Environmental Biology: Evaluating the Interaction between Invertebrates & Amphibian Chytrid *Batrachochytrium Dendrobatidis*

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Table of contents

iStock, Ken Griffiths

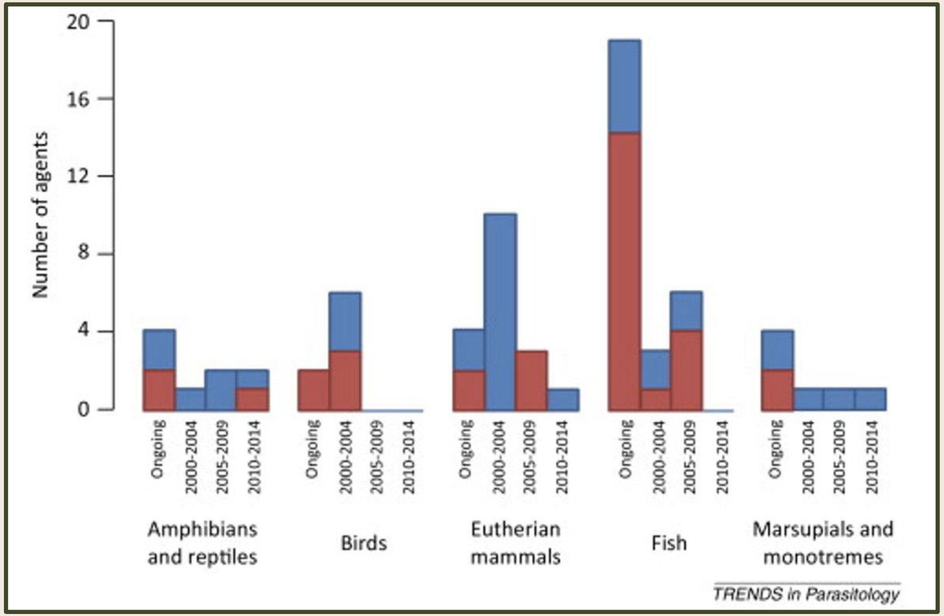
01

Introduction

What are infectious pathogens?

Infectious Disease & Biodiversity

- Blue: known pathogens
- Red: unknown pathogens
- Infectious diseases → rapid population decline → altered species interactions & **imbalances** in the ecosystem



Tompkins et al., 2015

Bd & Chytridiomycosis

Vance T. Vredenburg

Chytridiomycosis: the Most Ecologically Detrimental Amphibian Disease on Earth

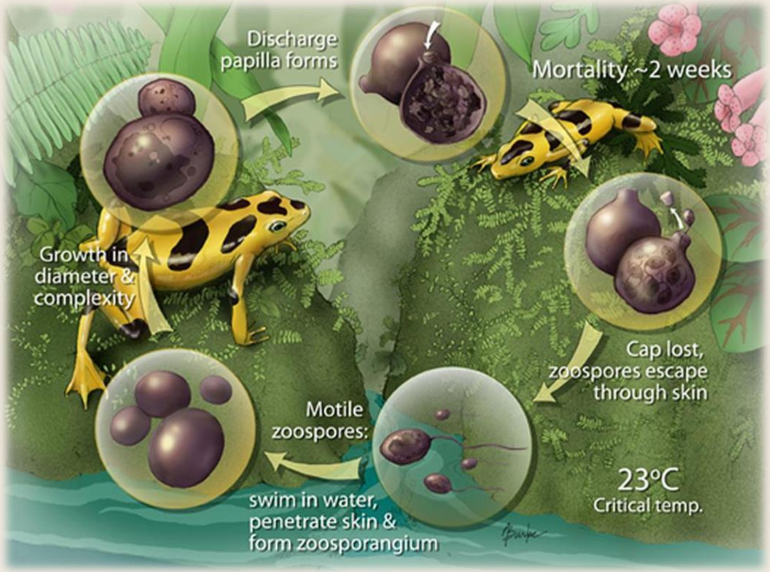
About the Pathogen

How it Infects

- First transmitted in form of aquatic, motile zoospores → penetrate into **keratin layer** of amphibians' skin

- Immotile phase of their life cycle: zoosporangia
  - can release up to **thousands of zoospores** within 2 days

- Triggers **osmotic imbalances** in juvenile and adult frogs



Alison Burke, Johns Hopkins School of Medical Illustration & the National Aquarium

About the Pathogen

Spread within Ecosystems

- *Bd* does **not** occur in all suitable amphibian populations due to different biotic and abiotic factors in the system
- Invertebrate composition has a **direct influence** on *Bd* incidence
- Physicochemical dissimilarity has an **indirect effect** on *Bd* incidence

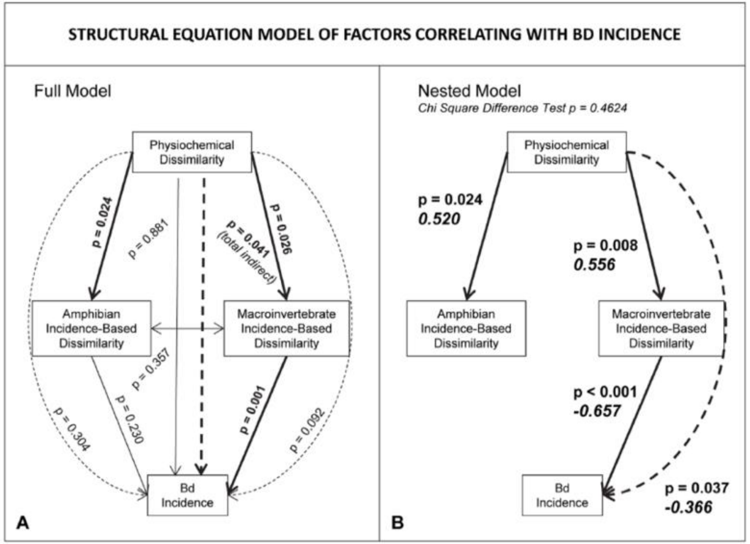


Figure 4. Structural equation modeling of factors correlating with Bd incidence. Structural equation modeling reveals that the correlation between physiochemical dissimilarity and Bd incidence is an indirect effect, mediated by macroinvertebrate community dissimilarity; macroinvertebrate community dissimilarity shares the only significant direct correlation with Bd incidence. Direct links are represented as solid lines and indirect links are represented as dashed lines. Significance of each correlation is reported as a p value, and significant links are emphasized in bold. Only significant links from the saturated model (A) were used to build the final, nested model (B). Standardized effect sizes for each link and a test of model fit are included (in italics) for the final model.
doi:10.1371/journal.pone.0076035.g004

Strauss & Smith, 2013

Alternative Hypothesis

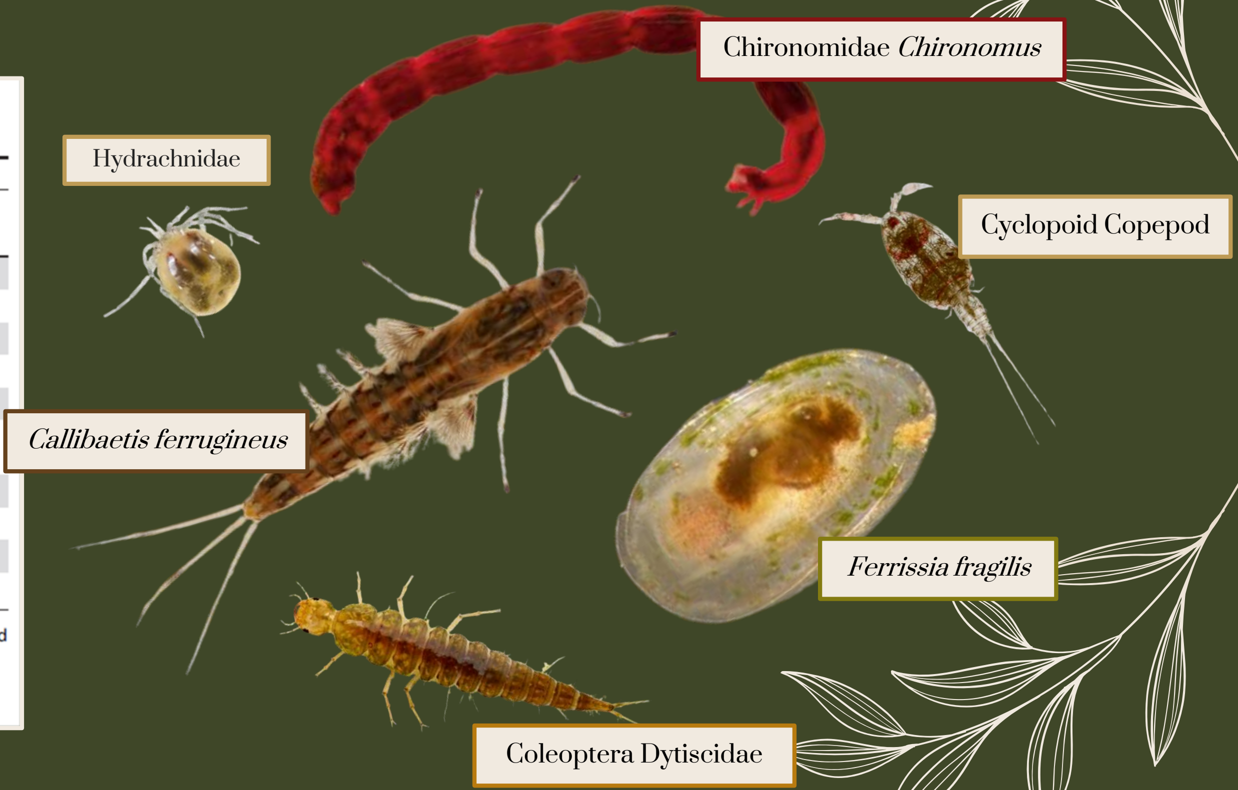
Invertebrates **will influence the abundance** of *Bd* within a closed environment.

Invertebrates Tested

Table 1. SIMPER analysis of macroinvertebrate communities in Bd and non-Bd ponds.

Macroinvertebrate Taxa	Cumulative Contribution	More Abundant In:
Chironomid sp.	9.028	Bd ponds
Physa gyrina	16.96	non-Bd ponds
Hesperocorixa spp.	24.23	Bd ponds
Chaoborus sp.	30.88	Bd ponds
Libellula semifasciata	34.87	Bd ponds
Pachydiplax longipennis	38.7	non-Bd ponds
Notonecta (juvenile)	42.3	Bd ponds
Buenoa spp.	45.51	Bd ponds
Dineutus sp.	48.52	Bd ponds
Callibaetes sp.	51.03	Bd ponds

Over 50% of the difference in macroinvertebrate communities between Bd and non-Bd ponds was explained by the abundances of 10 of the 68 total invertebrate taxa (listed in order of decreasing relative contribution).
doi:10.1371/journal.pone.0076035.t001



Strauss & Smith, 2013

02

Methods

Data Collection &
Experimental Design

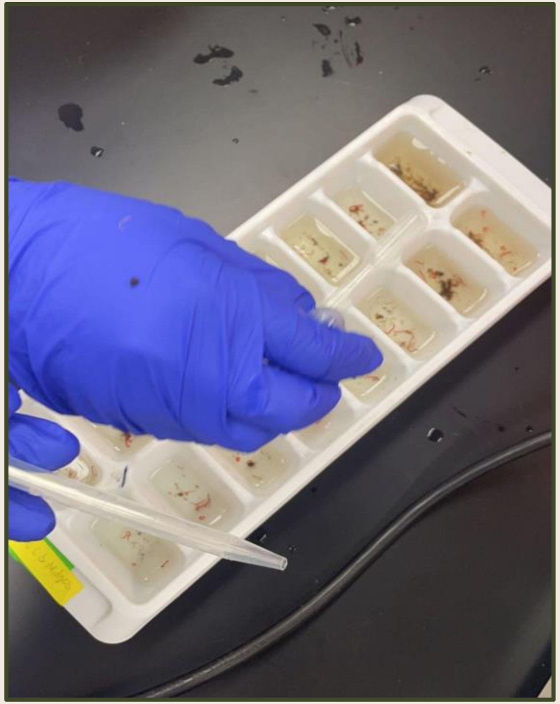
Collecting Invertebrates

Macros collected from ***Bd*-free** ponds with nets, scraped off sides & in leaf litter

Sorting Invertebrates

- Buckets **searched** for Chironomidae *Chironomus*, *Callibaetis ferrugineus*, *Ferrissia fragilis*, Cyclopoid Copepod, Coleoptera Dytiscidae, Hydrachnidae

- Animals sorted:
    - Midges of **same** color & instar
    - Mayflies of **same** genus & size
    - Freshwater limpets identified in leaf litter
    - Copepods, diving beetle larvae, water mites found in pond sample

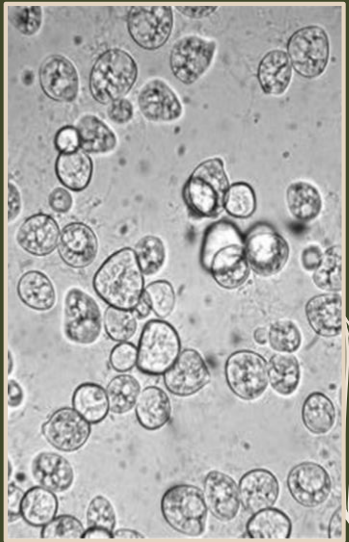


Invertebrate & *Bd* Preparation

- Each macroinvertebrate was isolated and fasted for 24 hours in 100 mL of spring water

- Initial zoospore concentration in a culture was measured & diluted to a concentration of **1000 zoospores/mL** using hemocytometer

- Remained in **zoospore-spiked water** for 18 hours before water collection



Voyles et al., 2012

qPCR Experiment

- Remaining *Bd* DNA in each sample was **extracted** using chemical reagents & physical bead-beating
- Nanodrop used to equalize DNA concentrations
- **Primer Sequences**: ITS1-3 Chytr, 5.85 Chytr, and ChytrMGB2
- qPCR used to quantify **relative abundance** of *Bd* DNA in the samples

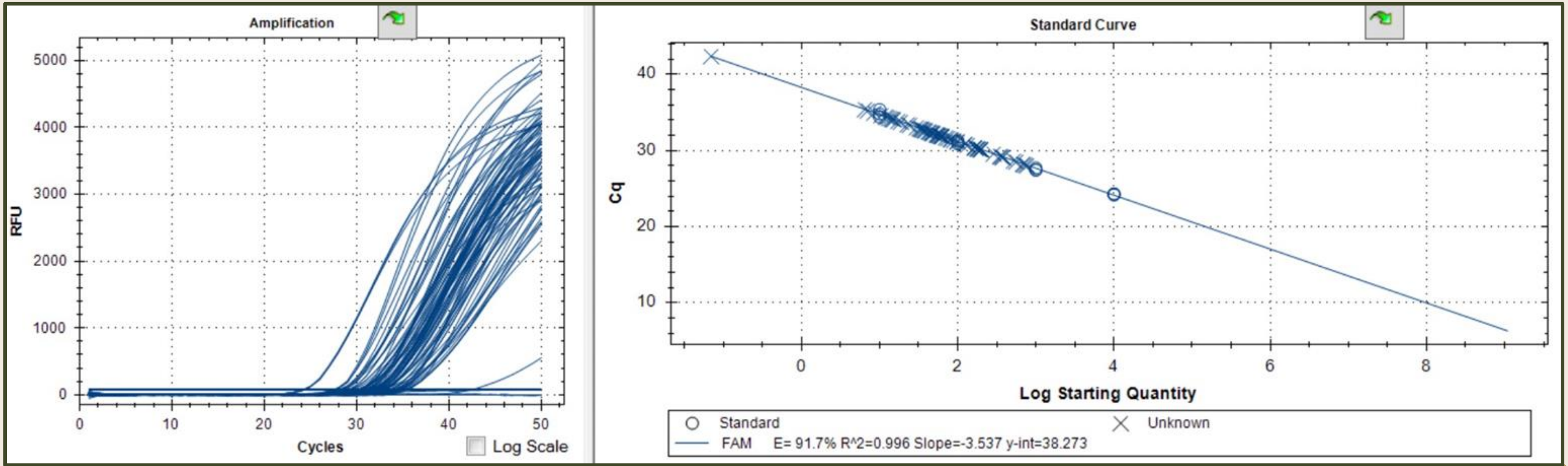
03

Results & Discussion

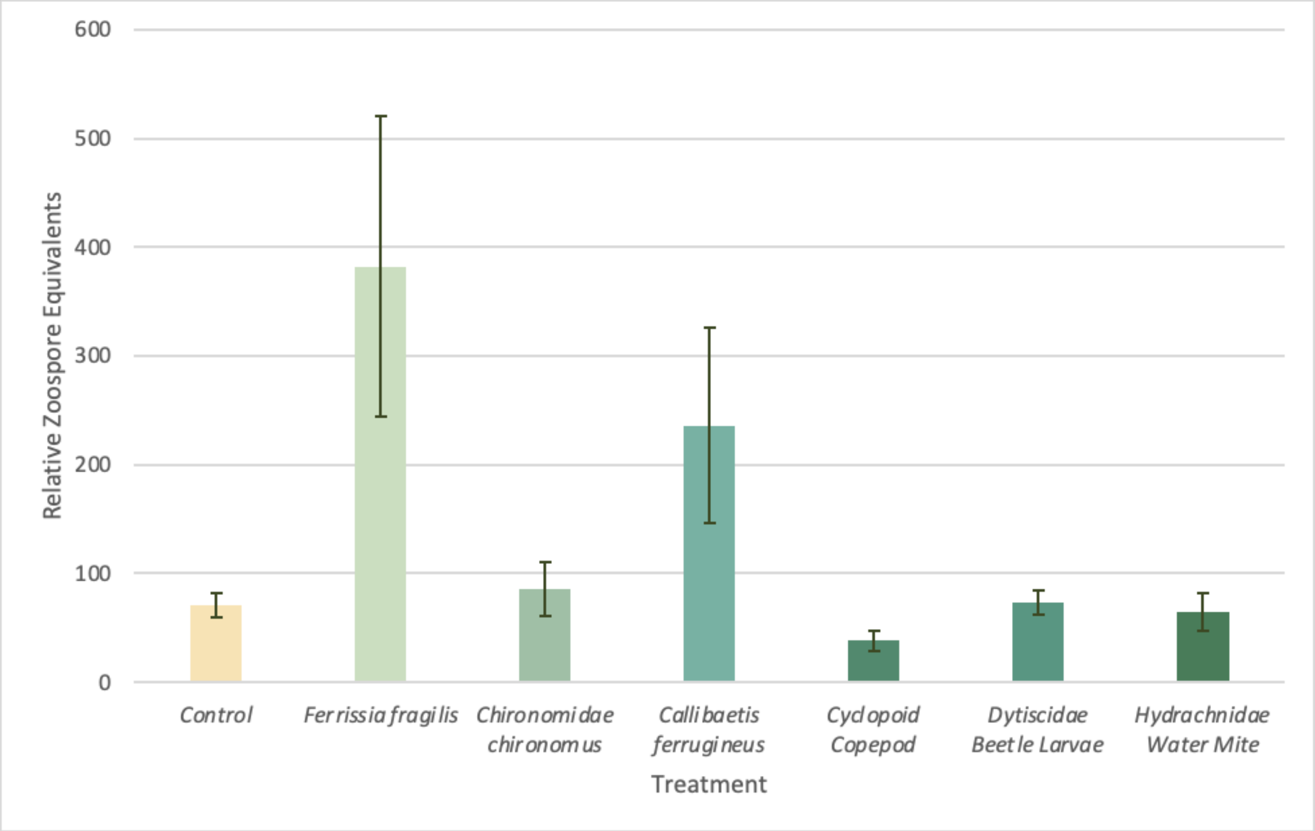
Findings & Statistical Significance

qPCR Results & Statistical Significance

qPCR Results



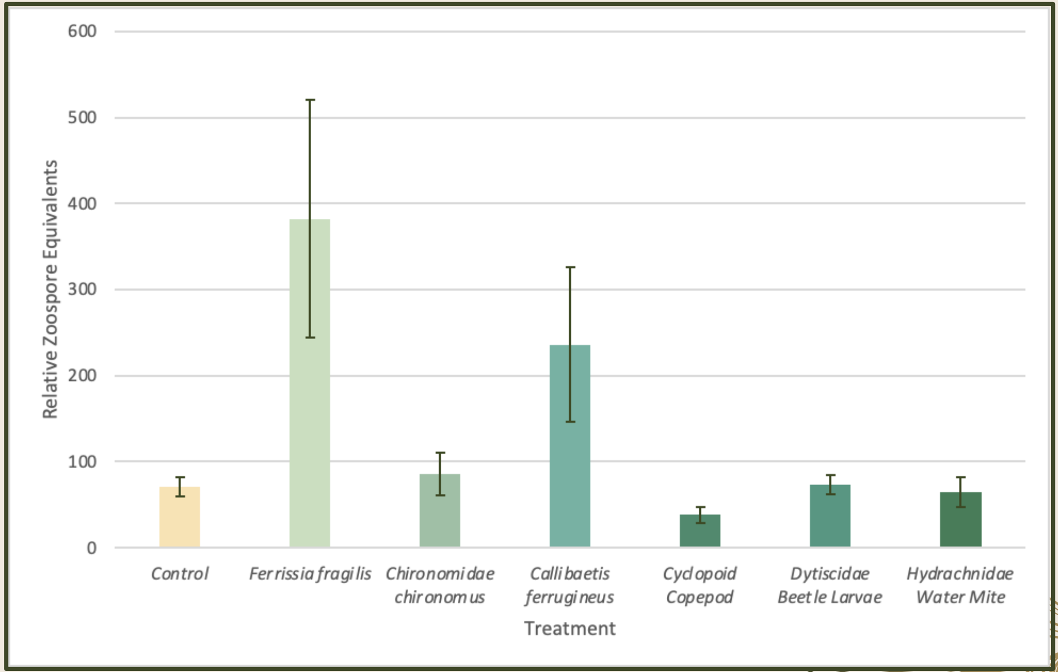
Primary Results



Primary Results

- Zoospore count between
- Two-Tailed T-Test
- Control 84 = outlier
  - Removed from data

- α = 0.05, p-value below 0.05
  - Accept the Alternative Hypothesis



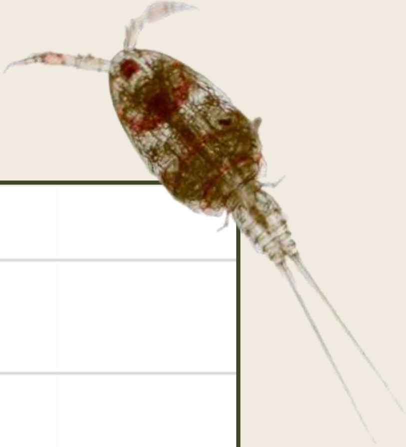
Controls

- Number of controls **based on** number of animals collected

- These invertebrates **were not** infected with *Bd*
  - Confirmed to have **no *Bd* contamination**

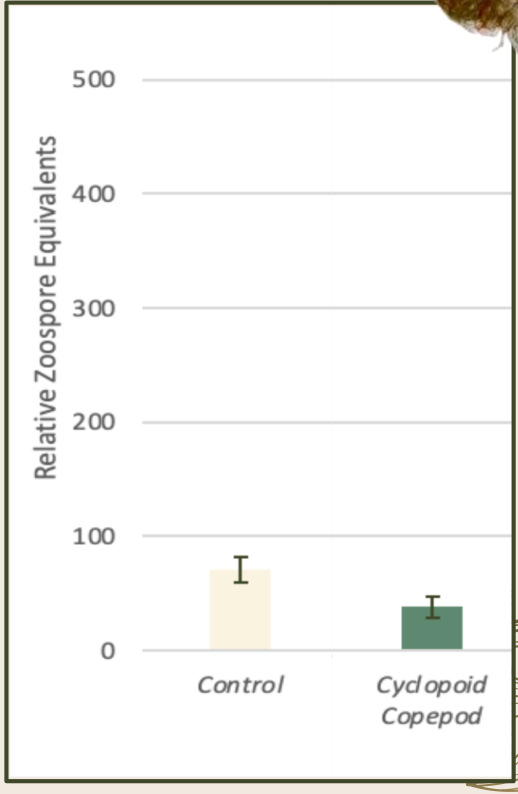
- **Levels of zoospore DNA compared** between the experimental cups (with *Bd*) and control (NO *Bd*)

Cyclopoid Copepod Results



- Lower zoospore expression than control
  - Cyclopoid Copepods: 38.16 ± 9.34;
  - Control: 71.29 ± 11.07
- P-value of ~0.046 - **statistically significant**
- Subjects 51 and 53 were removed
  - Conservative in data analysis

Cyclopoid Copepods **reduce** the presence of *Bd.*



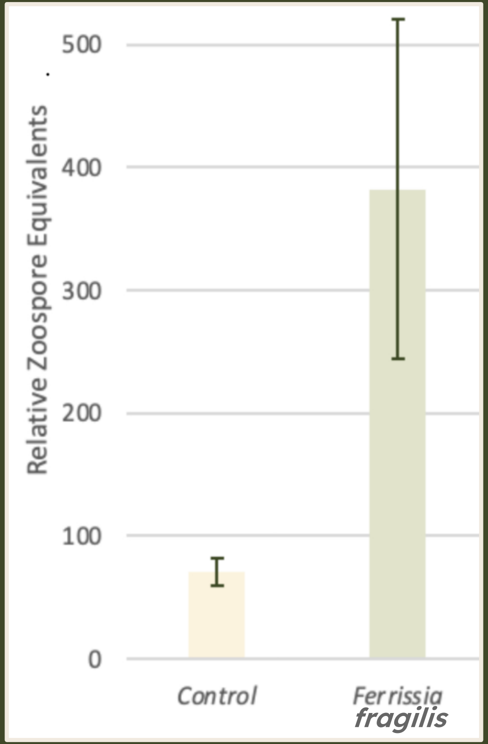
Summary: *Cyclopoid Copepod*

- Significantly decrease *Bd* presence ∴ **hypothesis accepted**

- Could be **consuming** *Bd* zoospores
  - Diet consists of detritus, cyanobacteria, and protists

U.S. Geological Survey

Ferrissia fragilis Results



- Higher zoospore expression than control
  - *F. fragilis*: 382.41 ± 138.40
  - Control: 71.29 ± 11.07

- P-value: 0.049 - **statistically significant**

Ferrissia Fragilis **increases** the abundance of *Bd*.

Summary: *Ferrissia fragilis*

- Large error bars indicate **high variability** within sample
- Limpet mucus may have inhibited qPCR results, but the samples were rerun
- Further research recommended to explore mechanisms

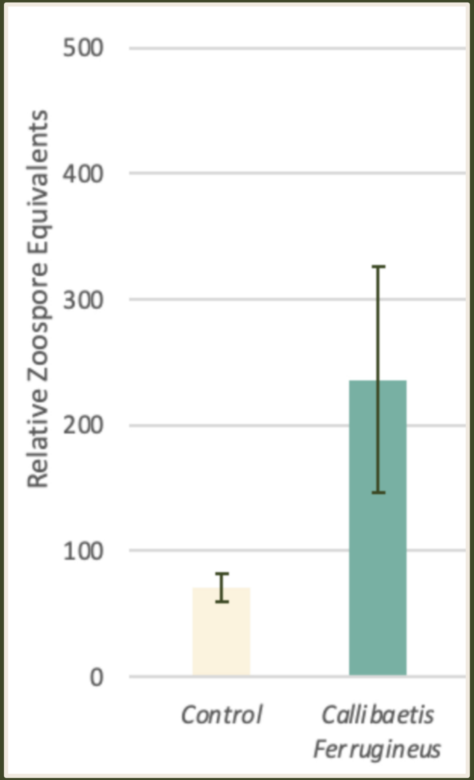
Mikhail O. Son, 2007

Summary: *Chironomidae chironomus*

- P-value: 0.603 - statistically **insignificant**
- Did not impact *Bd* abundance
    - 2023 GSNJS study **large variation** in Chironomidae *chironomus*
- Chironomidae *chironomus* possible **reservoirs** or **alternate hosts**

B. Schoenmakers

Callibaetis ferrugineus Results



- Increased zoospore expression
  - *C. Ferrugineus*: 236.14 ± 89.67
  - Control: 71.29 ± 11.07
- P-value: ~ 0.087
  - $0.05 < p < 0.1$
  - **marginally significant**

Callibaetis Ferrugineus **could influence** the abundance of *Bd*.

Summary: *Callibaetis ferrugineus*

- Increased *Bd* presence compared to control

- P-value: 0.087 - **marginally significant**
    - ∴ Null hypothesis **neither accepted nor rejected**
        - Large variance in zoospore expression → higher p-value

Rick Hafele

Summary: *Statistically Insignificant Invertebrates*

- P-values > 0.10
  - 0.901 - *Coleoptera Dytiscidae* (diving beetle)
  - 0.729 - *Hydrachnidae* (water mites)

- **No significant effect** on *Bd* presence ∴ do not warrant future research

NC State University

University of Guelph

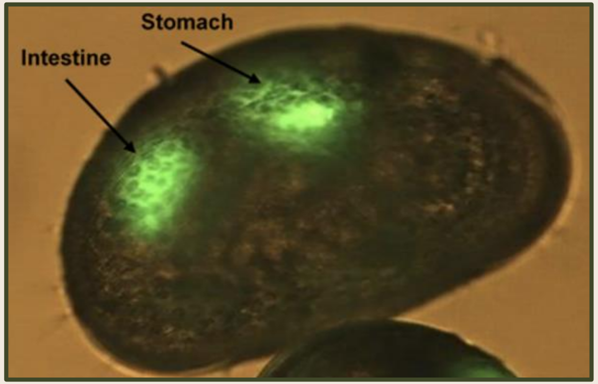
Limitations

- Some invertebrates did not survive
  - Relatively small sample sizes

35	34	8.3	Callibaetis Ferrugineus	Nymph	Bd	Alive
36	35	12.8	Callibaetis Ferrugineus	Nymph	Bd	Alive
37	36	10.2	Callibaetis Ferrugineus	Nymph	Bd	DEAD
38	37	11.3	Callibaetis Ferrugineus	Nymph	Bd	Alive
39	38	10	Callibaetis Ferrugineus	Nymph	Bd	Alive
40	39	12	Callibaetis Ferrugineus	Nymph	Bd	Alive
41	40	8	Callibaetis Ferrugineus	Nymph	Bd	DEAD
42	41	7.5	Callibaetis Ferrugineus	Subimago	NoBd	Alive
43	42	13	Callibaetis Ferrugineus	Nymph	NoBd	Alive
44	43	6.8	Callibaetis Ferrugineus	Nymph	NoBd	DEAD
45	44	12.6	Callibaetis Ferrugineus	Nymph	NoBd	Alive
46	45	8.4	Callibaetis Ferrugineus	Nymph	NoBd	Alive

Future Research

- Suggests some invertebrates interact with *Bd*
- Further research with mayflies and midges
- Need to determine how/why
    - Red nile
    - Fluorescent dye
- Interactions in Natural Environments



05

Acknowledgments

Thank you!

Acknowledgments

Office of the Secretary of Higher Education of New Jersey

The Overdeck Family Foundation

Novartis

Dr. Adam Cassano

Dr. Stephen Dunaway

GSNJS Alumnae & Parents of Alumnae

Dr. Jessica McQuigg

Chloe Dudonis, Brenna Hezel, Brynn McCarthy, & Danny Walker

Harris Naqvi & Jonah Fine

Drew University Faculty

Works Cited

- Bernardo-Cravo AP, Schmeller DS, Chatzinotas A, Vredenburg VT, Loyau A. 2020. Environmental Factors and Host Microbiomes Shape Host–Pathogen Dynamics. Trends in Parasitology. 36(7):616–633. doi:https://doi.org/10.1016/j.pt.2020.04.010. https://www.cell.com/trends/parasitology/fulltext/S1471-4922(20)30107-0?rss=yes&utm_source=researcher_app&utm_medium=referral&utm_campaign=RESR_MRKT_Researcher_inbound
- Mcquigg JL, Boone M. EVALUATING THE INFLUENCE OF ABIOTIC AND BIOTIC ENVIRONMENTAL CHARACTERISTICS IN AN AMPHIBIAN DISEASE SYSTEM [PhD Thesis]. Oxford (OH): Miami University. 2022. https://etd.ohiolink.edu/acprod/odb_etd/ws/send_file/send?accession=miami1657724856475325&disposition=inline
- Pappas G, Vrioni G. 2024 The Last of Us and the Question of a Fungal Pandemic in Real Life. Emerging Infectious Diseases journal. doi:10.3201/eid3003.230684. https://wwwnc.cdc.gov/eid/article/30/3/23-0684_article#:~:text=In%20recent%20years%2C%20a%20rising.
- O’Hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Bataille A, Kosch TA, Murray KA, Brankovics B, Fumagalli M, et al. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science. 360(6389):621–627. doi:https://doi.org/10.1126/science.aar1965. https://science.sciencemag.org/content/360/6389/621.
- Gladieux P, Byrnes E.J., Aguileta G, Fisher M.C., Billmyre R.B., Heitman J.H., Giraud T. 2017. 4 - Epidemiology and Evolution of Fungal Pathogens in Plants and Animals. https://doi.org/10.1016/B978-0-12-799942-5.00004-4. https://www.sciencedirect.com/science/article/abs/pii/B9780127999425000044

Works Cited Cont.

- Lips KR. 2016. Overview of chytrid emergence and impacts on amphibians. Philosophical Transactions of the Royal Society B: Biological Sciences. 371(1709):20150465. doi:https://doi.org/10.1098/rstb.2015.0465. https://royalsocietypublishing.org/doi/10.1098/rstb.2015.0465
- Rosenblum E, Fisher M, James T, Stajich J,Longcore J, Gentry L, Poorten T. 2009. A molecular perspective: biology of the emerging pathogen Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms. 92(3):131–147. doi:https://doi.org/10.3354/dao02179. https://pubmed.ncbi.nlm.nih.gov/21268975/
- Searle CL, Mendelson JR, Green LE, Duffy MA. 2013. Daphnia predation on the amphibian chytrid fungus and its impacts on disease risk in tadpoles. Ecology and Evolution. 3(12):4129–4138. doi:https://doi.org/10.1002/ece3.777. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853558/
- Kilpatrick AM, Briggs CJ, Daszak P. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol Evol. 25(2):109–118. doi:10.1016/j.tree.2009.07.011.
- Strauss A, Smith KG (2013) Why Does Amphibian Chytrid (Batrachochytrium dendrobatidis) Not Occur Everywhere? An Exploratory Study in Missouri Ponds. PLoS ONE 8(9): e76035. doi:10.1371/journal.pone.0076035 https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0076035

Works Cited Cont.

- Bay E.C. 2003. Chironomid Midges. https://s3.wp.wsu.edu/uploads/sites/408/2015/02/PLS-45-Chironomid-Midges.pdf
- Ponder, W. F., Hallan, A., Shea, M. E., Clark, S. A., Richards, K., Klunzinger, M. W., and Kessner, V. 2023. Australian Freshwater Molluscs. Revision https://keys.lucidcentral.org/keys/v3/freshwater_molluscs/key/australian_freshwater_molluscs/Media/Html/entities/ferrissia.html.
- About the COPEPOD Project. [accessed 2024 Jul 25]. https://www.st.nmfs.noaa.gov/copepod/about/#:~:text=A%20%22copepod%22%20is%20a%20type,habitat%2C%20even%20in%20underground%20caverns.
- Potter G. 2020. Cyclopoid Copepods (Apocyclops panamensis). Aquatic Live Food | Aqua Cultured Aquarium Foods. [accessed 2024 Jul 25]. https://www.aquaticlivefood.com.au/cyclopoid-copepods-apocyclops-panamensis/.
- Hamrsky J. Water mites - LIFE IN FRESHWATER. LIFE IN FRESHWATER - Macro photography of aquatic insects and other freshwater invertebrates. [accessed 2024 Jul 25]. https://lifeinfreshwater.net/water-mites/.

Works Cited Cont.

- Hafele R. 2017. Rick Hafele. [accessed 2024 Jul 23]. https://www.rickhafele.com/mayflies
- Bertone M. 2018. NC State Extension. Raleigh (NC): NC State Extension Publications; [accessed 2024 Jul 23]. https://content.ces.ncsu.edu/biology-and-control-of-non-biting-aquatic-midges
- Son MO. 2007. North American freshwater limpet Ferrissia fragilis (Tryon, 1863) (Gastropoda: Planorbidae) – a cryptic invader in the Northern Black Sea Region. In: Aquatic Invasions. Ukraine: European Research Network on Aquatic Invasive Species; [accessed 2024 Jul 23]. Vol. 2, Issue 1, p.55-58. https://www.semanticscholar.org/paper/North-American-freshwater-limpet-Ferrissia-fragilis-Son/790e22a37753d1dbe3fd070489fd5a00872910c6
- McQuigg J, Kissner K, Boone M. 2023 28. Exposure to Amphibian Chytrid Fungus Alters Terrestrial Growth and Feeding Rate in Metamorphic Anurans. meridianallenpresscom. https://meridian.allenpress.com/journal-of-herpetology/article/57/1/36/491752/Exposure-to-Amphibian-Chytrid-Fungus-Alters.
- Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira CA, Lee M, Zhao S, Wangen C, Ferreira CM, Hipolito M, Davies AJ, Cuomo CA, Daszak P, James TY. 2012. Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. 21(21):5162-77. doi: 10.1111/j.1365-294X.2012.05710.x.

Works Cited Cont.

- Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms.
- Pritschet L, Powell D, Horne Z. 2016. Marginally Significant Effects as Evidence for Hypotheses: Changing Attitudes Over Four Decades. In: Psychological Science. 7th ed. Sage Publications; [accessed 2024 Jul 23]. Vol. 27, p.1036-1042. Available from Sage Journals; https://journals.sagepub.com/doi/10.1177/0956797616645672
- Sirica R. The influence of aquatic macroinvertebrates on the abundance of Batrachochytrium dendrobatidis. [Bachelor’s Thesis]. Madison(NJ): Drew University. 2024.
- Galindo LJ, Milner DS, Gomes SL, Richards TA. 2022. A light-sensing system in the common ancestor of the fungi. Current Biology. 32(14):3146-3153.e3. doi:10.1016/j.cub.2022.05.034. [accessed 2024 Jul 26]. https://www.sciencedirect.com/science/article/pii/S0960982222007965.
- Herbert, Sm, Tlf Leung, and Pj Bishop. 2011. “Fluorescent Probes as a Tool for Labelling and Tracking the Amphibian Chytrid Fungus Batrachochytrium Dendrobatidis.” Diseases of Aquatic Organisms. https://doi.org/10.3354/dao02377.