Heterospecific Anural Eavesdropping Cues

Lucia Maldonado, Hayley Lunn, Max Sprute, Conor Kramer, Nolan Gentile, Andrew Wang, Ripley Conklin, Lee Kats, PhD.
Pepperdine University, 24255 Pacific Coast Highway, Malibu, CA 90265

Introduction

The ability to communicate within species is a trait utilized by every organism. Using cues conspecifically creates a better chance of survival for other members of the species and increases fitness overall. (Deng K. et al. 2021). However, using cues heterospecifically also poses a great advantage as animals can eavesdrop on cues released by another species. Organisms can additionally interpret eavesdropping cues from outside their own species to avoid harm and gather resources. Previous studies have recorded that eavesdropping is beneficial to prey species, such as squirrels reacting to bird calls and tadpoles reacting to visual and chemical cues to avoid predation. (Lily et al. 2019). Additionally, Deng K. et al. (2022) and Fouquet et al. (2021) suggest that the use of heterospecific cues could also aid in mate choice, further increasing the fitness of the species. The results of these studies pose the question of how one local and one exotic species of frog would respond to cues emitted by another local species of frog, especially considering that the commonly poisonous frog has no natural predators.

This experiment aims to examine how two amphibian species *Pseudacris cadaverina* (non-poisonous) and *Dendrobates tinctorius* (poisonous) will react to the chemical cues of a third species, *Pseudacris regilla*. Through studying the reactions of these frogs to the cues of a predator, we hope to gain insight into their communication abilities and their potential for fitness.

Hypothesis

Pseudacris cadaverina will react to the chemical cues released by an agitated *Pseudacris regilla* while *Dendrobates tinctorius* will not.

Materials and Methods

Materials:

- 4 inch in diameter pipe by 3 feet long
- Fine wire mesh
- Hot Glue
- 9 Popsicle Sticks
- 12 Rubber Bands
- 15 Binder Clips
- Black sharpies
- Black tape
- Timer
- Nitrile gloves
- 5 *Pseudacris regilla*
- 16 *Pseudacris cadaverina*
- 36 *Dendrobates tinctorius*

Laboratory Methods:

- Three pipes were modified so that there was an opening in the center. This is where the frogs responding to the stress cues would eventually be placed. Additionally, the inside of the pipe was marked with black tape to help indicate their movement towards or away from the agitated frog.
- 3 small frog enclosures were made using fine wire mesh, popsicle sticks for support, and hot glue to hold it all together.
- This experiment was overall conducted twice. Once for the *Pseudacris cadaverina* trials and another for the *Dendrobates tinctorius*.
- The *Dendrobates tinctorius* were handled with nitrile gloves and minimal contact.
- A cotton swab was used to stroke the *Pseudacris regilla* and agitate them to trigger stress.
- Once agitated, the *P. regilla* was enclosed within the makeshift mesh container which was secured with binder clips Then it was placed in the end of a 3 feet pipe where it was left alone for 10 min to acclimate.
- After the 10 minute period, an arbitrary frog from the *Pseudacris cadaverina* species or *Dendrobates tinctorius* was picked to be placed inside the tube.
- The opening was immediately covered after the frog was placed inside to prevent the frog from escaping and the timer for 2 minutes was started.
- The immediate location of the *P. cadaverina or D. tinctorius* was noted once placed in the tube.
- After 2 minutes, the final location of the frog was noted and the frog was placed back into its enclosure.
- After each trial, the plastic pipe was washed thoroughly with carbon filtered water to ensure minimal cross contamination.
- Once conclusions were made, *Pseudacris cadaverina* were safely returned to Arroyo Canyon. *Pseudacris regilla* and *Dendrobates tinctorius* remain in the lab under our care.

Analytical Methods:

The data was run through a Shapiro-Wilks and Anderson-Darling test to check for normality. Since the data was found to be non-normal, it was run through a Wilcoxon test.

Results

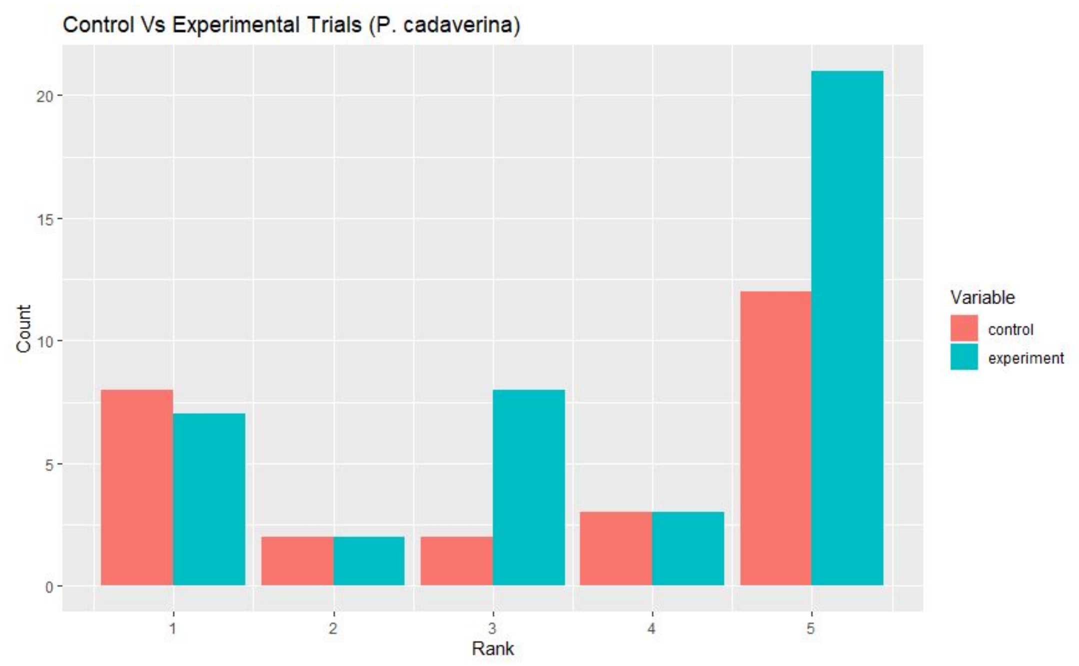

Figure 1: The bar graph represents the data collected on *P. cadaverina* in this experiment, comparing the control and experimental groups. The rank was the final location of the frog with 1 being very close to the agitated *P. regilla*, 5 being very far away, and 3 being no movement. The y-axis represents the number of frogs for each rank. A Shapiro-Wilks test (p=3.875e-09) as well as an Anderson-Darling test (p=4.285e-16) were run to determine that the null hypothesis cannot be rejected, and that the data is non-normal. A Wilcoxon test was run to determine that there is a significant difference between the control and experimental groups (w=74, p<2.2e-16).

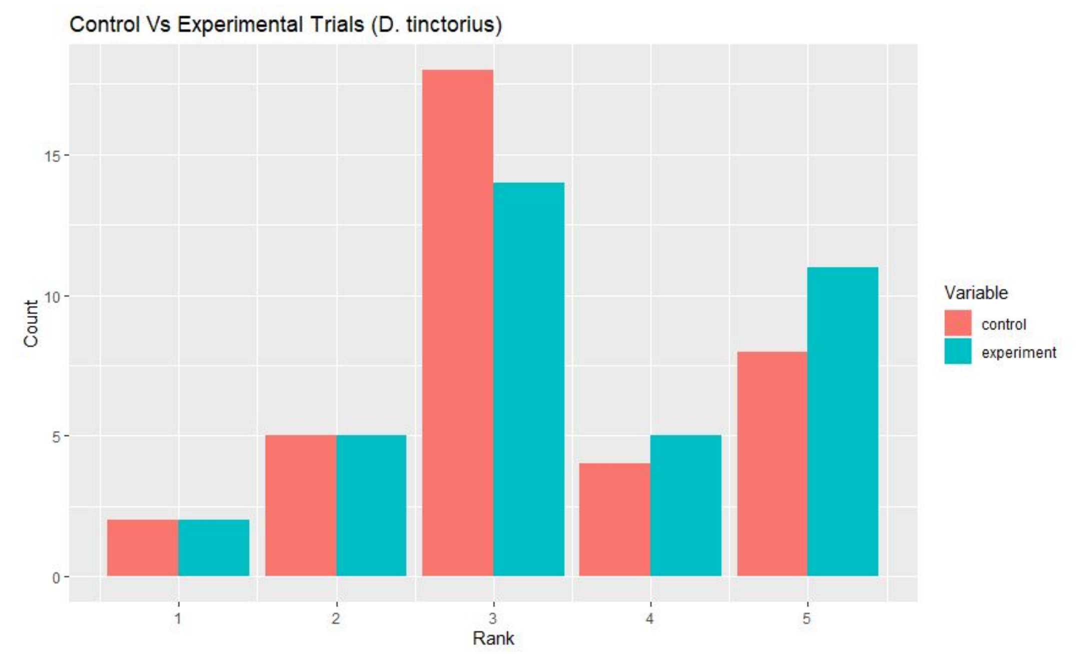

Figure 2: The bar graph represents the data collected on *D. tinctorius* in this experiment, comparing the control and experimental groups. The rank was the final location of the frog with 1 being very close to the agitated *P. regilla*, 5 being very far away, and 3 being no movement. The y-axis represents the number of frogs for each rank. A Shapiro-Wilks test (p=2.009e-06) as well as an Anderson-Darling test (p=3.675e-10) were run to determine that the null hypothesis cannot be rejected, and that the data is non-normal. A Wilcoxon test was run to determine that there is a significant difference between the control and experimental groups (w=307.5, p<2.2e-16).

	No movement	Far movement
P. cadaverina	7	27
D. tinctorius	16	14

Figure 3: This table shows the count of the experimental groups for *P. cadaverina* and *D. tinctorius*. The behavior of the frogs was split into no movement and far movement, where far movement was the sum of the count of frog that was ranked a 1 or a 5. A chi-square test was run to determine that there is a significant difference between the two species of frogs reaction to an agitated *P. regilla* disturbance cue (p=.0137).

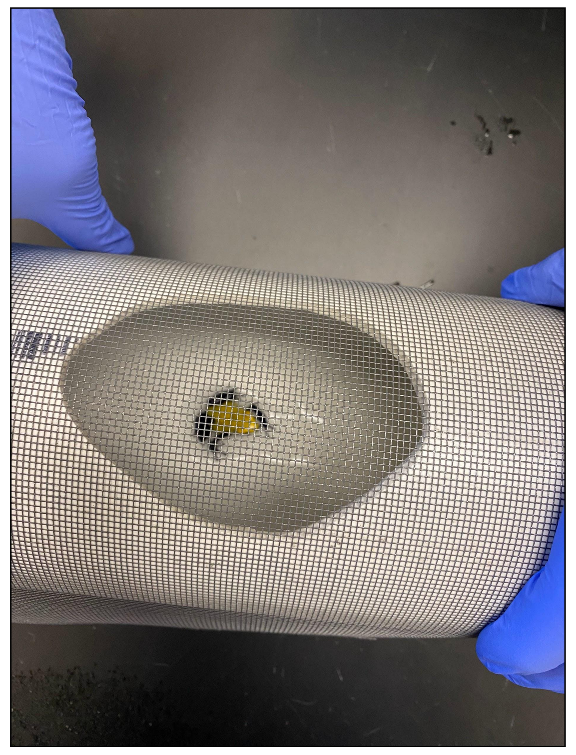

Image 1: *D. tinctorius* placed in the center of the pipe. A fine wire mesh was placed over the opening to prevent the frog from jumping out.

Image 2: Experimental setup of the plastic pipe with the fine wire mesh covering up the openings. A jug of carbon filtered water was used to rinse out the tubes.

Image 3: There were three separate *D. tinctorius* enclosures. After each trial, the frogs were placed back in their respective enclosures.

Discussion

From the results yielded in this study, there is a statistical difference between the control and experimental groups for either frog as well as a difference in reaction to cues between frogs. This data implies that there is a difference in reaction to *P. regilla*. We can conclude that *P. cadaverina* is more likely to move to either extreme and that *D. tinctorius* is less likely to move at all. These results support our initial hypothesis. The results could have occurred due to the fact that *P. regilla* is a close relative to *P. cadaverina* and a distant relative to *D. tinctorius*, so it makes sense for *D. tinctorius* not to respond to the disturbance cue since it would not know what the cue is. It is important to note that frogs will not want to be near a stressed frog as it could be sick or in danger, so *P. cadaverina* moving away from the cues makes sense but moving towards the cues does not. Additionally, not moving is characteristic of *D. tinctorius* as their reaction to suspected danger is to stay put, if they could interpret the cues at all. The bright coloration and patterns of this species are used to warn predators that they are poisonous as opposed to the use of camouflage and evasion by the local species. The results from this study can be used to make a better experimental design to test eavesdropping cues. Since it can be determined that *D. tinctorius* will not respond to the cues, it would be better to run this experiment with a species of frog that does not rely on toxicity and bright coloration. This would be to test if a species with similar evasion tactics are able to eavesdrop and respond to these cues.

Other studies done on disturbance cues yield similar conclusions to our experiment. For example, another study has shown that disturbance odors from one frog species did not trigger a response in another frog species, but the odor was exploited by a predator species of snake. (He, Q. L, et al. 2023). Additionally, researchers saw no reaction between species heterospecifically, even though one species of tadpole was predatory to another. They did find that the prey tadpoles were likely to respond to cues emitted from dragonfly larvae. Overall they determined that the tadpoles would benefit from using visual and chemical cues. (Szabo, B, et al. 2021). All of this information is pertinent to understanding the relationship of sympatric species, but what about allopatric species. Similar to our study, Klosinski et al. (2022) experimented with an invasive species. They found that invasive species and local species can respond to each other's cues, but the extent to which they respond heterospecifically is less than the conspecific response. Understanding how and why animals eavesdrop is vastly important to their conservation and the steps we can take to lessen the impact of invasive species.

There were a few limitations to our study that should be outlined. First and foremost, the data collection and how the experiment was run. As each frog needed to be handled before it was put into the tube or the mesh housing, they could have been already stressed out and released distressed cues or were too stressed out to overhear potential danger cues from other frogs. Additionally, the tubes were a new environment, which could have overstimulated the frogs, resulting in the possible release of unwanted disturbance cues in the control experiment.

A future experiment that could be done to produce conclusive results and further our study would be to to repeat the experiment. The experimental design should be changed to where there is no frog during the control trials, and to where the frogs being placed in the tubes have a longer acclimation period. With these changes, the experiment would be more sound. It would also be advantageous to use frog species that are closer in proximity and would have a reason to react to the cues being emitted by the local species of frog.

Conclusions

- Our original hypothesis was supported, where *P. cadaverina* had extreme responses to the cues, while *D. tinctorius* did not.
- There were flaws in the experimental design and limitations to the study, but it is important to note that there was a difference in behavior overall between the two species of frog.
- Ranked scores determined that *D. tinctorius* were less likely to move, and *P. cadaverina* were more likely to move in either direction than to sit still.

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Acknowledgments

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