

Investigating Antimicrobial Properties Released from Developing Amphibian Eggs

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Background

Early amphibian development often occurs in a hostile environment, abundant with potential predators and pathogens. While it is known that amphibian eggs are protected physically by 5-8 layers of glycoprotein jelly, additional **protection mechanisms used against water pathogens are largely unknown**. Previous research in our lab has indicated that amphibian eggs exhibit a unique microbiome on the egg surface which is distinct from the surrounding environment. This uniqueness may indicate **defensive/offensive antimicrobial properties by developing eggs to microbes**.

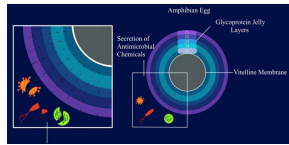


Figure 1. Representation of various glycoprotein layers of an amphibian egg (created by lab member Christian Sorensen).

Purpose

This project is investigating a novel method to elucidate the effects of potential antimicrobial chemicals released from developing amphibian eggs to heterotrophic microbes.



Figure 2. Wood frog egg mass used in this study (left) and developing wood frogs (right). Photos taken by the authors.

Methods

Characterization of Microbiota from Aquatic Ecosystems Used by Breeding Wood Frogs (*Lithobates sylvaticus*)

Streak for Isolation of Water Microbiota

- Microbes from water samples will be isolated to obtain pure cultures of various bacteria from the pond water where wood frog eggs were collected

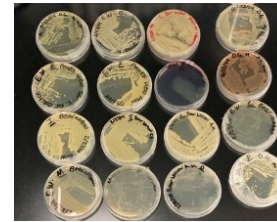


Figure 3. Pure cultured bacteria from Wood frog pond water. Image taken by Beth Williams.

Lyophilizing (Freeze-drying) of Egg Masses to Isolate Antimicrobial Properties Released from Developing Amphibian Eggs

- Eggs will be opportunistically collected and concentrated via a lyophilized method to concentrate chemicals in developing eggs
- The eggs will be moderately rehydrated, filtered through a syringe to remove any microbes, and then tested with a paper disk assay against isolated microbes

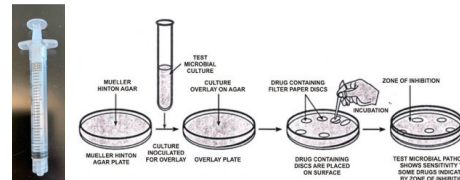


Figure 4. (left) Syringe used to concentrate antimicrobial chemicals from egg masses (Photo taken by authors) (right) Example Disk assay. *Mueller Hinton Disk Assay*. (2018).

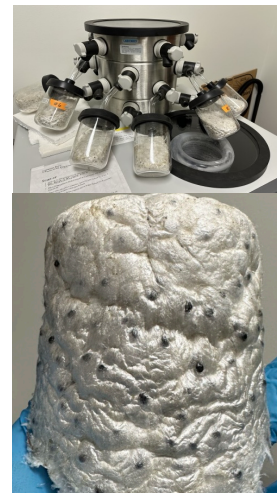


Figure 5. Lyophilizing of wood frog egg masses (top) and resulting freeze-dried wood frog egg mass (bottom) ready for testing. Photos by the authors.

Expected Results

It is hypothesized that the concentrated eggs on the paper disks will show zones of inhibition to pond water microbiota. Previous experiments in our lab indicated possible zones of inhibition, but eggs desiccated before we could draw strong conclusions.



Figure 6. Image showing egg mass with eggs being devoured by heterotrophic organisms (circled in red), and viable developing eggs (circled in white). The blue circle encompasses eggs that have already hatched.

Isolating antimicrobial chemicals released from amphibian egg masses can potentially be utilized in amphibian conservation efforts.

Future Work

Captive White's Tree Frog Egg Mass Antimicrobial Inquiry

- Currently raising White's Tree Frogs (*Litoria caerulea*) in the lab for breeding in a rain chamber.
- Repeat methodology refined from *L. sylvaticus* to continue investigating for antimicrobial properties released from developing amphibian eggs.



Figure 7. One of eight *Litoria caerulea* (left) being raised for the production of egg masses to support developmental investigations

Acknowledgements and References

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