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The Effect of Associative Learning on Antlion
Feeding Behavior and Fitness

by

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ABSTRACT

Previous research has demonstrated that a variety of insects are capable of responding to learned cues; however research has yet to analyze the effect of learning in predators that do not use active foraging methods to procure food. The current study examined the effect of learning on a sit-and-wait predator, the antlion (Neuroptera: Myrmeleontiadae). In the study 16 antlions were placed in individual plastic containers and allowed to build a pit. The subjects were assigned to learning (LRN) and control (CON) treatment groups. Each subject received one ant every 48-hour training day. Subjects in the LRN condition received a cue, namely 4.5 ml of sand dropping, immediately prior to the delivery of the ant, while CON subjects received the same sand dropping cue at an independent time. Feeding behavior (mass extracted, extraction time, extraction rate, extraction efficiency, extraction efficiency rate, visibility, and movement) as well as pit building behavior (pit volume and pit location) were measured each training day to determine if an association between the presentation of a cue prior to the presentation of an ant resulted in differences in feeding behavior. The study did not find evidence of differences in extraction measures; however, a response to the cue was demonstrated in increased visibility and greater pit volume in the LRN condition.

INTRODUCTION

Scientists seek to understand the mechanisms of and constraints on learning in animals. Early studies of animals focused primarily on mammals, birds and fish. However, learning has recently been observed in a variety of insect species including bees (Dukas & Real 1991; Dukas & Real, 1993; Dukas & Visscher 1994), wasps (Lewis & Takasu, 1990; Dukas & Duan, 2000), flies (MacGuire, 1984; Dukas, 1998), grasshoppers (Dukas & Bernays, 2000), cockroaches (Sakura and Mizunami, 2001), and locusts (Simpson & White, 1990). By expanding the research to include more diverse species, scientists may be able to determine if there are limitations to insect learning. A comparison of species that have demonstrated learning with those that appear not to learn would allow scientists to identify the physiological and behavioral differences that might account for learning in one but not the other.

The observation of learning in such a wide range of insects indicates that associative learning may be an adaptive trait that could increase the fitness, or reproductive success of the insect. Fitness refers to the ability of an individual to survive and reproduce successfully. Fitness is affected by the physical attributes of the individual, such as brightly colored wings that attract a mate, or by behavioral characteristics. Any behavior that increases the likelihood of survival and reproduction results in increased fitness. For example, the ability to locate food quickly might allow an insect to spend less time feeding and thus spend less time risking predation. This decrease in possible predation would increase the chances of survival and thus increase an individual's fitness.

If an insect were able to associate cues in the natural environment with biologically important events, such as the approach of a predator, mate, or prey, that insect should have a fitness advantage over an insect that does not associate the cue with the event. This advantage would lead to increased survival and reproductive success. Considering the possible fitness advantage of learning, one would assume that learning occurs in nature; therefore, some studies of insect learning conducted in laboratory settings attempt to resemble natural circumstances as closely as possible.

Although great strides are being made in further identifying learning in a wide variety of species, the limitations of learning are not fully known. Further research can determine what these constraints might be. For example, might behavioral constraints such as passive foraging techniques hinder learning? A review of associative learning in insects demonstrates that learning can greatly benefit insects in the areas of foraging (Simpson & White, 1990; Lewis & Takasu, 1990; Dukas & Real, 1991; Dukas & Real, 1993; Dukas & Visscher, 1994; Raubenheimer & Tucker, 1997; Daly & Smith, 2000; Dukas & Bernays, 2000), growth (Dukas & Bernays, 2000), predator avoidance (Dukas, 1998), and reproduction (Prokopy et al., 1982; Dukas & Duan, 2000).

Associative Learning

Associative learning in insects can be assessed through the use of the classical or operant conditioning models. Although these two types of associative learning are different from one another procedurally, all studies of associative learning contain the necessary conditions for both classical and operant

conditioning. In an operant conditioning procedure, a response to a stimulus is strengthened or weakened through reinforcement. In a Pavlovian conditioning procedure, an essentially neutral stimulus, known as the conditioned stimulus (CS), is presented with an unconditioned stimulus (US), which elicits a response. This response occurs without prior training and is known as an unconditioned response (UR). With repeated pairings, the two stimuli become associated and the CS elicits a conditioned response (CR). The development of new responses to novel stimuli is indicative of learning. Because Pavlovian conditioning allows for the observation of novel responses to previously neutral stimuli, it is a helpful paradigm for experiments in which identifying learning can be difficult, such as insect learning studies. Learning in insects was not thought possible due to a variety of constraints including short life spans and small brain size; however, studies of associative learning in insects have proven that despite these constraints, associative learning is possible in various insect species (MacGuire, 1984, Lewis & Takasu, 1990; Simpson & White, 1990; Dukas & Real, 1991; Dukas & Real, 1993; Dukas & Visscher, 1994; Raubenheimer & Tucker, 1997; Dukas, 1998; Daly & Smith, 2000; Simpson & White, 1990; Dukas & Duan, 2000).

One goal of studying associative learning in insects is to understand learning as it occurs in nature. For a laboratory experiment to replicate learning that occurs in nature, the experiment should reflect the natural environment as much as possible (Domjan 2005), which is done by placing emphasis on biologically significant events, such as the presentation of food, mates, or

predators (Hollis, 1982). Selecting ecologically relevant conditioned stimuli, in which the CS and US are naturally related, would further simulate learning as it occurs in nature (Domjan, 2005). In insect learning studies, the presentation of food is commonly presented with stimuli in the form of colors or odors, which would naturally accompany food. For example, Sakura and Mizunami (2001) presented cockroaches with desirable and undesirable solutions paired with distinct odors. The forming of an association between odor and food is frequently observed in animals. Sakura and Mizunami found that the cockroaches moved towards the odor that was associated with the desirable solution, indicating that cockroaches could use the odor cue to find the reward. The study found further that cockroaches associated the odor and solutions in as short a time as one training day.

The findings that cockroaches alter their foraging techniques to seek out novel stimuli that are associated with food was further demonstrated in locusts (Simpson & White, 1990). When deprived of protein, locusts moved towards the odor that was associated with a protein rich diet as opposed to a carbohydrate rich diet. Although Simpson and White did not test for any fitness effects in their study, it is possible to infer how their results might increase fitness in a natural setting. The ability of an insect to differentiate between two food sources, and actively seek out the food source that would best suit its nutritional needs, has clear benefits. That is, the insect could use odor cues to locate the most beneficial diet first, rather than wasting energy testing various food sources.

The ability to differentiate between food sources was further tested using

bees as subjects. Honeybees that were trained to associate blossom color with rewarding or nonrewarding blossoms learned to fly towards rewarding blossoms (Dukas & Real, 1991). A similar experiment determined that bumblebees, like honeybees, could use floral color to distinguish between rewarding and non-rewarding blossoms, indicating that various species of bees are able to associate visual cues with differences in nutritional value (Dukas & Real, 1993).

Research has shown that various insects are able to adjust foraging behavior according to nutritional need. However, insects are able to use cues in other contexts, too. For example, parasitoid wasps are able to associate odors with two different biological needs, namely the need for food and the need for a host. Wasps that were deprived of food moved towards the odor that was paired with food, while well-fed wasps moved towards the odor that had been paired with the host (Lewis & Takasu, 1990). These findings further indicate that insects are able to discriminate between cues, depending on their biological need for food or reproduction.

The ability to associate cues in the environment with biologically significant events, such as the presence of food or predators, was further studied using fruit flies as subjects. Fruit flies associate odors with high and low quality food (Dukas, 1998). Moreover, flies associate odor with safe environments and unsafe environments, using the odors to select the most beneficial situation. That is, the fruit flies learned to avoid stimuli that were associated with the appearance of a negative situation, in this instance, an unsafe environment. The ability to identify stimuli with positive fitness effects and avoid stimuli with negative

fitness effects is an adaptive behavior that could benefit insects in natural environments.

The study of associative learning using biologically significant events is particularly important. Hollis (1982) theorized that learned cues could allow animals to engage in behavior that optimizes subsequent interactions with biologically important events, thus giving the animal an advantage over other animals that did not form a CS-US association. The utilization of cues to optimize future interactions can be observed in insect learning studies. For example, parasitoid wasps were exposed to fruit that hosted fruit fly eggs while other wasps did not have that exposure (Dukas & Duan, 2000). When the wasps were allowed to search for fruit that hosted fruit fly eggs, those that had previous exposure to host fruit flew directly to the known fruit, resulting in significant time savings compared to wasps that did not have the previous exposure. In addition, previous exposure increased the fitness of the wasps, as wasps with previous exposure produced significantly more eggs and adult offspring than those that did not have previous exposure.

Studies of learning in flies also suggest that insects are capable of altering their behavior in a way that could optimize subsequent interactions (MacGuire, 1984). Blow flies, fruit flies, and house flies have been conditioned to extend their proboscis to cues paired with food. The proboscis is an appendage used specifically for feeding. Thus, learning to extend the proboscis at the presentation of a cue prior to the appearance of food could enable the fly to optimize the upcoming interaction with the food. For example, extension of the

proboscis after a cue might result in a decrease in time spent feeding, or more efficient feeding.

Although much research has demonstrated that insects are capable of responding to stimuli associated with the presence of biologically relevant events, the ways in which associative learning increases fitness have been more difficult to establish. Nonetheless, Dukas and Bernays (2000) found that the growth rate of grasshoppers in the learning condition was 20% higher than grasshoppers that were prevented from learning. Although Dukas and Bernays did not report that growth rate had a direct effect on reproductive success, one can hypothesize that the benefit of increased growth rate might benefit reproduction in a variety of ways, such as a faster escape from predators. Increased growth rate may also have direct reproductive benefits, such as an increase in the number of eggs laid or the number of eggs that hatch successfully. Thus, Dukas and Bernays' findings suggest that learning could have a significant effect on various aspects of fitness.

If learning is an adaptive trait that benefits insects in responding to biologically significant events, one would assume that the benefits of learning would increase over a lifetime of experiences. Therefore, measures of fitness based on a lifetime of learning would be reflective of learning in the natural environment. Supporting this prediction, Dukas and Visscher (1994) observed honeybees throughout their lifetime to determine the effect of learning and found that as the honeybees' experience with learning increased, their collection of pollen from foraging also increased. This increase in food collection with experience suggests that the bees were able to alter their foraging behavior based

on the information learned from past foraging experiences. An increase in foraging throughout the lifespan of an insect suggests that an insect that learned would have an adaptive advantage over insects that did not have that opportunity. Although this particular advantage was demonstrated in the uptake of food, it could also be manifested in decreased foraging time, which in turn would decrease predation risks and conserve energy.

The aforementioned studies have expanded our understanding of insect learning. Despite short life spans and small brain sizes, insects from a variety of species have demonstrated the ability to form associations between stimuli and respond to learned cues. In particular, these studies suggest that insects adjust their foraging to locate the most beneficial food. Although the insects used as subjects vary widely in their diets and methods of foraging, there is one similarity among these studies: All of these subjects are active foragers. Currently, research has not analyzed the effect of learning on predators that do not use active foraging methods to procure food.

Sit-and-Wait Predation

Sessile, or sit-and-wait, predators are passive foragers that remain stationary until prey is detected. Rather than expend energy in the search of prey, sit-and-wait predators remain immobile, until prey approach. Through this immobility, sit and wait predators conserve energy and remain hidden from predators. Thus, sit-and-wait predators rely on the prey to come within capture range (Baily, 1998). Once the predator has detected the prey, it can attempt a prey capture.

A sit-and-wait predator's dependence on the approach of prey requires that it detect the prey. It is therefore important for the predator to select a foraging site that does not hinder prey detection. The value of a sit-and-wait predator's foraging site is dependent on the availability of prey, and the ability to detect prey that comes within capture range (Metcalf et al., 1997). Once a predator has selected a foraging site, it is likely to conserve energy by remaining in that site.

Some sit-and-wait predators anticipate the approach of food and then exhibit brief bouts of movement to capture prey (Andrews, 1979; Metcalf et al., 1997). In other species, the predators construct traps for prey such as webs or pits and wait for the prey to encounter the trap (Olive, 1982; Bailey, 1998; Walker et al., 1999; Miyashita, 2005; Morse, 2006). Once the prey enters the trap, the predator can attempt a capture. With this form of trap-building foraging, the major energy expenditure is in the construction of the trap, rather than the actual prey capture. Trap-building predators are not able to select the prey that enters the trap; however, they may be able to detect cues to determine whether prey that entered the trap is worth capturing, and thus adjust their foraging behavior. One such trap-constructing, sit-and-wait predator is the antlion.

Antlions

Antlions (Neuroptera: Myrmeleontiadae) are insects that are so named because of their predation on small insects including, but not limited to, ants. There are more than 2000 species of antlions, and they are found on most continents (Mansell, 1992; 1994; 1999). Antlions can live in a variety of habitats but are most commonly found in dry, shaded substrates such as sand (Turner,

1915; McClure, 1976; Griffiths, 1985; Lucas, 1985; Mansell, 1992; Gotelli, 1996; Arnett & Gotelli, 1999; Crowley and Linton, 1999; Day & Zalucki, 2000).

Antlions exhibit a four part life cycle, beginning as an egg, hatching into the larval stage, continuing to the pupal stage and ending as winged adults (Turner, 1915). The larval stage consists of three instars, which are marked by changes in the size of the head capsule and mandibles (Griffiths, 1980; Lucas & Stange, 1981; Arnett & Gotelli, 1999). Antlions can remain in the larval stage for up to 2 years depending on food availability (Arnett & Gottelli, 1999). Upon the completion of the third instar, the antlion forms a round cocoon under the sand in which to pupate. Pupation can be initiated by various factors, including larval size and environmental factors. Griffiths (1985) found that up to 57% of third instar antlions that were fed for 10 days and then starved proceeded to pupate. The antlions remain in their cocoon for approximately one month before hatching into adults. Once hatched, the adult antlion has a short lifespan of approximately one month during which it reproduces (Arnett & Gotelli, 1999).

Pit Construction

In their larval stage, antlions are sessile, “sit-and-wait” predators (Griffiths, 1980; Cains, 1987; Gotelli, 1997). Although not all species of antlions construct pits (Mansell, 1992; Mansell, 1994, Mansell, 1999), pit building antlions have been studied extensively (Turner, 1915; McClure, 1976; Topoff, 1977; Griffiths, 1980; Griffiths, 1982; Boake & Visscher, 1984; Lucas, 1986; Cains, 1987, Mansell, 1992; Gotelli, 1996; Gotelli, 1997; Napolitano, 1998; Arnett & Gotelli, 1999; Crowley & Linton, 1999; Hauber, 1999; Day & Zalucki,

2000; Farji–Brener 2003). Antlions construct conical pits in the sand by plowing their bodies backwards in concentrically narrowing circles and throwing the loosened sand out of the pit with their mandibles, pincher-like appendages that serve a variety of functions in prey capture (Turner, 1915). Once the antlion reaches the vertex of the pit, it burrows under the sand so that only the mandibles are visible and waits for prey to enter the pit.

As a sessile predator, the antlion's area of predation is only as wide as the pit it constructs; therefore pit diameter is an important factor in capturing prey (Lucas, 1986; Crowley & Linton, 1999; Farji-Brener, 2003). Griffiths (1980, 1986) reported that a 2-mm increase in diameter results in a 10% increase in capture success. The depth of the pit is also an important feature in retaining prey that enter the pit (Farji-Brener, 2003). Considering that the size of the pit tends to increase with the antlion's size (McClure, 1976; Griffiths, 1980; Mansell, 1992; Crowley & Linton, 1999; Day and Zalucki, 2000) as well as with its instar (Lucas, 1986), it is possible to conclude that larger larvae should have greater capture success. The slope of the pit walls depends on the larval instar; that is, antlions in their first and second instar build pits with greater depth but smaller diameter than antlions in their third instar (Griffith, 1980). Greater depth benefits the larvae by making escape increasingly difficult. Larvae in their first and second instar benefit from capturing all prey that they encounter, making pit depth important. Increased pit depth is energetically costly to maintain, however, as steeper walls are less stable and thus require more maintenance. Larger larvae are better able to capture prey with their mandibles, and do not need the walls of their pits to be as

steep as first instar antlions (Griffith, 1980). Antlions frequently build pits in stages, initially building a smaller pit that is later enlarged by throwing sand out of the pit (Hauber, 1999).

The quality of an antlion's pit depends on the substrate in which it builds it. Although antlions can build pits in a variety of substances (Turner, 1915; Mansell, 1992), the size of the particles influences the shape and depth of the pit. Finer particles allow for larger pits (Lucas, 1986; Day & Zalucki 2000). Pits constructed in fine grained soil are 50% bigger and deeper, and 100% more efficient, than pits constructed in coarse grained soil (Frji–Brener, 2003); moreover, prey is able to escape pits constructed in coarse soil three times faster than pits constructed in fine grained soil.

The conical structure of the pits serves to bring the prey directly to the antlion's mandibles (Mansell 1992). The slope of the pit walls further serves to make prey escape more difficult. If a prey item is not immediately captured, it frequently slides back down the pit walls to the antlion. The antlion can further hinder prey escape by throwing sand against the pit walls, creating miniature landslides (Topoff, 1977; Griffiths, 1980). The unique structure of the pits allows the antlion to conserve energy that might otherwise be spent pursuing prey; however, the antlion is limited to prey that enters the pit (Mansell, 1992; Arnett & Gotelli, 2001). This limitation is ameliorated by the fact that antlions can live for as long as three months without food (Crowley & Linton 1999).

Prey Capture

Once an insect has entered the pit, the antlion uses its mandibles to capture

the prey. Typical capture behavior includes attacking, holding, submerging beneath the sand, prey beating, and feeding (Napolitano, 1998). However, the behaviors utilized for capture depend greatly upon the prey that enters the pit. Prey beating, used commonly with larger prey, is used to facilitate mandible penetration, and to disorient and subdue the prey. The antlion inserts its mandibles into the prey and injects a toxin that kills the insect. Once the prey has died, the mandibles are used again to inject enzymes into the body cavity of the prey; these enzymes externally digest the prey within its own exoskeleton (Griffiths, 1980). After the prey has been digested, the mandibles are used to extract the contents. The use of the mandibles to capture and consume prey eliminates the contact of the prey contents with the sand, which protects the food from contamination (Mansell, 1992). Externally digesting the prey processes the food so thoroughly that after the food is ingested the antlion does not excrete solid waste (Mansell, 1992).

When the antlion has finished consuming the prey, it uses its mandibles to throw the carcass out of the pit. After removing the carcass, the antlion rebuilds its pit, using the mandibles to remove sand that might have fallen to the center of the pit during the prey capture. This maintenance of the pit serves to prepare the pit for subsequent captures. After an initial capture, the success of subsequent captures is significantly reduced due to the disruption to the pit walls during the initial capture (Lucas 1986). The importance of pit maintenance for capture success explains why antlions that are in need of food maintain their pits more frequently than well-fed antlions (Arnett & Gotelli, 2001).

Pit Dispersion

The dispersion of antlion pits in areas has been one of the most extensively studied aspects of antlion behavior. In areas of high density, antlions face a variety of challenges, including competition for prey, disruption of pits due to the sand throwing from other antlions, and even the danger of cannibalism (McClure, 1976; Gotelli, 1997; Day & Zalucki, 2000). Gotelli (1997) found a high mortality rate of second and third instar antlions at high densities, due to cannibalism. The study was supported by the finding of Day and Zalucki (2000) who found cannibalism in densities of five antlions or higher in an area of 100 cm². However, cannibalism only occurred when lack of space prohibited the relocation of pits.

Relocation of pits is a common response to high densities. Relocation does not seem to be based on prey capture, as limiting food has no effect on pit relocation (Day & Zalucki, 2000). Rather, avoidance of other antlions and sand throwing seem to be the significant factors in pit relocation. However, in areas of especially high population densities, antlions forgo building pits (Boake and Visscher, 1984; Gotelli, 1997; Day & Zalucki, 2000). In such situations the energy costs of maintaining a pit or relocating a pit appear not to be worth the energy gain of securing prey.

Learning in Antlions

Although antlions have been the subject of research for decades, they primarily have been studied for their unique foraging behaviors. Studies have focused on where they build pits (Turner, 1915; McClure, 1976; Griffiths, 1985;

Lucas, 1986; Mansell, 1992; Gotelli, 1996; Arnett & Gotelli, 1999; Crowley & Linton, 1999; Day & Zalucki, 2000), where and why they relocate pits (Hauber, 1999; Day & Zalucki, 2000), how they build pits (Turner, 1915; Griffiths, 1980; Hauber, 1999), and how they capture and consume prey.

Currently, no research has been published regarding an antlion's ability to learn. Recently, Hayden (2005) addressed whether antlions are able to learn in an unpublished honors thesis. In that study, antlions in the learning condition were given a sand dropping signal immediately before being presented with a food item for 16 consecutive training days. During the same period, antlions in the control condition were also presented with a food item; however, the sand dropping signal was presented at another time, separate from feeding. Following this training period, antlions entered a test phase in which the sand dropping signal preceded a food item for animals in both groups. The food item was measured for the amount of mass extracted, the proportion of available mass extracted, and the proportion of available mass extracted per second. Measures throughout the training period revealed that subjects in the learning group had significantly greater extraction rate and pit volume than control subjects. On the test day, antlions in the learning group were found to have significantly greater extraction efficiency, extraction rate, pit diameter and pit volume than control antlions. Hayden further reported that one week after the test day, all of the subjects in the learning condition had molted, thereby entering another instar, while only one of the control antlions molted within the same time frame. The findings suggest that antlions are capable of learning. In particular, the significant difference in the

number of antlions that molted suggests that learning may have an effect on the fitness of antlions. An earlier molt might enable antlions to reach maturity and reproduction sooner than antlions that did not learn or, minimally, decrease the length of time that they remained in the vulnerable larval stage.

Although Hayden (2005) found some evidence that learning improved prey extraction, her study did not allow her to observe how subjects in the learning treatment may have responded to the signal for food. A subsequent study by Sun (2006) looked for evidence of a conditioned response. Procedurally, much of Sun's experiment remained the same as Hayden's; however, Sun paid particular attention to the effect of distance of the sand dropping cue on the antlions' behavior to the cue. In Sun's study, antlions were presented with the sand dropping cue at three distinct distances; 3 cm, 8 cm, and 13 cm from the edge of the pit. Sun found that antlions in the learning condition demonstrated significantly more mandible movement than those in the control condition, but only in instances in which the signal induced sand to fall into the pit. This observation of movement induced by sand fall was observed primarily when the signal was presented at the closest distance, 3 cm from the edge of the pit. However, in contrast to Hayden's findings, Sun did not find any differences in the amount of mass extracted or the rate of extraction between the learning and control condition. One possible reason for these somewhat contradictory findings is that, in both of these studies, no attempt was made to control for developmental stage of the antlion subjects. Griffith (1985) suggests that as antlions approach pupation they behave in a way that reduces energy expenditure rather than

optimizing foraging. If subjects were at different points in their developmental stage, it is possible that some would expend less energy on efficient prey extraction.

Current Study

The current study continues to examine the effect of associative learning on the feeding behavior of antlion (Neuroptera: Myrmeleontiade) larvae (Hayden, 2005; Sun, 2006). In addition to establishing that learning occurs, the current study attempts to control for a variety of factors that may have produced the contradictory findings of the previous two studies.

In those studies, antlions began training at various times in their larval stage. In the current study, all antlions enter training directly after molting to control for larval age. Preliminary studies also used a set number of days as a training period, before the testing phase began. Because antlions have not yet been used as subjects of learning experiments, it is not possible to know if the training period was long enough for the testing to be an accurate reflection of learning. The current study does not rely on a single testing day to assess learning. Rather, the current study assesses learning using measures of the feeding behavior of antlions throughout an entire instar.

In contrast with Hayden's findings, Sun did not find better food extraction or larger pits in the learning group. One possible explanation for the differing results is that differences in food items might have hindered measures of extraction. Sun used fruit flies as the food item whereas Hayden used much larger pill bugs. It is possible that Sun encountered a ceiling effect, wherein all

antlions were able to extract the maximum possible from the *Drosophila*. To control for this ceiling effect, the current study uses an intermediate sized food item, namely feeder ants.

The current study differs further from preliminary research by paying particular attention to possible fitness effects of learning in antlions. As an adaptive trait, learning should benefit the antlions by increasing their fitness. By measuring the feeding behavior throughout an entire instar, we hope to observe differences that would indicate increased fitness. Such measures might include an increased growth rate in the learning condition (Dukas & Bernays, 2000), or earlier molting or pupation in the learning condition.

This study examines similar measures of extraction, feeding behavior, and pit size as the previous studies (Hayden, 2005; Sun, 2006). I predicted that training antlions throughout an entire instar would result in differences in extraction: Subjects in the learning condition were predicted to extract more mass from the prey, extract in a shorter amount of time, extract more mass per unit of time, and extract more efficiently than control subjects. I predicted that there would also be differences in antlion feeding behavior, including antlion visibility and movement. Finally, I predicted that learning would result in differences in pit size; that is subjects in the learning condition would build larger pits than control subjects.

METHOD

Subjects and apparatus

Each of approximately 50 antlions (Neuroptera: Myrmeleontiadae), obtained from antlionfarms.com (Pensacola, FL), was placed in a 210 ml plastic cup (7.5 cm top diameter x 8.5 cm height x 4.25 cm bottom diameter) filled with 150 ml coarse sanitized sand. Each cup was placed in a 473 ml plastic container (11.5 cm top diameter x 8 cm height x 9 cm bottom diameter) that was filled with 200 ml of fine sanitized sand to stabilize the cup.

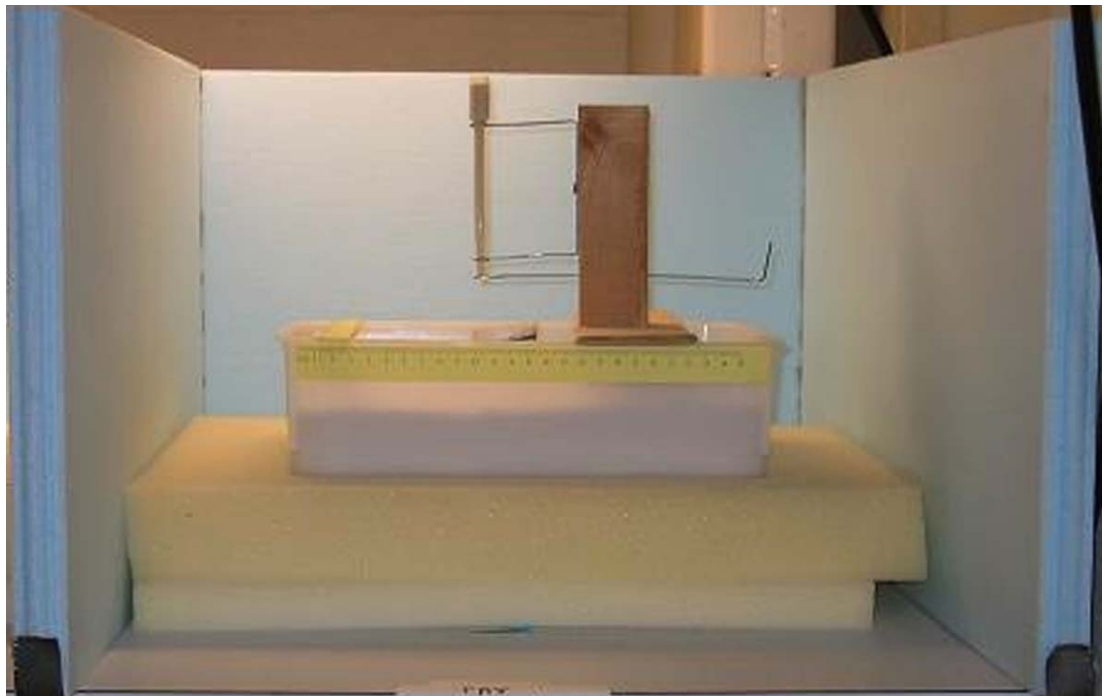
The antlions were monitored, unfed, for two days to determine if they would build pits. Following this two-day habituation phase the antlions received two fruit flies (*Drosophila*), delivered to the center of their pits, on six out of seven consecutive days (Sunday through Friday). The fruit flies were obtained from Connecticut Valley Biological Supply (Southampton, MA). Overhead lights were set on a 14:10-hr light: dark light cycle (on at 0900 hr, off at 2300 hr). Approximately 30 minutes separated the delivery of the second fly during each feeding session. Four antlions that did not eat consistently during the first six days were eliminated from the experiment. The remaining 46 antlions, which formed the pool from which the subjects were chosen, were monitored during feeding sessions, and any abnormal pits or behaviors were noted. Characteristics of abnormal pits included a flat-bottomed pit, no visible pit, or tracks (doodles) in the sand, which indicated that the antlion was continuing to search for a suitable pit site (Boake & Visscher, 1984). Abnormal behavior included antlions hiding under the sand or being seen outside of a formed pit.

Once an antlion in the subject pool was not visible for two consecutive days, the cup and surrounding area were monitored for the appearance of an exoskeleton as a sign of molting. Once an exoskeleton was observed and the antlion was again visible, it was given a 24 hour period to rebuild a pit, unfed. Nineteen antlions met these criteria during the same week. They were weighed to the nearest 0.1 mg and measured to the nearest mm. Of these 19 antlions, the 16 antlions with the closest weights were selected as subjects. Antlions were paired by weight to ensure that subjects were evenly distributed within the two conditions. Within each pair the subjects were randomly assigned to the LRN or CON condition. The sixteen antlions ranged in weight from 15.8 mg to 20.3 mg.

Each antlion subject was moved to a rectangular plastic container (28 cm long, x 17 cm wide x 17 cm deep) filled with one gallon (approximately 3.8 liters) of fine sanitized sand, at a depth of approximately 13 cm. The container was situated in a sound-attenuating compartment constructed of foam pads. The foam floor of the compartment was 1 cm thick, and the back and side walls of the compartment were 2 cm thick. Six compartments were placed adjacent to each other on each of four individual shelves. Of the six compartments on each shelf, the four center compartments were used, while the two end compartments were left vacant (see Figure 1).

A sand-delivery device was placed on the top of each plastic container. The device, which consisted of a plastic dropper and a metal release valve attached by wire to a wooden holder, was used to deliver a vibratory cue of 4.5 ml of dropping sand. A vibratory cue was selected because vibrations are known to

Figure 1. Illustration of the sound-attenuating compartment, including a sand delivery apparatus and a sand-receiving device.



be within an antlions range of detection (Devetak, 1985). A sand-receiving container, which was placed underneath the delivery device to contain the falling sand, consisted of plastic wrap (5 mils thick) attached to the bottom of a 3.8 cm (1.5 in) PVC pipe. The sand-delivery device and the sand-receiving container were placed so that the cue was delivered 4.5 cm from the edge of the pit (see Figure 2).

Procedure

Training. Each antlion in the LRN condition received the signal of 4.5 ml of sand dropping. Immediately following the signal, each LRN antlion was delivered a single live ant to the center of the pit, weighing between 1.6 mg and 5.2 mg, purchased from AntsAlive.com (Hurricane, UT). The signal, followed immediately by the food, was delivered at a randomly selected time between 0900 hr and 1700 hr during each 48 hour feeding session. The times of the feedings were randomized to ensure that the feeding time per se did not become an inadvertent cue. This 48-hour feeding session was repeated three times a week, and was followed by a 24 hour period during which training was suspended. This training schedule continued until each subject entered the pupal stage.

The antlions in the CON condition were fed one pre-weighed ant at the same randomly selected time as LRN subjects; however, the sand dropping signal was programmed at another randomly selected time between 0900 hr and 1700 hr within the 48 hour feeding session with the constraint that the cue could not be presented for four hours before or after the feeding period. The separate presentation of the sand dropping cue prevented the control subjects from

Figure 2. Top view of a single plastic container, including a sand delivery apparatus and a sand-receiving device, which is placed directly beneath the sand delivery apparatus.



associating the sand dropping cue with the presentation of food. The order in which subjects were fed was determined for each training day using an online random number generator (<http://www.random.org/nform.html>).

Feeding was considered complete when the antlion threw the ant carcass out of the pit. If the antlion did not throw the carcass out of the pit, feeding was considered complete when ten minutes had passed since the antlion's mandibles last touched the carcass. At the completion of a feeding session, the ant carcass was retrieved and weighed. If an ant escaped the pit during the feeding session, it was collected and the feeding session was considered complete.

During training, the pit diameter and depth were measured daily to the nearest 0.1 cm. The depth of the pits was measured using a ruler that was angled at one end. For both measures of diameter and depth, the ruler was held as close to the sand as possible without touching the sand, so as to get the most accurate measure without disrupting the pit or injuring the antlion. Training continued until each antlion stopped feeding in preparation for the cocoon stage. That is, the procedure for selecting subjects after they had molted, as described above, resulted in all subjects being in the third instar at the start of training. Thus, feeding sessions during training prepared each subject for the pupal stage (see Figure 3 for illustration of experimental design and procedure).

Data analysis

Pit volume was calculated using the following equation: $Volume(cm^3) = \frac{1}{3} \pi \times r^2 \times h$ where r is the pit radius (half of the diameter) and h is the pit height. The time when the food was delivered and the

Figure 3. Illustration of experimental design and procedure.

<p align="center">‘Prior to Training (the date received antlions through shedding of exoskeleton)</p>	<p align="center">Training (42 training days)</p>
<ul style="list-style-type: none"> • Placed in individual cups of coarse sand • Antlions monitored for 2 days, unfed, pit building • Antlions fed two drosophila a day • Monitored each antlion until it molted and rebuilt pit 	<ul style="list-style-type: none"> • Divided into LRN and CON • Placed in individual containers • Pits measured (depth, diameter and volume, walls touched) six days a week • Each antlion received either LRN or CTL treatment once in a 48-hour training day: <p>LRN: CS → US CTL: CS / US</p>

time when the antlion threw the carcass were recorded using video recorders. The carcass was weighed immediately after the completion of a feeding session. The amount of mass extracted from the ant was calculated by subtracting the final weight (W_f) of the carcass from the initial weight (W_i). The extraction

efficiency, or proportion of prey mass ingested, was calculated by dividing the

mass extracted by the initial weight: $\frac{W_i - W_f}{W_i}$. The extraction rate, or mass

consumed per unit of time, was calculated by dividing the extraction by the

amount of time: $\frac{W_i - W_f}{\text{Extraction Time (s)}}$. Extraction time, or the time it took to throw

the carcass, was measured in seconds from the time that the prey was captured by the antlion to the time when the carcass was thrown or retrieved from the pit.

Extraction efficiency rate, or the efficiency of extraction per unit of time, was calculated by dividing the extraction efficiency by the extraction

time: $\frac{\left(\frac{W_i - W_f}{W_i} \right)}{\text{Extraction Time (s)}}$.

Behavioral measures. During training, feeding and stimulus sessions were recorded onto 60 minute Panasonic Mini DV tapes using six Panasonic PV-GS31 Mini DV Digital Camcorders. The Camcorders were stationed approximately .75m from the antlions. The subjects in the LRN condition were taped 20 seconds prior to the presentation of the sand dropping cue. Immediately following the sand dropping cue, the ant was dropped into the center of the antlion pit. The taping continued until the carcass was thrown. If the prey item was not thrown

from the pit, taping continued for 10 minutes from the last time the antlion touched the carcass. The same recording procedure was used with the subjects in the CON condition; however, the subjects in the CON condition were taped again when they were presented with the sand dropping cue. The cameras were set up in the same way for the cue delivery sessions and the food delivery sessions. The camera recorded the subject for 20 seconds before the sand dropping cue was presented and continued for 10 minutes after the presentation of the stimulus.

The tapes were later scored by 6 experimenters who were trained in scoring techniques. Evaluated behaviors included visibility prior to the presentation of the ant, any movement prior to the presentation of the ant, the speed of capture, any difficulties in capture, the duration of the feeding and any attempted throws. Inter-observer agreement was calculated to ensure the reliability of behavioral measurements. A two-way mixed model intraclass correlation using an absolute agreement model demonstrated very little variability within scores across raters, $ICC = .93$, $p > .05$, where Cronbach's Alpha = .99.

To reduce observer bias, video sessions were taped without any identifying information. Prior to each taping session, 10 s of a reference letter (A-H) was recorded, which allowed the person viewing the tape to reference the session with an identification sheet. Identification sheets were attached to the cassette case inserts and were used to record the condition, subject, date, and training day of all feeding or stimulus sessions. Each tape was labeled with the condition, subject, and tape number. Experimenters were trained to look only at the identifying information after viewing the entire video tape. To test whether

this “blind” viewing, per se, introduced bias, experimenters scored 5 tapes that had all identifying information removed. These tapes were separated from their identification sheet, and were viewed without sound. The tapes were labeled only with a reference letter (A-E). The scores from these blind tapes were then compared with the experimenters’ scores from the same tapes when they included the identifying information. Agreement between the blind and not blind scores varied between 92% and 100%.

RESULTS

Prior to training, sixteen antlions were paired by weight and randomly assigned to either the learning or the control group. To ensure that all antlions were of the same weight after assignment to the learning or control treatment group, an independent *t*-test was performed. The analysis revealed that initial weight (mg) was not significantly different between the LRN treatment group ($M = 18.78, SD = 1.41$) and the CON treatment group ($M = 18.75, SD = 1.10$), $t(14) = .04, p > .05$. Another independent *t*-test was performed to insure that the antlions assigned to the treatment did not differ in the volume (cm³) of their initial pit construction. That analysis revealed that pit diameter (cm) was not significantly different between the LRN treatment group ($M = 5.01, SD = 1.19$) and the CON treatment group ($M = 5.80, SD = .89$), $t(14) = -1.33, p > .05$; however, pit depth (cm) was significantly greater in the CON group ($M = 2.29, SD = .23$) than the LRN group ($M = 1.80, SD = .57$), $t(14) = -2.259, p < .05$. Due to greater pit depth in the CON treatment group, pit volume was also significantly larger for subjects in the CON condition ($M = 7.45, SD = .98$) than in the LRN condition ($M = 4.93, SD = 2.75$), $t(14) = -2.44, p > .05$. These differences in pit size between the LRN and CON group were due to the measure of a pit in the LRN group that was in the process of being constructed at the time of measurement. A second independent *t*-test was performed for the first day that all subjects had complete pits, and this analysis showed not only that pit depth did not differ significantly between the LRN ($M = 2.32, SD = .54$) and CON ($M = 2.29, SD = .36$) treatment groups, $t(14) = .163, p > .05$, but also that pit volume did not differ significantly between the

LRN ($M = 9.07$, $SD = 4.60$) and CON ($M = 8.78$, $SD = 3.05$) treatment groups, $t(14) = .15$, $p > .05$.

The training period of this study was designed to follow the subjects throughout an entire instar. The result was that the training period extended past six months. Time constraints limit the analysis of this study to the first 14 weeks, or 42 training days.

Training Phase Data

I hypothesized that the delivery of a cue prior to the presentation of food would elicit a difference in the behavior of antlions in the learning and control treatment groups. I further hypothesized that subjects in the LRN group would be better at prey extraction, exhibit feeding behaviors more frequently and build larger pits than CON subjects. Extraction was recorded using 5 measures: a) the amount extracted from the prey; b) the extraction length, from the time when the antlion captured the prey to when the carcass was thrown or dropped; c) the extraction efficiency, or proportion of prey mass that was extracted; d) the rate of extraction, or the amount of extraction per unit of time; and, e) the extraction efficiency rate, or the efficiency of extraction per unit of time. Feeding behavior included measures of: a) antlion visibility at the presentation of food; b) antlion visibility at the presentation of the sand dropping cue; c) antlion movement; and, d) prey escape. Pit building behavior referred to measures of: a) pit size, or volume of the pits; and b) the shape and location of pits in the container, as many subjects built pits that at some point during training encountered one or more walls of the container.

Extraction Measures

A mixed analysis of variance (ANOVA) was performed to analyze each measure of extraction. These ANOVAs included one between-subjects variable, namely treatment group (LRN vs. CON), and one within-subjects variable, namely 14 blocks of 3 training days. The analysis revealed that the LRN group ($M = 1.76$, $SEM = .03$) did not extract significantly more mass (mg) from prey than the CON ($M = 1.69$, $SEM = .03$) treatment groups, $F(1, 13) = .18$, $p > .05$. The amount of mass extracted did change over blocks, $F(1, 13) = 2.84$, $p < .05$, however there was not a group by block interaction, $F(1, 13) = .95$, $p > .05$ (see Figure 4). These findings indicate that presenting subjects in the LRN treatment group with a cue immediately prior to the presentation of food did not enable them to extract more mass from the prey as predicted. Subjects in the LRN treatment group ($M = 2805.79$, $SEM = 124.79$) also did not extract in significantly less time (s) than subjects in the CON treatment group ($M = 2845.36$, $SEM = 124.79$), $F(1, 13) = .83$, $p > .05$, nor did extraction time change over blocks, $F(1, 13) = 1.77$, $p > .05$. Furthermore, there was not a significant treatment group by block interaction for extraction time, $F(1, 13) = 1.37$, $p > .05$, indicating that the presentation of the cue prior to feeding did not result in faster extraction (see Figure 5). The lack of significant findings in extraction and extraction time is mirrored by analysis of extraction rate, or the amount of mass extracted per unit of time (mg/s), which showed that LRN ($M = .04$, $SEM = .00$) subjects did not extract at a greater rate than the CON ($M = .04$, $SEM = .00$) subjects, $F(1, 13) = .25$, $p > .05$, nor was there a group by block interaction for extraction rate, $F(1,$

Figure 4. Amount of mass extracted (mean milligrams \pm SEM) for LRN and CON treatment groups across blocks of three training days.

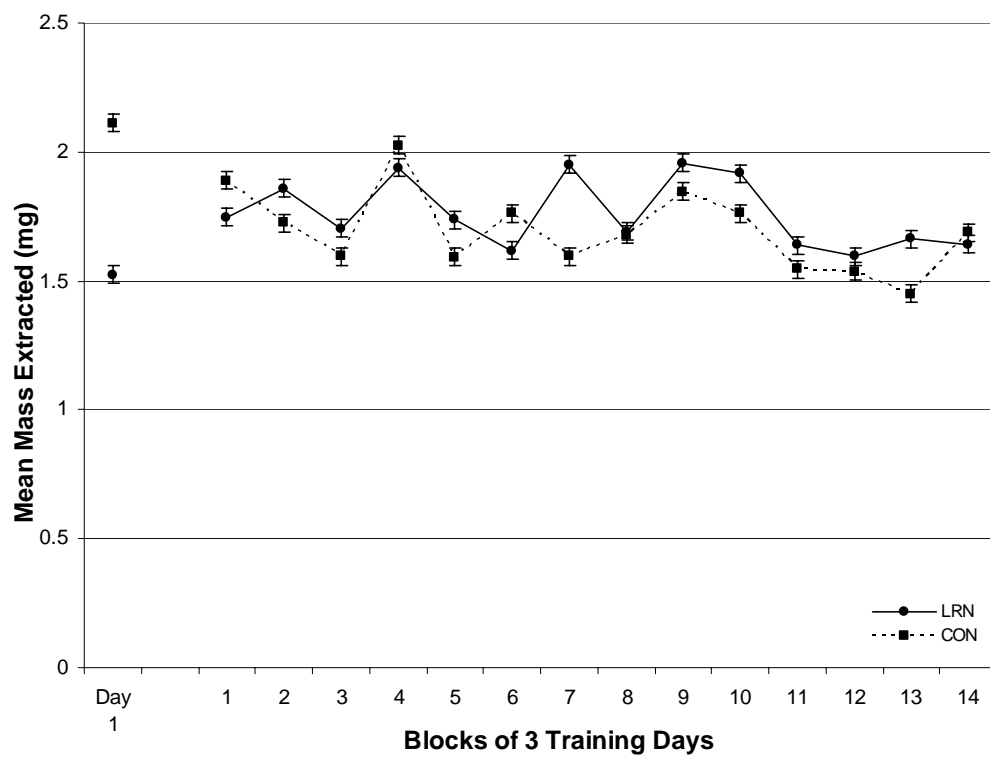
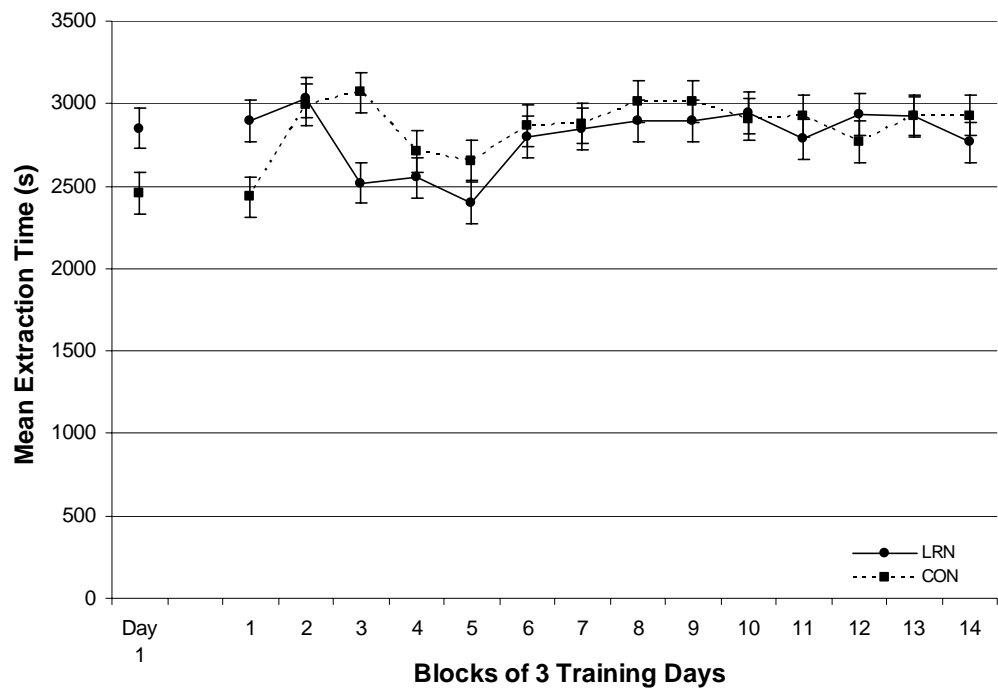


Figure 5. Extraction time (mean seconds \pm SEM) for LRN and CON treatment groups across blocks of three training days.



13) = .95, $p > .05$. There was, however a change in extraction rate over blocks, $F(1, 13) = 2.89, p < .05$ (see Figure 6).

The other measures of extraction were used to determine what proportion of the available mass was extracted, or how efficiently the subjects extracted the prey mass. The LRN group ($M = .62, SEM = .01$) did not extract a significantly greater proportion of prey mass (%) than the CON group ($M = .59, SEM = .01$), $F(1, 13) = 3.13, p > .05$, nor did subjects extract a greater proportion of prey mass over blocks, $F(1, 13) = 1.48, p > .05$ (see Figure 7). Furthermore, there was not a significant group by block interaction for extraction efficiency, $F(1, 13) = 1.02, p > .05$. Thus, the presentation of a cue prior to the presentation of food did not lead to LRN subjects extracting a greater proportion of mass from the food item than subjects that were not given a cue prior to the presentation of a food item. Similarly, the LRN treatment group ($M = .01, SEM = .00$) did not have a significantly greater extraction efficiency rate, the efficiency of extraction per unit of time (%/s), than the CON treatment group ($M = .01, SEM = .00$), $F(1, 13) = .35, p > .05$, nor did the extraction efficiency rate change over blocks, $F(1, 13) = 1.34, p > .05$. Furthermore, there was not a group by block interaction for extraction efficiency rate, $F(1, 13) = 1.33, p > .05$ (see Figure 8). However, this lack of significant findings for extraction efficiency rate using parametric analyses may be a result of the large variability between subjects. That is, a non-parametric Mann-Whitney U -test comparison revealed that LRN subjects did extract a greater proportion of mass per second than did CON subjects, $U(8, 8) = 50, p < .05$. The Mann-Whitney U -test explored groups effect, as demonstrated in

Figure 6. Extraction rate (mean grams per second \pm SEM) for LRN and CON treatment groups across blocks of three training days.

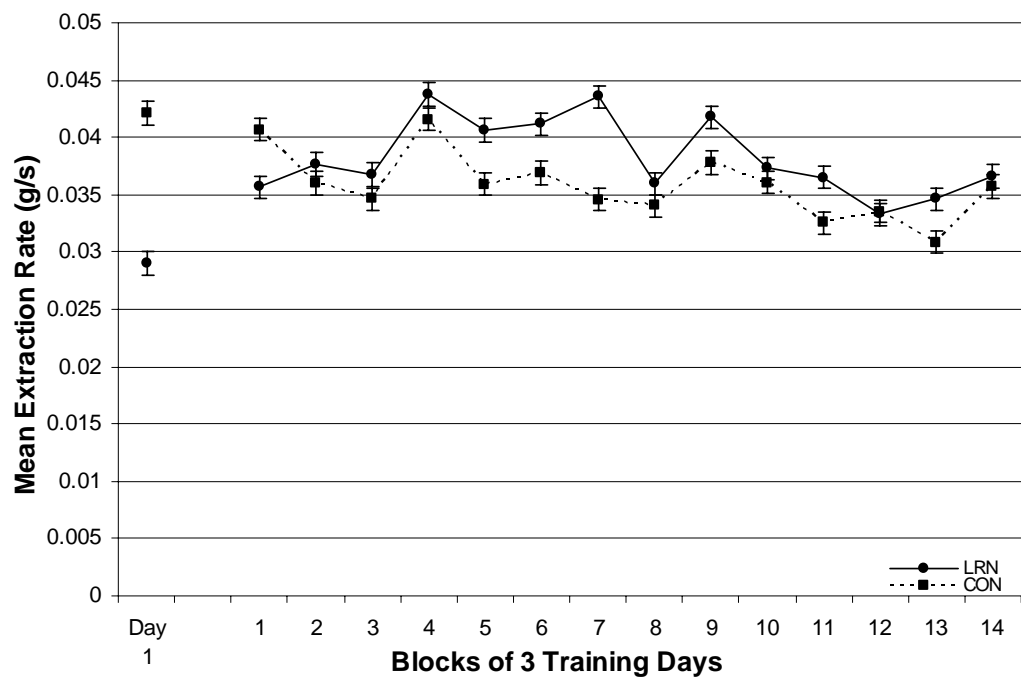


Figure 7. Extraction efficiency (mean proportion of mass \pm SEM) for LRN and CON treatment groups across blocks of three training days.

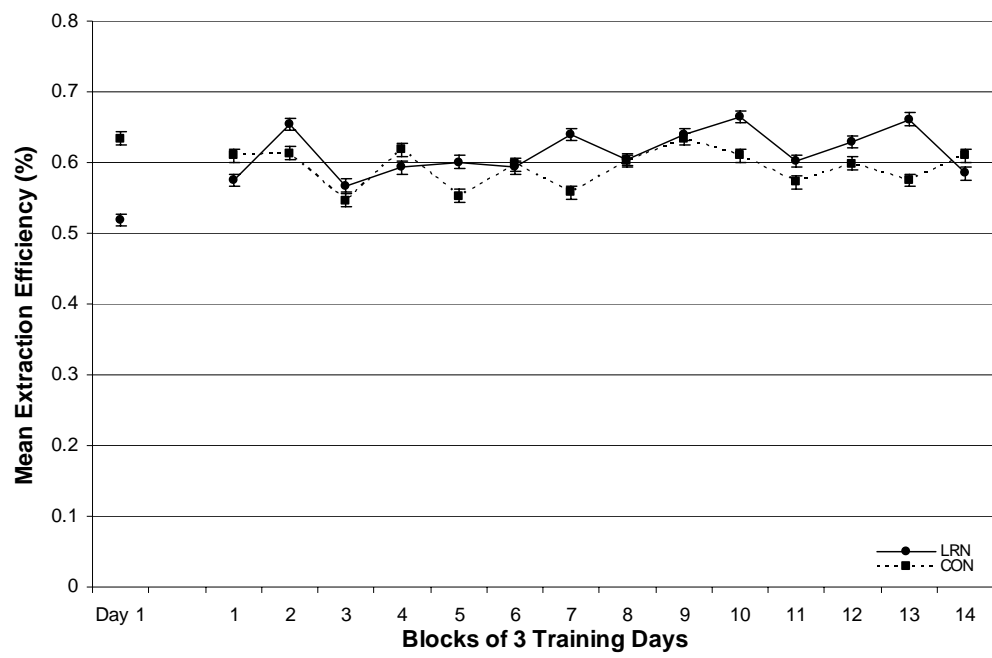


Figure 8. Extraction efficiency rate (mean proportion of mass per second \pm *SEM*) for LRN and CON treatment groups across blocks of three training days.

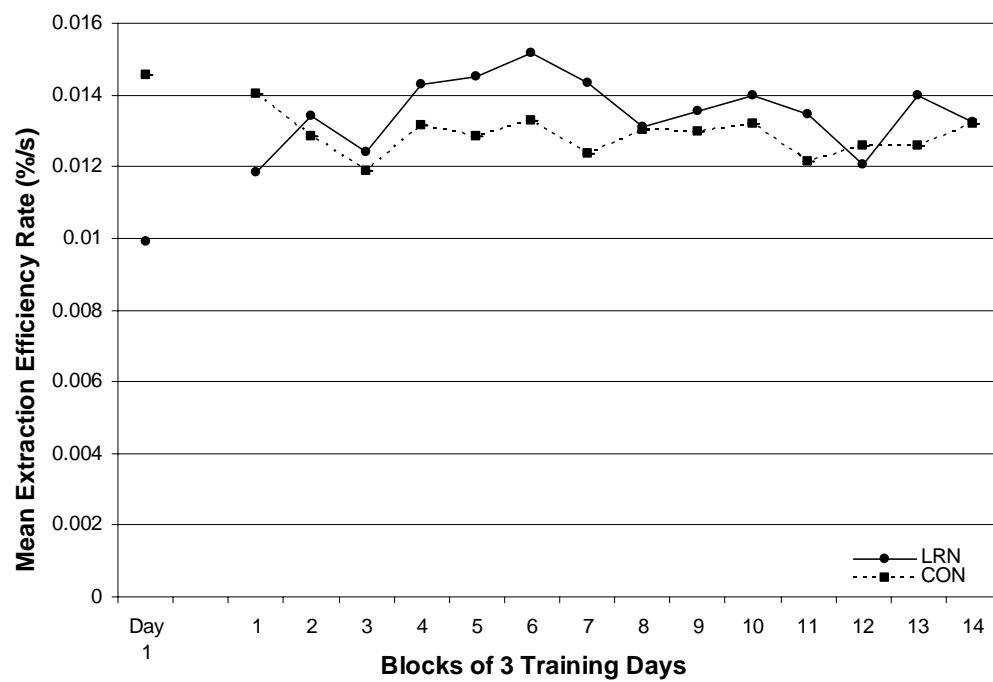


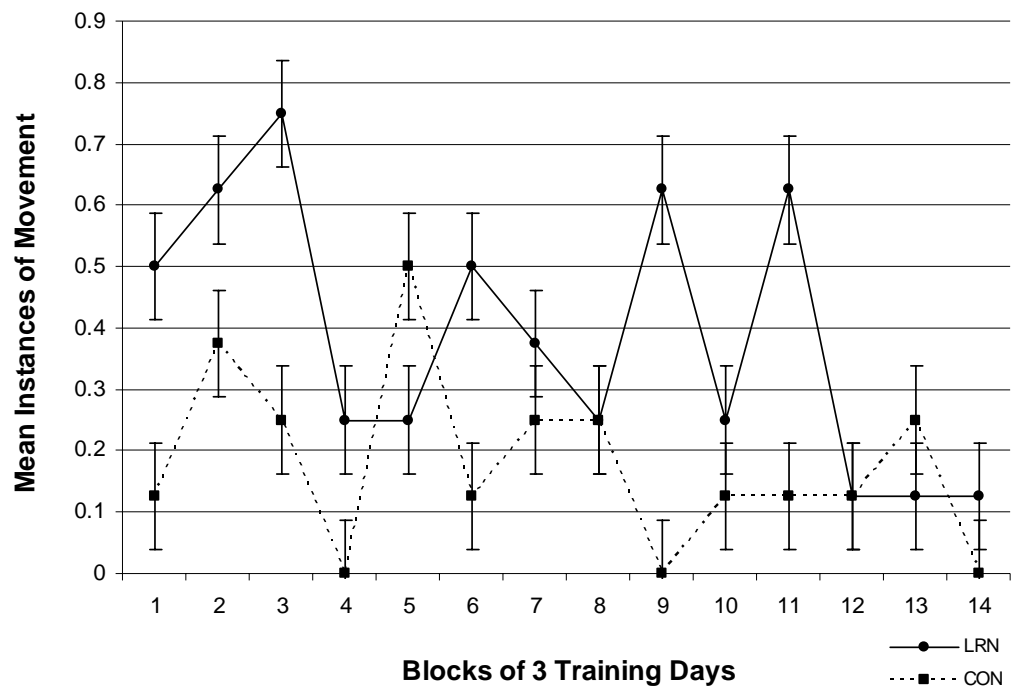
Figure 8, reveals that if anything, the CON group was better at extraction on Day 1. Thus, the significant Mann-Whitney *U*-test suggests that learning occurred.

Behavioral Measures

Feeding behavior, namely measures of movement prior to the delivery of food, antlion head or mandible visibility at the time of food delivery, and prey escape, were analyzed using mixed ANOVAs. These ANOVAs included one between-subjects variable, namely treatment group (LRN vs. CON), and one within-subjects variable, namely 14 blocks of 3 training days. I hypothesized that the presentation of a cue prior to the presentation of food might elicit movement prior to the presentation of food. However, the instances of movement did not differ significantly between the LRN ($M = .38$, $SEM = .09$) and CON ($M = .18$, $SEM = .09$) treatment groups, $F(1, 13) = 2.79$, $p > .05$, nor did movement change over blocks, $F(1, 13) = 1.18$, $p > .05$. Furthermore, there was not a significant group by block interaction for movement, $F(1, 13) = 1.06$, $p > .05$ (see Figure 9), indicating that the presentation of a cue prior to the presentation of food did not result in LRN subjects moving more than CON subjects.

The measure of visibility during cue delivery was used to determine the effect of learning on antlion visibility. Visibility was measured as the number of instances that each subject was visible at the beginning of the presentation of the cue. Subjects in the LRN condition ($M = 2.88$, $SEM = .16$) were significantly more visible than subjects in the CON condition ($M = 2.20$, $SEM = .16$) $F(1, 13) = 8.92$, $p < .05$. Visibility at the presentation of the cue changed significantly over blocks, $F(1, 13) = 2.56$, $p < .05$, and there was a significant group by block

Figure 9. Instances of movement (mean \pm SEM) for LRN and CON treatment groups across blocks of three training days.



interaction of visibility, $F(1, 13) = 1.89, p < .05$ (see Figure 10). This difference in visibility indicates that subjects in the LRN condition had their heads and/or mandibles on top of the sand more often than did CON subjects at the presentation of the cue.

Another measure of visibility, namely visibility at the time of prey delivery, was used to compare the number of instances when the CON subjects were visible at the delivery of a prey with the number of instances when the LRN subjects were visible at the delivery of prey. If there were no difference in the visibility of LRN and CON subjects immediately prior to feeding, it could indicate that subjects in the CON condition were utilizing inadvertent cues from the environment to distinguish between feeding sessions and stimulus sessions. However, subjects in the CON treatment group ($M = 2.40, SEM = .13$) were significantly less visible than subjects in the LRN treatment ($M = 2.88, SEM = .13$) group during feeding, $F(1, 13) = 7.16, p < .05$, indicating that CON subjects were not adjusting their visibility in response to food delivery (see Figure 11).

During feeding sessions there were occasional instances when the prey would escape the pit. Although the number of escapes in the LRN condition ($M = .05, SEM = .02$) did not differ significantly from the number of escapes in the CON condition ($M = .06, SEM = .02$), $F(1, 13) = .07, p > .05$ (see Figure 12). , the occurrence of prey escapes could be influenced by the location of the pit. That is, many subjects built their pits so that the pit touched one or more walls. In doing so, the area of predation was reduced, which in turn decreased the prey capture potential of their pits. In particular, pits that touched two walls faced a

Figure 10. Instances of visibility during cue delivery (mean \pm SEM) for LRN and CON treatment groups across blocks of three training days.

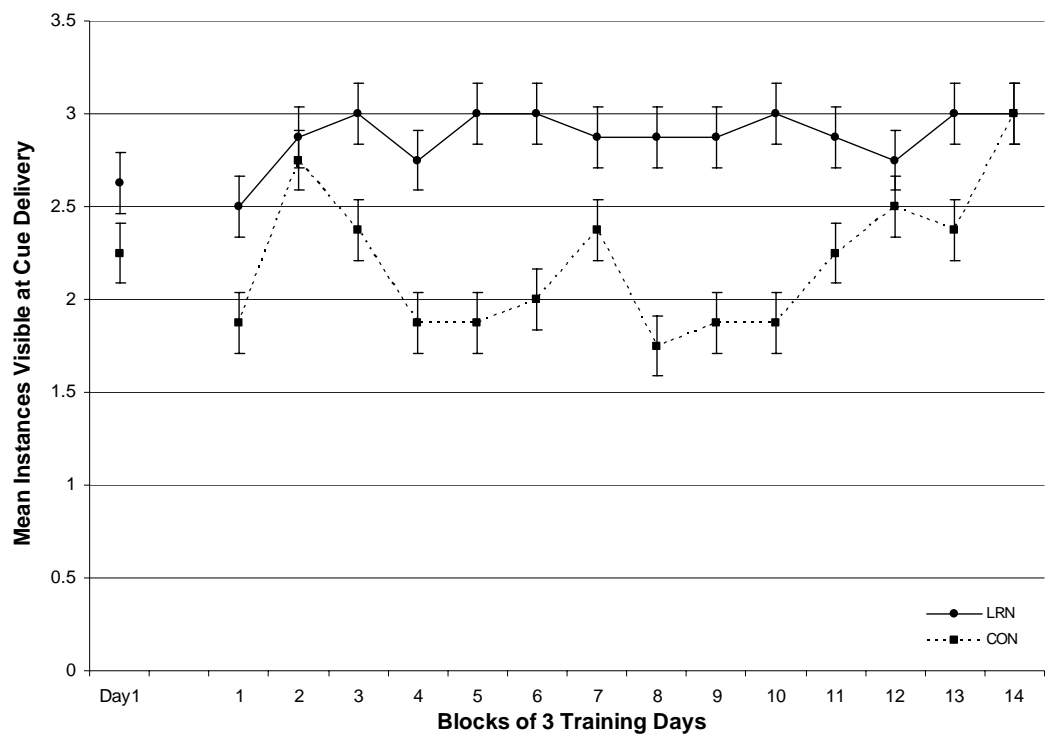


Figure 11. Instances of visibility prior to prey delivery (mean \pm SEM) for LRN and CON treatment groups across blocks of three training days.

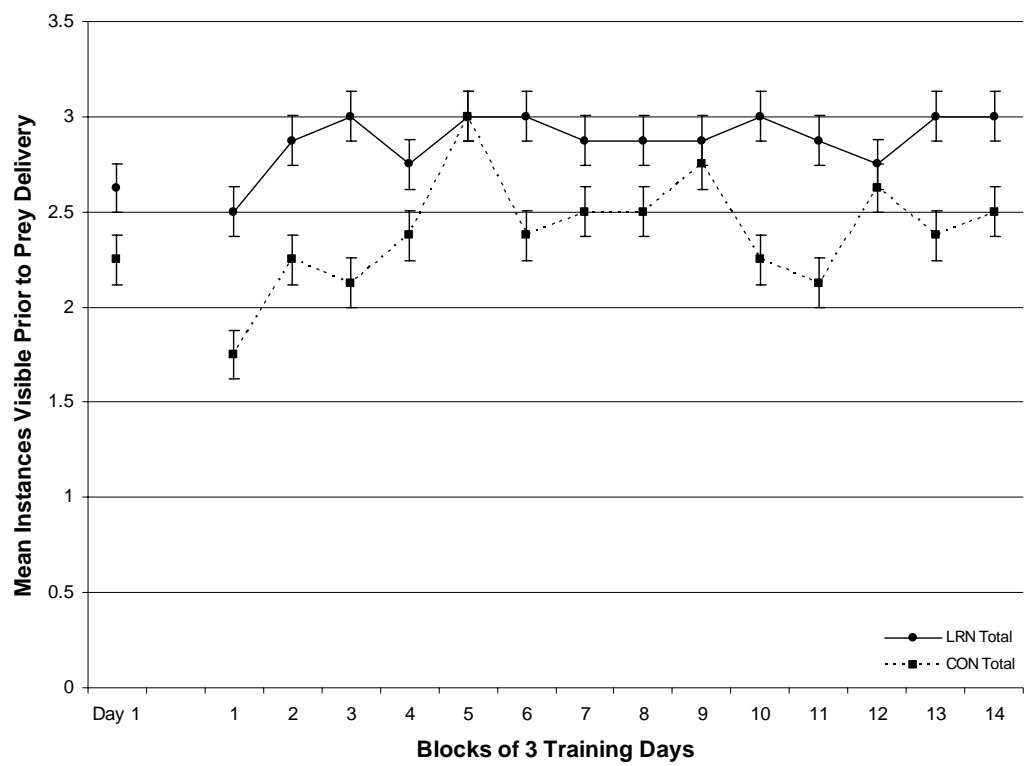
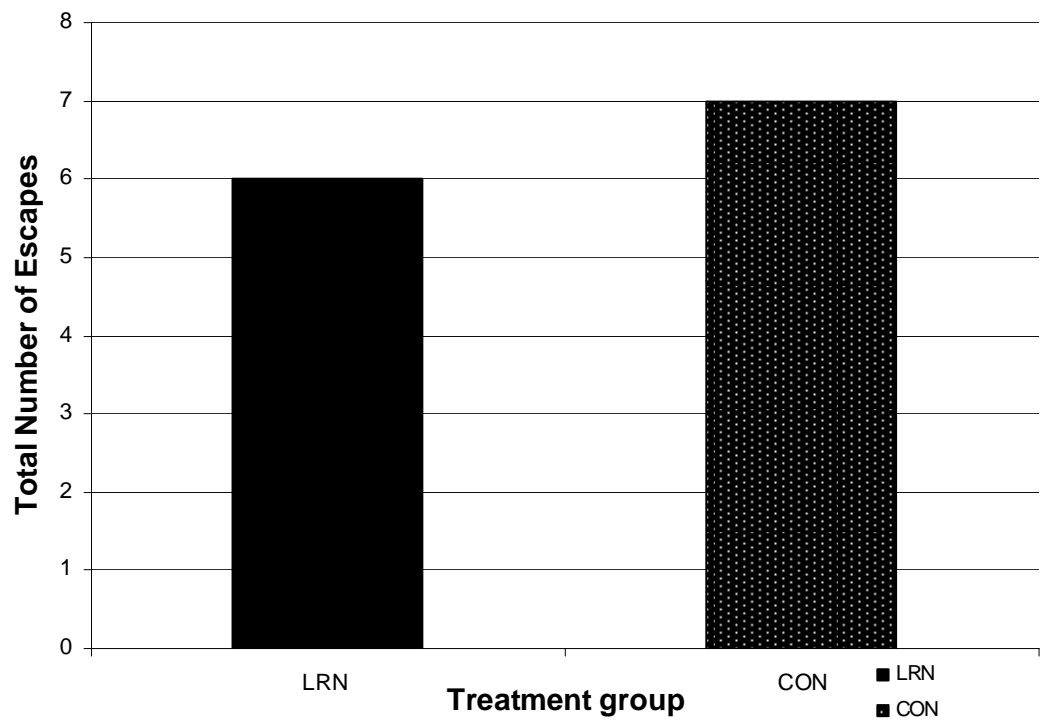


Figure 12. Total number of prey escapes for LRN and CON treatment groups.



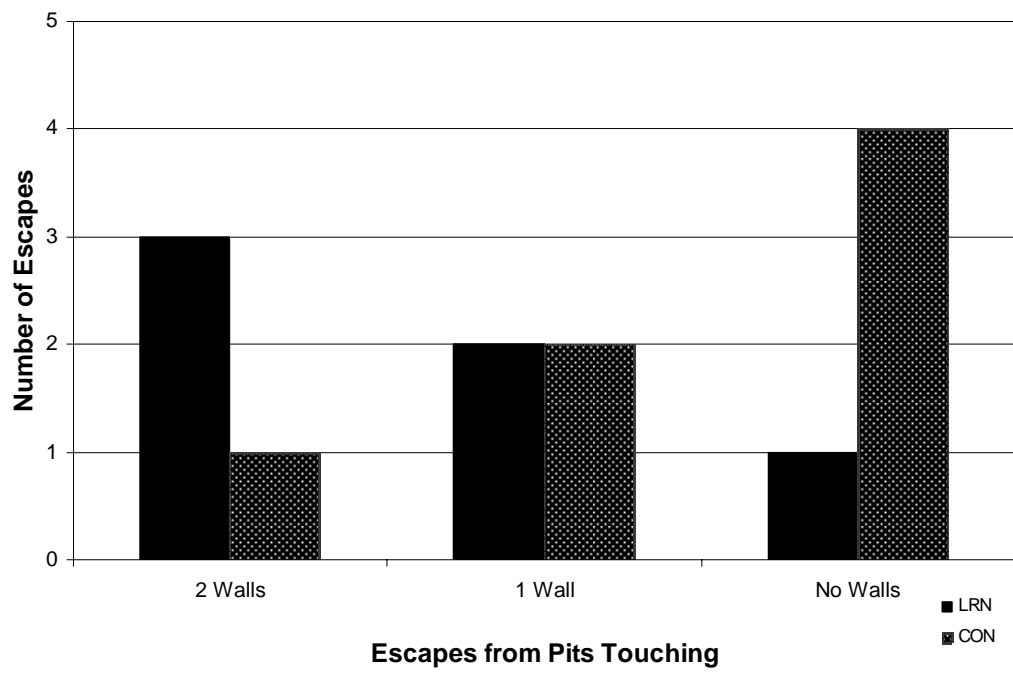
reduction of predation area in two directions. Between the treatment groups, there was a difference in the number of escapes from pits that touched one or more walls. A chi-square test showed that the number of escapes were not significantly different between treatment groups, $\chi^2(1 N=13) = 2.24, p > .05$; however, 83% of escapes in the LRN group occurred in pits that touched one or more walls, while only 43% of CON escapes were from pits that touched at least one wall (see Figure 13).

Pit Measures

As an antlion increases in size, its pit also increases (McClure, 1976; Griffiths, 1980; Mansell, 1992; Crowley & Linton, 1999; Day & Zalucki, 2000). Because I was not able to measure the size of an antlion during the experiment without disturbing the pit and potentially injuring the subject, I used pit size as a measure of growth. I hypothesized that subjects in the learning condition would build larger pits than subjects in the control condition. Pit measurements were taken six days a week to record the depth and diameter of each subject's pit, along with records of pit location.

Mixed ANOVAs were conducted to determine if there were differences in pit size between groups. The ANOVAs included one between-subjects variable, treatment group (LRN and CON), and one within subjects variable (73 days of pit measurement). The pits of three subjects (two in the LRN condition and one in the CON condition) were excluded from this analysis because their pits were built in the corner of the container, and it was not possible to calculate pit volume reliably. Analysis showed that pit depth was not significantly greater in the LRN ($M = 3.12$,

Figure 13. Number of prey escapes from pits touching container walls for LRN and CON treatment groups.



$SEM = .13$) than in the CON ($M = 3.09$, $SEM = .13$) treatment group $F(1, 72) = .02$, $p > .05$, nor was there a significant group by day interaction for pit depth, $F(1, 72) = .95$, $p > .05$ (see Figure 14). Although pit size did change significantly over days, $F(1, 72) = 24.83$, $p < .05$, these changes in pit depth over days might have been due to instances where pit measures followed a feeding session in which pits were disturbed by the prey .

Pit diameter also did not differ significantly between the LRN condition ($M = 8.05$, $SEM = .43$) and the CON condition ($M = 7.87$, $SEM = .43$), $F(1, 72) = .09$, $p > .05$; however, pit diameter did change over days, $F(1, 72) = 51.19$, $p < .05$ and there was a significant treatment group by day interaction for pit diameter $F(1, 72) = 1.32$, $p < .05$ (see Figure 15). The finding of a significant group by day interaction indicates that as the study progressed, LRN subjects were building wider pits than CON subjects. From Day 37 on, the mean width of the LRN subjects' pits was greater than the mean pit width for CON subjects.

The pit volume of the LRN treatment group ($M = 25.62$, $SEM = 2.13$) also did not differ significantly from pit volume of the CON treatment group ($M = 22.62$, $SEM = 1.97$), $F(1, 72) = 1.07$, $p > .05$ but again pit volume did change over days, $F(1, 72) = 33.14$, $p < .05$; and there was a significant treatment group by day interaction for pit volume $F(1, 72) = 1.33$, $p < .05$ (see Figure 16). On Day 1, subjects in the LRN condition had smaller pits ($M = 6.92$, $SEM = 1.72$) than CON subjects ($M = 8.16$, $SEM = 1.02$). An independent t -test revealed that the volume of LRN group pits were not significantly different than CON pits on Day 1, $t(11)$

Figure 14. Pit depth (mean centimeters \pm SEM) for LRN and CON treatment groups across days. Pit depth (mean centimeters) for LRN and CON treatment groups across days.

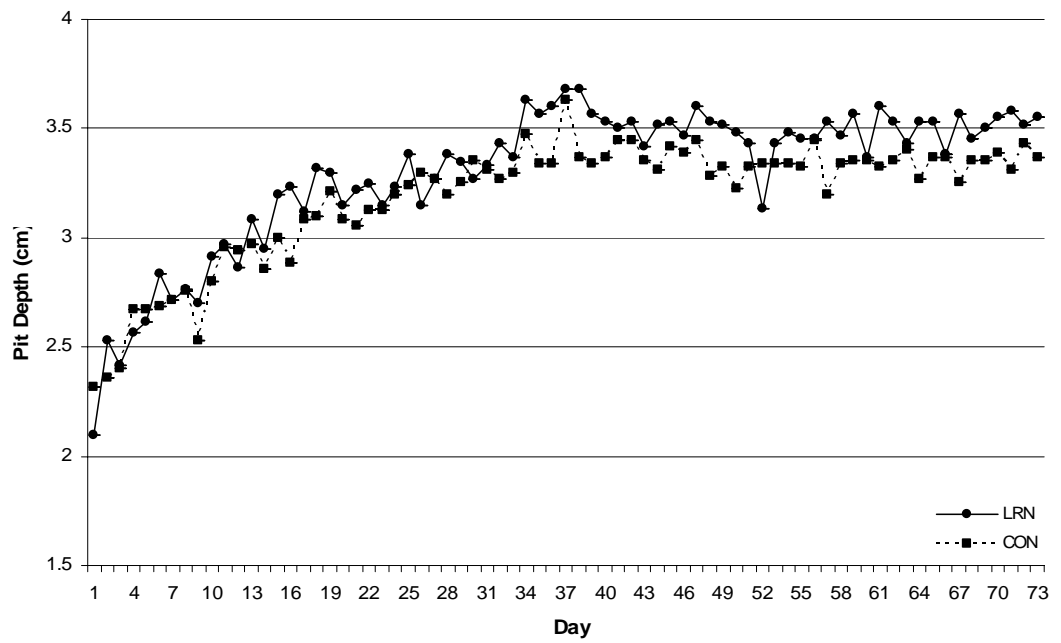
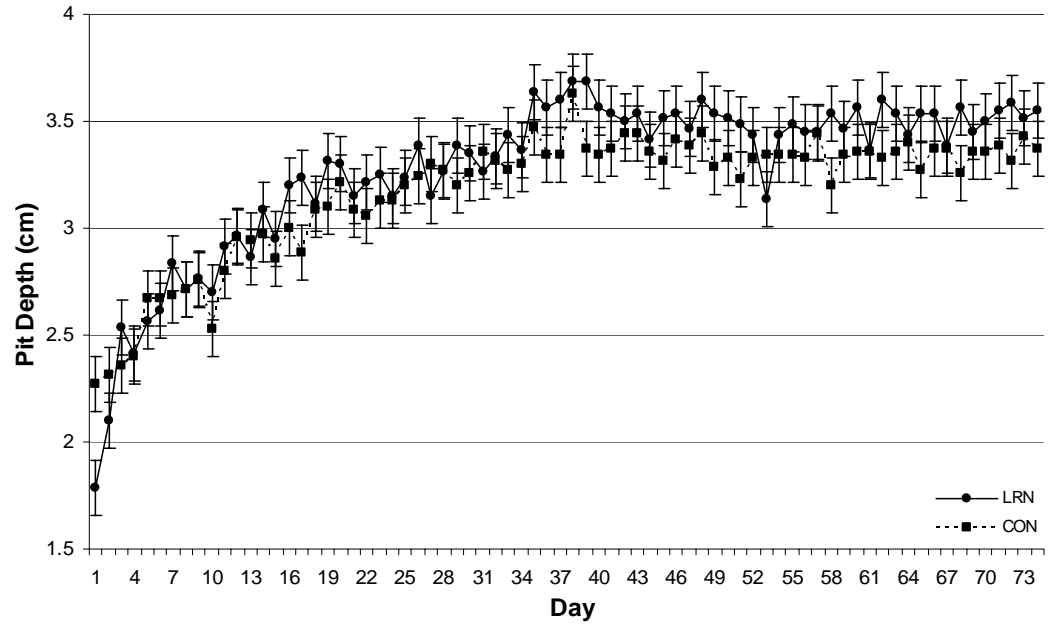


Figure 15. Pit diameter (mean centimeters \pm SEM) for LRN and CON treatment groups across days. Pit diameter (mean centimeters) for LRN and CON treatment groups across days.

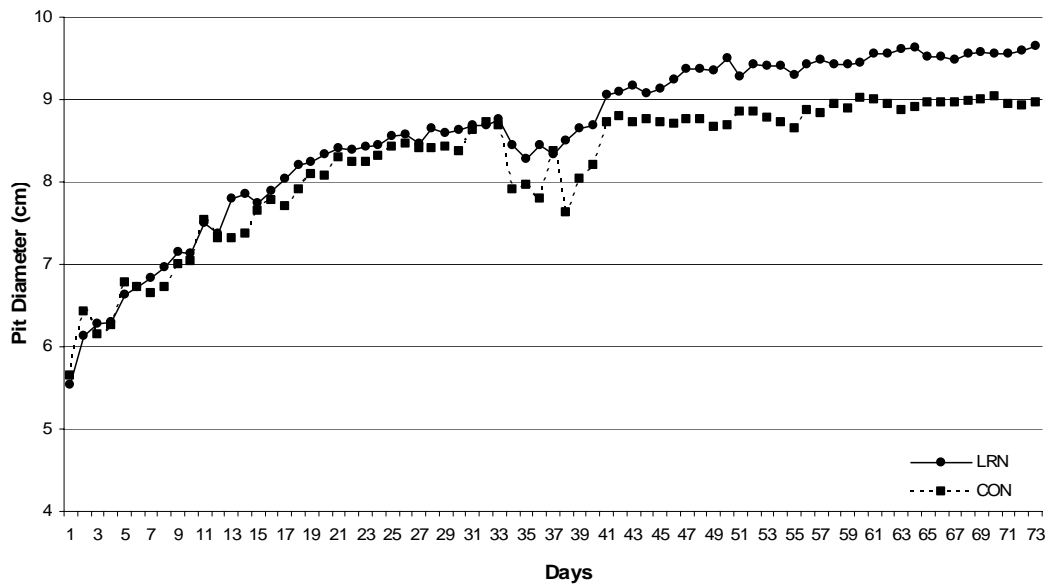
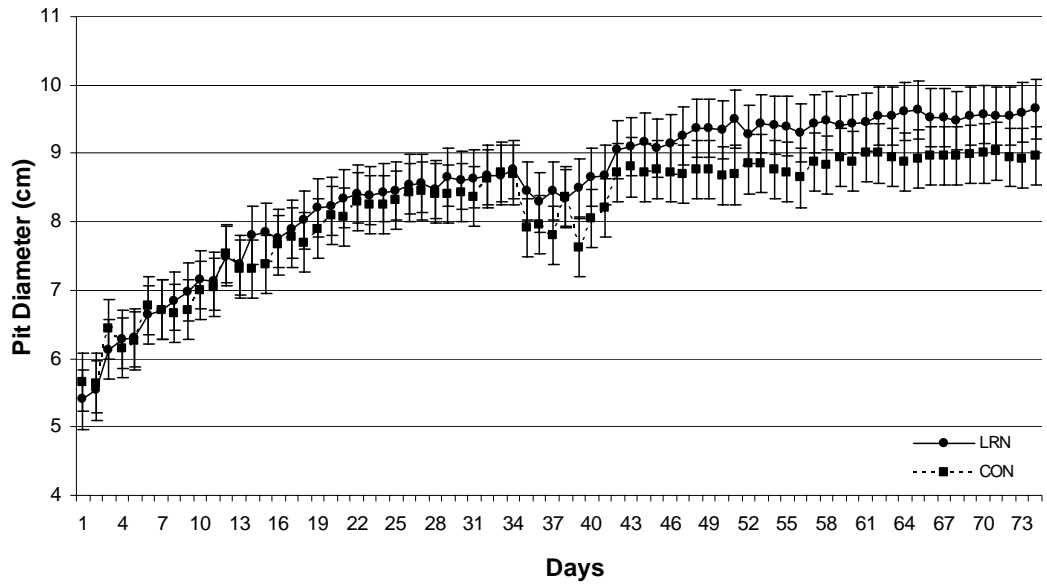
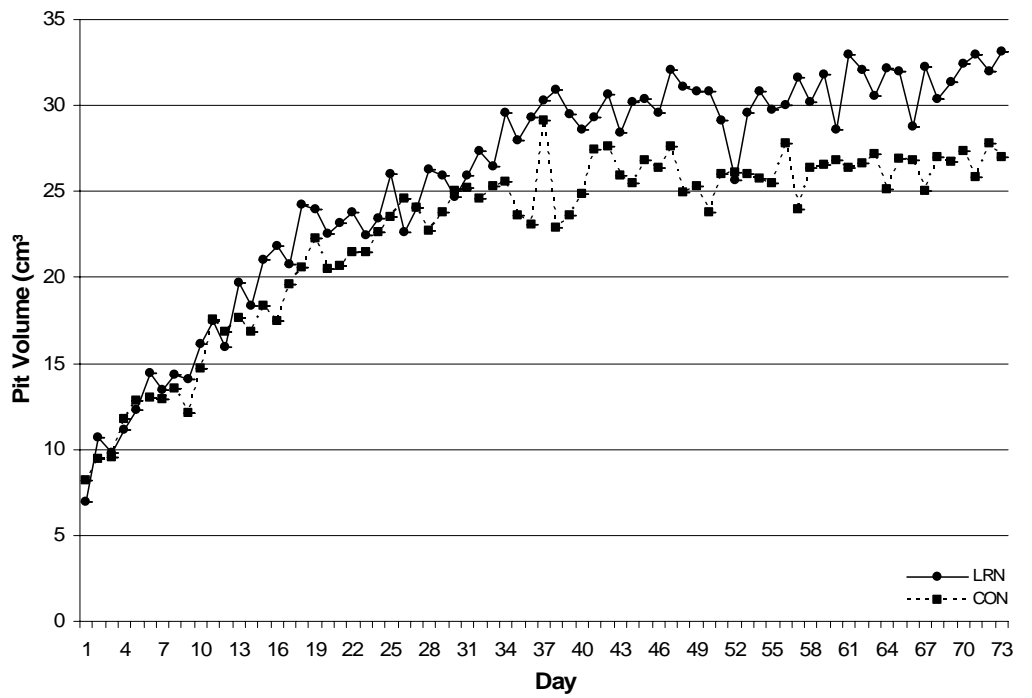
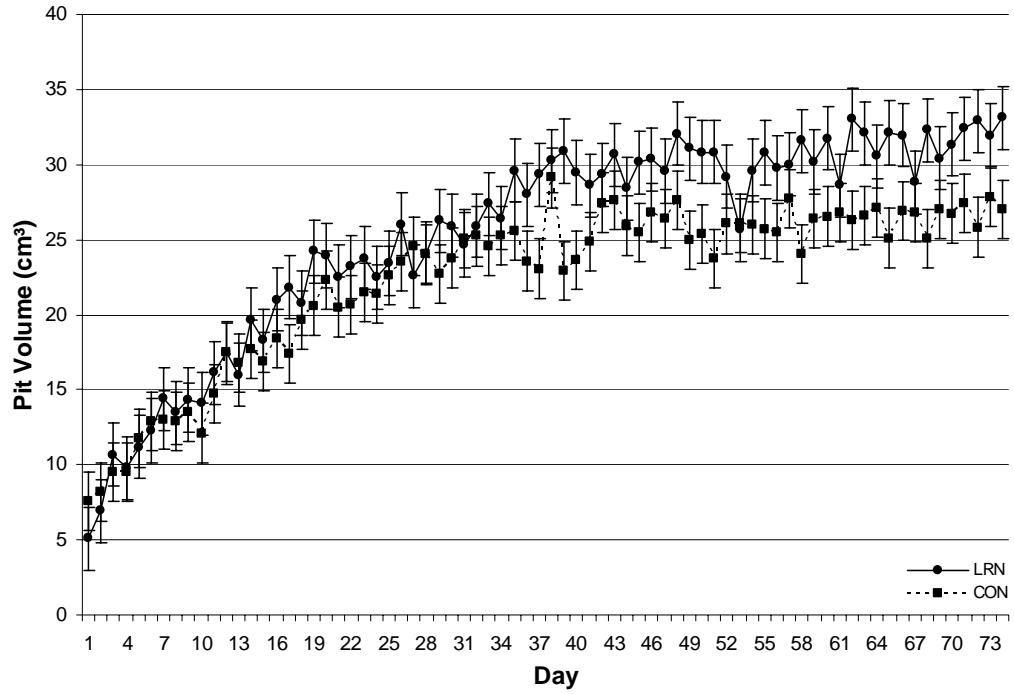


Figure 16. Pit volume (mean centimeters³ \pm SEM) for LRN and CON treatment groups across days. Pit volume (mean centimeters³) for LRN and CON treatment groups across days.



= -.641, $p > .05$. On the last day of analysis, however, an independent t -test revealed that the LRN subjects had significantly larger pits ($M = 33.10$, $SEM = 12.60$) than the CON subjects ($M = 26.99$, $SEM = 5.47$), $t(11) = 1.17$, $p < .05$. This analysis supports the prediction that LRN subjects would build pits of larger volume than CON subjects.

DISCUSSION

I hypothesized that differences in the behavior of the two treatment conditions, the learning condition and the control condition, would appear in three areas, namely extraction behavior, feeding behavior, and pit building behavior. Although I observed significant differences between the treatment groups in feeding behavior and pit building behavior, measures of extraction, for the most part, did not differ between the LRN and CON treatment groups. Associating a cue with the presence of food should result in behaviors that would allow an individual to optimize the upcoming event, and one way to optimize foraging would be through better extraction. Dukas and Visscher (1994) found that, as honeybees learned, their net uptake from foraging increased. I hypothesized that this study would find similar results, whereby the subjects in the LRN condition would increase their net uptake through increased extraction, decreased extraction time, increased extraction rate, increased extraction efficiency, or increased extraction efficiency rate. The findings of this study did not support these hypotheses, as there were no differences in the amount extracted, the extraction time, or extraction rate between the LRN and CON treatment conditions. Hayden (2005) found that LRN subjects had a greater extraction efficiency and extraction rate than CON subjects. Although the results of this current study do not support Hayden's findings, the difference in findings may be a result of the small sample which reduced statistical power. Due to physical limitations, only 16 subjects could be observed during a single replication. Furthermore, the unexpected length of the study did not allow for additional replications, which would have increased

the sample size. With such a small sample size it is possible that trends observed in the data are simply not able to reach significance.

For example, the effect of a small sample size can be seen in the measures of extraction efficiency rate. Parametric analysis did not reveal a difference between the LRN and CON groups, yet the mean rank of the LRN condition was greater than the CON condition, indicating that subjects in the LRN group had a greater extraction efficiency rate than CON subjects. Greater extraction efficiency rate in LRN subjects indicates that the presentation of a cue prior to the presentation of food led subjects in the LRN condition to extract a greater proportion of the prey per second than CON subjects. Hayden (2005) did not find differences in extraction efficiency rate; however, she did find that LRN subjects extracted more efficiently, and extracted more mass per minute than control subjects.

The presentation of a cue prior to the presentation of food may have elicited a response that was not revealed in the measures of extraction. This study measured extraction in terms of total mass extracted from the prey; however, these measures did not take into account how much energy was expended during extraction. Some parts of the prey are more nutritionally profitable than others (Griffith, 1982). Thus, antlions in the LRN condition may have extracted more mass from these profitable parts of the prey and less mass from the less profitable parts. If subjects in the LRN condition extracted more mass from profitable parts than subjects in the CON condition, although the amount of mass extracted was not different, the way in which it was extracted may have benefited the LRN

subjects. If subjects are able to expend less energy during extraction, they would benefit from greater energy gains than subjects that extract equal amounts of mass. The head and thorax of prey are more difficult to consume than the abdomen (Griffiths, 1982). If an antlion primarily extracted mass from the abdomen it would require less manipulation of the carcass than if the antlion were extracting the same amount of mass from the head, legs, and thorax of the prey. The less an antlion manipulates the carcass, the less energy it expends, If subjects in the LRN and CON condition extracted the same mass from different parts of the prey, the LRN subjects may have expended less energy feeding, and thus had a higher energy gain.

Furthermore, LRN subjects may have metabolized food differently, so as to better benefit from similar amounts of extraction. Of the extracted nutrients, lipids are the most energetically valuable (Van Zyl et al., 1997), and are needed particularly in the third instar for the antlion to reach a critical weight and enter pupation and emerge as an adult (Griffith, 1985). Although the current study did not have a way to measure how the antlions metabolized the prey, the presentation of a cue prior to the presentation of food might have led subjects in the LRN condition to metabolize the consumed mass differently, so as to maximize lipid storage. If the way in which antlions extract and metabolize food differs between the LRN and CON treatment groups, the antlions in the LRN condition may have had greater energy gains than control subjects, despite the fact that the measures of extraction did not differ.

The possibility that the presentation of a cue immediately prior to feeding

may have led to differences in the extraction and metabolism of food between the two treatment groups is supported by the measures of pit size, because subjects in the LRN condition built larger pits than those in the CON condition over the 42 training days. The subjects in the LRN and CON treatment groups initially built pits that were the same size, but by day 30 the mean volume of the LRN pits was larger than that of the CON pits. This observation is supported by the findings of a significant interaction of pit volume and training day. By the last day of analysis, the mean pit volume for the LRN treatment group was 6 cm³ greater than the mean pit volume for the CON treatment group. These findings support Hayden's (2005) findings, namely that LRN subjects constructed pits with greater diameters and pit volumes than CON subjects.

Past studies of antlions have reported a correlation between pit size and larval size (McClure, 1976; Griffiths, 1980; Mansell, 1992; Crowley and Linton, 1999; Day & Zalucki, 2000). The larger pits of subjects in the LRN condition indicate that the subjects in the LRN condition may be larger than those in the CON condition. This study did not measure larval growth rate, per se, but if the observation of greater pit size in LRN subjects reflects previous research, the presentation of a cue immediately prior to feeding may have had an effect on antlion growth, even though the LRN group did not extract more mass. Differences in larvae size could be attributed to differences in how the prey was extracted or how it was metabolized, as described above. Thus learning in antlions may result in greater growth.

The effect of learning on insect growth was observed by Dukas and

Bernays' (2000), who found that grasshoppers that were presented with a cue prior to feeding had a 20% higher growth rate than control grasshoppers that were not presented with a cue. If the larger pit size observed in the current study is indeed a reflection of larger growth, our findings would support Dukas and Bernays' finding that learning increases growth rate of insects. The implications of increased growth rate on increased fitness, such as earlier pupation (Griffiths, 1985), could not be tested in this current examination, however.

The LRN condition did differ from the CON condition in measures of feeding behavior. In particular, subjects in the LRN condition were found to be more visible than subjects in the CON condition prior to the presentation of food. Visibility indicates that the antlion's mandibles or head were visible above the sand. Subjects that were not visible had their heads and mandibles covered by sand, which could inhibit prey capture. Antlions that are visible would have an advantage in prey capture over those that are not visible, by having their mandibles available for prey capture immediately upon the delivery of food

The increased visibility of heads and mandibles in the LRN group can be viewed in the same light as the conditioned extension of the proboscis in flies (McGuire, 1984). Such behavior would prepare the insects for the appearance of food, and should allow the insects to optimize their interaction with it. On the initial day of the study, seven antlions in the LRN group were visible after the delivery of a cue and six CON subjects were visible. By the next training day, seven LRN subjects remained visible but only four CON subjects remained visible.

Antlions that are not visible would be at a disadvantage if they needed to free their heads and mandibles from the sand before capturing prey; however, not being visible above the sand could serve as antipredator behavior, by concealing the antlion from predators. Morse (2006) found that this tradeoff between protection from predation and foraging changes as a response to hunger. Because both treatment groups were fed at the same time it seems unlikely that hunger would cause a difference in visibility. Rather, it is more likely that subjects in the LRN condition used the cue prior to feeding to anticipate future feedings, and thus behaved in favor of foraging by remaining visible.

Decreased visibility, on the other hand, would appear to favor predator avoidance. The fact that CON subjects were less visible than the LRN subjects suggests that CON subjects might favor predator avoidance to optimizing foraging. One possible reason for this difference is that the additional set up of cameras for the taping of stimulus sessions resulted in the CON subjects having one additional interaction with researchers that did not result in food. This interaction might have led to predator avoidance behavior taking precedence over foraging. By remaining under the sand, CON subjects would not be visible to predators, yet, as mentioned above, there are consequences for remaining under the sand, such as an increased possibility of prey escaping the pit.

This study did not measure if prey escapes were a result of antlion visibility; however, a trend appeared in the occurrences of prey escapes in pits that touched one or more walls. This was particularly true in LRN subjects, as all but one escape occurred in a pit that encountered a wall. Farji-Brener (2003)

observed that the characteristics of antlion pits, including depth of pits and particle size of the sand, can affect the probability of prey escapes. Thus, it does not seem surprising that the shape of pits, due to encountering walls, would also affect prey capture success. The importance of pit construction on prey capture poses the question, why would antlions form a pit against objects that would impede their foraging area, especially when the foraging area is so important for sit-and-wait predators (Baily, 1998).

Of 16 subjects, only three built pits that did not touch a pit wall during the first 14 blocks, which seems to indicate that the subjects somehow sought out the container edge. Similar behavior was observed by Boake and Visscher (1984), where antlions placed in the center of a sandy plot moved towards the edges of the plot. This movement to the edges of an area might have been an effort to find shelter, as most pit-building antlions are found in sheltered areas (Morrison, 2004). Gotelli (1996) found that antlions built pits along cliff edges that provided shelter from wind and rain. Thus, the benefits of protection provided by pit walls might overcome the costs of incomplete pits, including the cost of prey escapes.

Prey escapes from pits that touched one or more walls was observed in CON subjects as well; however, over half of the prey escapes in the CON condition escaped from pits that did not touch a wall. This trend seems to indicate that prey escapes, which can be viewed as errors on the part of the subject, were not caused primarily by pit location for CON subjects, whereas location seems to be a key factor in escapes from LRN group pits. Thus, the delivery of a cue prior to prey delivery may have enabled LRN subjects to retain more prey in complete

pits--those that were not hindered by container walls--than CON subjects, that did not receive a cue.

The objective of this study was to determine if the presentation of a cue prior to food delivery would affect the feeding behavior of antlions, and thus benefit antlions in the learning condition. Although this study did not find differences in most measures of extraction, LRN subjects extracted a greater proportion of prey per unit of time than CON subjects. A series of behavioral differences also indicated that antlions in the LRN condition did benefit from the presentation of a cue. In particular, the increased visibility of antlions in the LRN condition can be seen as a means of optimizing feeding. Antlions in the learning condition appeared to have greater capture success in complete pits, than antlions that were not presented with a cue prior to food delivery. Thus, the presentation of a cue prior to food appears to enable antlions to optimize the capture of prey, if not the extraction.

The observation of larger pits constructed in the LRN condition also suggests that the presentation of a cue prior to the appearance of food can result in greater growth. One possible fitness effect of greater growth includes earlier pupation. In their larval stage, antlions are vulnerable, and thus earlier pupation is an escape from that state of vulnerability (Griffith, 1985). Earlier pupation would further benefit fitness by leading to earlier reproduction. The aim of this experiment was to observe the subjects throughout pupation. Due to time constrictions, this analysis was limited to the first fourteen weeks of the study. Therefore this analysis was not able to include a measure of antlions' fitness;

however future analysis will look at the effect of learning throughout the entire instar.

Limitations and Directions for Future Research

The current study was limited by a few constraints, the first of which, and perhaps the most limiting, was the small sample size, which may not have provided the statistical power necessary to find conclusive results. A variety of factors contributed to the selection of only 16 subjects, including: limited space in the laboratory, limited resources, and a limited number of researchers available to conduct the experiment. The subjects that participated in the training portion of the study were selected because they molted and remerged within the same week, allowing all 16 subjects to begin training at the same time. Although all of our subjects were matched for weight, the size of subjects within each treatment group varied, ranging in weight from 15.8 mg to 20.3 mg. Future research should attempt to overcome these limitations through an increased sample size of subjects that are more consistent in weight.

The duration of the training phase of this experiment further limited the study, as subsequent replications were not able to be conducted at this time. The study could have been conducted in a shorter time frame by presenting the subjects with larger prey, and presenting the prey more frequently within the week. Heinrich and Heinrich (1984) reported that antlions observed in the field captured on average one prey every 12 h. In the current study antlions only received one prey item in a 48 hour period. Wilson (1974) reported that antlions grow until they reach their optimal size for metamorphosing. The more food

antlions receive, the faster they should reach their optimal size.

Future research may also benefit from utilizing different methods of recording the feeding and cue delivery sessions. In the current study, video recorders on tripods were positioned near each subject in order to record the sessions. The cameras and tripods were set up away from the antlions and then moved into place as an attempt to minimize the disruption to the antlions; however, this set up may have delivered inadvertent cues to the subjects. To control for these inadvertent cues, future research should use one camera for each subject that remains in a permanent position.

Measures of visibility in particular may have been affected by the set up of the cameras prior to taping. Although this study tried to minimize the effect of camera set up, the act of setting up the camera may have served as an inadvertent cue. If the cameras did provide a cue, subjects in both the LRN and CON condition may have adjusted their visibility as a response to the cameras. Thus, the current study's measure of visibility might have been elicited by camera set up instead of the sand dropping cue. Again, if future research is able to use permanently stationed cameras the possibility of inadvertent cues would be decreased.

Due to the physical structure of the antlion pits, measurements of pit depth, diameter, and volume could not be exact because the rulers used to measure pit size could never touch the sand without disturbing the pits and subjects. To make the measures of pit size as exact as possible, despite this limitation, all measurements were taken by two researchers, who were trained to take

measurements the same way. Thus, measurements were taken as consistently as possible during the experiment; however, future research may wish to utilize different means of measuring pit size. Fertin and Casas (2006) reported using a scanner system which allowed them to make digital representations of pit shape and size. Use of a similar method would allow for more accurate measures of pit size, especially in pits that encountered one or more walls, and could possibly result in fewer disturbances to the subjects.

The use of sit-and-wait predators as subjects for a study of associative learning offers a unique contribution to the study of insect learning. Although learning has been observed in many insect species, all are actively foraging predators. By studying a sessile predator, the antlion, this study is pushing the study of insect learning in new directions. In addition, the design of this experiment, to follow the subjects through an entire instar and through pupation, could potentially observe a connection between learning and increased fitness in sit-and-wait insects. Thus far, antlions have been the subjects of numerous studies, but research has yet to be published regarding antlions as subjects in insect learning studies. The findings of this study suggest that learning may have an effect on certain aspects of antlion behavior including visibility and pit volume. The current experiment must be completed, however, before this study can conclusively state whether learning has an effect on fitness. Further research should also be conducted, using a larger sample size, in order to fully determine what might be the effect of associative learning on the feeding behavior and fitness of antlions.

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Appendix A

Table 1. Behavioral Patterns and Operational Definitions

Behavioral Pattern	Operation Definition
Visibility	The head, mandibles, or any part of the antlion is visible anywhere in or near the pit.
Movement	The antlion moves mandibles, head, or body anytime before the food is delivered to the center of the pit
Extraction Time	The time from when the antlion has two mandibles on the ant and does not let go, until the carcass is thrown or 10 minutes from the last time the antlion touched the carcass with its mandibles.
Prey Escape	An ant is delivered to the center of the pit and climbs outside of the pit. The ant is delivered to the center of the pit and is not touched by the antlion for 10 minutes.