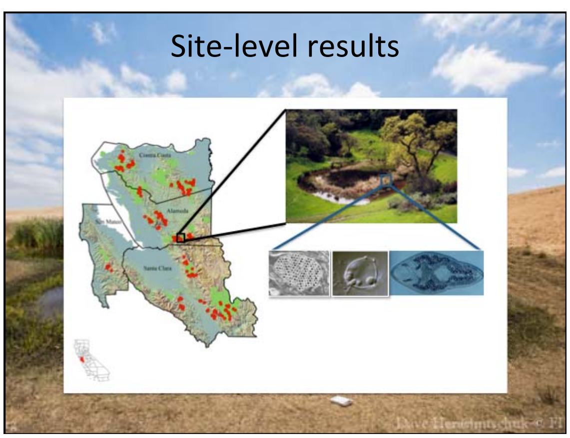
- Identify most frequent parasite communities
- Test for associations between parasites

Individual-level patterns of coinfection

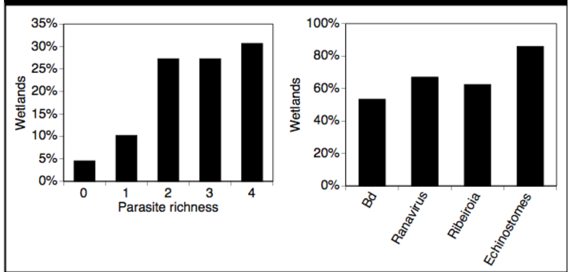
- Identify within-host parasite communities
- Test for predictors of infection status


Field survey
Bay area of California
88 total wetlands
• <2 ha
• <2 m deep
• Fishless
• Biotic variables
• Abiotic variables
• Landuse


Field survey
Pseudacris regilla
n = 627
Lithobates catesbeianus
n = 123
Anaxyrus boreas
n = 219
Taricha torosa
n = 464
Taricha granulosa
n = 125


Site-level results

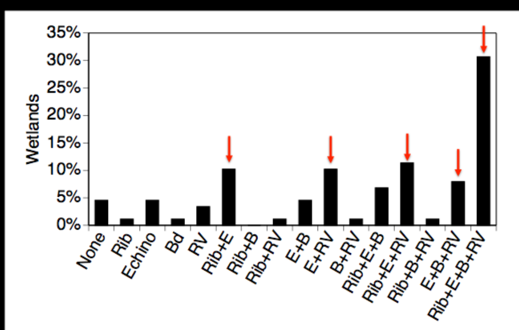
## Amphibian parasites are common



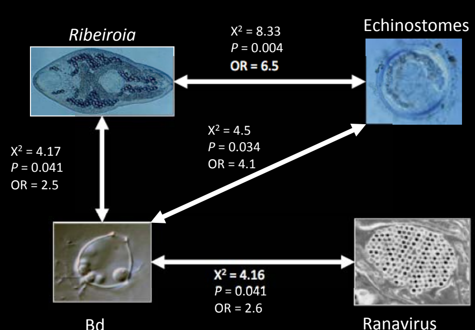

95% had parasites
85% had ≥ 2 parasites

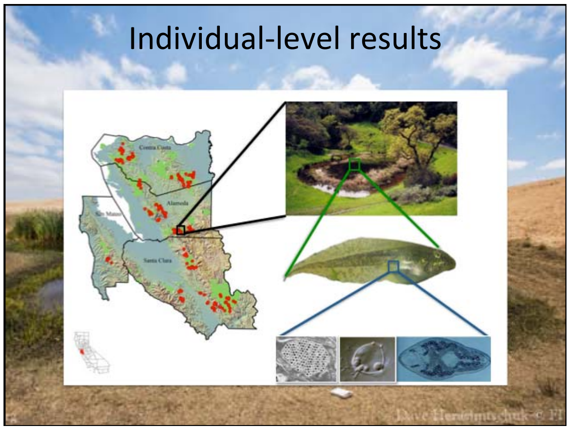
> 50% site-level prevalence for each parasite

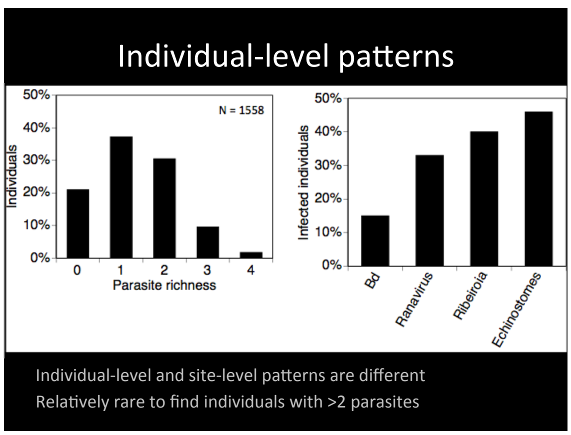
## Frequency of parasite communities

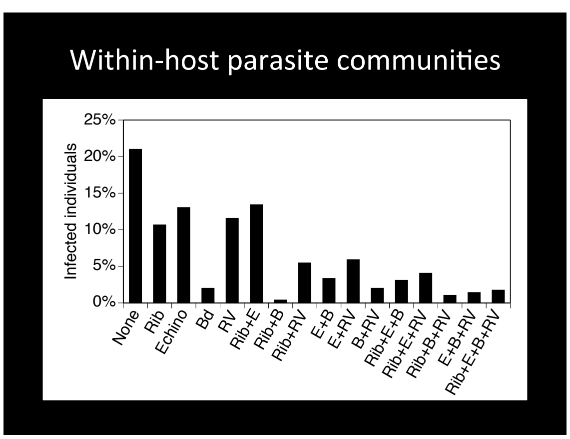


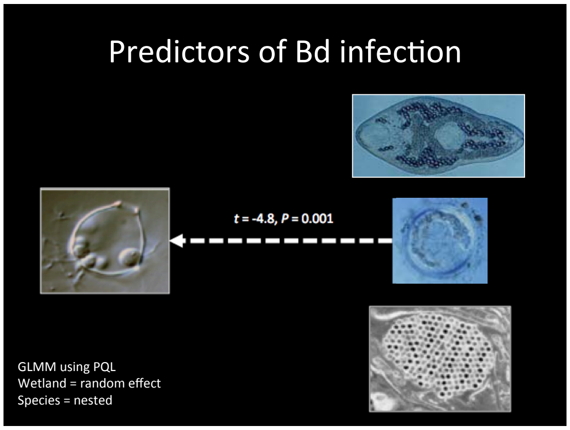

## Associations at the site-level

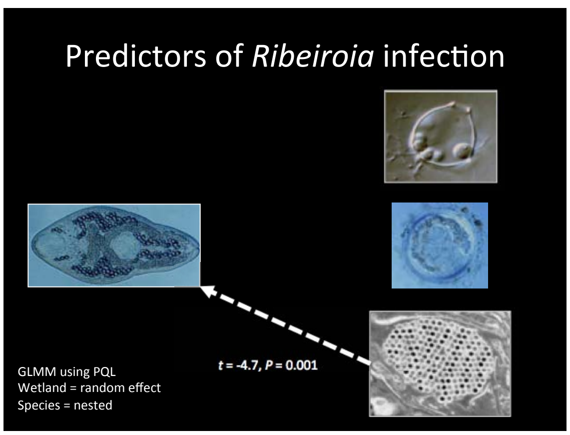


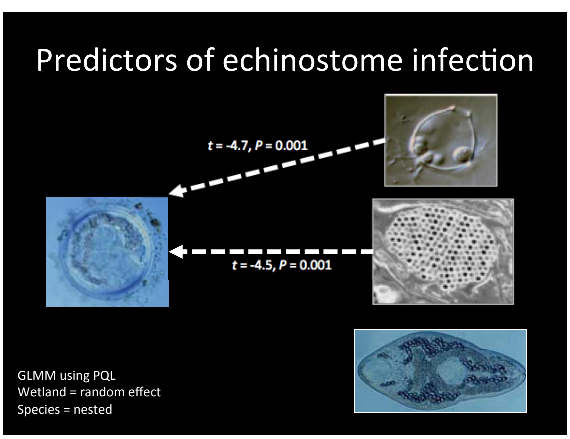

Individual-level results
Contra Costa
Alameda
San Mateo
Santa Clara


Individual-level patterns
Individuals
50%
40%
30%
20%
10%
0%
N = 1558
0
1
2
3
4
Parasite richness
Infected individuals
Bd
Ranavirus
Ribeiroia
Echinostomes
Individual-level and site-level patterns are different
Relatively rare to find individuals with >2 parasites

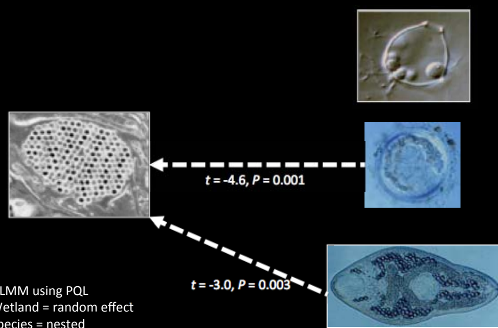

Within-host parasite communities
Infected individuals
25%
20%
15%
10%
5%
0%
None
Rib
Echino
Bd
RV
Rib+E
Rib+B
Rib+RV
E+B
E+RV
B+RV
Rib+E+B
Rib+E+RV
Rib+B+RV
E+B+RV
Rib+E+B+RV


Predictors of Bd infection
t = -4.8, P = 0.001
GLMM using PQL
Wetland = random effect
Species = nested


Predictors of *Ribeiroia* infection
t = -4.7, P = 0.001
GLMM using PQL
Wetland = random effect
Species = nested


Predictors of echinostome infection
t = -4.7, P = 0.001
t = -4.5, P = 0.001
GLMM using PQL
Wetland = random effect
Species = nested

## Predictors of ranavirus infection




GLMM using PQL
Wetland = random effect
Species = nested

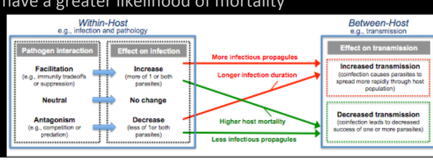
## Importance of scale

Results suggest contrasting patterns across scales

- Positive at the site level
- Negative at the individual level

What is the mechanism?

- Antagonistic interactions could prevent coinfections
- Facilitation could be occurring but coinfected individuals could have a greater likelihood of mortality



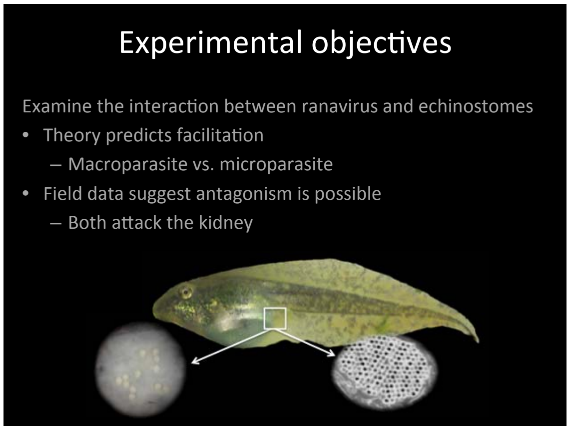

## Next steps

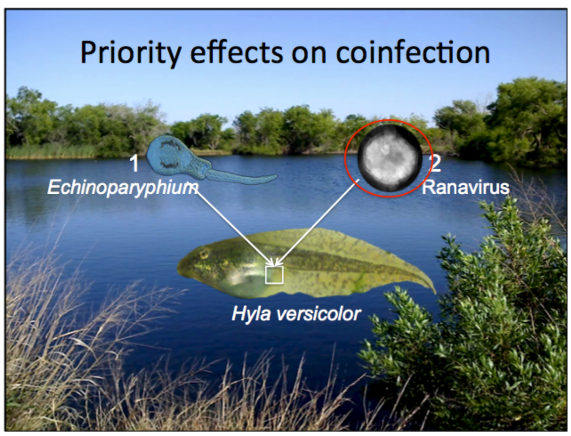
Field studies can suggest patterns but are limited

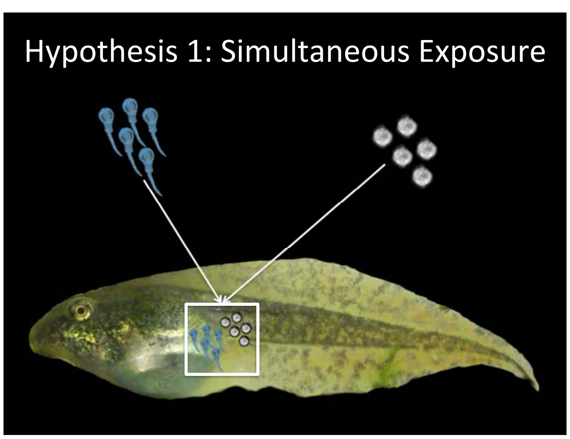
- Exposure
- Host density and richness
- Environmental factors
- Host mortality

Experimental studies can directly test the field patterns

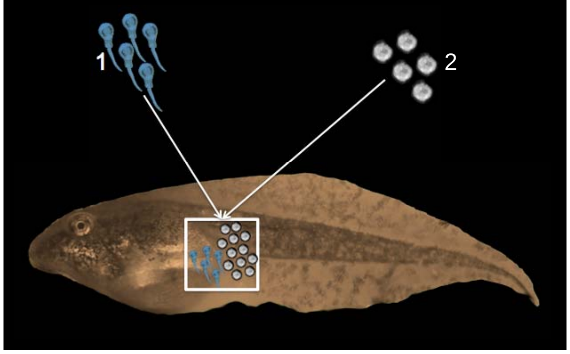
- Controlled environment
- Eliminates confounding variables
- Track disease outcomes


Experimental objectives
Examine the interaction between ranavirus and echinostomes
• Theory predicts facilitation
– Macroparasite vs. microparasite
• Field data suggest antagonism is possible
– Both attack the kidney

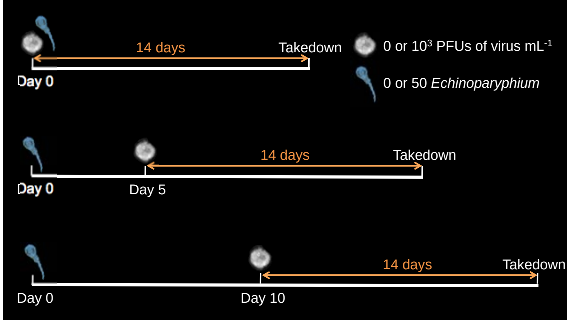

Priority effects on coinfection
1
Echinoparyphium
2
Ranavirus
Hyla versicolor


Hypothesis 1: Simultaneous Exposure

## Hypothesis 2: Separated Exposures




## Experimental Design




## Experimental Design

12 treatments x 20 replicates = 240 experimental units

Fed every 2 days

Water changed every 5 days

Monitored daily for signs of infection and mortality

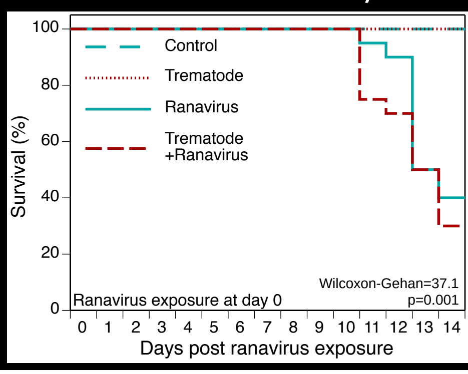
## Processing and Analyses

*Echinoparyphium*

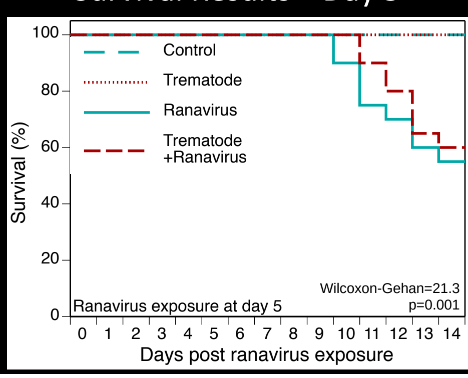
Ranavirus

| Response Variables | Analyses |
|---|---|
| Survival | Wilcoxon-Gehan Test |
| Ranavirus prevalence | Fisher's Exact Test |
| Ranaviral load | Mann-Whitney U test |
| *Echinoparyphium* infection | Two-way ANOVA |

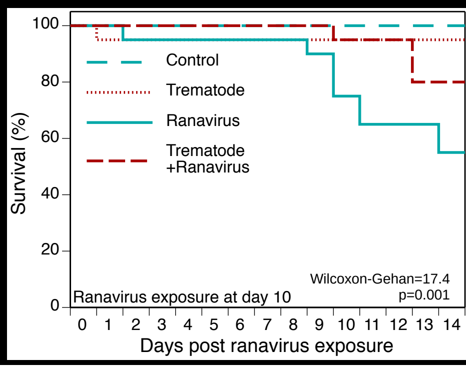
## Survival Results—Day 0



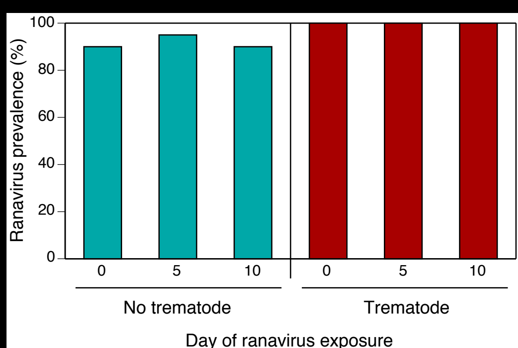

## Survival Results—Day 5



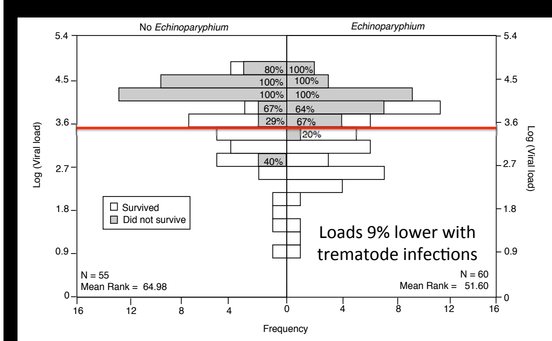
## Survival Results—Day 10



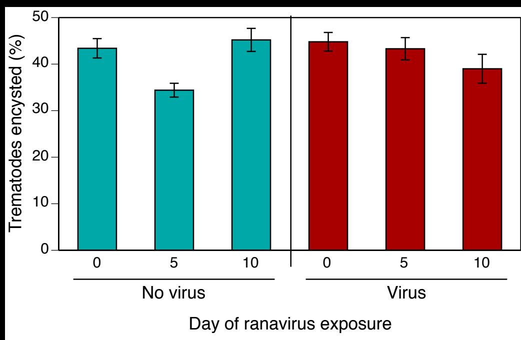

## Ranavirus Prevalence




## Ranavirus load



## *Echinoparyphium* Infection




## Laboratory summary

Echinostome infection reduced ranavirus loads

- No evidence priority effects

Disease outcomes (mortality) were sensitive to the timing of exposures

- Priority effects were important
- Developmental stage and viral load could be driving this pattern → viral load threshold

Suggests cross-reactive immunity

- Evidence for antagonism rather than facilitation

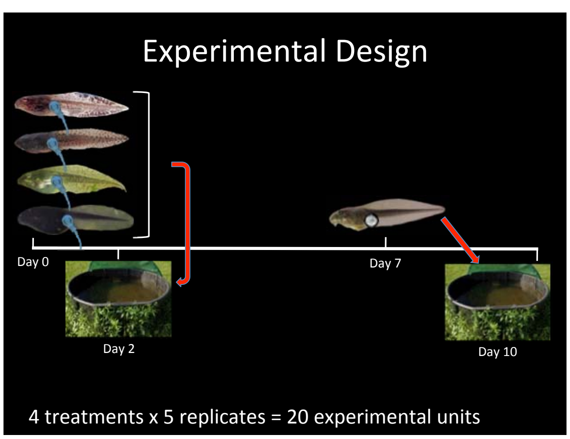
## Coinfection in communities

Can we extend these results to communities?

- Experiments under more natural conditions
- Multiple amphibian species - generality
- Natural routes of ranavirus transmission

Semi-natural mesocosm experiments will use to explore dynamics at the scale of communities


Objective


Experimental Design
Day 0
Day 2
Day 7
Day 10
4 treatments x 5 replicates = 20 experimental units

## Experimental Design

Monitored once daily for signs of infection

Sampling occurred 2 weeks after addition of infected wood frogs

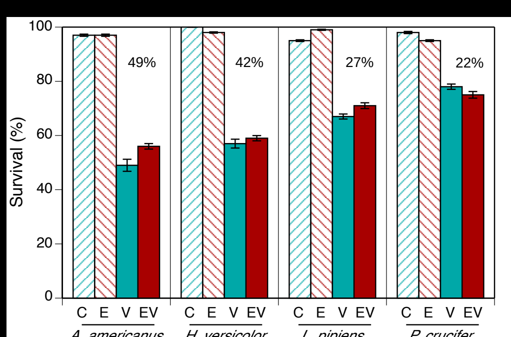
## Processing and Analyses

*Echinoparyphium*

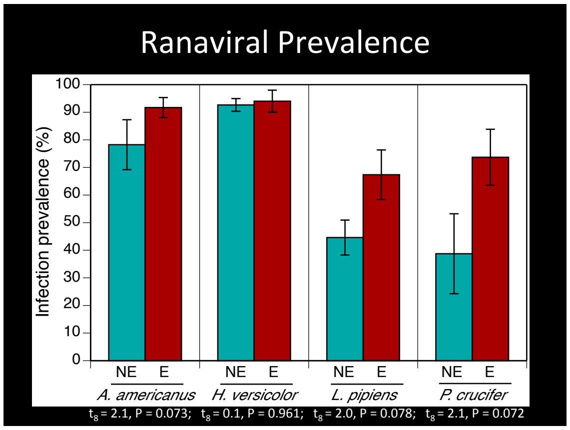
Ranavirus

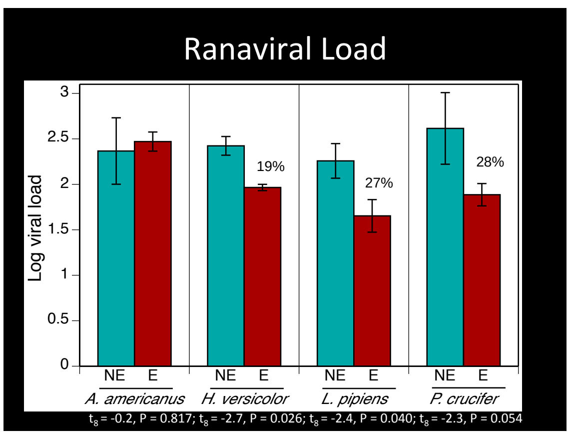
| Response Variables | Analyses |
|---|---|
| Echinoparyphium infection | Generalised linear mixed effects |
| Ranavirus prevalence | Generalised linear mixed effects |
| Ranaviral load | Linear mixed effects |
| Final survival | Multivariate general linear model |

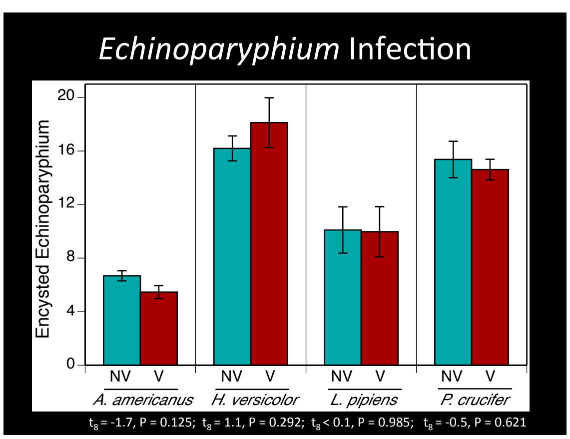
## Survival




Virus ($F_{1,16}$=96.2, P<0.001); *Echinoparyphium* ($F_{1,16}$=0.1, P=0.745); Interaction ($F_{1,16}$=0.2, P=0.691)


Ranaviral Prevalence
Infection prevalence (%)
100
90
80
70
60
50
40
30
20
10
0
NE E
NE E
NE E
NE E
A. americanus
H. versicolor
L. pipiens
P. crucifer
$t_8$ = 2.1, P = 0.073; $t_8$ = 0.1, P = 0.961; $t_8$ = 2.0, P = 0.078; $t_8$ = 2.1, P = 0.072


Ranaviral Load
Log viral load
3
2.5
2
1.5
1
0.5
0
19%
27%
28%
NE E
NE E
NE E
NE E
A. americanus
H. versicolor
L. pipiens
P. crucifer
$t_8$ = -0.2, P = 0.817; $t_8$ = -2.7, P = 0.026; $t_8$ = -2.4, P = 0.040; $t_8$ = -2.3, P = 0.054


*Echinoparyphium* Infection
Encysted Echinoparyphium
20
16
12
8
4
0
NV V
NV V
NV V
NV V
A. americanus
H. versicolor
L. pipiens
P. crucifer
$t_8$ = -1.7, P = 0.125; $t_8$ = 1.1, P = 0.292; $t_8$ < 0.1, P = 0.985; $t_8$ = -0.5, P = 0.621

## Mesocosm summary

Prior *Echinoparyphium* infection had minimal effects on ranavirus transmission

*Echinoparyphium* reduced viral loads by 19-28% in 3 of the 4 species

Mortality did not differ between ranavirus only and coinfected treatments

## Conclusions

While multiple parasite species are present in amphibian communities, research addressing the influence of coinfection on disease outcomes is limited

Future needs

- Importance of scale and patterns of co-occurrence and coinfection
- Environmental factors underlying patterns
- More experimental studies to confirm mechanisms including immunological responses
- Modeling approaches to develop predictions