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Social feeding preferences of cane toad tadpoles (Rhinella marina)

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ABSTRACT

Cane toad tadpoles (*Rhinella marina*) form aggregations in shallow water while feeding. To investigate their social feeding preferences, this study sampled tadpoles from a human-made pond on the property of Estación Biológica Monteverde. These tadpoles were given choices of feeding alone or in a group, and feeding with different group sizes. Individual tadpoles were placed in an aquarium with a built-in choice chamber, with a starting chamber that had two pathways that lead to the two different options. Overall, the tadpoles spent a greater amount of time remaining in the start of the aquarium than by the given options. These preferences of the tadpoles suggest that they prioritize their own safety over the option of exploration of a new situation.

Preferencias sociales de alimentación en renacuajos de *Rhinella marina*

RESUMEN

Los renacuajos de sapo de caña (*Rhinella marina*) forman agregaciones en aguas superficiales mientras se alimentan. Para investigar sus preferencias sociales de alimentación, en este estudio muestreé renacuajos de una laguna artificial en la Estación Biológica Monteverde. Les di a los renacuajos a escoger entre alimentarse solitariamente o en grupo, así como a escoger alimentarse en grupos de diferente tamaño. Puse a cada renacuajo en un acuario en el que tenía dispuesto una cámara inicial, con dos caminos, en cada uno dispuse cada opción. En general, los renacuajos permanecieron más tiempo en la cámara inicial del acuario que dirigiéndose a las opciones que les ofrecí. Esta preferencia de los renacuajos sugiere que ellos priorizan su propia seguridad sobre la opción de explorar una nueva situación.

An aggregation is typically classified as a group of conspecific organisms that occupies a densely populated area relative to the immediate surroundings (Jeanson et al., 2005). One of the motivations behind aggregating is seeking safety and cover from predators (Alexander 1974). This "selfish herd strategy" decreases the instances of being caught (Hamilton 1971). Across numbers of anuran species, tadpoles also form defensive aggregations, and some aggregations are composed of individuals that are kin to one another (Beiswenger 1975). In addition to social motivation, other studies have found that individual tadpoles have shared interest for certain resources such as light (Wassersug et al., 1981). When the light intensity of the sunlight is fairly strong, tadpoles gather in shallow areas of the water body, where it is warmest. As the light intensity decreases across the day, tadpoles retreat from the shores and swim to the deeper areas (Beiswenger 1977). Light is important for maintaining body temperatures, and therefore the availability of light directs the behavior of tadpoles (Adler 1970).

Cane toads (*Rhinella marina*), large toads native to Central and South America, were introduced to Australia as a form of biological pest control (Lever 2001). Their tadpoles form aggregations as well, and they are more likely to occur in brightly lit areas, especially if the area has physical structures, such as rocks or floating algal mats. (Raven et al., 2017). In a binary choice type experiment based in northern Australia, tadpoles were observed to move to other tadpoles that were feeding, as opposed to not feeding (Raven et al., 2017). Understanding their aggregation behavior provides insight for possible methods of controlling their population and mitigating their ecological impact in the areas they have invaded.

In this study, a series of binary choice experiments were designed to understand the social feeding preferences of cane toad tadpoles. In the first experiment, tadpoles were given a choice to swim to one of two chambers, one with food and one without food. This experiment sets the basis on whether tadpoles can detect food in the experimental setting. The second experiment investigates whether, when given a choice, tadpoles choose to feed with others or on their own. Finally, the third experiment investigate whether tadpoles prefer to feed with a densely populated group or a less populated group. I predict that when given the options of feeding alone or with others, tadpoles will choose to feed with others, and subsequently also choose to be with a bigger group for safety.

MATERIALS AND METHODS

Study Organism

Rhinella marina tadpoles were collected from a human-made pond on the Estación Biológica Monteverde property (1200m elevation) in Monteverde, Puntarenas, Costa Rica. Before experiments, at least thirty tadpoles were caught on the mornings of May 18th, 20th, and 21st, 2019 with the exception of May 23rd, where over sixty tadpoles were caught to run two sets of experiments. The tadpoles were kept in a 35 x 35 cm square aquarium with an air pump to oxygenate the water. Algae collected from the same pond was used to feed the tadpoles.

Study site

Schools of cane toad tadpoles in the human-made pond by the green gate of the Estación Biológica Monteverde were observed in the late morning of May 15th, 2019. For this experiment, a school was determined as a group of tadpoles where each tadpole is within a distance of three body lengths of at least two other tadpoles. Photographs of the naturally occurring schools were taken, and the temperature as well as water depth were measured at the center of each school (**Fig. 1**). For a big school that covered a greater surface area of the pond, multiple points were chosen for temperature and water depth measurements. These centers were roughly determined on site. The center of each school was treated as the average of the area that the school covered. The water depths recorded provided a reference for the experimental water depth of two centimeters. The temperatures provided insight into previously mentioned literature on aggregation behavior correlated with water temperature, but were not used for experimental conditions.

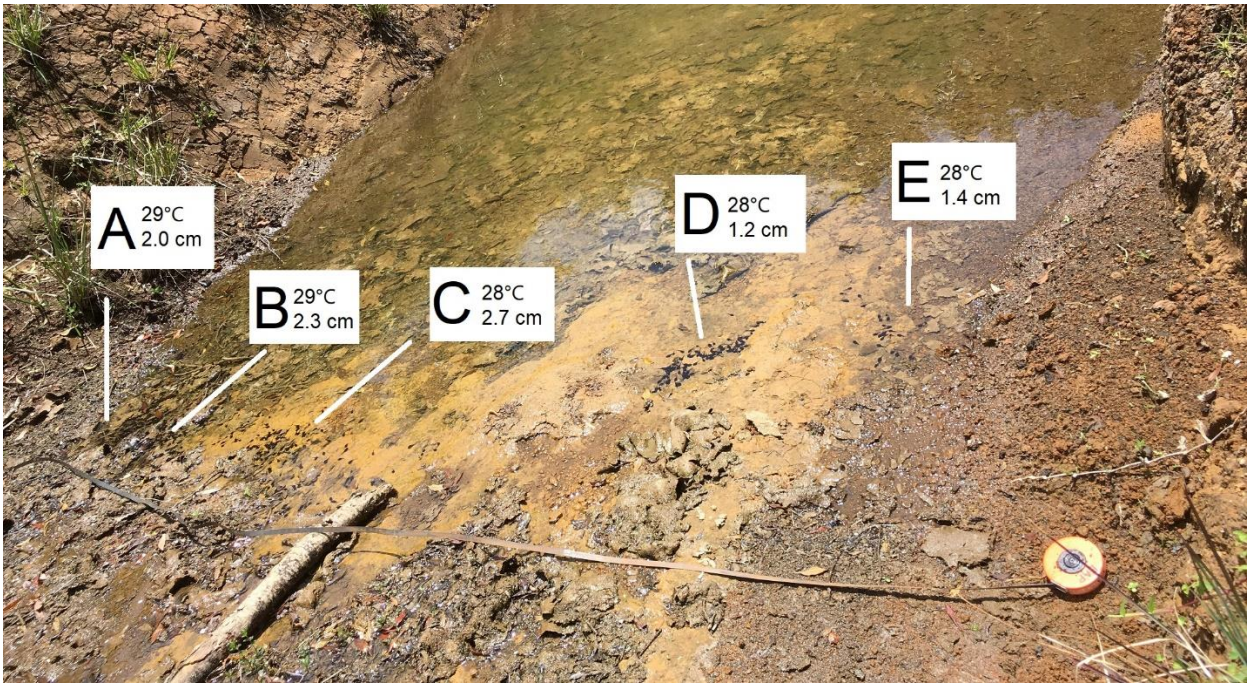

FIGURE 1. Photo of the observed site and the location of each school of *Rhinella marina* tadpoles at the human-made pond of the Estación Biológica Monteverde. Each letter represents epicenters of a school of tadpoles, with A and B representing two points within a large school. The letters are followed by the depth of the water in centimeters and the temperature of the water in Celsius at the respective points. (A= 29 ℃, 2.0 cm; B=29 ℃, 2.3 cm; C=28 ℃, 2.7 cm; D=28℃, 1.2 cm; E=28 ℃, 1.4 cm)

Preparations for the experiment

Water from the pond was collected for the experiments to assure that the tadpoles were tested in water they were acclimated to. The water was poured through a sieve (size 500 µm) to filter out particles that may obstruct my vision. When the tadpoles were caught, the temperature of the water of their catch site was recorded. This temperature was kept in experimental conditions by setting prepared water under sunlight. This ensured that the tadpoles would not experience a sharp change in temperature during the experiments. Sheets of algae from the pond were also collected and stored in a container with pond water. The algae were prepared by cutting the algae sheet into pieces that roughly weighed ten grams while wet.

Experimental aquarium

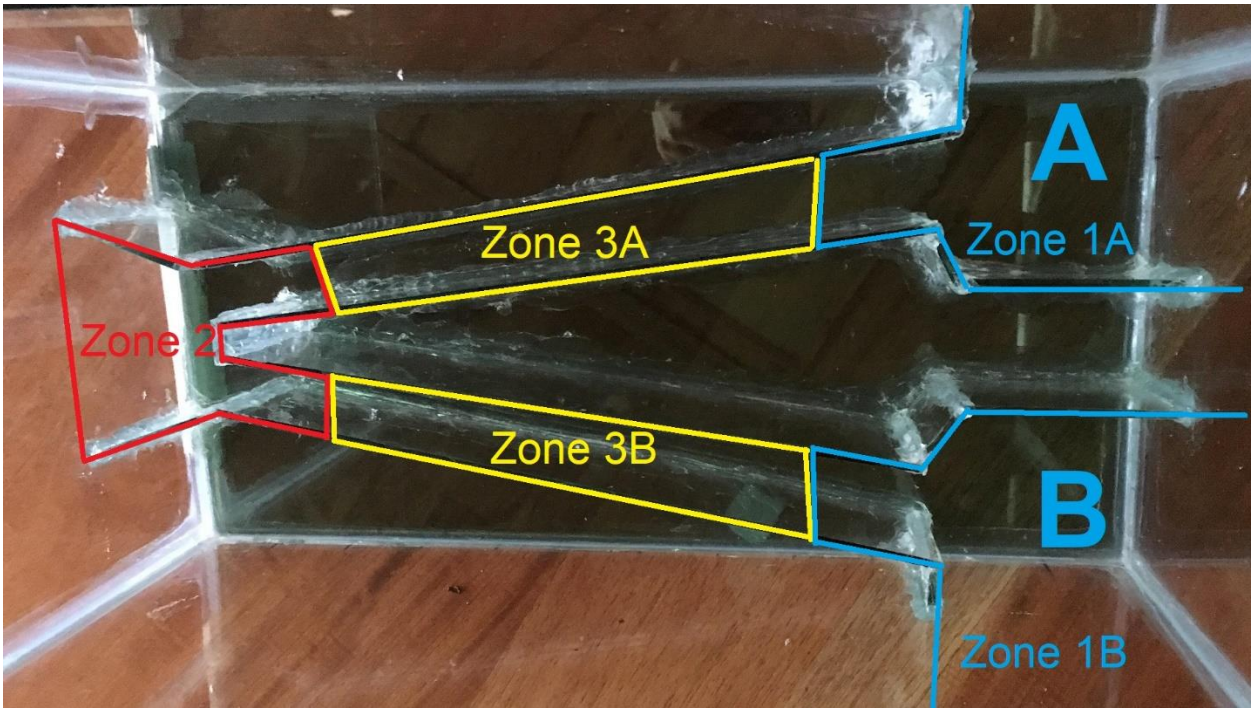

FIGURE 2. Diagram of the different zones in the experimental aquarium. The aquarium was divided into five zones which are classified as follows. Zone 1A: The chamber of choice A, and 5 cm within the entrance of chamber A. Zone 1B: The chamber of choice B, and 5 cm within the entrance of chamber B. Zone 2: The starting chamber and 5 cm within the entrance of the start. Zone 3A: The pathway between zone 1A and 2. Zone 3B: The pathway between zone 1B and 2.

General experimental process and categorization of behavior

A rectangular 49-centimeter by 24-centimeter aquarium was used for all experiments. A starting chamber, two pathways branching out from the starting chamber, and two choice chambers at the end of the pathways were built inside the aquarium using custom ordered pieces of glass and silicone glue (**Fig. 2**). Thick wire and an acetate sheet with strategically placed holes were used to create a removable barrier between the starting chamber and the pathways. Barriers made of wire mesh and thick wire were used to separate the pathways and the choice chambers. These barriers were used to prevent tadpoles from escaping the chambers.

Every test tadpole was allowed to acclimate to the experimental set up in the starting chamber for one minute. During this acclimation period, the behavior of the tadpole was classified as S or F. F signified “frantic” or “fidgety” behavior, where the tadpole did not remain still for more than five seconds at a time consistently. S signified “still” behavior, where the tadpole remained still for more than five seconds at a time, and generally did not change its position. After this minute, the algae and/or tadpoles were promptly added in the appropriate choice chambers of A or B (**Fig. 2**) according to the experiment that was conducted. Their scents were allowed to permeate the water for thirty seconds. Promptly, the starting divider was

removed, beginning the two-minute observation period. The amount of time the tadpole spent in each of the zones was recorded as a measure of preference.

Water in the testing tank was changed out after each run to ensure that the water will not carry organic material and chemical cues from the previous run. Additionally, the positions of the two options were varied to ensure that they were located in chambers A and B for an equal number of runs. Each day that the experiments were conducted, the order of the experiments were changed, due to the observation that tadpoles began to show lethargic behavior a few hours after being caught.

Experiment 1: Food detection

In experiment 1, the tadpoles being tested were given the options of a chamber with algae, and a chamber without algae. This experiment established the premise of whether tadpoles can sense the presence of algae in the experimental setting. Six tadpoles from the thirty tadpoles were chosen from the aquarium and individually tested. The tadpoles were caught haphazardly from the tank they were kept in with a net or small plastic cup. The individual being tested was placed in the starting chamber (**Fig. 2, Zone 2).** The tadpole was allowed to acclimate to the setting for one minute. During this minute, the tadpole was observed and characterized as showing F or S type behavior. After this minute, a piece of algae was placed in either chamber A or B, and it was allowed to permeate the water for thirty seconds. After the thirty seconds, the acetate divider was then removed, and the time the tadpole spent in each of the designated zones was recorded for two minutes. The tadpole was then removed from the tank and placed in a prepared bucket to assure that the same tadpole was not used twice in a single day. Between each run, the water in the tank was cleared out and replaced.

Experiment 2: Social feeding preference

In experiment 2, the tadpoles being tested were given the options of a chamber with algae and five other tadpoles, and a chamber with algae only. This experiment investigated whether the tested individual prefers to feed with others or alone. Six tadpoles from the remaining tadpoles were chosen haphazardly for this experiment. An individual tadpole was placed in the starting chamber (**Fig. 2, Zone 2**) and allowed to acclimate to the setting for one minute. During this time, the tadpole was observed and characterized as showing F or S type behavior. After this minute, a piece of algae and five tadpoles were placed in their appropriate chamber, behind a wire mesh divider which prevented the tadpoles from swimming out. Another piece of algae was placed behind a wire mesh divider in the other chamber. After thirty seconds, the acetate divider was then removed, and the time the tadpole spent in each of the designated zones was recorded for two minutes. The tadpole was removed from the tank and placed in a prepared bucket to assure that the same tadpole was not used twice in a single day. Between each run, the water in the tank was cleared out and replaced.

Experiment 3: Group size in social feeding preference

In experiment 3, the tadpoles being tested were given the options of a chamber with algae and ten other tadpoles, and a chamber with algae and five other tadpoles. This experiment investigated whether the tadpole prefers to feed with a bigger group of a smaller group. Six tadpoles from the remaining tadpoles were chosen haphazardly for this experiment. An individual tadpole was placed in the starting chamber (**Fig. 2, Zone 2**) and allowed to acclimate

to the setting for one minute. During this time, the tadpole was observed and characterized as showing F or S type behavior. After this minute, a piece of algae and ten tadpoles were placed in their appropriate chamber, behind metal mesh divider, which prevented them from swimming out. Another piece of algae and five tadpoles were placed in the other chamber, also behind a metal mesh divider. After thirty seconds, the acetate divider was then removed, and the time the tadpole spent in each of the designated zones was recorded for two minutes. The tadpole was removed from the tank and placed in a prepared bucket to assure that the same tadpole was not used twice in a single day. Between each run, the water in the tank was cleared out and replaced.

Statistical Analysis of Data

A simple ANOVA test was conducted on Microsoft Excel to analyze the average time tadpoles spent in each zone for each experiment type. This was followed by a Tukey-Kramer test to compare the significance of each of the averages. A Chi-square test was used to analyze the significance of the effects of the number of tadpoles that chose to leave the chamber and those that did not. Finally, to determine whether there is a relationship between the tadpole behavior type and the total time tadpoles spent in Zone 2, a T-Test was run.

RESULTS

Experiment 1

Thirty different tadpoles were tested in experiment 1. Fifteen were tested with the food option (algae) located in chamber A, and fifteen were tested with the food option located in chamber B. The averages of these two groups were separated to see whether the position of the options influenced the amount of time the tadpoles spent in a particular zone. The average time the tadpoles spent in Zone 2 for when the food option was in chamber A was 78.6 seconds, and 85.5 seconds for when the food option was in chamber B (**Fig. 3**). These averages were significantly higher than the average time the tadpoles spent in the zones of 3A, 3B, 1A or 1B (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). The averages of 3A, 3B, 1A, 1B were not significant from each other (Tukey-Kramer, $p < 0.05$).

To understand the high averages for Zone 2, the number of tadpoles that chose to leave Zone 2 and those that did not leave at all were calculated. When the food option was in chamber A, ten of the fifteen tadpoles left Zone 2, and when the food option was in chamber B, nine of the fifteen tadpoles left Zone 2 (**Fig. 4)**. These occurrences, however, were not significant (Chi-square value = 0.14, $p > 0.05$).

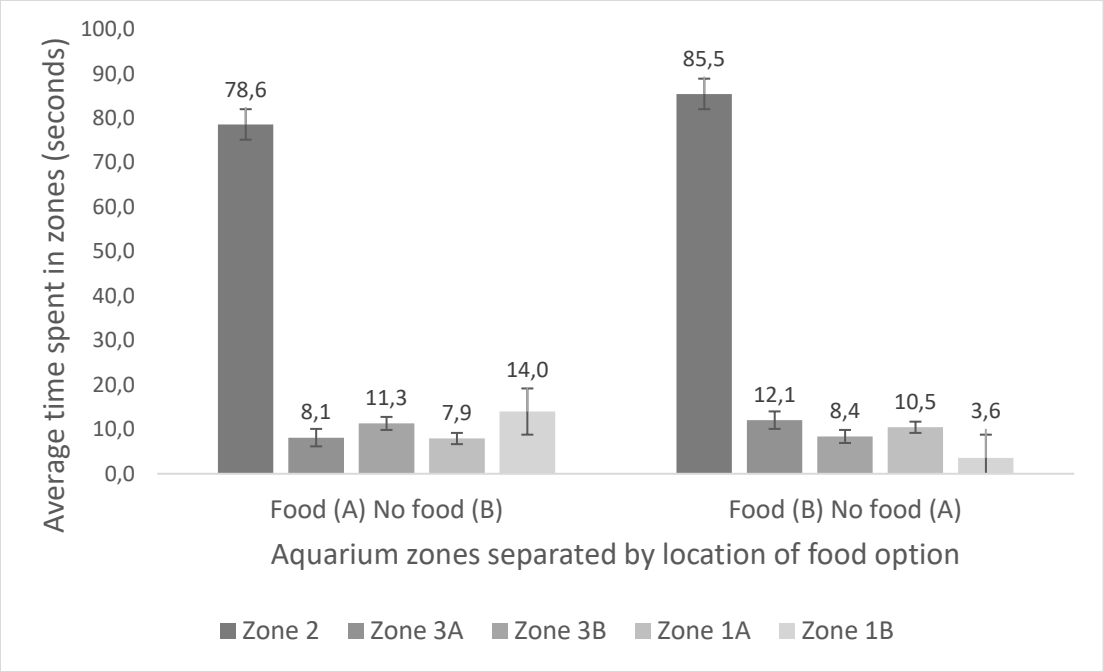

FIGURE 3. Average time tadpoles spent in each zone in Experiment 1 (food vs. no food) separated by the location of the food option (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). Difference between the means only significant when comparing to the mean time spent in Zone 2 (Tukey-Kramer, $p < 0.05$). Bars represent standard error. The numbers represent the value of the bars. n=15 for each.

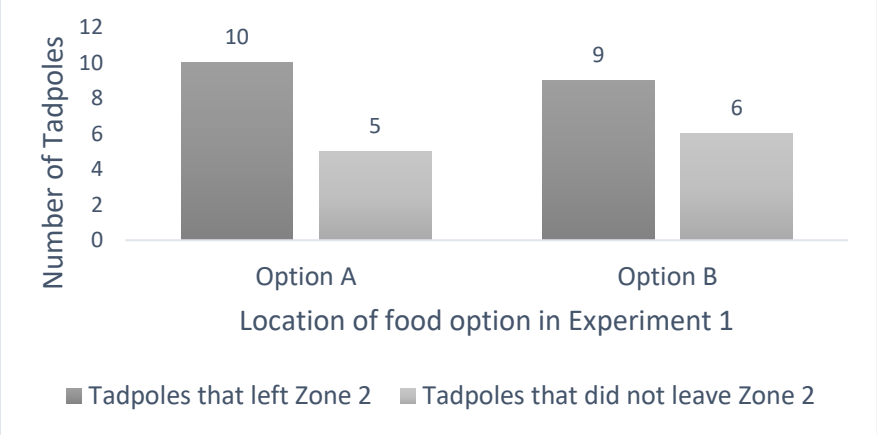

FIGURE 4. Number of tadpoles that left Zone 2 and did not leave Zone 2 in Experiment 1 (Food vs. No Food) separated by the location of the food option. (Chi-square value = 0.14, $p > 0.05$). n=15 for each.

Experiment 2

Thirty different tadpoles were tested in experiment 2. When the food and five tadpoles located in chamber A, twelve of the fifteen tadpoles left Zone 2, and when the food and five tadpoles located in chamber B, five of the fifteen tadpoles left Zone 2 (**Fig. 6**). These occurrences were statistically significant (Chi-square value = 6.65, $p < 0.05$). However, the average time the tadpoles spent in Zone 2 for when the food option was in chamber A was 97.7 seconds, and 107.6 seconds for when the food option was in chamber B (**Fig. 5**). These averages were significantly higher than the average time the tadpoles spent in the zones of 3A, 3B, 1A or 1B (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). The averages of 3A, 3B, 1A, 1B were not significant from each other (Tukey-Kramer, $p < 0.05$).

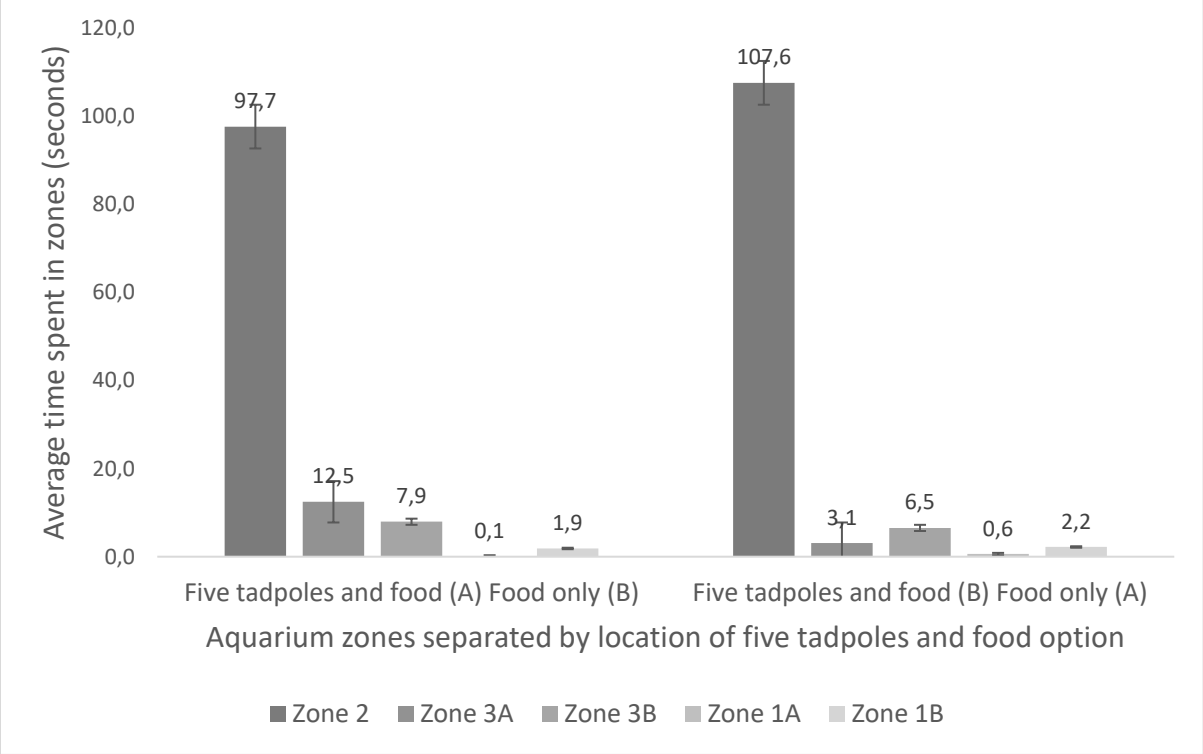

FIGURE 5. Average time tadpoles spent in each zone in Experiment 2 (five tadpoles and food vs. food only) separated by the location of five tadpoles and food (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). Difference between the means only significant when comparing to the mean time spent in Zone 2 (Tukey-Kramer, $p < 0.05$). Bars represent standard error. The numbers represent the value of the bars. n=15 for each.

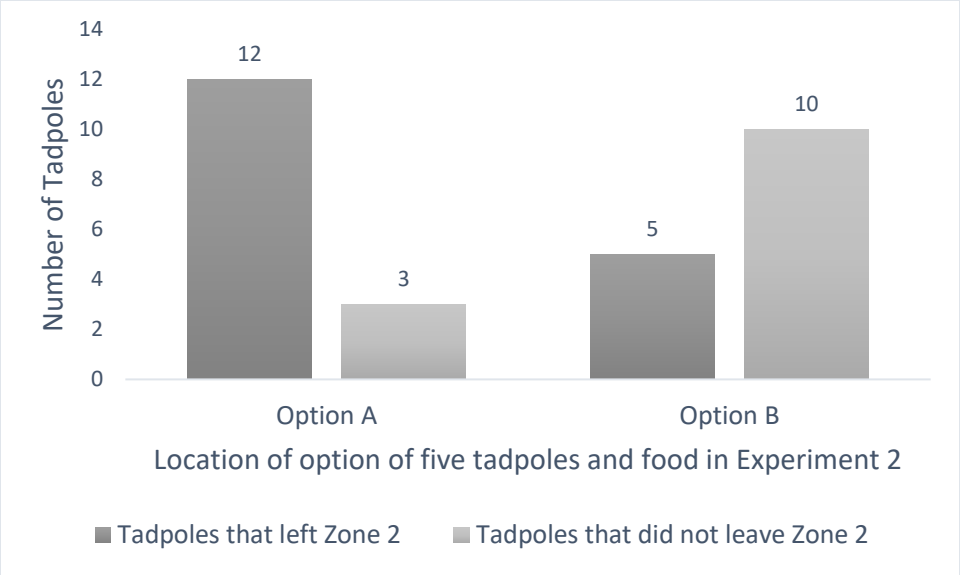

FIGURE 6. Number of tadpoles that left and did not leave Zone 2 of the aquarium in Experiment 2 (five tadpoles and food vs. food only) separated by the location of the five tadpole and food option. These occurrences were significant from each other (Chi-square value = 6.65, $p < 0.05$). n=15 for each.

Experiment 3

Thirty different tadpoles were tested in experiment 3. When the food and ten tadpoles located in chamber A, six of the fifteen tadpoles left Zone 2, and when the food and ten tadpoles located in chamber B, six of the fifteen tadpoles left Zone 2 (**Fig. 8**). These occurrences were not statistically significant (Chi-square value = 0, $p > 0.05$). However, the average time the tadpoles spent in Zone 2 for when the food option was in chamber A was 105.1 seconds, and 104.7 seconds for when the food option was in chamber B (**Fig. 7**). These averages were significantly higher than the average time the tadpoles spent in the zones of 3A, 3B, 1A or 1B (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). The averages of 3A, 3B, 1A, 1B were not significant from each other (Tukey-Kramer, $p < 0.05$).

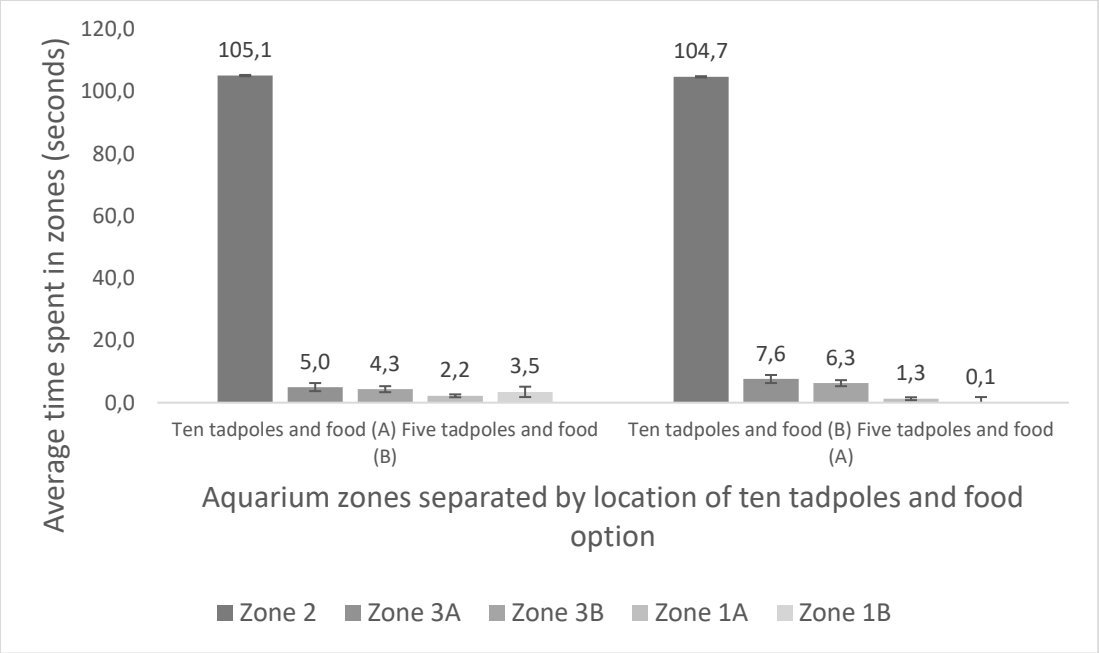

FIGURE 7. Average time tadpoles spent in each zone in Experiment 3 (ten tadpoles and food vs. five tadpoles and food) separated by the location of the ten tadpoles and food. Bars represent standard error. The numbers represent the value of the bars (ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$). Difference between the means only significant when comparing to the mean time spent in Zone 2 (Tukey-Kramer, $p < 0.05$). Bars represent standard error. The numbers represent the value of the bars. n=15 for each.

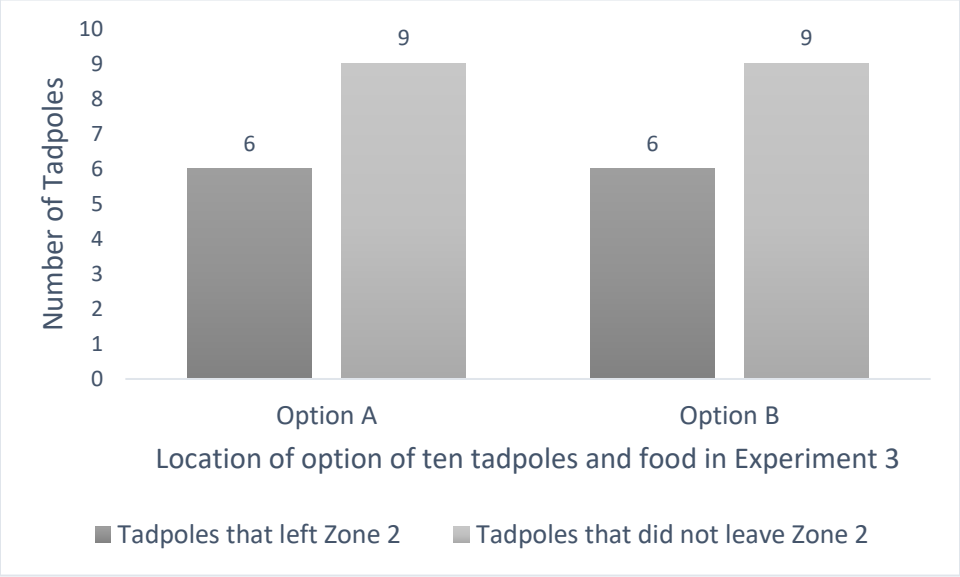

FIGURE 8. Number of tadpoles that left and did not leave Zone 2 of the aquarium in Experiment 1 (Ten tadpoles and food vs. five tadpoles and food) separated by the location of the ten tadpoles and food option. These occurrences were not significant from each other (Chi-square value = 0, $p > 0.05$). n=15 for each.

Average time spent in Zone 2 in relation to behavior type

The average amount of time spent in Zone 2 by behavior type F tadpoles was 72.0 seconds and 92.0 seconds for behavior type S tadpoles in experiment 1. In experiment 2, behavior type F tadpoles spent 97.5 seconds in Zone 2 on average, and behavior type S tadpoles spent 106.1 seconds on average. Finally, in experiment 3, behavior type F tadpoles spent 107.2 seconds in Zone 2 on average, and behavior type S tadpoles spent 103.2 seconds on average. Comparing the averages between the behavior types across each of the experiments, no significant difference was found (T-test, $p > 0.05$).

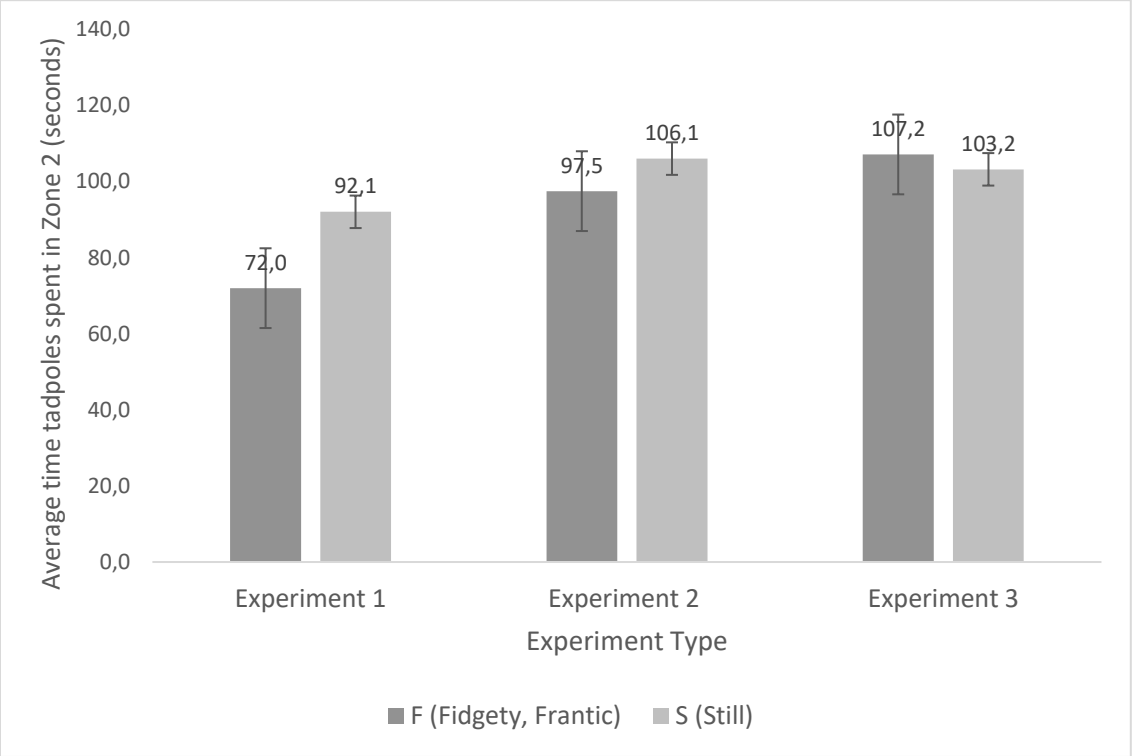

FIGURE 9. The average time that tadpoles spend in the starting chamber (Zone 2) for each experiment, with tadpoles separated by their behavior type. Each of the paired averages were not significantly different from each other (T-test, $p > 0.05$). Bars represent standard error. The numbers represent the value of the bars. N= 30 each.

DISCUSSION

Throughout each of the experiments, the tadpoles did not spend a significant amount of time outside of the Zone 2 of the aquarium (**Fig. 3, 5, 7,** ANOVA, $F_{(4,70)} = 2.50$, $p < 0.05$, Tukey-Kramer, $p < 0.05$). Therefore, a distinct preference was not expressed when given the options of food or feeding with other tadpoles across all experiments. During the two-minute observation period, most tadpoles exhibited a behavior that I described as “fascination with corners.” Oftentimes tadpoles that exhibited this behavior swam back and forth between the two acute corners (**Fig. 2**) of the starting chamber for a majority of the two-minute period before the

considered exploring the pathways. I also observed that tadpoles classified as type F (frantic) began swimming between the two corners earlier than tadpoles classified as type S (still). To determine whether this difference in behavior could explain the high averages for Zone 2 of the aquarium (**Fig. 3,5,7**), the average amount of time spent in Zone 2 was graphed against the different behavior types for each experiment (**Fig. 9**). However, the high average amount of time spent in Zone 2 was not explained by the observed tadpole behavior type, as the differences between each of the paired averages were not significant from each other (T-test, $p > 0.05$). This suggests that the prior to the decision of choosing a pathway to explore in the aquarium, the tadpoles had other prioritizations for themselves that did not encourage them to leave the starting chamber.

In general, it appears that the tadpoles displayed a behavior favoring safety. Rather than exploring a new situation and joining other tadpoles, the individual considered its own safety first in a "better safe than sorry" fashion (Haftorn 2000). The frantic, back-and-forth, movement may have been attempts to find a way to escape the unfamiliar situation. Additionally, this behavior may have been encouraged, by the design of the starting chamber. The acute angles of two of the corners provided narrow physical structures that cover two sides of the tadpoles' body. This may have provided the tadpoles with a sense of safety or a notion that they will lead to a body-tight pathway, as *Rhinella marina* tadpoles have been studied to prefer to be in settings with physical structures (Raven et al., 2017).

A starting chamber with more open corners (greater than 90 degrees) or a rounded chamber may have encouraged the tadpoles to leave the chamber earlier, as it would not provide as secure structure as acute corners. Overall it appears that tadpoles, when placed in a foreign environment, will not prioritize exploring new areas, and will instead favor searching for an escape to a familiar environment, or a place for hiding itself.

ACKNOWLEDGEMENTS

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