Background Research:

To design the experiment and understand the organisms' underlying behaviors which might affect it, extensive background research was required—specifically on their spatial distribution patterns. First, a previous study analyzing the spatial patterning and structure of termite mounds in an African savanna was examined to better understand the procedure of the experiment. This study examined how different termite colonies in the African savanna positioned themselves in relation to one another, and uncovered that termite mounds each neighbor sic other termite mounds at a relatively constant distance, creating uniform hexagons of termite mounds through the savannah. Furthermore, this study uncovered that termite mounds must maintain a constant distance from each other to prevent conflict between termite colonies, which would limit the overall success of the species. These results helped guide and shape this study that was conducted by providing insight to the possible intraspecies competition that could result from close antlion contact, leading to the prediction that antlions (Myrmeleon immaculatus) would have to space themselves in order to prevent competition for food. Lastly, this study determined that a change in available space could affect the spatial patterns of termites as well as their behavior, which was later used in designing the conducted experiment.

Next, several studies regarding the anatomy and behavior of antlions were used in order to better understand the insects. These studies determined that antlions stay in their larva form, in which they make pits, for 6-8 weeks and develop slower when exposed to less food. This helped determine the timeline of the experiment and determine the intervals at which the antlions would be fed, as in order to keep results consistent the antlions would have to be the same throughout the course of the experiment, which would require the participating antlions to be fed less in order to stay in their larva stage to make pits. Furthermore, these studies examined terms such as pit depth and width as well as the feeding patterns and behaviors of antlions, which became crucial areas of study throughout the experiment, as these studies determined that pit depth and width can signify the dominance and success of antlion settlement. This helped determine the dependent variable—size/density constraints—to examine over the course of the study. Finally, these studies determined that antlions have a tendency to cannibalize each other in times of food shortage and significant competition. This provided another dependent variable to track over time and examine as size decreased, as cannibalized antlions were unsuccessfully metabolized and evident in pits.

Lastly, a series of studies about antlion dispersal pattern called the "Doughnut theory" were examined to better understand the current scientific knowledge surrounding antlion dispersal patterns. These papers determined that antlions naturally position themselves in a "doughnut," in which a ring of antlions circle a center point or food source to limit competition for ants, as each antlion has equal access to the food source. This study also concluded that when antlions are introduced one by one the same results occur, which confirmed that the procedure could introduce one antlion at a time without interfering with results and spatial patterns, helping further perfect and standardize the procedure, as well as provide a better understanding of antlions behavior patterns. These studies provided a better understanding of antlion settlement patterns and gave a guideline for what to expect as trials continued. Finally these studies provided scientific procedures that could be tested and confirmed throughout the experiment, allowing for a source to cross-check results and procedures with in order to perfect the procedure of the experiment.

Works Cited

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Essential Question:

How do antlion spatial patterns, such as pit depth, width, and nearest neighbor, as well as behaviors, such as cannibalism and eating habits vary with respect to spatial constraints and temporal change?

Hypothesis:

As the space available to antlion groups decreases, each claims less territory, and the populations tend towards more extreme behaviors, such as cannibalism and reclusivity, to limit competition for ants as an emergent feature of individual interactions.

Materials:

First, a 32x33 container was used to house antlions. A cardboard barrier (and sufficient tape) was constructed to restrict it to a 24x24, 16x17, and 8x7 spaces. Approximately 200 pounds, or four 50lb bags, of quartz sand were needed as a substrate. Next, 40 antlions were obtained and 160 ants for food. 40 plastic circular containers (at a six inch diameter and a four inch depth) housed the antlions and 1 housed the ants. One meter stick, a six inch ruler, and a sharpie were obtained to measure and obtain data from trials. Then, 40 toothpicks and a small plastic cup with a diameter of 2 inches were applied to record and manage the locations of antlions throughout the study. A sieve was used to move the antlions from place to place and secure them.

Independent and Dependent Variables:

Throughout the experiment the independent variable was the size of the container, which changed from trial to trial, but did not change due to any other variable. Furthermore, the dependent variable throughout the experiment was the settlement patterns and behaviors of the antlions (myrmeleon immaculatus), which was quantified through the nearest neighbor calculation, pit depth and width, and the number of cannibalized antlions. The control trial of the experiment was the 32x32 trial, as it shows the spatial patterns and behaviors of the antlions with the most available space, limiting the effect of competition on settlement patterns, which qualifies it to be a good control group.

Procedure:

To start the procedure the materials needed to be obtained. Once materials were obtained the 160 ants were kept in one of the 6 inch plastic containers, and 200 grams of native sand was poured into each of the 40 remaining six inch containers. Next, each of the 40 antlions (Myrmeleon immaculatus) were placed in one of the plastic containers containing sand, with each antlion getting its own container. Following this each noticeable antlion pit was given two ants as food once every week, starting the Friday after the antlions were introduced to their temporary containers. Then, the remaining amount of sand was placed into the 32x32 container and spread out using a meter stick until the surface of the sand was level. Next, a meter stick was used to mark the sides of the 32x32 container with inch markers starting from the bottom right of the container on its lid. Much like a coordinate plane, marks were made one inch apart going vertically from the bottom of the lid of the container and subsequently labeled with their position away from the bottom of the box in inches, this acted as the y-axis. After this, marks were made one inch apart going horizontally from the right most section of the lid of the container and subsequently labeled with their position away from the right of the box in inches, this functioned as the x-axis. Next, the 2 inch cup was placed at the center of the container and buried under 3cm of sand. After this 4 antlions were introduced to the container every 24 hours until 30 antlions had been introduced, starting at 3:30 pm. This was done by using the sieve to obtain four random antlions from their temporary containers and place them on the center of the container, where the plastic cup was. Antlions were moved by using the sieve to remove the antlion from its six inch holding container. As new antlion pits appeared toothpicks were inserted next to them to signify their presence. Following the introduction of all 31 antlions a 24 settling period was allotted, after which the location of each antlion was measured using the grid system created earlier. Following this a program was used to find the nearest neighbor and a ruler was used to find the pit depth and width in cm. After data was taken the antlions were transferred back to their temporary containers by using a sieve to obtain the antlions from pits, where they were later placed in their temporary containers, dead antlions were kept in a freezer. Following this a barrier was inserted to reduce the available space to 24x23 inches using cardboard dividers and sealed using making tape, to prevent antlions from escaping the enclosure. After this another hour introductory period every 24 hours was repeated, will all remaining antlions, as some died in the previous trial. Once all antlions were introduced another 24 hour settling period was allocated and pit depth, width and location were found using the same methods as above, and the antlions were returned to their temporary containers. Following this the area of the box was reduced to 16x15 inches and all antlions were again introduced, 4 every 24 hours until all remaining antlions were placed in the pit. Then another 24 hour settlement period was allotted and all data was collected the same was as the previous two trials, and the antlions were returned to their temporary containers. Lastly, the area of the box was reduced to 8x7 inches and all antlions were again introduced, 4 every 24 hours until all remaining antlions were placed in the pit. Then another 24 hour settlement period was allotted and all data was collected the same was as the previous three trials, and the antlions were returned to their enclosures. To further understand the relevance of the study, the species of the antlions were examined through DNA barcoding. Using min PCR and a gel barcoding system the antlion DNA was extracted, and used a strand of mitochondrial DNA, cytochrome C in order to identify the antlions using a national protein database. The observed genus and species was Myrmeleon Immaculatus.

Graphs:

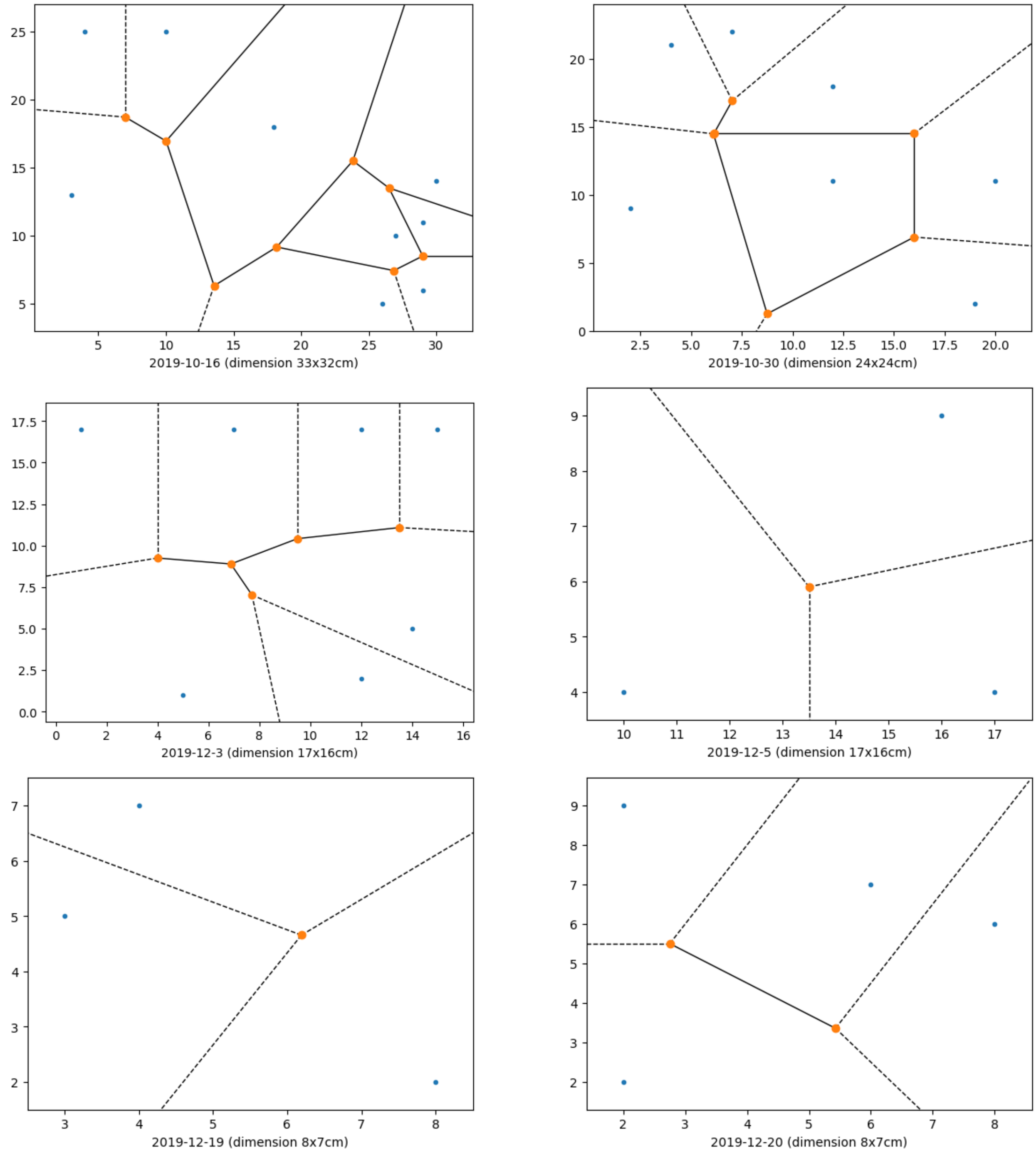

Fig 1: Voronoi diagrams showing the territory of each antlion that formed a pit and well as the location, depth, and width of each pit

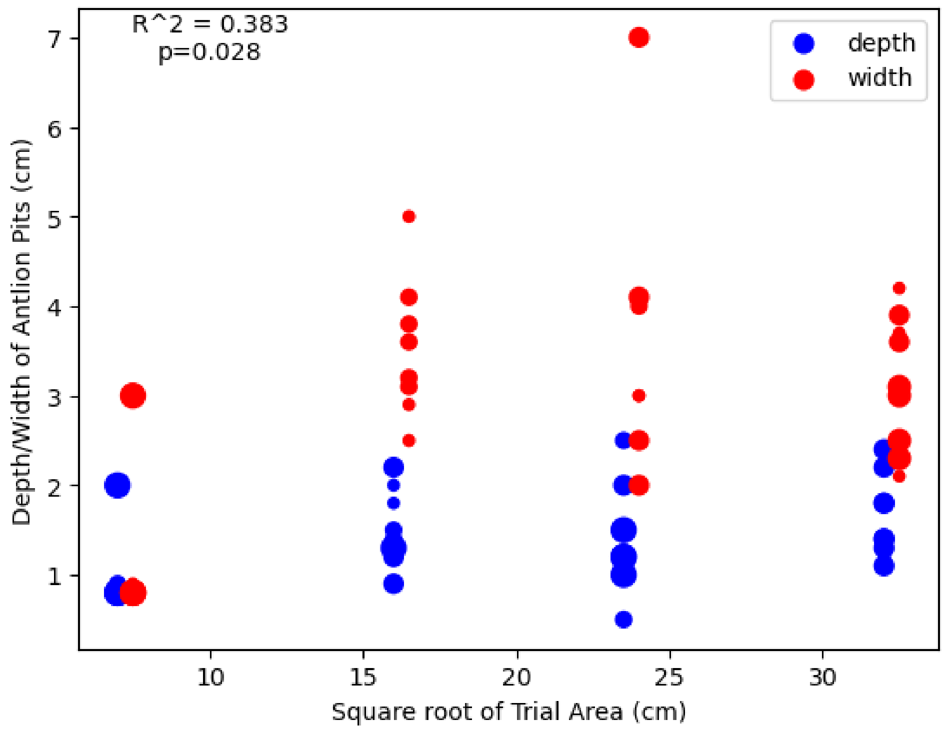

Fig 2: Shows pit depth and width in relation to the square root of the trial area (bigger dots mean more pits of that size)

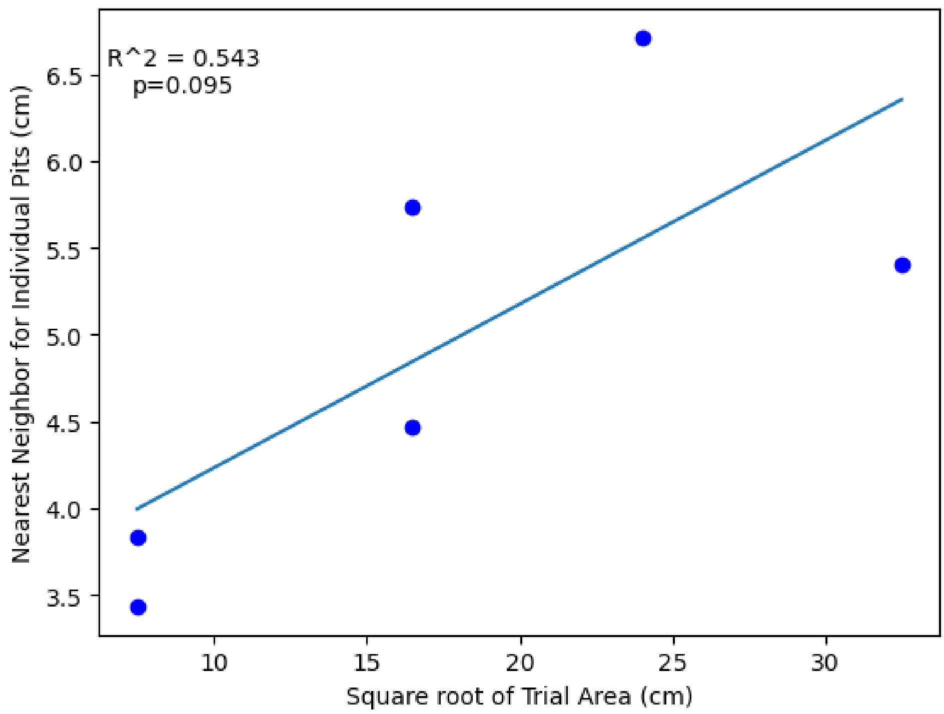

Fig 3: Shows the average nearest neighbor calculation for each trial group in relation to the square root of trial area, to create a ratio

Tables:

Trial Size	Date	Introduced	Deaths	Pits formed
33×32	2019-10-16	31	6	9
24×24	2019-10-30	27	3	7
17×16	2019-12-3	19	3	7
17×16	2019-12-5	10	0	3
8×7	2019-12-19	12	4	3
8×7	2019-12-20	5	0	4

Fig 4: Number of Deaths and Pits Successfully Formed in Each Trial/Subtrial

Dimensions (in)	Pit Depth (cm)	Pit Width (cm)	Nearest Neighbor (cm)
33×32	1.3	4.2	6.00
	1.4	3.7	12.04
	1.1	3.0	6.00
	1.8	2.3	10.63
	2.2	3.1	3.16
	1.4	2.5	2.24
	1.2	2.1	2.24
	2.4	3.9	3.16
	1.8	3.6	3.16
24×24	2.0	7.0	3.16
	2.5	4.1	3.16
	0.5	2.0	10.20
	1.2	2.5	6.40
	1.2	3.0	7.00
	1.0	3.0	8.00
	1.5	4.0	9.06
17×16	1.3	4.1	3.61
	1.2	3.8	3.61
	0.9	3.2	7.07
	2.2	3.8	3.00
	1.2	2.5	3.00
	2.0	5.0	5.00
	1.8	3.6	6.00
17×16	1.3	3.1	5.10
	1.5	3.1	7.00
	1.4	2.9	5.10
8×7	0.8	0.9	2.24
	0.9	0.8	2.24
	2.0	3.0	5.83
8×7	0.8	0.8	2.24
	0.8	0.8	6.40
	0.8	0.8	2.24
	0.8	0.8	4.47

Fig 5: The Pit Depths, Widths, and “Territory,” Observed in Each Trial

Conclusion:

Pit depth and width correlate strongly with trial area, as demonstrated by graph one, which relates the two. The pit positioning of antlions (as a group and as individuals) likely varies solely to maximize ant capture. Therefore, this phenomenon is observed because antlions' (myrmeleon immaculatus) pits don't need to be as big when the main constraint on ants falling into the pit is simply having a pit available for them to fall into. This is also observable by the trials' decreasing number of visible pits (versus total antlions introduced) with respect to size: they start to hide underground because rather than simply having smaller pits than stronger antlions, they have to rest underground, possibly to preserve group wellbeing. Graph 2 indicates a similar trend—antlions' territory as described by the nearest neighbor calculation is much lower in smaller containers. This is the natural consequence of less area being available but demonstrates that the effects of hiding don't completely level the density of antlion pits based on population per area. Additionally, deaths remain minimal even in highly crowded conditions like the 8x7, which means that deaths are probably accidental at worst and antlions work to preserve the group's chances of surviving. The earlier hypothesis was proven to be correct, as the correlation between a smaller trial size and more extreme behaviors (such as cannibalism and reclusiveness) is supported by the data, as an increase in cannibalism was seen in lower treatment groups, hinting towards more aggressive behavior at lower trial groups, thereby proving the hypothesis.

Experimental Notes:

- Keeping track of the antlions became challenging as the experiment progressed, especially in the later trials when some of the antlions began to hide passively beneath the sand

- Removing the antlions from the enclosure after each trial became tedious, as it was difficult to find antlions that were under the sand or evaded capture

- The setup and introductory periods both went well with each trial, as we came into not major issues when setting the trials or when introducing the antlions

- Depth and width of the pits were smaller in trials with smaller enclosures, which could be due to increased interaction between antlions within smaller enclosures

- Measurement of the antlions' pits became difficult, especially as the pits decreased in size (namely in the <1cm range), because parallax and 'bumping' of the pits could introduce error

- Antlions had roughly the same density across all sizes because they would become 'dormant' if sufficient area was not readily available.

- Cannibalism and death occurred at a relatively constant rate across all trials, meaning it was more a function of time than a result of overcrowding.