

Discrimination Learning in Antlions (Neuroptera: Myrmeleontidae): Learning to
Differentiate Prey from Non-prey

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Abstract

Associative learning has been demonstrated in many insects, including pit-digging larval antlions (Neuroptera: Myrmeleontidae). Nonetheless, the role of discrimination learning in antlions has yet to be explored. This study was an attempt to provide further support for the role of associative learning in antlion predation, while also examining discrimination learning. Antlions were assigned to either a learning or control group. Antlions in the learning condition were taught to associate a vibratory cue, namely falling sand of a particular density, with the delivery of food, and another vibratory cue, namely falling sand of a different density, with the delivery of an inert object. I hypothesized that antlions in the learning group would learn to associate the arrival of both the prey and the non-prey item with the delivery of a specific vibratory cue, which would lead to better prey handling and, in turn, faster pupation. That is, I predicted that, compared to the control group, antlions in the learning group would extract nutrients from prey more efficiently, construct larger pits, and discard the inert object from their pits more quickly. Although the results of this study could not support the hypotheses, they provide hope for future studies investigating discrimination learning in antlions with some simple procedural modifications.

**Discrimination Learning in Antlions (Neuroptera: Myrmeleontidae):
Learning to Differentiate Prey from Non-prey**

Like vertebrates, many insects are capable of associative learning. Nearly fifty years of research and experimentation have shown that many species of insects, from every major insect order, possess the ability to form associations. Such research has indicated that associative learning is an adaptive trait that ensures the reproductive success of that insect. Foraging insects, those that actively seek out their prey, such as fruit-flies, grasshoppers, crickets, wasps, butterflies, and honeybees, comprise the vast majority of insects used in previous studies. For example, fruit flies are able to associate an odor with a shock stimulus. These tiny insects learned to avoid an odor that they associated with a shock stimulus and move towards areas of a maze containing a neutral odor (Beck, Schroeder, & Davis, 2000).

Grasshoppers are capable of associative learning as well. Those in a learning group were presented with a balanced diet dish consistently paired with a specific color card and flavor, while those in a control group were presented with random pairings of the color cards and the dish flavors so that they would not be able to make associations. The results were as expected: The grasshoppers in the learning group were able to quickly find the dish containing a balanced diet and therefore spent more time eating. As a result, their growth rate was twenty percent higher than that of grasshoppers in the control group (Dukas and Bernays, 2000, as cited in Dugatkin, 2004, p. 14), suggesting that learning increased fitness.

Crickets can learn to pair an olfactory stimulus with a reward. After learning to associate a peppermint odor with a water reward and a vanilla odor with saline solution, the crickets developed a preference for the peppermint odor. In addition, crickets are capable of retaining this preference even up to four days later (Matsumoto and Mizunami, 2002), which could be advantageous in nature by increasing fitness.

Wasps can learn to associate certain colors with a food reward. They have an innate preference for yellow colored stimuli, but can learn to switch their preference for yellow and, instead, associate orange-colored stimuli with a food reward. After training, wasps given a choice to fly towards either a yellow-colored stimulus or an orange-colored stimulus chose to go to the orange-colored stimulus, associating it with the food reward (Lucchetta et al., 2008).

Butterflies also can learn to associate certain colors with a food reward. They have an innate preference for red/orange, blue/white, and yellow. However, the specific color preference depends on the species of butterfly. Butterflies can learn to fly to a colored flower that they have been trained to associate with a food reward. Interestingly, it has been found that species of larger butterflies have higher associative learning abilities, most likely because of the increased size of their brains (Kandori et al., 2009).

When honeybees were presented with two different colored petri dishes, one containing sucrose and the other containing water as a control, they were able to learn which color to approach for a nutritious meal. In flight to a window sill for a meal, honeybees could discriminate between the colors of the petri dishes in

each corner and, after training, those in the learning group flew directly to the one containing the sucrose, ignoring the dish of water completely (Couvillion, Leiato, & Bitterman, 1991).

Sit-and-wait predation

The previously discussed species are all active foragers, in that they search for their food and therefore must discriminate between what is suitable prey and what is not. This skill is vital for these foragers because without it, they would not be able to efficiently capture food. Like these foraging insects, birds also search for and capture their prey, discriminating between food items and inert items. Two species of birds, stonechats (*Saxicola torquata* L.) and wheatears (*Oenanthe oenanthe* L.) use predation tactics that differ from those of most birds in that they wait at one specific vantage point until they spot an obtainable prey item - they do not actively forage for their food. This method has been appropriately called “sit-and-wait” predation. These birds use the movements of their prey below them as the cue for detection and then swoop in for the capture (Lawrence, 1985), unlike most species of birds who fly sometimes vast distances to ensure the capture of their next meal.

Spiders are a common sit-and-wait predator that remain in the center of a carefully spun web until a prey item becomes trapped in the web. A spider is able to pinpoint the exact location of the prey in the web by plucking to feel where it is stuck, and then moves in that direction. It has been found that spiders actually pay more attention to the areas of their webs directly above and below the central hub, where they sit and wait, than the areas to the left and right of the hub. This is

because they have a better chance of capturing prey that land above and below them and so they learn to pay specific attention to those locations (Nakata, 2009).

A large amount of research has examined the predation tactics of antlions (Neuroptera: Myrmeleontidae) because they are an exceptional example of a sit-and-wait predator (see Figure 1). Antlions are very different from other insects, even other “sit-and-wait” predators, because they sit almost completely motionless at the bottom of a sand pit and wait, ready to capture a prey item that walks along the sand and happens to fall in. They are excellent insects to study in the laboratory because they are easy to feed, house, and care for. In addition, their feeding behavior is easy to observe and measure, as the entire process takes place in the sand pit. However, most importantly for the current study, these sessile insects can learn to associate a vibratory sand cue, which does not initially elicit any predatory behavior, with the arrival of prey (Guillette, Hollis, & Markarian, 2009). In that study, antlions were able to extract more nutrients from signaled prey and constructed larger pits over time, which was correlated with increased growth (Guillette, Hollis, & Markarian, 2009).



Figure 1. An image of a larval antlion out of its pit.

Antlion development

Antlions move through four separate stages of development. In the first stage, an egg is singly deposited by the female adult in a relatively sheltered area, typically under bushes or over-hangings of large rocks, or next to a wall or other barrier. The eggs are partially buried under the sand to remain hidden and protected (Scharf & Ovadia, 2006).

Upon hatching, the second, larval stage is reached during which larvae build and live in the sand pit. Antlions consume food exclusively during the larval stage; in no other stage of development do antlions consume nutrients (Burgess, 2009). This stage consists of three instars during which an antlion does not change in overall physical appearance (see Figure 1), but grows larger with each instar. Two molts occur in the larval stage, one between the first and second instar and the next between the second and third instar. After each molt, an antlion sheds an exoskeleton and then tosses it from its pit. Antlions are usually buried under the sand during a molt for approximately ten days. Under optimal condition and when prey is plentiful, the entire larval stage can be completed in under a year, but can also last as long as three years (Scharf & Ovadia, 2006).

Following the third instar, an antlion pupates in a round cocoon under the sand for approximately four weeks (Farji-Brenner, 2003). The external surface of this cocoon is sticky so that sand adheres to it, forming a sealed space in which an antlion can undergo the necessary changes for pupation. Upon completion of pupation, an antlion emerges as a winged adult, during which its only purpose is to reproduce. As an adult, the winged insect does not consume any nutrients, is

nocturnal, and is not an exceptionally skilled flyer (Farji-Brenner, 2003). It survives in the adult stage for approximately 28 days (Arnett and Gotelli, 2001).

Antlion pits

Larval antlions of some species, out of the approximately two thousand in total, construct pits in the sand that they use as traps to capture their prey (Mencinger-Vracko & Devetak, 2007). Most often in dry, shaded, sandy areas, they tend to position their pits far enough away from those of other antlions, so as to ensure that an optimal number of prey fall in. Commonly referred to as “doodlebugs”, they create winding tracks in the sand while searching for a location for the pit, which allows them to sample the environment surrounding their future home. It is more important for an antlion to construct a pit in a suitable environment - one in which temperature, space between other pits, sand particle size, rainfall amount, and a light:dark cycle is optimal - rather than in an area where prey is plentiful (Arnett and Gotelli, 2000).

However, prey capture is necessary for survival and a pit not only enables an antlion to capture prey, but also provides a way for an antlion to assess the amount of prey activity in a particular location (Crowley and Linton, 1999). Pit building is a strategic and intricate process that serves an important purpose for the antlion once it is finished. During the construction process, the sand forms an angle of repose on the pit walls which is to an antlion's advantage, as it causes prey items to slide down towards the predator. Antlions generally prefer a finer grain of sand (Scharf, Subach, & Ovadia, 2008) so as to make it even more unstable for the walking prey. Generally, the finest grains of sand line the most

external walls of the pit so that prey will efficiently slide down into the bottom of the pit (Griffiths, 1986). Given a choice between fine- and coarse-grained sand, antlions always choose fine-grain sand because they can construct bigger pits faster and capture prey more efficiently (Farji-Brener, 2003).

Antlions often make alterations to their pits, as sometimes sand can get dislodged during a prey's descent or from natural disturbances such as wind and rain. In addition, as antlions grow, they increase the size of their pit. These alterations generally occur at night because antlions are most active in the dark. Pit volume is correlated with antlion size (Scharf & Ovadia, 2006), as well as with prey size because antlions do not construct pits that are larger than the size of prey that they can successfully capture (Guillette, Hollis, & Markarian, 2008). This sessile process of prey capture makes antlions unique in the world of insects.

In a functional sense, the pit allows an antlion to bury itself, almost completely under the sand at the bottom, with only a small portion of its head and prey-grabbing mandibles sticking out. It is able to sit and wait, for months if necessary, for a prey item to land within its open jaws. However, if an extended period of time goes by without food, an antlion will most likely relocate to assess a different location (Day and Zalucki, 2000).

If conditions are suboptimal, antlions tend to move their pits often, construct small pits, or not build any pit at all. A suboptimal environment could include extreme high or low temperatures, excessive rain or water, an overpopulation of antlions, or possibly, frequent disturbances to the pit. Arnett and Gotelli (2001) found that antlions in a high-food condition actually had

smaller, shallower pits than those in a low-food condition because they would be more likely to encounter prey, regardless of how conical they made their pits.

Although the effect of pit disturbance has not been a common topic of research involving antlions in the past, some studies have shown that with increased disturbance to a pit, antlions will have a tendency to construct smaller pits and possibly even relocate their pits more frequently. Youthed and Moran (1969) found that pit size decreased with increased disturbance over time. Therefore, it is possible that when presented with regular pit disturbances, such as a small, inert object, antlions will construct smaller pits over the course of an experiment. It is also possible that antlions subjected to regular pit disturbances might attempt to avoid the disturbance by relocating to an area far enough away from the suboptimal location (Griffiths, 1986).

In nature, it may be common for inert items, such as twigs, stones, or leaves, to accidentally fall into an antlion's pit. There are currently no studies investigating the behavior of antlions upon the delivery of a non-prey item into the pit. It is well known that antlions toss the drained carcass of their most recent meal out of the pit so as to be ready for the next prey item that happens to walk along. However, are antlions just as capable of tossing out a rock or twig that might accidentally fall into the pit or would they simply burrow out and build a new pit elsewhere? Furthermore, upon frequent introduction of an inert object into a pit in a controlled location, would that antlion consistently move its pit so as to avoid an encounter with the inert object, or simply maintain a small pit for easy expulsion of the object?

Antlion prey capture and feeding

Antlions feed on a number of small terrestrial arthropods. Many species of ants are available in antlions' natural environment and so become the most frequently consumed prey (Griffiths, 1980). However, antlions can also feed on mealworms (Guillette, Hollis, & Markarian, 2008), spiders, beetles (Loria et al., 2008), fruit flies, and termites (Van Zyl, Westhuizen, & Van Der Linde, 1997). An antlion will usually feed on anything actively moving and of suitable size that falls into its pit.

Once a prey item walks within a few inches of the pit, an antlion senses its movement via small hairs containing mechanoreceptors that can detect vibrations in the sand. The prey continues to walk until it slides into the pit and down into an antlion's wide-open mandibles. If a prey item does not fall directly into the mandibles and instead attempts to climb out of the pit, antlions will flick sand at it using the head and mandibles until the prey slides back down to the bottom of the pit (Mencinger-Vracko & Devetak, 2007). If the prey item is extremely active at the bottom of the pit, antlions will flick it back and forth in order to disorient it, making it easier for the predator to insert its feeding tubes (Napolitano, 1998). If the item in the pit is indeed suitable prey, antlions inject an enzyme to paralyze and kill it, which slowly takes effect as the predator-prey struggle comes to an end (Griffiths, 1980).

A twelve-step depiction of antlion eating is shown in Table 1 (Napolitano, 1998). Antlions usually grab the midsection of the prey with their mandibles, which protrude from each side of the head, and acquire a firm hold before

inserting both feeding tubes, located within the mandibles (Griffiths, 1980). Next, they typically drag the prey under the sand, eventually reemerging with it sometime during feeding (Napolitano, 1998). Through the feeding tubes, antlions are able to suck out the prey's protein-rich body fluids. They extract the nutritious components from the prey, essentially draining it to an empty carcass. Sometimes, antlions will only partially consume the nutrients from a given prey item, therefore not fully draining the carcass (Lucas, 1985). Finally, antlions will rapidly flick their prey up and down while holding it in the mandibles and then toss the prey carcass out of the pit (Napolitano, 1998). The tossing of the prey carcass marks the end of feeding, after which pit maintenance usually occurs to fix any sand displacement done to the pit during feeding.

Table 1. A twelve-step depiction of antlion eating (adapted from Napolitano, 1998).

Step #	Description
1	<i>Attack.</i> Head is moved rapidly, sand flicked from pit.
2	<i> Holding.</i> Prey is gripped in mandibles.
3	<i>Submergence.</i> Antlion pulls prey down into sand.
4	<i>Emergence.</i> Antlion brings prey back out above sand.
5	<i>Prey Beating.</i> Antlion rapidly flicks head up and down with prey in mandibles.
6	<i>Feeding.</i> Fluids are extracted from prey via feeding tubes.
7	<i>Pit Clearing.</i> Sand accumulated on head and then tossed back and out of pit.
8	<i>Head Roll.</i> Head sweeps along pit walls in a circular pattern, accumulating sand in the center
9	<i>Prey Clearing.</i> Prey is scooped onto head and mandibles and then the head is flicked back to expel prey from pit.
10	<i>Grooming.</i> Tip of one mandible moves along inside edge of other mandible for cleaning purposes.
11	<i>Quiescence.</i> Antlion remains motionless for more than 7 seconds.
12	<i>Jaw Set.</i> Antlion fully opens mandibles and submerges under sand with only eyes, antennae, and mandible tips visible

Discrimination learning

The vast quantity of previous research in the area of insect learning has focused on associative learning. However, almost all of the formed associations made by the insects in these studies requires some amount of discrimination learning as well. Insects that actively seek out their prey in an unpredictable environment must be able to differentiate between what is suitable prey and what is an inert object. Past studies, such as that involving the discriminatory behavior of male courtship in fruit-flies (Dukas, Clark, & Abbott, 2006), have demonstrated that insects are indeed capable of this kind of discrimination, which in turn, increases fitness. Therefore, the majority of the previous research in foraging insects contains a component of discrimination learning, under the assumption that this discriminatory behavior is necessary for the future success of the insect.

Previous studies examining the associative learning abilities of antlions did not contain a discrimination learning component. Because prey items literally walk right into antlions' mandibles and, thus, they are not forced to interact with an unpredictable environment, antlions do not necessarily need to have a discriminatory cognitive function. However, because antlions rely on the vibrations produced by a prey item as it walks along the sand, this sessile insect might benefit if it could distinguish between the vibrations produced by walking prey and those of a leaf blowing across the sand in the wind. This elevates the question of whether antlions possess the ability to discriminate to a greater relevance with respect to the field of insect learning as a whole.

Current study

The goal of the current study is to expand our understanding of antlions' associative learning abilities as well as to explore antlions' discrimination learning abilities. Although discrimination learning has been studied in many other insects, such as honeybees and wasps, it has never been studied in antlions. The implications of this kind of study could reveal the limits of antlions' cognitive abilities and provide insight into how these sessile insects are capable of interacting with their surroundings, even though they do not forage for food. Furthermore, it could provide support for the question of why antlions would build shallower pits or move them frequently in a condition in which they experience constant disturbance to the pit (Youthed and Moran, 1969).

The current experiment involved two vibrational cues that were used to signal two different events, namely the arrival of a prey item and the arrival of an inert object. I hypothesized that antlions would be able to associate one specific vibrational cue with the arrival of prey and the other vibrational cue with the arrival of an inert object. In turn, antlions would demonstrate an increased rate of extraction efficiency, larger pit volumes, and a decreased latency to throw both the prey item and the inert object. In addition, I hypothesized that antlions in the learning group would demonstrate greater fitness and therefore either molt or pupate sooner than those in the control group.

Method

Subjects and apparatus

Prior to selection as subjects, larval antlions (Neuroptera: Myrmeleontidae), which were obtained from AntLionFarms.com in Pensacola, Florida, were individually placed into 354 mL white plastic dessert bowls, three-quarters filled with Estes Marine Sand. These bowls were kept on shelves in a vivarium at a constant temperature of 20° C with a 16:8-hr light:dark cycle (on at 0700 hr, off at 2300 hr).

Antlions were monitored for an initial two days without being fed to ensure that they built pits. Those that constructed pits were each fed two wingless fruit flies (*Drosophila melanogaster*) obtained from Connecticut Valley Biological Supply (Southampton, MA) per day, the second given approximately 30 minutes after the first, until molting occurred. The process of molting began with the appearance of a flat-bottomed pit and a small bulge in the sand under which the antlion was buried. It remained buried for at least three days during which time the bowl was checked daily for an exoskeleton.

After antlions molted and dug new pits, they were extracted and weighed. Antlions of similarly matched weights were designated as pairmates, with one member of the pair randomly assigned to the learning condition and the other assigned to the control condition. Each antlion was transferred into a rectangular plastic container (28 cm long x 17 cm wide x 17 cm deep) filled with 16 cups of sanitized Estes Marine Sand at a depth of approximately 13 cm, and located in a separate room. Each container was placed on top of, and nested within, a pocket

cut into the top piece of a three-layer unit of foam padding. In addition, each antlion container was separated by 2.54 cm thick styrofoam walls that surrounded the container on three sides. Containers for the learning condition were placed on one shelf and containers for the control condition were placed on an adjacent shelf at the same height. Within the container, each antlion was confined to a small circular area in the center using the rim of a plastic cup. This rim was removed with minimal disturbance to the antlion once the pit was completely constructed, the night before the start of testing.

Each of two different cues, both vibratory, produced by two different densities of falling sand, were delivered to each antlion via a small wooden device resting on top of the short side of the rectangular container and mounted over a sand collecting container (see Figure 2). Each delivery device consisted of a plastic dropper suspended from two wires and mounted on a block of wood. A lever, which protruded from the back side of the wooden device, enabled experimenters to control the release of the sand, which fell into the small collecting container just underneath. Two types of sand and collecting containers were used as the two different cues. A very fine sand was used with a mesh-bottomed collecting container and a heavier grain sand was used with an aluminum foil-bottomed collecting container. The sand falling from the plastic dropper lasted 3 seconds as it fell into the collecting container. These collecting containers allowed vibrations to travel from the falling sand to the antlion's pit



Figure 2. Top-view of the sound-attenuating apparatus, showing an antlion in its conical pit, the two collecting containers, and a wooden cue delivery device.

without adding to the sand in antlion's rectangular container. The mesh-bottomed collecting container was always located on the right side of the rectangular container while the aluminum foil-bottomed collecting container was always located on the left side.

Procedure

For each antlion, training occurred for four consecutive days (a block of training), followed by a rest day, and then another four consecutive training days, followed by another rest day, until either molting or pupation occurred, or until 14 blocks of training were completed (see Table 2). Each antlion in the experiment was fed the head of a mealworm (*Tenebrio*, obtained from Connecticut Valley Biological Supply, Southampton, MA) weighing between 0.0025 and 0.0045 g on the assigned food days of each training block. On the non-food days of each training block, each antlion was presented with an inert object instead of a mealworm head. For the inert object, a small colored bead weighing approximately 0.0110 g was used. One bead was assigned to each antlion and only that bead was given to that antlion for the duration of the experiment.

All antlions received the food stimulus on the same day of training. The learning and control groups differed only in terms of the cue delivered. Each of the two cues for antlions in the learning group was paired with a particular item (mealworm or inert object); however, cue type was counterbalanced across subjects in the learning condition. The cues for the control group were randomized with respect to mealworms and the inert object, so that on any given

training day, each control antlion had a fifty percent chance of receiving either cue. During each four-day training block, each antlion was presented with food twice and the inert object twice. The six possible patterns of food (F) and inert object (I) are: F I F I, F I I F, I I F F, I F I F, I F F I, F F I I. The order of these six combinations was chosen randomly for each period of six blocks.

On both food and inert days, the cue apparatuses were set up first. The correct type of sand (fine- or heavy-grained) was funneled into the pipet in the wooden apparatus and placed over the corresponding collecting device (mesh- or aluminum foil-bottomed) for each antlion. Next, the cameras were set up so that an average of four antlions were taped each training day (only four working cameras were available to the experimenters). Then, on a food day, the mealworm heads were cut, one at a time. Each one was immediately weighed and then delivered to the antlion so that it was still moving during the descent into the pit. The camera was turned on to record, the cue was delivered, and the mealworm head was immediately placed into the pit to begin that day's training. On an inert day, the same procedure was used, but without the need for cutting and weighing. After the camera had been turned on, the bead was gently rolled down the side of the pit immediately following the delivery of the sand cue.

Table 2. Illustration of design and procedure.

Pre-Training	Training
Duration: about 1 month	Duration: 14 five-day blocks
Antlions housed in individual bowls of fine sand	4 days of training followed by a rest day
<p>Pre-molting: Monitored for pit construction and normal behavior</p> <p>Fed two wingless <i>Drosophila</i> per day</p> <p>Monitored for presence of an exoskeleton to signify molting</p>	<p>Learning group antlions: Food day: specific cue delivered, mealworm placed into pit</p> <p>Inert day: specific cue delivered, bead placed into pit</p> <p>Behavior and time recorded</p>
<p>After molting: Weighed and matched with antlion of similar weight</p> <p>Brought into testing room to begin experiment</p>	<p>Control group antlions: Food day: random cue delivered, mealworm placed into pit</p> <p>Inert day: random cue delivered, bead placed into pit</p> <p>Behavior and time recorded</p>

The camera remained on during the entire training session. On a food day, each antlion was monitored for the first few minutes to make certain eating had begun and then watched closely for the remaining time, to look for a tossed carcass. The experimenters were careful to watch each antlion to ensure the recording of an accurate tossing time. The carcass was removed from the container, weighed, and discarded. If an antlion had not tossed the prey carcass after two hours, the experimenters stopped watching and recorded the time as 120 minutes. The carcass was left in the pit and weighed later that night after the antlion had tossed it. On an inert day, the experimenters watched each antlion very closely as some of them tossed the bead in less than a minute. Again, if an antlion took over two hours, the experimenter could stop watching and remove the bead from the container later that night.

Each evening at least three hours after training was completed, the diameter of the top and the depth of each antlion's pit was recorded to the nearest 0.1 cm. The experimenters were not blind during these measurements because clear labeling of all of the antlion containers was absolutely necessary so as to ensure that each antlion received the correct training. All of the experimenters had been previously trained during a pilot study by one experimenter to measure in a specific way, so as to minimize experimenter error. Finally, each antlion container was checked and prepared for the next training day.

Data analysis

The dependent measures under observation in the current study were quantitative. The quantitative measures included: extraction efficiency rate, the

amount of food (g) extracted over time (sec) (Van Zyl et al., 1997); pit volume, the depth and diameter of each antlion's pit calculated in the equation for the volume of a cone [$Volume (cm^3) = (1/3\pi) \times r^2 \times h$, where r is pit radius and h is pit height (depth)]; and latency to throw, the amount of time (min) it takes each antlion to toss either the carcass or the inert object out of the pit.

Extraction efficiency was calculated by subtracting the weight of the prey after feeding from the weight before delivery into the pit. Extraction efficiency rate was obtained by dividing extraction efficiency by time in seconds. Pit depth and diameter was measured with a ruler configured to fit into the pits. Latency to throw was recorded in minutes and calculated by subtracting the time the object was placed into the pit from the time the antlion threw it out.

All data were analyzed using paired samples t -tests which were generated using SPSS version 17.0 to compare the differences between the learning and control groups across days. A t -test was also performed to compare the final weights of the learning versus the control antlions. Because antlions were brought into the experiment as weight matched pairs, the results were analyzed as a within-subjects factor for both antlion pair and days. Some of the data were transformed into logs because the standard deviations were not normal. A log transformation was chosen for the data because it created the clearest graphs. Since subjects remained in the experiment for varying periods of time, measurements from the first, middle, and last days were used to ensure that the results being analyzed were comparable.

Results

I hypothesized that, over the course of the experiment, antlions in the learning group would construct larger pits, exhibit a higher rate of extraction efficiency, show a decreased latency to throw both the prey item and the inert object, and grow larger than antlions in the control group.

Pit volume

A paired samples *t*-test was performed to compare pit volumes of the learning and control groups on each of three separate occasions (first, middle, and last day) of the experiment. The analysis revealed no significant difference ($p > .05$) between the learning ($M = 5.35 \text{ cm}^3$, $SEM = 1.35 \text{ cm}^3$) and control ($M = 6.49 \text{ cm}^3$, $SEM = 1.89 \text{ cm}^3$) antlions on the first day ($n = 6$ for both groups). No significant difference ($p > .05$) was found between the learning ($M = 33.49 \text{ cm}^3$, $SEM = 2.18 \text{ cm}^3$) and control ($M = 33.33 \text{ cm}^3$, $SEM = 7.39 \text{ cm}^3$) antlions on the middle day ($n = 6$ for both groups). Finally, no significant difference ($p > .05$) was found between the learning ($M = 50.36 \text{ cm}^3$, $SEM = 7.09 \text{ cm}^3$) and control ($M = 51.45 \text{ cm}^3$, $SEM = 9.13 \text{ cm}^3$) antlions on the last day ($n = 5$ for both groups). Log values were calculated for the pit volume data because standard deviations on the last day were more than three times larger than those on the first day, and were used in Figure 3. However, no significant difference was found in the analysis using log transforms. Individual antlion data points were graphed in Figure A1.

Extraction efficiency rate

A paired samples *t*-test was performed to compare extraction efficiency rate of the learning and control groups on each of three separate occasions (first, middle, and last day) of the experiment. The analysis revealed no significant difference ($p > .05$) between the learning ($M = .000155$ g, $SEM = .0000194$ g) and control ($M = .000157$ g, $SEM = .0000237$ g) antlions on the first day ($n = 4$ for both groups). No significant difference ($p > .05$) was found between the learning ($M = .000122$ g, $SEM = .0000242$ g) and control ($M = .000149$ g, $SEM = .0000374$ g) antlions on the middle day ($n = 5$ for both groups). Finally, no significant difference ($p > .05$) was found between the learning ($M = .000151$ g, $SEM = .0000154$ g) and control ($M = .000197$ g, $SEM = .0000168$ g) antlions on the last day ($n = 5$ for both groups). Log values were calculated for the extraction efficiency rate data because the data was not normally distributed and some standard deviations were more than three times larger than others, and were used in Figure 4. However, no significant difference was found in the analysis using log transforms. Individual antlion data points were graphed in Figure A2.

Latency to throw the prey

A paired samples *t*-test was performed to compare the latency to throw the prey for the learning and control groups on each of three separate occasions (first, middle, and last day) of the experiment (see Figure 5). The analysis revealed no significant difference ($p > .05$) between the learning ($M = 74.00$ min, $SEM = 6.81$ min) and control ($M = 67.75$ min, $SEM = 6.70$ min) antlions on the first day ($n = 4$ for both groups). No significant difference ($p > .05$) was found between the

learning ($M = 74.20$ min, $SEM = 10.99$ min) and control ($M = 76.20$ min, $SEM = 12.81$ min) antlions on the middle day ($n = 5$ for both groups). Finally, no significant difference ($p > .05$) was found between the learning ($M = 66.20$ min, $SEM = 5.36$ min) and control ($M = 75.60$ min, $SEM = 12.17$ min) antlions on the last day ($n = 5$ for both groups). Individual antlion data points were graphed in Figure A3.

Latency to throw the bead

A paired samples t -test was performed to compare the latency to throw the bead for the learning and control groups on each of three separate occasions (first, middle, and last day) of the experiment. The analysis revealed no significant difference ($p > .05$) between the learning ($M = 12.25$ min, $SEM = 5.25$ min) and control ($M = 17.00$ min, $SEM = 6.19$ min) antlions on the first day ($n = 4$ for both groups). No significant difference ($p > .05$) was found between the learning ($M = 9.40$ min, $SEM = 3.78$ min) and control ($M = 36.6$ min, $SEM = 21.04$ min) antlions on the middle day ($n = 5$ for both groups). Finally, no significant difference ($p > .05$) was found between the learning ($M = 4.40$ min, $SEM = 1.12$ min) and control ($M = 33.00$ min, $SEM = 22.10$ min) antlions on the last day ($n = 5$ for both groups).

Log values were calculated for the latency to throw the bead data, because the data was not normally distributed and because the standard deviations on the last day were more than three times larger than those on the first day, and were used in Figure 6. With log transforms of the data, a near significance ($p = .073$) was found between the learning ($M = .5873$ min, $SEM = .11009$) and control ($M =$

1.1590 min, $SEM = .27012$) antlions on the last day of training ($n = 5$). Individual antlion data points were graphed in Figure A4.

Final antlion weights

A paired samples t -test was performed to compare the final weights of the learning ($n = 5$) and control ($n = 5$) antlions. The analysis revealed no significant difference ($p > .05$) between the weights of the learning ($M = .0338$ g, $SEM = .00185$ g) and control ($M = .0353$ g, $SEM = .00255$ g) antlions at the end of the experiment.

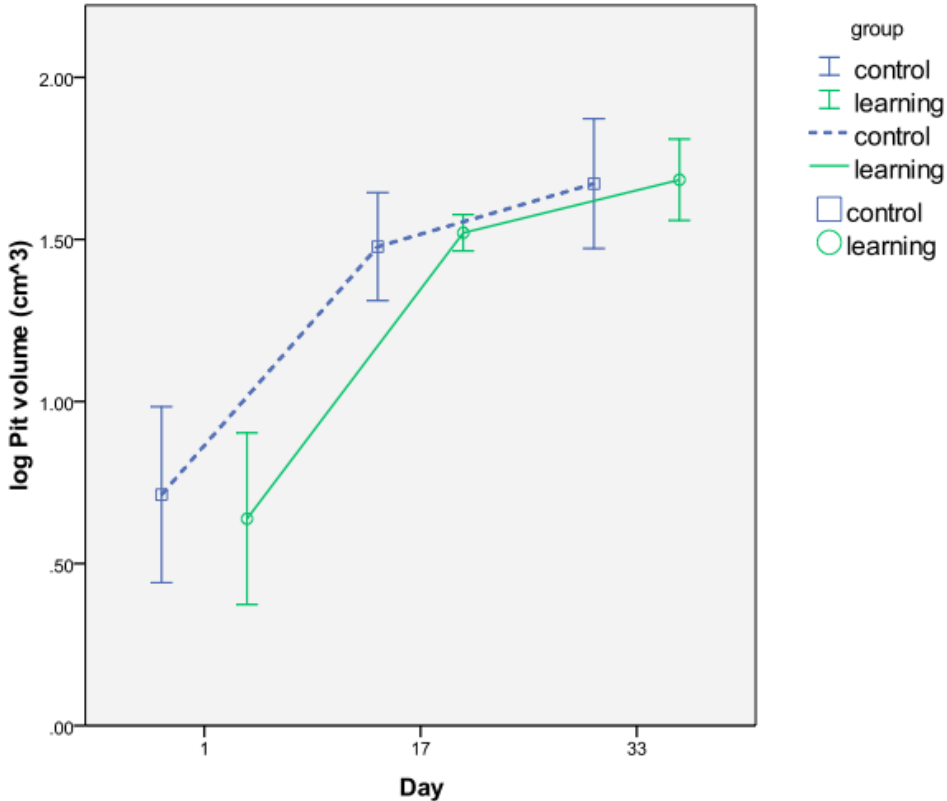


Figure 3. A graph of the means (cm³ ± SEM) of pit volume across first, middle, and last days for all learning and control subjects.

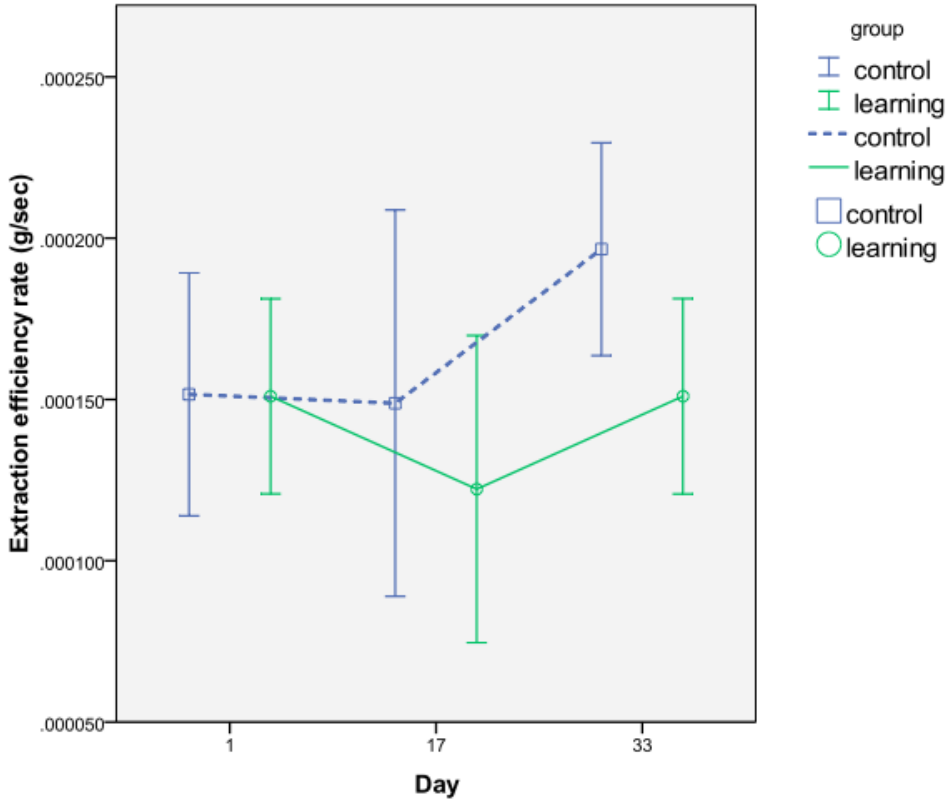


Figure 4. A graph of the means ($g \pm SEM$) of extraction efficiency rate across first, middle, and last days for all learning and control subjects.

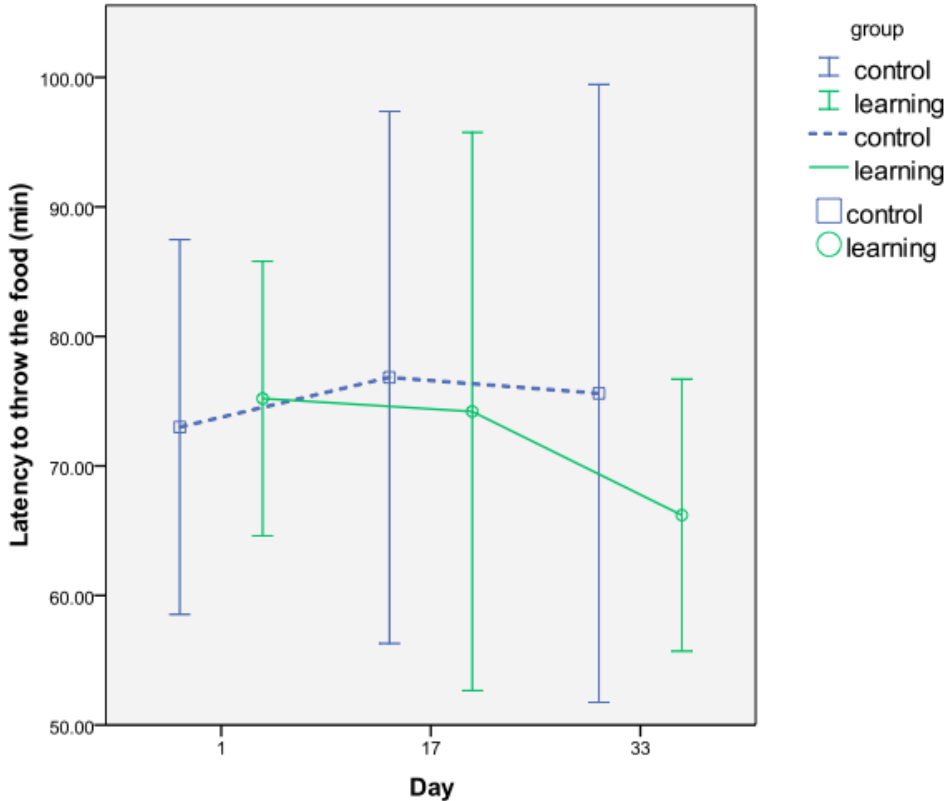


Figure 5. A graph of the means (min ± SEM) of the latency to throw the prey across first, middle, and last days for all learning and control subjects.

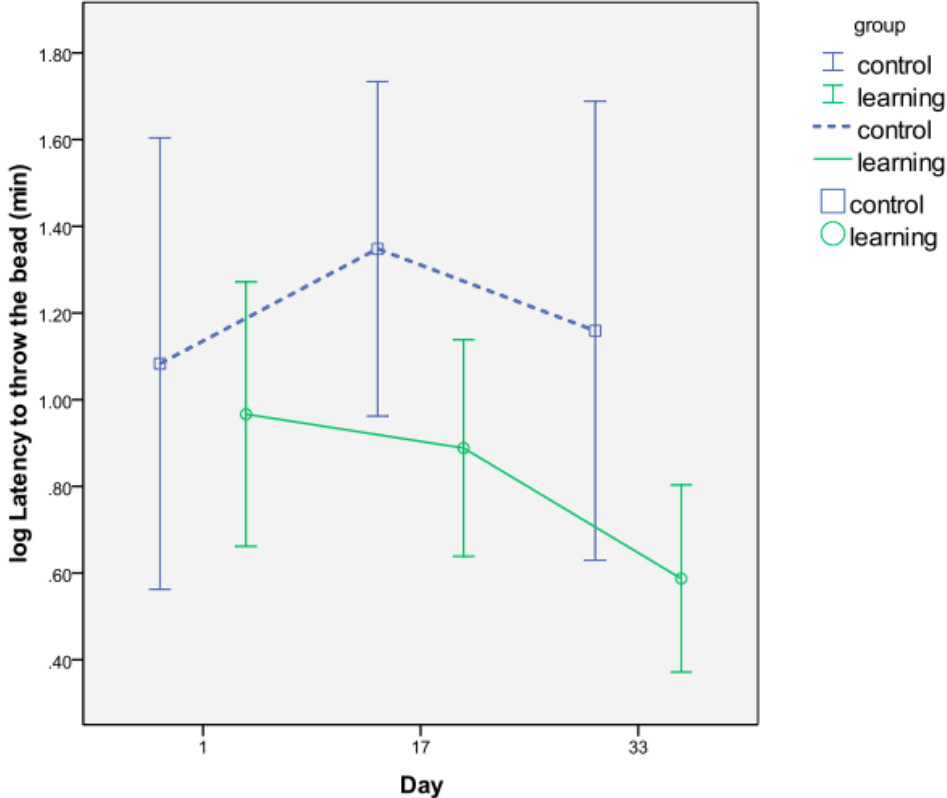


Figure 6. A graph of the means (min ± SEM) of the latency to to throw the bead across first, middle, and last days for all learning and control subjects.

Discussion

The ability of antlions to form associations when presented with a cue followed by the delivery of a prey item into the pit (Guillette, Hollis, & Markarian, 2009) makes them fascinating insects to study. Because of their sit-and-wait predation pattern, they are very different from active predators: They do not need to search for food in an unpredictable environment. Due to this fact, the associative learning abilities of these sessile insects is quite remarkable. It would prove even more remarkable if antlions are also capable of a task involving a higher level of cognition, such as discrimination learning. Discrimination learning has been studied in other insects, such as fruit-flies, with regard to such behaviors as male courtship (Dukas, Clark, & Abbott, 2006) and has revealed that these small insects can learn to distinguish between a potential mate and an inanimate object. An array of other insects are also capable of discrimination learning, as a vast amount of research has shown, because these active predators rely on discrimination as a means of surviving in their environments. Antlions are different in this respect because they do not interact with their environment on a daily basis. However, because research has shown that they are capable of associative learning (Guillette, Hollis, & Markarian, 2009), I hypothesized that antlions would be able to discriminate between the arrival of a prey item versus the arrival of a non-prey item through the use of two different vibratory cues.

It is possible that in nature, antlions need to be able to distinguish between the sand vibrations caused by a prey item walking nearby and the sand vibrations caused by falling leaves. If they were capable of this kind of discrimination, they

would be able to prepare for whichever event was about to occur, and would therefore have the advantage in their environment. The current experiment had hoped to expand upon previous findings not only by studying associative learning, but also by studying antlions' ability to discriminate between different cues that indicate different events. I hypothesized that antlions in the learning condition would differ from antlions in the control condition with respect to such dependent measures as prey extraction efficiency rate, pit volume, latency to throw, and final weight at the end of the experiment.

Extraction efficiency rate

Prey extraction efficiency rate corresponds to the rate at which the antlion is able to extract the contents from a prey item over time. Because the size of the prey item delivered remained constant across both groups and between all subjects, I hypothesized that antlions in the learning group would extract a greater amount more efficiently than those in the control group, because they would learn to anticipate the arrival of the prey item and would therefore prepare for the feeding process. With no anticipation of feeding, antlions in the control group would take longer to ready themselves for a meal, in that they would have to correctly position their mandibles, grab a hold of the moving prey, prepare to inject the toxin, and begin extracting. I did not find evidence to support my hypothesis regarding extraction efficiency rate as there were no significant differences between the learning and control antlions over time.

There are some possible explanations that could account for the fact that no significant differences arose during the analysis of extraction efficiency rate.

The antlions in this experiment moved their pits frequently throughout the training period (a possible reason as to why this would happen is discussed in the following subsection). Therefore, there were days during training when some antlions were tested while others were not. Because the design required the pit to be somewhat in the center of the large container, between the two collecting containers, an antlion that had relocated to a corner or next to a wall overnight could not be tested the following day. If that following day happened to be a food day, during which all antlions received the prey item, the antlion(s) that had moved overnight would not eat. This frequency of pit movement seemed to have occurred equally over both the learning and control groups but most likely affected the process of association in antlions in the learning group.

Pit volume

I hypothesized that as a result of extracting nutrients from prey more efficiently, antlions in the learning group would grow larger than those in the control group. Because pit size is directly proportional to the size of the antlion, I expected to see greater pit volumes for antlions in the learning group. My results did not support my hypothesis for pit volume as there were no significant differences between the learning and control antlions over time.

However, disturbed antlions tend to build smaller pits than those left undisturbed (Scharf & Ovadia, 2006). Because all antlions in the current study were subjected to unnecessary pit disturbances (from the delivery of the inert object), they may have chosen to maintain small pits as a natural reaction to this experimental disturbance. Also, as discussed previously, all of the antlions in this

experiment tended to move their pits often - more than what would be considered typical for continuously fed antlions - which could be a result of the consistent delivery of the inert object. This frequent movement of pits required the experimenters to physically relocate the antlion back to the center of the container, which in turn, forced that antlion to construct an entirely new pit. Often, this new pit would be substantially smaller than the one it had built previously, even if a greater pit size was achieved in a short amount of time. As a result of the relocation of pits, there are some missing data points from the training days when pit measurements could not be obtained from an individual antlion due to the lack of a pit in the center of the large container. This missing data could have decreased the power in the analysis and caused higher statistical p-values.

Asymmetry of the pit walls might have been affected by the constant disturbance of the inert object. Antlions generally build a steeper wall directly in front of where their mandibles lie open, waiting for prey (Lucas, 1989). In the current study, this wall may have become less steep over time because of the need to toss something bulky out on a regular basis. This asymmetry can affect the pit measurements taken by the experimenters each night because it becomes more difficult to accurately judge the depth of the pit as the walls level out. The lack of significance in the pit volume data most likely can be attributed to one or all of these factors.

Final weights

Similar to pit volume, the final weight of each antlion as compared to its initial weight is indicative of growth during the experiment. Although all of the antlions should have increased in size, I hypothesized that antlions in the learning group would grow larger than those in the control group because they would have been extracting more from prey. The results did not support my hypothesis as there were no significant differences between the final weights of the learning and control antlions. Although the antlions grew throughout the experiment, they all grew at approximately the same rate and so it can be concluded that the learning group antlions did not increase fitness.

Latency to throw

I hypothesized that antlions in the learning group would exhibit a decrease in the latency to throw both the inert object and the prey carcass out of their pit over time. Because subjects in the learning group received a consistent cue that preceded the arrival of the inert object into the pit, they should have become more efficient in preparing to toss it out and, therefore, in the action of tossing itself. Keeping the pit clean, neat, and obstacle free is important for an antlion to be able to capture any prey that might subsequently fall in. The results of this study do not support my hypothesis as the learning antlions were not significantly faster than the control antlions at throwing the prey item or the inert object.

It is possible that the learning group was not significantly faster than the control group at tossing out the prey carcass or the bead because the antlions could not distinguish between the two vibratory cues used. If this were the case,

the learning group was essentially no different from the control group in that the antlions experienced a cue that meant nothing to them and therefore could not predict which object they were about to receive. The data reveal that all of the antlions, in both groups, became faster at tossing out both the prey and the bead. However, with no significant differences between the learning and control groups, the results suggest that all of the antlions were progressing at the same pace and all of them were “learning” that the small, round, plastic bead was not edible and so should be tossed out.

Although no significant conclusions could be drawn from the latency to throw the bead, the p-value for this measure on the last day approached a significant difference between the two groups. The means of the learning and control groups on the last day of the experiment suggest that there is a difference between these two groups by the conclusion of the experiment. Unfortunately, this difference is not quite significant; however, these results are promising for a future study implementing some simple modifications (described below) to the current procedure. These modifications might result in a significant difference between learning and control groups for the latency to throw the bead, which would indicate the occurrence of discrimination learning.

General limitations and future directions

As the first discrimination study performed with antlions, this experiment can be thought of as a pilot study. There were some limitations and so some modifications should be made in future experiments. One factor that could have affected the results of this study was the lack of power. Although this experiment

began with twelve antlions, two (a set of pairmates) molted in the middle of the training period and so were eliminated from further analysis. In addition, an antlion in the control condition buried under the sand towards the end of the training period and did not resurface. As a consequence, some data were missing for that antlion and a final weight was not obtainable, forcing the experimenters to disregard the final weight for that antlion's pairmate as well.

Another general limitation could have been that the antlions in the learning group were unable to discriminate between the two cues. Although all of the antlions became faster in both the feeding and tossing process as training continued, the learning group did not progress in speed beyond the control group and so may not have formed any associations between the specific cue and the subsequent prey delivery event.

In the future, a similar study examining the discriminatory learning abilities of antlions should include three important modifications to the current study. First, a greater number of subjects would instantly increase the power and give more validity to the data. Second, more distinct cues might help the antlions to discriminate between them and therefore induce learning. One possible way to increase the discriminability of the two cues used in the current study would be to change the medium used to line the bottom of the collecting containers. The collecting container used in the current study that had a mesh bottom has been used in previous studies (Gullette, Hollis, & Markarian, 2009), and so can be used with confidence for one of the cues in a discrimination study. However, the collecting container that had an aluminum foil bottom had not been used in

previous studies and was designed by the experimenters of the current study. It is possible that the antlions in this study did not receive adequate vibrations through the sand when tested with the aluminum foil collecting container because the foil limited the transmission of vibration. A different medium, such as thin paper, could possibly be used for the bottom of the second collecting container in a future study to test whether a limited transmission of vibration had an effect on the subjects' discrimination abilities.

Third, because only two of the twelve antlions in the current study molted and none pupated during training, it is possible that they were not receiving enough food to encourage a molt or pupation within about a month of time. Had this study continued for many more weeks, the majority of the antlions probably would have molted/pupated; however, time did not allow for that. In a future study, all antlions could be tested twice a day, once receiving the prey item and then, at some other time, receiving the inert object. This might help to speed the subjects along in the molting/pupation process because more food would cause them to grow faster. In addition, testing subjects twice per day would give the experimenters more data to analyze.

It also might be interesting to perform this study (with the suggested modifications) with a third experimental group that would not experience the inert object at all. This group would receive a sand cue immediately before the delivery of the food item, as would all of the other subjects, but would not be trained a second time each day with the inert object. This would allow for a comparison of pit size, frequency of pit movement, and general behavior between

antlions that experienced a constant pit disturbance and antlions that did not experience any unnecessary disturbances. This would eliminate the question of whether the lack of significance with regard to pit volume in the current study was due to the delivery of the inert object or whether it was due to the small sample size.

The purpose of this experiment was to replicate the findings of similar associative learning studies involving antlions and to begin to explore discrimination learning in these sessile insects. This study aimed to contribute to the fields of neuroscience and animal behavior because both associative and discrimination learning are foundations for understanding cognition in vertebrate and invertebrate animals. Only through experimentation on simple nervous systems, such as that of an antlion, can we understand how the brain functions to create cognition. This understanding can then be applied to larger, more complex nervous systems, such as that of a human, to advance our current knowledge of how we think and function. The lack of significance in this study was disappointing; however, the near-significant p-value for the latency to throw the bead was an encouraging first step in discovering the discrimination learning abilities of antlions. The finding of a clear difference in means between the learning and control groups allows for the further exploration of the topic of discrimination learning, suggesting modifications for specific procedural details, which might help future studies find the answers that this study sought.

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