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The Role of Associative Learning in the Feeding Behavior, Development, and Fitness of Antlions

by

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ABSTRACT

Previous studies have established the importance of associative learning in actively foraging insects, including bees, wasps, flies, grasshoppers, cockroaches, and locusts. This observation of learning in such a wide range of species has revealed the significance of associative learning for increasing an individual's fitness, that is, the individual's ability to survive and successfully reproduce. To date, no published study has investigated the role of associative learning in sessile predators, like antlions (*Neuroptera: Myrmeleontidae*). Thus, the purpose of the current study was to determine the role of associative learning in antlion feeding behavior, development, and fitness. In the current study 56 antlions were assigned to either a learning (LRN) (*n* = 28) or a control (CON) (*n* = 27) condition. One prey item was delivered to each subject once per 24-hour training day until the subject molted or pupated, which concluded the experiment. Data were analyzed through 70 training days. Subjects in the LRN condition received a vibrational cue, namely 4.5 mL of dropping sand, immediately prior to delivery of the mealworm. CON subjects received the same cue at a time independent of the feeding. Feeding behavior (extraction efficiency rate) and pit volume were measured each training day. In addition, sand-throwing behavior was observed. Third instar LRN subjects demonstrated increased sand-throwing behavior in response to the cue. In addition, a survival analysis revealed that antlions in the LRN condition to demonstrated greater fitness as measured by faster development and pupation.

INTRODUCTION

Associative learning has been demonstrated in a variety of insect species including bees (Dukas & Real, 1991 & 1993; Dukas & Visscher, 1994), wasps (Lewis & Takasu, 1990; Dukas & Duan, 2000), flies (MacGuire, 1984; Dukas, 1998), grasshoppers (Dukas & Bernays, 2000), cockroaches (Sakura & Mizunami, 2001), and locusts (Simpson & White, 1990). However, these previous studies investigated learning in foraging insects that actively seek out their food. No published studies have analyzed the effect of learning on predators that use sessile methods, such as traps, webs, or pits, to procure food. The current study investigated the role of associative learning in sessile insects, specifically, antlions. Many behavioral and morphological commonalities, including associative learning, exist between insects and vertebrates. While insects and vertebrates are not true homologies they "may represent universality of an even higher order: that of fundamental principles of brain functionality" (Greenspan, 2007, p.649).

Antlions

Antlions (*Neuroptera: Myrmeleontidae*) 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 & Linton, 1999; Day &

Zaluki, 2000). There are more than 2000 species of antlions (Mansell, 1992), and are found on all continents except Antarctica.

Antlions are commonly known as “doodlebugs” because many larval species leave tracks in the sand as they move on or just beneath the surface of the sand, resulting in winding furrows. This doodling allows the larvae to sample the environment and find suitable places to construct pits (Topoff, 1977).

The majority of research focuses on antlions that reside in sand and dig pitfall traps to catch prey; however, only a few larval antlion species build pitfall traps. Some families become covered in lichens, live in detritus, bark, under rocks or sheltered overhands, while others live on top of the soil, blending into their environment by covering themselves with loose leaves and soil (Grimaldi & Engel, 2005). Even without a pit, these antlions remain motionless, hidden by their surroundings, for long durations before suitable prey passes by; therefore, all antlion larvae are considered sit-and-wait predators.

Antlion larvae feed on small terrestrial arthropods that accidently fall into their pit. As the species’ common name implies, antlions’ diets consists predominately of the many species of ants that forage in the environment. This particular eating habit isn’t the result of unique feeding specialization, but rather results from the fact that ants are usually the most abundant wingless arthropods in antlions’ environment. Antlion larvae will readily consume a wide variety of arthropods, including spiders, sowbugs, mealworms, caterpillars, and beetles (Topoff, 1977).

Antlion Development

Antlions pass through a four-part life cycle. First, adult antlions oviposit eggs singly in the sand. Antlion eggs are relatively large; they are found in open areas or tree holes, under bushes, in caves, under rock overhangings, or in areas sheltered by buildings (Tauber, Tauber & Albuquerque, 2003). The eggs are covered with a glandular secretion that facilitates adhesion of sand or soil particles to the egg to provide camouflage and protection for the eggs, which hatch after 28 days (Mansell, 1994).

The eggs hatch into the second, larval, stage. The larvae of most antlion species are sit-and-wait predators that live beneath the soil surface. Only a small number of genera construct pits in the sand or soil (Tauber et al., 2003). The sit-and-wait predation that occurs during the larval stage is crucial to antlion development because the larval stage is the main part of the life cycle in which antlions consume food. Thus, their feeding behavior during this second stage has major implications for development of sexually mature adult antlions. The larval stage consists of three instars, which are marked by changes in the size of the head capsule and mandibles (Griffiths, 1980; Arnett & Gotelli, 1999). Two molts occur, namely between the first and second instar and again between the second and third instar. During a molt period, antlions cease feeding and move approximately a centimeter down into the sand below the bottom of the pit. After roughly ten days antlions emerge, toss their exoskeletons, and resume feeding in the same pit (personal observation).

The third stage of the antlion lifecycle is the pupal stage. Upon the completion of the third instar, antlions form round cocoons in which to pupate under the sand. Antlion larvae generally spin a single-walled cocoon produced by the hindgut and malphigian tubules (Tauber et al., 2003). The exterior of the cocoon is sticky and nearby sand or debris becomes attached to the cocoon providing protection and shelter for roughly thirty days, after which the adult emerges. Pupation can be initiated by various factors, including larval size, environmental factors, and food availability. Based on previous laboratory studies, adults emerge from the cocoon in four weeks (Gotelli, 1993).

The adult form is the final stage in the life cycle. Adult antlions, with their long, slender bodies and delicate outstretched wings (ranging between 30 – 170 mm), resemble damselflies (Mansell, 1999) but, unlike damselflies, adult antlions are nocturnal and feeble flyers (Farji-Brenner, 2003). Once hatched, adult antlions have a short lifespan of approximately one month during which their primary function is to reproduce (Arnett & Gotelli, 1999).

The development from first instar larva to adult takes approximately 6 months (Gotelli, 1993); although some individuals might complete their life cycle in a single dry season, most require two dry seasons (9-10 months) (Griffiths, 1985).

Pit Construction

The pit serves several functions: It funnels prey to the antlion, increasing the “striking distance” over which the predator can capture prey; and, it increases prey escape time and, therefore, the probability of capture (Lucas, 1982). Antlions build their pits by moving backward in a circle, using their oval-shaped abdomens as a plow and their flat heads as a shovel for flicking sand upward (Topoff, 1977). Antlions start on the surface of the sand and move in a backward spiraling motion. The circumference of the pit is outlined first, and then antlions spiral inwards to excavate sand from the pit. With each inward spiral, antlions digs deeper, throwing excess sand out of the way by using their mandibles as a shovel, and flicking sand out of the pit (Resh & Cardé, 2003; Masnsell, 1999). Once the pit is completed, antlion larvae remain motionless at the bottom, hidden under the sand, with only a portion of their head or mandibles visible. The pits are enlarged sporadically, and at night (Griffiths, 1986).

Antlions stop pit building once the angle of repose of the pit walls is reached; the angle of repose is the point when the addition of a grain or two of sand causes the side to cave in. The condition of the soil, including dampness, particle size, and particle consistency, can dramatically affect the construction and morphology of the pit because the effects of the soil alter the stability of the sloping sides of the pit. The angle of repose is often achieved by lining the front walls of the pits with a higher density of fine sand (Lucas, 1989).

For overall construction, finer grain soils are preferred by antlions because these more readily dislodge prey (Farji-Brener, 2003).

Pits are typically built in areas that are sheltered from rain and other elements to minimize disturbance, and hence the need for constant maintenance or re-location. Crowley and Linton (1999) found that antlions track shade and cover more strongly than prey movement; antlions rarely relocated their pits for the remainder of a feeding season after constructing a pit in a shaded spot that also was protected from wind and rain. Constructing a pit in shade lowers temperature and allows for longer uninterrupted foraging periods because antlions need not dig deeper in the cooler soil during hotter parts of the day.

Once an antlion constructs a pit, it often does not move. However, starved, disturbed larvae were more likely to move their pits than well fed ones, the average probability of movement varying with larval size (Griffiths, 1986). In starved *Macroleon* larvae, pit movement was relatively frequent in 40-140 mg, mid-sized, larvae but infrequent in the first two instars (0-40 mg) and in large (> 140 mg) larvae (Griffiths, 1986).

Another factor that affects pit relocation is the density of antlions in an area. Several studies have investigated the effects of high population densities on pit building activities of antlions. At low densities, pits are randomly distributed within areas favorable for pit construction (McClure, 1976). Under high-density regimes, where pits are close together, sand throwing during pit construction or maintenance by one antlion may disturb neighboring individuals (Day & Zalucki, 2000). As a result, at high densities, antlions will relocate pits, fail to construct pits, or cannibalize neighboring antlions. However, pit diameter is not affected by high density situations because antlions can flick sand up to 50 cm out of their pits (McClure, 1976).

As antlions grow, their pits increase in size; thus, pit diameter is closely correlated with antlion size (Heinrich & Heinrich, 1984). In addition, studies have found that pit size is selective for prey size; the size of the pit dug by each antlion does not exceed the size of the prey that an individual antlion is physically able to capture (Griffiths, 1986; Day & Zalucki, 2000; Mansell, 1994). Pit morphology and prey capture strategies vary between the three instars of antlion larvae. Griffiths (1986) found that first and second instar larvae construct steeper walled pits than third instar larvae. As a result, capture success in the first two instars increases rapidly with pit size but in the third instar the relationship is weaker. Third instar larvae construct pits with larger diameters, and less steep walls to maximize encounters with larger prey. As little as a 2 mm increase in pit diameter causes a 10% increase in capture success, regardless of prey species (Griffiths, 1980). Griffiths (1986) has proposed a cost-benefit model of pit construction. According to this model, an antlion can benefit (i) by increasing pit circumference, and hence the probability of encountering prey; or (ii) by increasing the slope of the pit, and hence the probability of capture. For a given pit volume, an antlion can maximize either capture success or prey encounters but not both since an increase in pit circumference necessitates a decrease in pit slope, and *vice versa*.

Prey Capture and Feeding

After pit construction is complete, antlions will position themselves at the vertex of the pit with their mandibles open and will remain stationary until a prey item falls into the pit. Antlions have several techniques for detecting the presence of prey. They detect substrate vibrations using mechanoreceptor setae located on their bodies. Substrate vibrations are signals commonly used in insect courtship behavior, but they also serve as cues for prey recognition or localization of a prey. Sand strongly attenuates low-frequency waves and, thus, antlions have morphological adaptations that allow them to detect these vibrations and capture prey. The bodies of antlions are covered in numerous hair tufts that project anteriorly. The dominant mechanoreceptors are long bristles, but antlion bodies are also covered with short bristles and feathered hairs (Devetak, 1985). When antlions move backward, the hairs bend and offer little resistance. But when a struggling prey item attempts to pull the antlion forward, the hairs flare outward and anchor into the substrate (Topoff, 1977).

Antlions can effectively capture prey over a range of only 0.2 mm (Cain, 1987); therefore, it is crucial that the pit funnel the prey directly to the antlion’s mandibles. If, however, a prey item is not immediately delivered to the open mandibles when it falls into the pit, antlions will begin to flick sand at the prey, creating a barrage of sand that will either cause the prey to lose footing or cause the walls of the pit to collapse, delivering the prey to an antlion. Antlion mandibles consist of a broad basal region bearing three teeth and a number of stout setae which, along with the flat head, is used as a shovel for digging the pit or flicking sand. The mandibles have a smooth, curved, tapering region which is inserted into the prey (Griffiths, 1980). Usually only one mandible is inserted into the prey, and the second mandible is pushed back and forth along the outer side of the prey until a space between cuticular plates can be found to anchor the second mandible (Griffiths, 1980). Once antlions capture the prey in their mandibles, they inject an enzyme, ALBM-toxin, via the mandibles. ALBM-toxin is concentrated in the thorax of antlions and present only during its larval life (Yoshida, Sugama, Gotho, Matsuda, Nishimura, & Komai, 1998). ALBM-toxin paralyzes or kills the prey within minutes. After the prey is dead, enzymes are introduced; the body contents are digested extra-orally and later extracted (Griffiths, 1980; Griffiths, 1982; Van Zyl, Van Der Westhuizen, & Van Der Linde, 1998).

As soon as an antlion has grabbed the prey with its mandibles, the antlion moves backward, dragging the victim deeper into the sand and, using its mandibles, sucks out the prey’s protein-rich body fluids (Topoff, 1977). The antlion’s mandibles are suctorial and have adjacent grooved surfaces which form a feeding tube. The mandible feeding tubes attach at the side of an antlion's head and connect to the mouth (Tauber et al., 2003). The digestive tract is composed of three main parts, namely the foregut, midgut, and hindgut. The foregut, which begins at the mouth slit, consists of the pharynx, into which a pair of salivary glands secrete enzymes into the esophagus. The midgut is the stomach, which the food enters via valve constriction (Wheeler, 1930). Antlion larvae do not have contiguous intestinal tracts; instead, the midgut and hindgut remain separate until pupation. As the larva feeds, feces accumulate in the midgut, and are expelled only after pupation from the larval stage to the adult. The feces are expelled in the form of a meconial pellet (Tauber et al., 2003). Once antlions have finished feeding, the exoskeleton of the prey is maneuvered onto the back of the head and mandibles and then flicked out of the pit.

Feeding can disrupt the pit in several ways. If the prey slides down the wall of the pit it will cause sand to collapse down into the pit bottom. If the prey puts up a struggle during capture, antlions may need to flick sand to knock the prey back down to the center of the pit. Finally, if the prey puts up a struggle while it is in the antlion's grasp, but before the ALBM-toxin has taken effect, it may disrupt the conical structure of the pit. Therefore, antlions perform routine pit maintenance after feeding to restore the pit to a perfect conical shape.

Associative Learning

Associative learning in insects is most commonly assessed through classical or Pavlovian conditioning. In Pavlovian conditioning, an essentially neutral stimulus, called the conditioned stimulus (CS), is presented immediately before an unconditioned stimulus (US), which elicits a response. This response occurs without prior training and is called the unconditioned response (UR). With repeated pairings, the CS elicits a conditioned response (CR). A CR is a response that emerges under the conditions of the experiment and is sometimes similar to the UR. This development of new responses to novel stimuli is indicative of associative learning.

The current study employed a Pavlovian conditioning procedure to study associative learning in antlions, recognizing that implicit instrumental contingencies could account for an observed CR. To be precise, Pavlovian conditioning was used to describe the experimental *procedure*, not the mechanism of associative learning.

Previously, learning in insects was not thought to be possible due to a variety of constraints, including short life spans and small brain size. However, studies of associative learning in insects have demonstrated that despite these constraints, learning is possible in many species. The fact that learning occurs in such a wide range of insects indicates that associative learning is an adaptive trait that increases the fitness, or reproductive success, of the insect. Fitness, which refers to the ability of an individual to survive and successfully reproduce, is affected by both the physical attributes and behavioral characteristics of the individual. Any behavior that increases the likelihood of survival and reproduction would result in increased 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 would 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.

Active Foraging Predators

Given that associative learning may provide a fitness advantage, one goal of studying associative learning in insects is to determine how learning occurs in nature. While ecologically relevant stimuli are not a requirement for learning, researchers tend develop laboratory experiments that are as reflective of the natural environment as possible (Domjan, 2005) by utilizing ecologically relevant conditioned stimuli, where the CS and US are naturally related (Domjan, 2005).

For example, Sakura and Mizunami (2001) presented cockroaches (*Periplaneta americana*) with a desirable sucrose solution, and an undesirable saline solution. Each solution was paired with a distinct odor. Under these conditions, cockroaches moved toward the odor that was associated with the sucrose solution, which indicates that the cockroaches could use an odor cue to find food. Moreover, cockroaches were able to associate the odor and food stimuli in as short a time as one trial.

Wantanabe, Kobayashi, Sakura, Matsumoto, and Mizuami (2003) expanded on the previous study by Sakura and Minaunami (2001). They used the same technique to train cockroaches (*Periplaneta americana*) to discriminate between a rewarding sucrose solution paired with a peppermint odor and a punishing saline solution paired with a vanilla odor. However, during the subsequent four-day test phase, cockroaches were moved to a naturalistic enclosure with four odor locations; two site locations each smelled of either peppermint or vanilla. The cockroaches approached the peppermint (reward) odor more often than the vanilla odor. This study demonstrates that cockroaches not only can retain learned associations for several days, but also can transfer learning from a training situation to a naturalistic testing environment.

Similar findings were demonstrated in locusts (Simpson & White, 1990). Locusts were presented with two distinct odors, each of which was paired with one of two diets. One diet lacked protein but was rich in carbohydrates, while the other lacked carbohydrates but was rich in protein. When deprived of protein, the locusts would move towards the odor that was associated with the protein rich diet as opposed to the carbohydrate rich diet. Although Simpson and White did not test for any fitness effects in their study, 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, would greatly enhance that insect's ability to grow and reproduce. The insect could use odor cues to locate the most beneficial diet first, rather than wasting energy testing various food sources.

These predictions are supported in a study of conditioned behavior in fruit flies, *Drosophila melanogaster* (Quinn, Harris, & Benzer, 1974). During training, fruit flies were exposed to two odorants, one of which was coupled with an electric shock. On testing, the flies avoided the shock-associated odor. In a second experiment, the researchers paired quinine sulfate powder, a substance flies have been shown to avoid, with an odor. Consistent with the first set of results, during testing the flies avoided the odor that was paired with quinine. This study suggests that fruit flies are able to learn associations between odors and negative stimuli and thus avoid those specific odors.

Associative learning also has been demonstrated in a study of growth rate in grasshoppers, *Schistocerca americana* (Dukas & Bernays, 2000). Grasshoppers were placed in an environment that contained two artificial food sources, one providing a balanced diet of protein and carbohydrates, which maximizes growth, and the other being carbohydrate-deficient, which is unsuitable for growth. Grasshoppers in the learning condition were able to associate the nutritious food with a specific spatial location, taste, and color whereas grasshoppers in the control condition did not have the benefit of learning which location, taste, and color predicted the presence of nutritious food. Instead, the spatial location, taste, and color cues were randomly alternated twice each day for grasshoppers in the control condition. The feeding behavior and body mass of each grasshopper were monitored throughout the experiment. Not only did the grasshoppers in the associative learning condition visit the nutritious food site more often than controls, but also they spent more time eating the nutritious food. Grasshoppers in the associative learning group had 15% higher fat mass and 11% higher non-fat mass than controls. Furthermore, the duration of the final instar was 7% shorter for the learning condition versus the control condition. Previous studies in grasshoppers (Atkinson & Begon, 1987) have shown that larger body mass is positively correlated with the number and size of eggs laid and that less time spent traveling to obtain nutritious food also translates into a lower predation risk. Thus, associative learning conveys a fitness advantage in grasshoppers because it allows for faster development, increased reproductive success, and decreased foraging time and, thus, decreased predation risk.

The potential fitness consequences of associative learning have also been studied in parasitoid wasps (*Biosteres arisanus*) (Dukas & Duan, 2000). This study sought to quantify not only the number of eggs parasitized by wasps, but also the number of offspring that reached adulthood. Wasps in the learning condition were able to associate a specific fruit substrate with the presence of fruit fly eggs, which the wasps could parasitize. In contrast, wasps in the control condition did not have the advantage of learning this association. When the wasps were given a chance to lay their eggs over a two day period, the wasps in the learning group were able to successfully parasitize more host eggs than control wasps. In addition, wasps in the learning group produced more offspring that reached adulthood than wasps in the control condition. These results suggest that learning could have a positive effect on fitness if certain conditions are met, namely, (i) the environment must consist of patterns that can enhance performance if learned; (ii) the animal must possess sufficiently robust sensory, learning, and memory abilities that allow it to make clear associations between a stimulus and an environmental state and remember that association until the next time that association is relevant; and, (iii) learning must result in significant time savings that can be used directly or indirectly for additional reproduction (Dukas & Duan, 2000). For example, the fact that more offspring reached adulthood in the learning condition than in the control condition suggests that learning conveys a fitness advantage because it allows more subjects to reach sexually mature adulthood.

Taken together, studies of learning in insects have shown that, despite short life spans and small brain sizes, insects from a wide variety of species demonstrate the ability to respond to learned cues. In particular, these studies have found that insects adjust their foraging to immediately locate the most beneficial food. However, all of the subjects used in these previous experiments were active foragers. No studies have analyzed the effect of learning on predators that use extremely sedentary means of obtaining food, like sit-and-wait predators.

Sit-and-wait Predators

Sessile, or sit-and-wait predators are passive foragers that remain stationary until prey is detected. Sessile predators employ a variety of techniques to capture prey. Not all sessile predators build traps, for example, in lieu of building a trap, some sit-and-wait predators, such as the iguanid lizard (Andrews, 1979) and juvenile salmon (Metcalfe, Valdimarsson & Fraser, 1997) anticipate the approach of food and then exhibit brief bouts of movement to capture prey. In other species, the predators construct traps for prey such as webs or pits and then wait for the prey to encounter the trap.

Trap building has been studied extensively in web building spiders (Olive, 1982; Walker, Marshall, & Rypstra, 1999; Takahiro & Miyashita, 2005; Morse, 2006). Spiders are able to approach and capture the prey once it has become ensnared in the web. With this form of trap-based foraging, the major energy expenditure is in the construction of the trap, rather than the actual prey capture. Trap-based foraging allows the sit-and-wait predator to conserve energy and remain hidden from predation. Despite the fact that sit-and-wait predators are immobile, they are still able to forage by detecting potential prey in the vicinity and deciding whether or not to attempt to capture the prey.

Construction of traps limits the size of prey that the predator can capture, and sit-and-wait predators do not necessarily pursue all prey that enters the trap. For example, iguanid lizards are selective when capturing potential prey; they capture only larger arthropods and allow smaller prey to enter the foraging area without attempting to capture it (Andrews, 1979). This selective foraging indicates that lizards utilize cues to detect the size of the prey, and consequently adjust their foraging. 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 or not a potential prey is worth capturing and, thus, adjust their foraging behavior. These data suggest that perhaps sit-and-wait predators, such as antlions, are able to adjust their foraging behavior based on learned cues.

Over the decades, many studies have investigated the unique foraging behavior of antlions, including where antlions build pits (Turner, 1915; McClure, 1976; Griffiths, 1985; Lucas, 1986; Mansell, 1992; Gotelli, 1996; Arnett & Gotelli, 1999; Crowley & Linton, 1999; Day & Zalucki, 2000), how they build pits (Turner, 1915; Griffiths, 1980; Hauber, 1999), where and why they relocate pits (Hauber, 1999; Day & Zalucki, 2000), and how they capture and consume prey. However, no published research has investigated associative learning in antlions.

Recently, Hayden (2005) addressed whether antlions are able to learn. In this 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 presented with a food item; however, the sand dropping signal was presented at another time, independent of 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. 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 demonstrated significantly greater extraction efficiency, extraction rate, pit diameter, and pit volume than control antlions. Hayden further reported that one week after the conclusion of the experiment, all of the subjects in the learning condition molted, while only one of the control antlions molted during the same time period. Hayden's results suggest that antlions may be capable of learning. In addition, the significant difference in the number of antlions that molted suggests that learning may affect the fitness of antlions; that is, an earlier molt might enable antlions to reach maturity and reproduction sooner than antlions in the control condition, thus decreasing the length of time that they remained in the vulnerable larval stage.

Although Hayden (2005) found some evidence of learning and improved prey extraction, her study did not allow her to observe behavioral responses to the food cue for subjects in the learning condition. Sun (2006) attempted to look for a conditioned response. Sun used the same training paradigm as Hayden; however, Sun paid particular attention to the effect of distance of the sand dropping cue on antlions' behavioral response to the cue. Antlions were presented with the sand dropping cue at three distinct distances, namely 3 cm, 8 cm, and 13 cm from the edge of the pit. Antlions in the learning condition demonstrated significantly more mandible movement than those in the control condition, but only in instances in which the signal itself induced sand to fall into the pit. However, in contrast to Hayden's findings, Sun did not find any significant effect of learning on prey extraction measures. A possible explanation for these contradictory findings is that neither Hayden nor Sun controlled for the developmental stage of antlion subjects at the start of the experiment.

In a follow-up study, Guillette (2007) waited until antlions molted and then distributed them into learning and control conditions. Both the learning and control groups received food every day. In the learning condition,12 antlions were given a sand dropping signal immediately before being presented with a food item for 42 consecutive training days. Antlions in the control condition also were presented with a food item; however, the sand dropping signal was presented at another time, independent of the feeding. Feeding behaviors (mass extracted, extraction time, extraction rate, and mandible visibility and movement during feeding) as well as pit building behaviors (pit volume and pit location) were measured each training day. Animals in the learning group displayed a conditioned behavioral response, namely, moving during the vibratory cue. Learning animals also constructed larger pits and extracted prey contents more quickly than control animals. Taken together, these experiments demonstrate that larval antlions utilize cues that predict the availability of food to gain a fitness advantage. Guillette had a small sample size (*N* = 12) and, thus, her survival analysis did not reveal a significant difference in the rate at which subjects molted to finish the experiment.

Current Study

The goal of the current study was to replicate the experimental design of Guillette (2007) with a greater number of antlions. I hypothesized that antlions in the LRN condition would demonstrate increased sand-throwing behavior and greater pit volume than the CON condition. I expected antlions in the LRN condition to demonstrate greater extraction efficiency rate than antlions in the CON condition. In addition, I expected antlions in the LRN condition to demonstrate greater fitness as measured by faster development and pupation.

METHOD

Subjects and Apparatus

Prior to training, 73 larval antlions (*Neuroptera: Myrmeleontiade*) obtained from AntLionFarms.com (Pensacola, FL) were placed, individually, in 210 mL plastic cups (7.5 cm top diameter x 8.5 cm height x 4.25 cm bottom diameter) filled with 150 mL Estes Marine 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 300 mL silica play sand (Pakmix, Inc., Toledo, OH) to stabilize the cup. Subjects were kept in a room at a constant temperature of 20ºC and overhead lights were set on a 14:10-hr light:dark light cycle (on at 0900 hr, off at 2300 hr).

Antlions were monitored, unfed, for two days to ensure that they built pits. Following this two-day habituation phase, antlions were fed two wingless fruit flies per day, (*Drosophila melanogaster*) obtained from Connecticut Valley Biological Supply (Southampton, MA), delivered to the center of their pits, Sunday through Friday. During each feeding session, the second fly was delivered to the antlion approximately 30 minutes after delivery of the first fly. Any antlions that did not build pits or did not eat consistently during the first six days were eliminated from the subject pool (n = 3). The remaining antlions were monitored during feeding sessions and any abnormal pit behavior (i.e., a flat-bottomed pit, no visible pit, or tracks in the sand) or aberrant behavior (i.e., concealment under the sand or being seen outside of a formed pit) was noted. Pit depth and pit diameter were measured approximately once a week for each subject.

If an antlion in the subject pool developed a flat-bottomed pit and was not visible for two consecutive days, most likely the antlion had begun molting. The cup and surrounding area then were monitored for the appearance of an exoskeleton. Once an exoskeleton was found, the antlion was weighed to the nearest 0.1 mg and measured to the nearest 0.1 mm.

Fifty-six of the 73 antlions molted, which ensured that all antlions entered the experiment at the same developmental stage. The 56 antlions ranged in weight from 4.0 mg to 22.8 mg. Upon molting, antlions were paired by length, weight, and pit volume. Within each pair, the subjects were randomly assigned to the learning (LRN) (*n* = 28) or control (CON) (*n* = 27) condition. One antlion in the CON condition was removed from the experiment after six days because it was not eating, leaving a total of 55 subjects. Each subject was moved to the testing room and housed individually in a rectangular plastic container (28 cm long x 17 cm wide x 17 cm deep) filled with 16 cups of fine sanitized Estes Marine Sand, at a depth of approximately 13 cm (see Figure 1). An 18 oz. Solo Grip® cup (9 cm diameter, approximately 3.0 cm tall as measured from the top rim, 0.05 cm

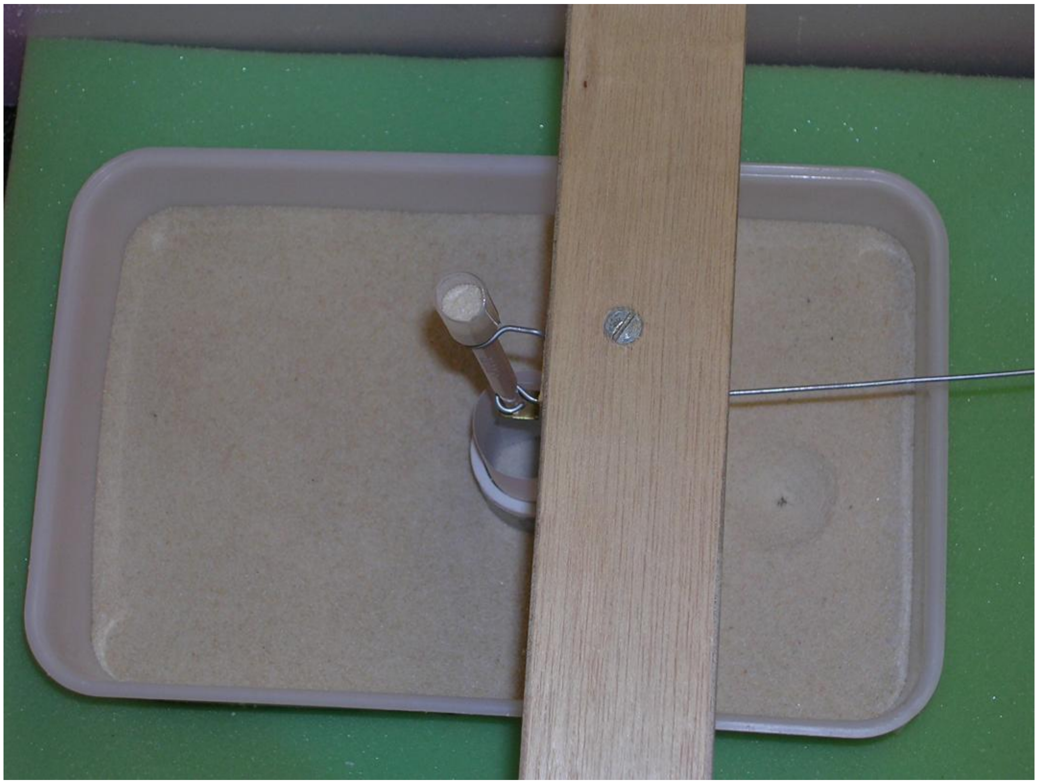
Figure 1. Illustration of the sound-attenuating compartment, including a sand delivery apparatus and a sand-receiving device.

thick) was used to confine each antlion to a small circular area in the center of the plastic container; this procedure ensured that antlions did not build a pit along the wall of the plastic container. Once the pit had been built, the ring was carefully removed so as not to disturb the pit.

Each container was situated in a sound-attenuating compartment constructed of foam pads. The foam floor of the compartment was 2.54 cm thick and the plastic container fit into a 5.08 cm deep pocket, cut in the middle of a foam block. The back and side walls of the compartment were 2.54 cm thick styrofoam. The sound-attenuating compartments were placed adjacent to each other in the same training room; 25 of the subjects were on a lower shelf and 25 of the subjects were on an upper shelf. Subjects from the PAV and CON groups were evenly distributed between the two shelves. The testing room was kept at the same temperature and at the same light:dark cycle as prior to training.

Prior to the first day of training, a sand-delivery device, which consisted of a plastic dropper suspended from two wires extending from a block of wood, was positioned over each plastic container. Another wire, which also extended from the block of wood, served as a lever to control the release of sand contained in the dropper (see Figure 2). A round wooden stick (26 cm long, 1 cm diameter) with a groove in one end was used to turn the lever and release the sand; the use of the stick minimized the presence of the trainer’s hand over the pit during training. The sand-delivery device was used

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



to deliver a vibratory cue, namely 4.5 mL of sand (approximately 5 s duration of vibratory cue). The sand fell into a sand-receiving container, which consisted of plastic wrap attached to the bottom of a 3.8 cm tall section of 1.5 inch PVC pipe, and which was placed underneath the delivery device. The sand-delivery device and the sand-receiving container were positioned so that the cue was delivered 4.5 cm from the center of the pit.

Procedure

An illustration of the experimental design and procedure is presented in Figure 3. On every training day, each antlion in the LRN condition received the sand-dropping cue immediately followed by the delivery of approximately ¼ of a single live mealworm (*Tenebrio* larvae) obtained from the Connecticut Valley Biological Supply Co., Inc. (Southampton, MA). The mealworms were cut so that they weighed between 0.0025 g to 0.0045 g; only the head-portion of the mealworm was fed to the antlions. The signal, followed immediately by the food, was delivered at a selected time (between 0930 hr and 1630 hr) each day of training. Training days occurred six days each week and was followed by one 24-hour period during which training was suspended. The times of the feedings were randomized within the selected time range using an online random number generator (http://www.random/org/nform.html) to ensure that the feeding time, itself, did not become an inadvertent cue.

Antlions in the CON condition were fed one pre-weighed mealworm at the same selected time as LRN subjects; however, the sand dropping signal was programmed at another time (between 0930 hr and 2030 hr) within each training day. I used a variation of an explicitly unpaired control group. In a classic explicitly unpaired procedure, the CS and US are presented in the same context but do not co-occur or occur closely together in time. In the current experiment, the sand dropping signal was presented at least four hours before or after a feeding period so that the two events never coincided. Signal presentation in the current experiment had more variation than in a classic explicitly unpaired control because in the current experiment the signal occurred at variable times, equally as often before and after the training.

A variation of an explicitly unpaired control group was chosen for the current study because it was the most feasible option in terms of scheduling lab time. This group controlled for extra cues that may have surrounding presentation of the vibratory cue (i.e., hand movement or shadows over the pit). However, a stimulus that is negatively correlated with the US may generate an inhibitory response, in which antlions learn that the cue never predicts food and so they learn not to respond to the cue.

Feeding was considered complete when each antlion threw the mealworm carcass out of the pit. If an antlion did not successfully 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 mealworm carcass was retrieved and weighed. The amount of food extracted from the mealworm was determined by weighing it before presentation to the antlion and weighing it again after it was thrown from the pit. These latter weighings were done within 15 minutes of carcass throwing so that weight loss by evaporation was minimal.

Feeding sessions during training continued until each antlion stopped feeding in preparation for either the cocoon stage or a second molting. If an antlion molted it was weighed (within 24 hours of the molt) to the nearest 0.1 mg and measured to the nearest 0.1 cm.

Each night (at least 1.5 hours after the last training or sand drop), the depth, diameter, and location of the pit was recorded. In addition, the sand collection container was removed and emptied into a graduated cylinder to check for the correct sand volume and then poured back into the sand delivery device to prepare it for the next training day. The empty sand collection container was then reset at the original distance from the pit.

To prevent antlions from moving their pits to the perimeter of the rectangular plastic container, clear plastic rings (18 cm diameter, 10.5 cm height, and 0.01 cm thickness) were placed around the pits after pit measuring on Friday night until Sunday morning. The rings were removed at least 1.5 hours before the first training or sand drop session.

Figure 3. Illustration of experimental design and procedure.

Prior to Training (date received through completed molt)	Training (70 training days)
• Placed in individual cups of fine sand • Monitored antlions for pit building • Fed antlions two *Drosophila* per day • Measured pit size once a week • Monitored each antlion until it molted and rebuilt pit	• Measured pits (depth, diameter and volume, walls touched) six days a week • Delivered either LRN or CON treatment once each training day: LRN: CS → US CON: CS/US • Videotaped antlions on the first day of training and then every 12 days thereafter for 70 days

Data Analysis

Data for quantitative extraction measures, qualitative behavioral measures, pit volume, and survival analysis were analyzed over 70 training days. Extraction efficiency rate and pit volume were analyzed using a mixed 2 x 2 x 3 (Condition x Instar x Block) factorial design. The between-subject independent variables were condition (LRN vs. CON) and instar (second vs. third). Blocks, which were computed by taking the mean of three training days, were a repeated measure. A mixed analysis of variance (ANOVA) was conducted using SPSS version 15.0 to calculate differences between experimental groups. Sand-throwing behavior was analyzed via a chi-square test and survival analysis was analyzed by a Mantel-Cox log rank chi-square. An alpha level of .05 was used for all analyses.

Due to the large sample size and the nature of the experiment, subjects could not be feasibly videotaped during every training session. Therefore, measurements of extraction efficiency rate and sand-throwing behavior were observed and recorded by the experimenter and, hence, not blind.

To minimize inter-rater measurement differences, all experimenters participated in a rigorous training phase with 12 practice antlions for approximately seven weeks prior to the start of the current experiment. In addition, during the experiment, observed feeding times were verified with videotapes that were scored blind and collected every 12th training day. While scoring videotapes, observers were naïve with respect to condition; there was no identifying information on the videotape screen that revealed the treatment condition. A paired *t*-test was conducted to ensure that there were no differences in observed versus actual (as recorded blind form the videotape) feeding times. For example, the observed capture latency (s) ($M = 31.97$, $SEM = 24.17$) was not significantly different from the actual (as recorded from the videotape) capture latency time ($M = 9.38$, SEM = 4.92), $t(14) = 1.05$, $p > .05$.

Pit volume. During training, pit depth and diameter were measured daily to the nearest 0.1 cm. It was not possible for experimenters to be blind with respect to experimental condition during pit measuring. However, prior to the experiment, experimenters were trained on practice pits until all experimenter measurements were consistently the same. Pit volume was calculated using the equation for the volume of a cone: $Volume\ (cm^3) = \frac{1}{3}\pi \times r^2 \times h$, where r is the pit radius (half the diameter) and h is the pit height (depth).

Extraction efficiency rate. The latency to capture prey was measured in seconds, from the time the prey was introduced to the center of the pit until the antlion captured the prey. The amount of mass extracted from the mealworm was calculated by subtracting the final weight (W_f) of the carcass from the initial prey weight (W_i). The extraction efficiency, the proportion of prey mass ingested, was calculated by dividing the mass extracted by the initial weight: $(W_i - W_f)/W_i$. Extraction time, the time it took antlions to consume the prey, was measured in seconds from the time an antlion captured the prey until the prey carcass was thrown from the pit or discarded by the antlion inside the pit for 10 minutes. Extraction efficiency rate, or efficiency of extraction per unit of time, was calculated by dividing the extraction efficiency by the extraction time: $((W_i - W_f)/W_i)/\mathit{Extraction\ Time}$ (see Table 1).

Scoring of feeding behavior. Each antlion received one score per day, during presentation of the vibratory cue, for the following measures: (1) movement, (2) visibility, and (3) sand fall (see Table 2). If an antlion moved its mandibles or head it received a nominal score as follows: 0 = no movement; 1 = mandibles open; 2 = mandibles closed; 3 = antlion moves backwards under the sand; 4 = antlion moves forward out of the sand; and, 5 = antlion throws sand. For subjects in the LRN and CON groups, visibility during the vibratory cue was assessed by the following nominal scale: 0 = not visible; 1 = entire head visible; 2 = two mandibles open and visible; 3 = two mandibles closed and visible; 4 = head, two mandibles (open) and visible; 5 = head, two mandibles (closed) and visible; 6 = head, part of body, two mandibles (open) and visible; 7 = head, part of body, two mandibles (closed) and visible; and, 8 = tip of head visible. Finally, if any sand fell into the pit of the subject during the vibratory cue, it received a score of 1; if no sand fell during that time, it received a score of 0.

Video recording. During training, both feeding and the sand dropping

Table 1
Definitions of computed extraction measures

Extraction Measure	Definition	Equation
Amount of mass extracted (g)	Amount of mass extracted from the prey	$W_i - W_f$
Extraction Efficiency (%)	Proportion of prey mass ingested	$\frac{(W_i - W_f)}{W_i}$.
Latency to capture prey (*CapLat*) (s)	Time to capture prey	*Capture Time – Feeding Start Time*
Extraction Time (s)	Time to consume the prey	*(Prey Discard Time – Feeding Start Time)* - *CapLat*
Extraction Efficiency Rate (%/s)	Efficiency of extraction per unit of time	$\frac{((W_i - W_f)/W_i)}{\text{Extraction Time}}$

Table 2

Nominal scoring definitions of antlion movement, visibility, and sand fall during presentation of the vibratory cue

Movement	Visibility	Sand Fall
0 = No movement	0 = Not visible	0 = No sand fell into pit
1 = Mandibles open	1 = Entire head visible	1 = sand fell into pit
2 = Mandibles closed	2 = Two mandibles (open) visible	
3 = Antlion moves backwards under the sand	3 = Two mandibles (closed) visible	
4 = Antlion moves forward out of the sand	4 = Head, two mandibles (open) visible	
5 = Antlion throws sand	5 = Head, two mandibles (closed) visible	
	6 = Head, part of body, two mandibles (open) visible	
	7 = Head, part of body, two mandibles (closed) visible	
	8 = Tip of head visible	

signal were recorded using Panasonic PV-GS31 and PV-GS39 Mini DV Digital Camcorders onto 90 minute (LP) Panasonic Mini DV tapes. The camcorders were positioned approximately 0.75 m from each container, at a 45º angle to avoid casting a shadow over the pit. The viewfinder was adjusted so that the entire pit of the antlion was visible, and filled the view. There was no identifying information in the viewfinder of the videotapes. While scoring videotapes, observers were naïve with respect to condition; there was no identifying information on the videotape screen that revealed the treatment condition. Antlions were videotaped on the first day of training and then every 12th day thereafter. Subjects in the LRN condition were videotaped for approximately five seconds prior to the presentation of the vibratory cue (approximately five seconds duration), followed immediately by delivery of the prey to the center of the pit. Videotaping continued until the feeding session terminated, either because the prey carcass was thrown out of the pit by the antlion, or because the prey carcass was discarded by the antlion but remained in the pit untouched for 10 min.

Subjects in the CON condition were videotaped in the same manner during a feeding session, except the vibratory cue was not present. However, the CON subjects were also videotaped when the vibratory cue was delivered independently of the food. During these sessions, each CON subject was videotaped for five seconds prior to the vibratory cue presentations, and five seconds after the cue.

Survival analysis. A Kaplan Meier survival analysis is used on quantitative data corresponding to the time from a well-defined time origin (i.e., the start of the experiment) until the occurrence of some particular event of interest or end-point (i.e., molting or pupating) (Chan, 2004). A survival analysis differs from non-parametric tests, like chi-square, because it accounts for censored data. Censored data include cases in which the critical event has not yet occurred (i.e., the subject has not molted or pupated), the subject was lost to follow-up, other interventions were offered, or the event occurred but was unrelated to the cause of interest (Chan, 2004). In the current study, censored data was solely the result of subjects who had not yet molted or pupated to finish the experiment.

In the current study, the number of days from the start of the experiment to molting/pupating were calculated for each subject. If a subject was still in the experiment at day 70, then total days in the experiment were calculated through day 70 and that subject was marked as censored data. The output is a plot of the proportion of cases surviving at each day of the experiment. While survival analyses are often used in the medical profession to track patient death rates resulting from certain medical interventions, and thus have a negative connotation, for the current study, molting/pupating to finish the experiment was a positive result because it indicated faster development and growth.

RESULTS

To ensure that the initial weights (mg) of antlions in the two treatment groups were not different from one another, an independent t-test was performed, which revealed no difference between the CON ($n = 27$) treatment group ($M = 13.98$, $SEM = 1.19$) and the LRN ($n = 28$) treatment group ($M = 14.36$, $SEM = 1.02$), $t(54) = .24$, $p > .05$. Another independent t-test revealed that the initial length (cm) of animals in the CON treatment group ($M = .98$, $SEM = .04$) was not significantly different from animals in the LRN treatment group ($M = 1.01$, $SEM = .04$), $t(54) = .48$, $p > .05$. Finally, an independent t-test was performed to ensure that the antlions assigned to the two treatments did not differ in the volume (cm^3) of their initial pit construction. The analysis revealed no significant difference in pit volume between the CON treatment group ($M = 12.11$, $SEM = 2.61$) and the LRN treatment group ($M = 11.78$, $SEM = 2.58$), $t(54) = -.09$, $p > .05$.

I hypothesized that antlions in the LRN condition would demonstrate behavior that increased the effectiveness of prey capture and show greater pit volume than the CON condition. In addition, I predicted that antlions in the LRN condition would demonstrate greater fitness as measured by faster development and pupation.

Differences between CON and LRN groups were analyzed with respect to four subgroups: a) all second instar subjects (I2), (n = 19); b) second instar subjects that molted and thus completed the experiment ($I2_M$), (n = 16); c) all third instar subjects (I3), (n = 36); and, d) third instar subjects that pupated and thus completed the experiment ($I3_P$), (n = 21). Since subjects remained in the experiment for varying durations of time, it was important to break the measurements into portions (i.e., first, middle, last) to ensure that I was comparing analogous measures between subjects. For groups $I2_M$ and $I3_P$, blocks of training days were computed by taking the mean of three days; the first block, middle block and final block of the experiment were analyzed.

Extraction Measure

Extraction efficiency rate was analyzed with a 2 x 2 x 3 (Condition x Instar x Block) mixed ANOVA, with condition (LRN or CON) and instar (second or third) serving as a between-subjects factors and with block serving as repeated measures. Extraction efficiency rate was only analyzed for $I2_M$ and $I3_P$ subjects, that is, subjects that had molted/pupated to finish the experiment. The original extraction efficiency rate data was not normally distributed; therefore, a natural log transform was conducted to normalize the data, as normal data is assumed in parametric analyses. A Kolmogorov-Smirnov test showed that the natural log transformed data did not differ from normal, $p > .05$.

I2$_M$ and I3$_P$ subjects. The natural log of extraction efficiency rate (mean %/s ± *SEM*) across first, middle, and last blocks for I2$_M$ and I3$_P$ LRN ($n = 23$) and CON ($n = 14$) subjects is presented in Figure 4. No significant difference in extraction efficiency rate was found between CON ($M = -9.24$, $SEM = .13$) and LRN ($M = -9.29$, $SEM = .12$) conditions, $F(1, 34) = .31$, $p > .05$. A significant main effect for time was found; extraction efficiency rate significantly decreased from the first block of training ($M = -9.27$, $SEM = .09$) to the last block of training ($M = -9.02$, $SEM = .07$), $F(2, 68) = 7.74$, $p < .05$. No significant interaction effect for condition and time was found, $F(2, 68) = .94$, $p > .05$, indicating that there was no significant change in extraction efficiency rate across CON and LRN conditions over the three blocks of training days.

I2$_M$ subjects. The natural log of extraction efficiency rate (mean %/s ± *SEM*) across quarters of training for I2$_M$ subjects is shown in Figure 5. No significant difference in extraction efficiency rate was found between CON ($M = -9.54$, $SEM = .15$) and LRN ($M = -9.33$, $SEM = .11$) conditions, $F(1, 14) = .30$, $p > .05$. No significant main effect for time was found; extraction efficiency rate did not significantly change from the first block of training ($M = -9.43$, $SEM = .13$) to the last block of training ($M = -9.32$, $SEM = .13$), $F(2, 28) = 2.99$, $p > .05$. No significant interaction effect for condition and time was found, $F(2, 28) = .34$, $p > .05$, indicating no significant change in extraction efficiency rate across CON and LRN conditions over the three blocks of training days.

$I3_P$ subjects. The natural log of extraction efficiency rate (mean %/s ± *SEM*) across three blocks of training for $I3_P$ LRN ($n = 15$) and CON ($n = 6$) subjects is presented in Figure 6. No significant difference was found in extraction efficiency rate between CON ($M = -8.68$, $SEM = .06$) and LRN ($M = -8.82$, $SEM = .09$) conditions, $F(1, 18) = 1.55$, $p > .05$. A significant main effect for time was found, $F(2, 36) = 3.87$, $p < .05$, indicating a significant decrease in extraction efficiency rate over time from the first block of training days ($M = -9.15$, $SEM = .14$) to the last block of training ($M = -8.78$, $SEM = .07$). No significant interaction effect for condition and time was found, $F(2, 36) = 1.68$, $p > .05$, indicating no significant change in extraction efficiency rate across CON and LRN conditions over three blocks of training days.

Figure 4. Extraction efficiency rate (mean %/s ± *SEM*) across first, middle, and last blocks for $I2_M$ and $I3_P$ LRN (*n* = 23) and CON (*n* = 14) subjects.

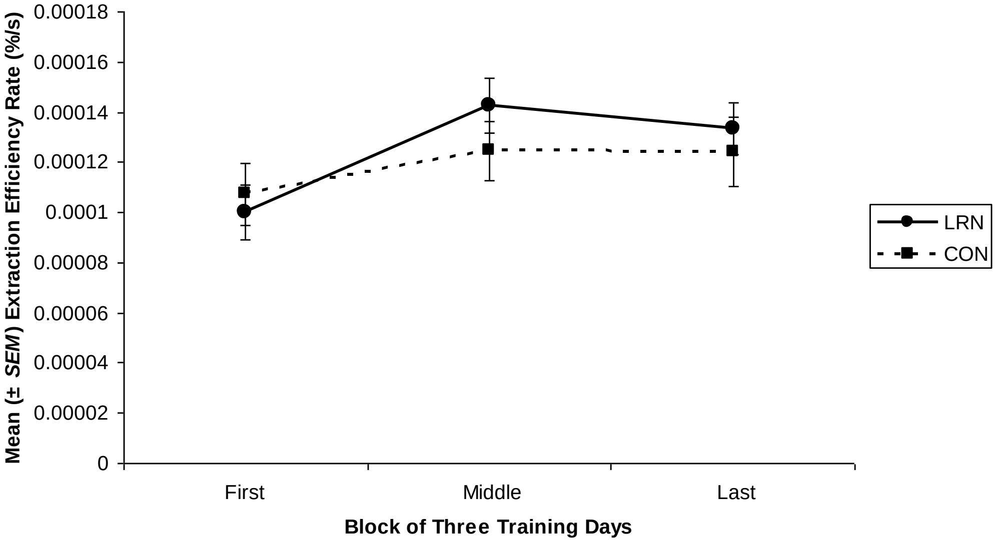

Mean (± *SEM*) Extraction Efficiency Rate (%/s)
0.00018
0.00016
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
First
Middle
Last
Block of Three Training Days
LRN
CON

Figure 5. Extraction efficiency rate (mean %/s ± *SEM*) across first, middle, and last blocks of training for $I2_M$ LRN ($n = 8$) and CON ($n = 8$) subjects.

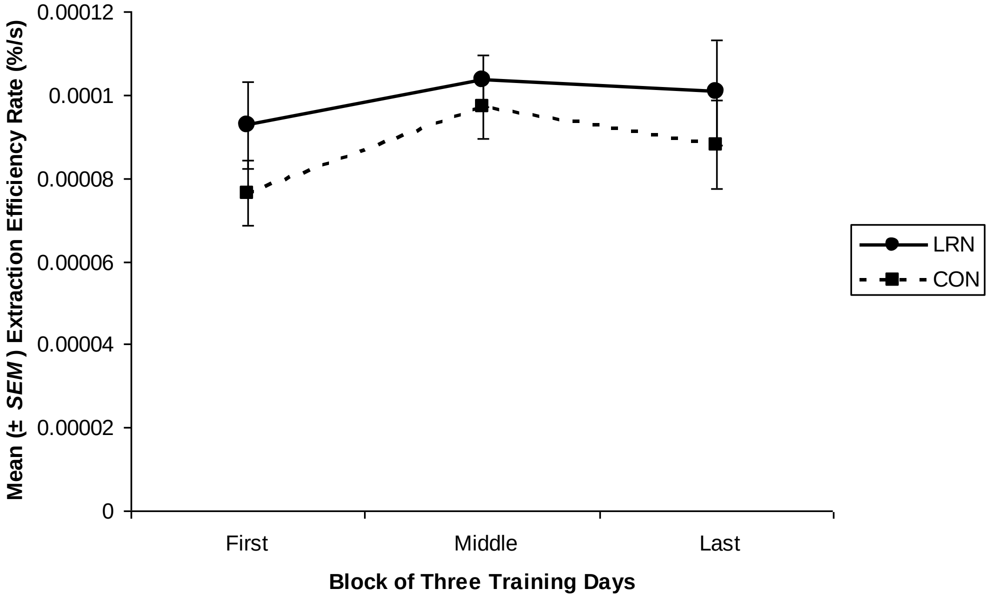
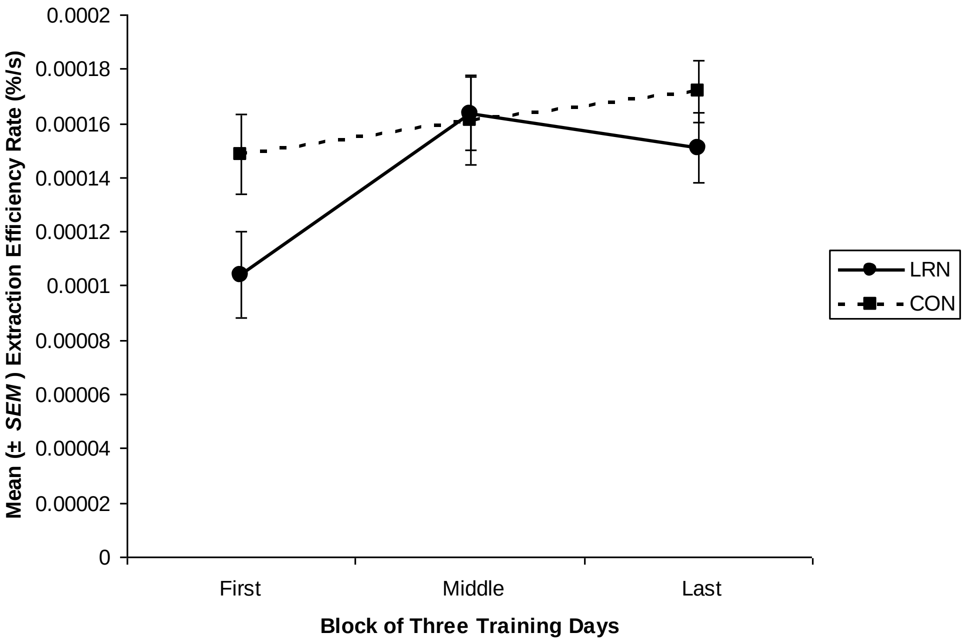

0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
Mean (± *SEM*) Extraction Efficiency Rate (%/s)
First
Middle
Last
Block of Three Training Days
LRN
CON

Figure 6. Extraction efficiency rate (mean %/s ± *SEM*) across first, middle, and last blocks of training for $\mathrm{I3_P}$ LRN (*n* = 15) and CON (*n* = 6) subjects.


0.0002
0.00018
0.00016
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
Mean (± *SEM*) Extraction Efficiency Rate (%/s)
First
Middle
Last
Block of Three Training Days
LRN
CON

Sand-Throwing Measure

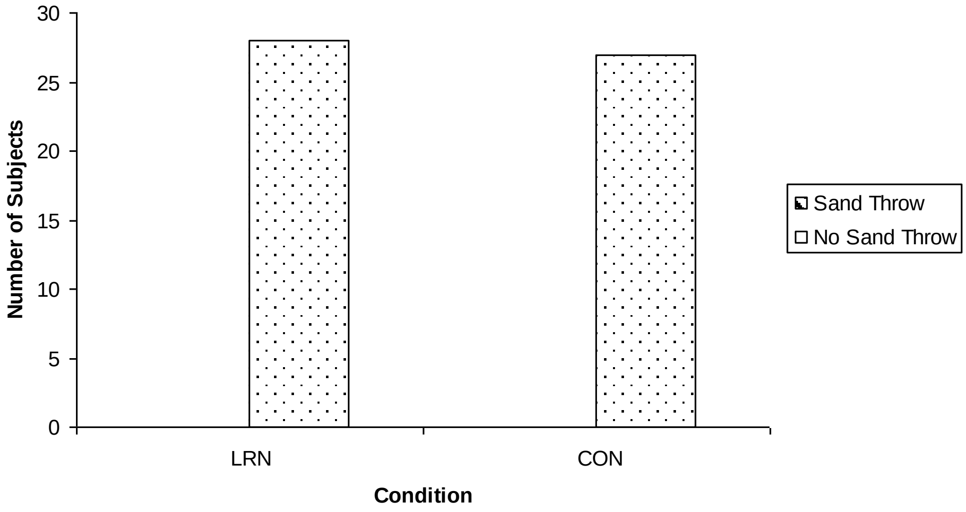
A conditioned response, namely, sand-throwing during cue delivery, was analyzed using chi-square analysis. I hypothesized that antlions in the LRN condition, in which the vibratory cue immediately preceded food presentation, would demonstrate behavior that increased the effectiveness of prey capture, such as increased sand-throwing.

To ensure that there were no differences in sand-throwing behavior between conditions at the start of the experiment, sand-throwing behavior during the first six training days was analyzed. The number of subjects throwing sand on the first day of training is presented in Figure 7a. No significant difference was found between CON and LRN sand-throwing behavior during presentation of the vibratory cue, $\chi^2(1, N = 55) = .89, p > .05$. The number of subjects throwing sand during the first six days of training is presented in Figure 7b. No significant difference was found between CON and LRN sand-throwing behavior during presentation of the vibratory cue, $\chi^2(1, N = 55) = .39, p > .05$. The number of subjects throwing sand during the 70 training days is presented in Figure 8a. A Chi-square test revealed a significant difference between CON and LRN sand-throwing behavior during presentation of the vibratory cue. Significantly more LRN subjects threw sand at least once during training compared to CON subjects, $\chi^2(1, N = 55) = .03, p < .05$.

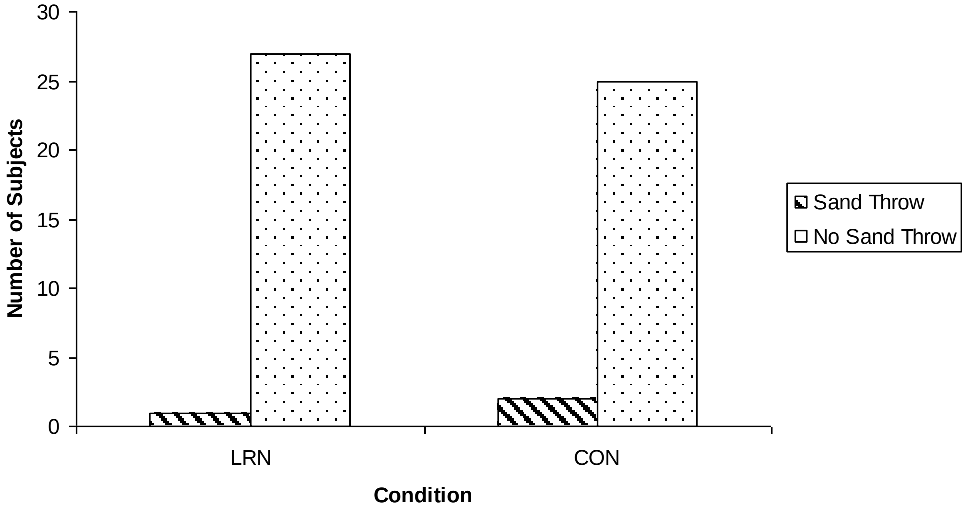
The number of I2 subjects throwing sand during the first 70 training days is presented in Figure 8b. No significant difference was found between CON and LRN sand-throwing behavior during presentation of the vibratory cue, $\chi^2(1, N = 19) = .58, p > .05$.

The number of I3 subjects throwing sand during the first 70 training days is presented in Figure 8c. A significantly greater number of I3 subjects in the LRN condition threw sand compared to subjects in the CON condition, $\chi^2(1, N = 36) = .04, p < .05$.

Figure 7. The number of subject to throw sand (*N* = 55). A) On the first training day; B) During the first six training days.



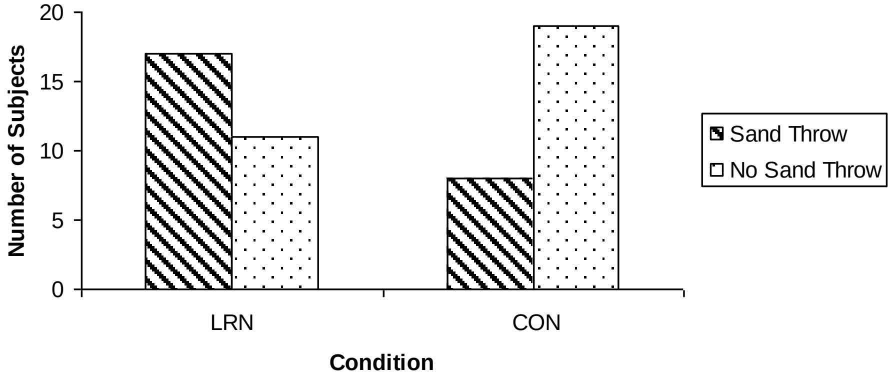
A. Subjects to throw sand on the first training day



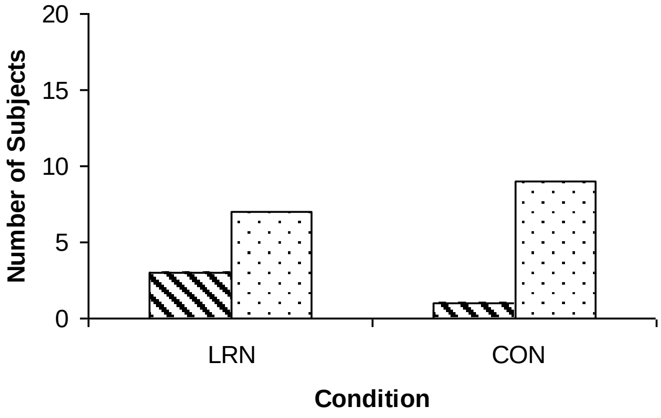
B. Subjects to throw sand during first six training days

Figure 8. The number of subjects throwing sand during the 70 training days. A) All second and third instar subjects (*N* = 55); B) All second instar subjects (*n* = 19); C) All third instar subjects (*n* = 36).

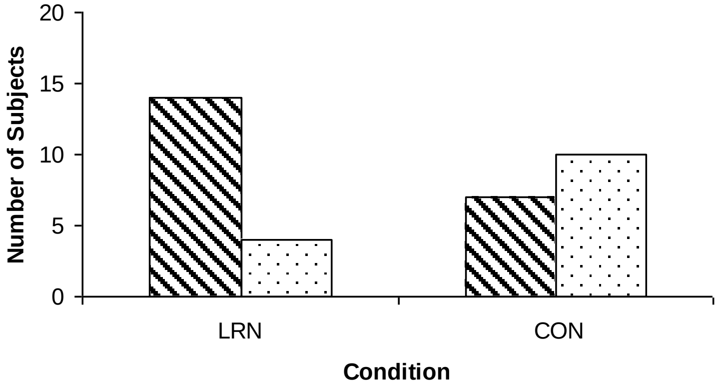
A. All second and third instar subjects



B. All second instar subjects



C. All third instar subjects



Survival Analysis

Kaplan-Meier survival curves were compared with a Mantel-Cox log rank chi-square to analyze different molting and pupating rates between LRN and CON treatment groups (see Figure 9). At the terminus of the study 8 of 9 animals in the I2 LRN group molted and 15 of 19 animals in the I3 LRN group pupated. Eight of 10 animals in the I2 CON group molted and 6 of 17 animals in the I3 CON group pupated.

The plot of survival rate for all subjects is presented in Figure 10a. The plot for LRN animals falls under the plot for CON animals and constantly stays lower after approximately 30 days, suggesting that overall, LRN animals were molting/pupating more quickly than CON animals. A chi-square analysis reveals that LRN animals (M = 40.73, SEM = 2.80) were molting/pupating in significantly less time than CON animals (M = 50.42, SEM = 3.88), $\chi^2(1, N = 55) = 6.79$, $p < .05$.

The plot of survival rate for all I2 subjects is presented in Figure 10b. The plot of I2 LRN animals falls under the plot of CON animals and stays lower after 20 days, suggesting that overall, I2 LRN animals were molting more quickly than I2 CON animals. However, a chi-square analysis reveals that I2 LRN animals (M = 27.33, SEM = 5.67) were not molting in significantly less time than I2 CON animals (M = 37.00, SEM = 7.49), $\chi^2(1, N = 19) = .96$, $p > .05$.

The plot of survival rate for all I3 subjects is presented in Figure 10b. The plot of I3 LRN animals falls under the plot of CON animals and stays lower for the entire experiment, suggesting that overall, I3 LRN animals were pupating more quickly than I3 CON animals. A chi-square analysis reveals that I3 LRN animals ($M = 47.20$, $SEM = 1.91$) were pupating in significantly less time than I3 CON animals ($M = 57.1$, $SEM = 2.60$), $\chi^2(1, N = 36) = 7.66$, $p < .05$

Figure 9. Kaplan-Meier survival curves for all second and third instar subjects (*N* = 55).

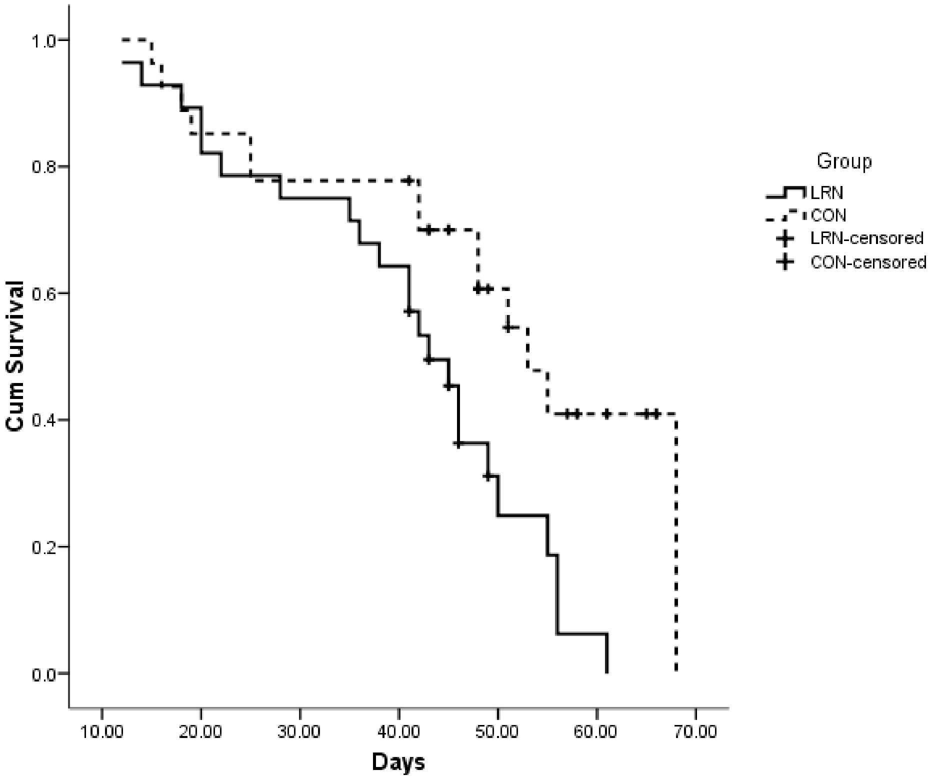
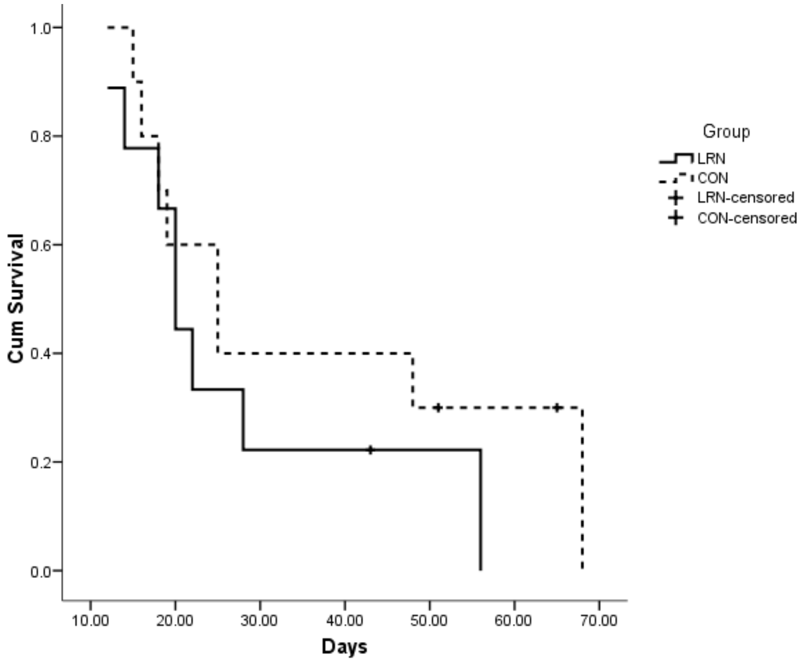
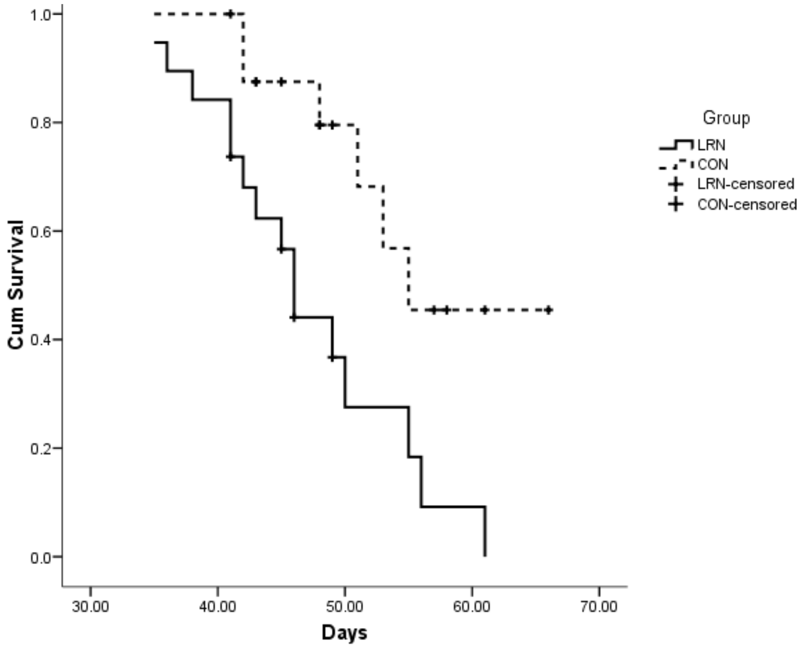

1.0
0.8
0.6
0.4
0.2
0.0
Cum Survival
10.00
20.00
30.00
40.00
50.00
60.00
70.00
Days
Group
LRN
CON
LRN-censored
CON-censored

Figure 10. Kaplan-Meier survival curves for A) All second instar subjects (*n* = 19); B) All third instar subjects (*n* = 36).

A. Second instar subjects



B. Third instar subjects



Pit Measures

Pit volume was analyzed with a 2 x 2 x 3 (Condition x Instar x Block) mixed ANOVA, with condition (LRN or CON) and instar (second or third) serving as a between-subjects factors and with block serving as repeated measures. Pit volume (cm^3) was only analyzed for $I2_M$ and $I3_P$ subjects, that is, subjects that had molted/pupated to finish the experiment. The original pit volume data was not normally distributed; therefore, a natural log transform was conducted to normalize the data, as normal data is assumed in parametric analyses. A Kolmogorov-Smirnov test showed that the natural log transformed data did not differ from normal, $p > .05$.

Pit measurements, including pit depth and diameter, were recorded six days a week. Pit location was also recorded due to pit construction interference that occurred when pits encountered one or two walls; two LRN and three CON subjects were excluded from analysis because of pit obstruction from the walls of the container. I hypothesized that subjects in the LRN condition would grow larger than subjects in the CON condition. While the size of antlions cannot be measured during the experiment without risking pit disruption and antlion injury, antlion size is correlated with pit size. Therefore, the pit size is used as a measure of growth.

$I2_M$ and $I3_P$ subjects. Pit volume (mean cm^3 ± *SEM*) across first, middle, and last blocks for all LRN ($n = 20$) and CON ($n = 11$) subjects is presented in Figure 11. While subjects in the LRN ($M = 3.44$, $SEM = .19$)

group appear to have larger pit volumes than subjects in the CON (M = 3.19, SEM = .31) group, this difference was not significant, $F(1, 29) = .73$, $p > .05$. Pit volume significantly increased across conditions from the first block (M = 1.60, SEM = .15) to the last block (M = 3.81, SEM = .14), $F(2, 58) = 222.96$, $p < .05$. However, no significant change in pit volume across LRN and CON conditions over the three blocks was observed, $F(2, 58) = .11$, $p > .05$.

I2$_M$ subjects. Pit volume (mean cm^3 ± SEM) across three blocks of training for I2$_M$ LRN (n = 7) and CON (n = 6) subjects is presented in Figure 12. While subjects in the LRN (M = 2.47, SEM = .23) group appear to have larger pit volumes than subjects in the CON (M = 2.37, SEM = .24) group, this difference was not significant, $F(1, 11) = .62$, $p > .05$. Pit volume significantly increased across conditions from the first block (M = 1.02, SEM = .20) to the last block (M = 3.00, SEM = .13), $F(2, 22) = 90.16$, $p < .05$. However, no significant change in pit volume across LRN and CON conditions over the three blocks was observed, $F(2, 22) = .37$, $p > .05$.

I3$_P$ subjects. Pit volume (mean cm^3 ± SEM) across three blocks of training for I3$_P$ LRN (n = 13) and CON (n = 5) subjects is presented in Figure 13. While subjects in the LRN (M = 3.96, SEM = .09) group appear to have smaller pit volumes than subjects in the CON group (M = 4.17, SEM = .05), this difference was not significant, $F(1, 16) = 2.07$, $p > .05$. Pit volume significantly increased across conditions from the first block (M = 2.02, SEM = .16) to the last block (M = 4.40, SEM = .04), $F(2, 32) = 142.74$, $p < .05$.

However, no significant change in pit volume across LRN and CON conditions over the three blocks was observed, $F(2, 32) = .22$, $p > .05$.

Figure 11. Pit volume (mean cm^3 ± *SEM*) across first, middle, and last blocks for all LRN (*n* = 20) and CON (*n* = 11) subjects.

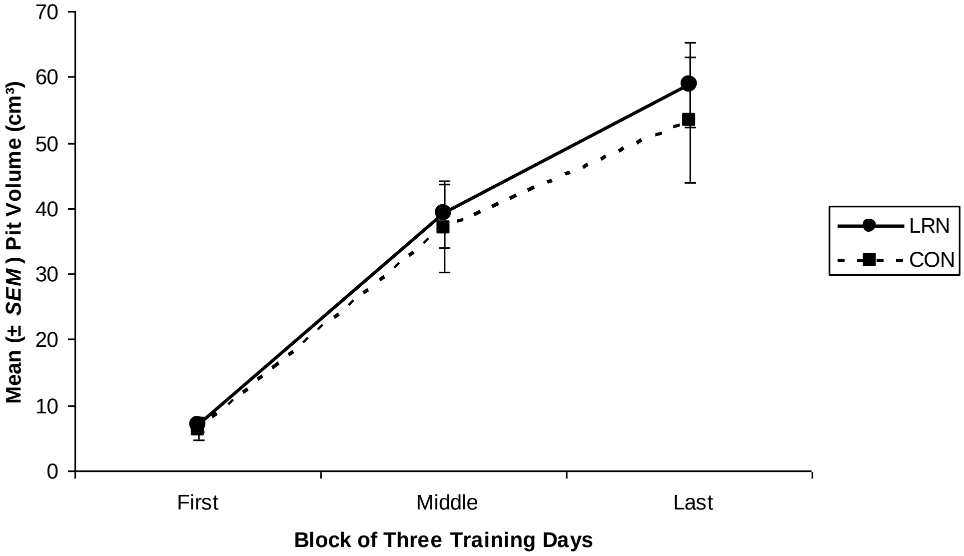

70
60
50
40
30
20
10
0
Mean (± SEM) Pit Volume (cm³)
First
Middle
Last
Block of Three Training Days
LRN
CON

Figure 12. Pit volume (mean cm^3 ± *SEM*) across three blocks of training for $I2_M$ LRN ($n = 7$) and CON ($n = 6$) subjects.

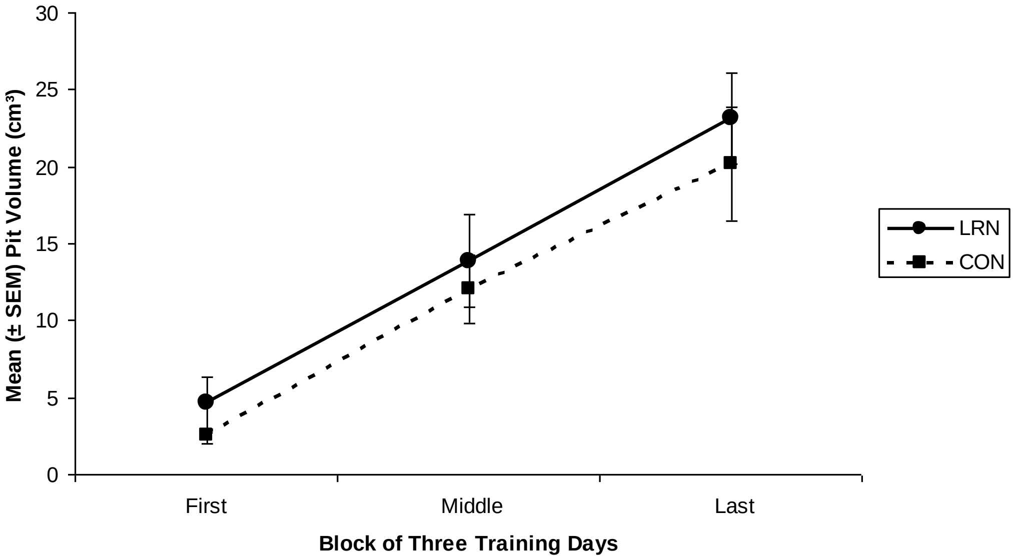
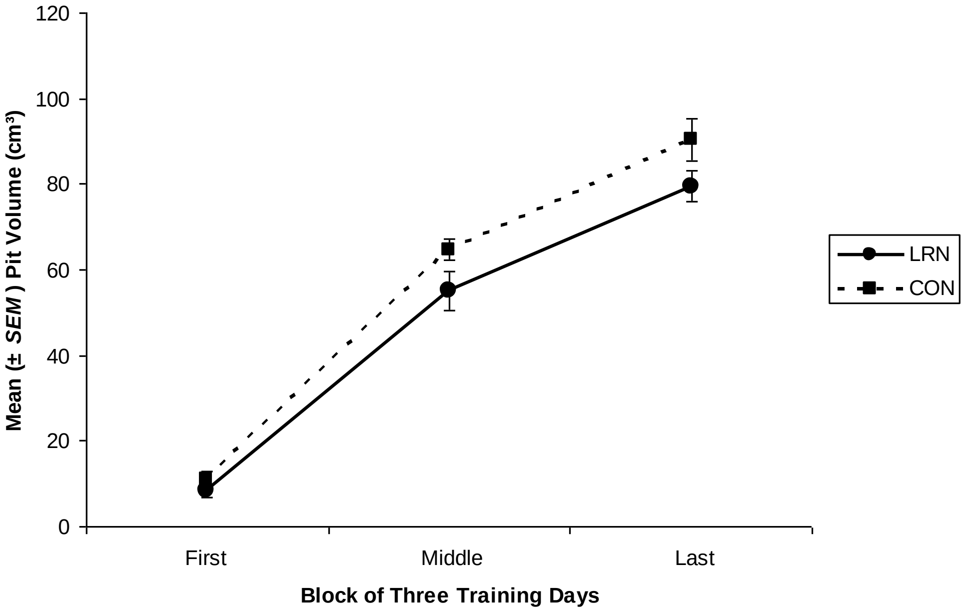

30
25
20
15
10
5
0
Mean (± SEM) Pit Volume (cm³)
First
Middle
Last
Block of Three Training Days
LRN
CON

Figure 13. Pit volume (mean cm³ ± *SEM*) across three blocks of training for I3$_P$ LRN (*n* = 13) and CON (*n* = 5) subjects.



120
100
80
60
40
20
0
Mean (± *SEM*) Pit Volume (cm³)
First
Middle
Last
Block of Three Training Days
LRN
CON

DISCUSSION

I hypothesized that subjects in the LRN condition would differ from subjects in the CON condition with respect to extraction efficiency rate, sand-throwing behavior, pit volume, and survival analysis. Significant differences between treatment groups were found with respect to extraction efficiency rate in third instar subjects, sand-throwing behavior, and survival analysis. Measures of pit volume were not significantly different between treatment groups.

Although, prior to training, all subjects were paired by size, weight, and pit volume and then assigned to the LRN and CON conditions, I was not able to determine the current instar of each subject at the start of the experiment. The changes in body size, head shape, and mandible shape between instars are minimal and difficult to determine with the untrained eye. All subjects molted at least once prior to training, so I was able to determine that all subjects were either second or third instar; however, I had to wait until subjects completed the experiment to classify them according to developmental stage or instar. This classification was based on whether antlions molted or pupated, the former indicating second instar subjects and the later indicating third instar subjects. Overall, the experiment consisted of 36 subjects in their third instar and 19 subjects in their second instar.

Extraction Measures

I hypothesized that antlions in the LRN condition would demonstrate a greater extraction efficiency rate as compared with CON subjects. This hypothesis stems from a study in which Dukas and Visscher (1994) found that associative learning in honeybees increased their net food intake. My hypotheses were not completely supported by the data. Extraction efficiency rate was not significantly different between LRN and CON subjects, regardless of instar. Extraction efficiency did not significantly change from the first block of training to the last block of training for $I2_M$ subjects, however, extraction efficiency rate did significantly decrease across blocks for $I3_P$ subjects.

The lack of difference in extraction efficiency rate across condition and time for $I2_M$ subjects suggests that perhaps $I2_M$ subjects simply weren't in the experiment long enough for learning to occur. One reason why antlions are great subjects in an associative learning experiment is that antlions are a long lived species; the larval stage may last for two or three years. The longevity of the antlion species may also mean that associative learning takes longer to occur. When fed the same sized prey, large larvae grow slower than small larvae because of higher maintenance costs (Griffiths, 1980), hence, when fed the same diet, the third instar stage lasts longer than the second instar stage. In the current experiment, almost all second instar subjects had molted by day 30, whereas, third instar subjects didn't begin to pupate before day 30. Perhaps differences in extraction efficiency rate would have been evident it $I2_M$ subjects if they had remained in the experiment for a longer period of time.

For $I3_P$ subjects, extraction efficiency rate increased significantly over time from the first training block to the last. While I expected extraction efficiency rate to increase across time for all subject, I expected a differentially greater increase in extraction efficiency rate in LRN subjects. These results do not support my hypothesis.

For $I3_P$ subjects, no significant changes in extraction efficiency rate were found across condition. These results are surprising, considering that a survival analysis revealed that $I3_P$ LRN subjects were pupating at a faster rate than $I3_P$ CON subjects. One would expect that faster pupation would result from more efficient extraction and use of nutrients from food. These anomalous results are most likely due to the small sample size in this subset of third instar antlions; although the LRN condition consisted of 15 antlions, the CON condition consisted of only 6 antlions. This small subject pool may not have provided adequate power to uncover potential differences between extraction efficiency rate across condition and time.

Extraction efficiency rate appears to increase at a greater rate from the first to middle block in $I3_P$ LRN subjects, in contrast, extraction efficiency in $I3_P$ CON subjects remains relatively constant between the first and middle block of training. While not significant, this change suggests increased extraction efficiency rate in LRN subjects during the first half of the experiment. Perhaps extraction efficiency rate isn't the highest at the end of training; as subjects prepare to molt/pupate they may spend less time eating and, consequently, extraction efficiency rate may decrease or plateau. With the exception of $I3_P$ CON subjects, all extraction efficiency rates for the last block were lower than the middle block. In this case, the final block of training wouldn't be representative of each subject's maximum extraction efficiency rate. A comparison of the highest extraction efficiency rate for each subject might produce more significant results.

A larger increase in extraction efficiency rate between first and middle blocks in $I3_P$ LRN subjects is evidence of learning. Rates of prey digestion depend, among other things, on the quantity of enzyme present; the release of enzyme is usually stimulated by the presence of food (Griffiths, 1982). Therefore, extraction efficiency is primarily determined by the amount of enzyme that is injected into the prey upon first capture. Feeding efficiency rate depends on initial injection of a sufficient amount of enzyme because extraction becomes increasingly difficult as the feeding progresses and, therefore, limits an antlion's ability to inject a second round of enzymes (Griffiths, 1982). Feeding time consists of three periods: a) injection of enzymes, b) followed by a digestive pause, and c) extraction of the food (Griffiths, 1982). Feeding time should depend on the amount of material to be digested and not the quantity to be extracted (i.e., the rate at which food is sucked out of the prey is not limiting). In light of this information, if antlions learn to associate a cue with forthcoming food, then one would expect that antlions in the LRN condition should be better prepared for prey capture, enzyme injection, and prey digestion. More efficient feeding could result in more food extraction and nutrition, which in turn would enable antlions in the LRN group to develop faster.

Several instances exist in which learning may have occurred, but wasn't detectable using the measure of extraction efficiency rate. For example, anticipation of the forthcoming prey, as a result of learning, might have caused antlions to inject more enzymes into the prey. A sufficient enzyme injection upon first contact with the prey would result in more efficient and thorough extraction. As a result of faster prey consumption, the antlions in the LRN condition would be able to consume more prey per day and this would confer the advantage of increased growth resulting in faster pupation and development to sexual maturity. Third instar antlions are more developmentally advanced which corresponds to larger body size, allowing them to capture larger prey. Capture success of prey varies; larger antlions with larger pits can generally capture a wider variety of prey types and sizes than can smaller antlions (Crowley & Linton, 1999). If third instar antlions have the capacity to produce larger volumes of enzyme to capture larger prey items, then, as a result of learning, they may be able to learn to produce the same larger amount of enzyme for any prey item that follows presentation the vibratory cue, regardless of size. This capacity to produce larger amounts of enzyme may result in the observed increases in extraction efficiency rate across blocks. On the other hand, there may be a ceiling effect for the volume of enzyme that second instar subjects can produce, thereby limiting the range over which extraction efficiency rate can increase.

Feeding time and extraction efficiency are functions of prey density (Griffiths, 1982). If the density of the prey items differs uncontrollably, then extraction efficiency rate may vary from day-to-day, depending on prey density, and not be representative of the amount of nutrients extracted. In the current experiment, differences in prey density and nutritional value may have been confounded by the fact that antlions were fed mealworms, which undergo a series of molts as they grow and develop. As the experiment proceeded, changes in prey size and structure may have affected the nutritional value and ease of extractability of the prey. Other factors may have affected extraction efficiency rate. For example, some parts of the prey are more nutritionally profitable than others; the head and thorax of prey are more difficult to consume than the abdomen (Griffiths, 1982). Although antlions in the current experiment were fed only the head portion of a prey item, antlions in the LRN condition may have learned to extract more mass from the profitable parts of the head and less mass from the less profitable parts. If the mass extracted by the LRN subjects was more nutritionally profitable, then this increased nourishment conveys an advantage, regardless of whether or not a difference in the amount of mass extracted existed. In addition, if LRN subjects were able to expend less energy during prey extraction, then this energy conservation would convey an advantage because they would have greater energy gains from equal amounts of mass extracted.

It is possible that subjects in the LRN condition metabolized prey differently and were able to extract more nutrients from the same prey mass. However, in a starvation experiment in which different ages of antlion larvae were fed consistently for 12 days and then unfed for eight days, Griffiths (1981) did not detect any age related metabolic responses in *Macroleon*.

In future experiments, care should be taken to ensure that all subjects receive prey items of the same developmental stage across the entire experiment.

Sand-Throwing Measure

I hypothesized that antlions in the LRN condition, in which the vibratory cue immediately preceded food presentation, would demonstrate an anticipatory conditioned response, such as increased sand-throwing movement, that would increase the effectiveness of prey capture. The data support my hypothesis. Overall, significantly more LRN subjects threw sand at least once during presentation of the vibratory cue as compared with CON subjects.

Sand-throwing behavior is commonly documented during prey attack and capture. Napolitano (1998) describes 12 discrete predatory behaviors exhibited by antlions, the first of which is “attack”. During the “attack” phase, an antlion’s head rapidly moves forward while closing the mandibles, and is often flicked rapidly back, expelling sand from the pit. Griffiths (1980) also documents this sand-throwing behavior in conjunction with prey capture; should prey prove difficult to capture and start to climb out of the pit, antlions toss sand with violent flicks of their heads, thereby causing miniature landslides that carry the prey back to the vertex of the pit where an antlion is waiting. Topoff (1977) further discusses how the antlion responds to the mechanical stimulation that results when prey try to escape from the pit by flicking its head upward and showering the prey with lose sand to help aid capture.

The vibratory cue used in the current experiment mimicked sounds that might be detected in nature; however, this cue was not initially associated with forthcoming food. At the start of the experiment, antlions had no unconditioned response to the vibratory cue, as supported by the fact that there were no significant differences in sand-throwing between LRN and CON conditions on the first day of the experiment and for the subsequent five days. The purpose of this experiment was to determine if antlions could learn to associate the vibratory cue with forthcoming food. The fact that significantly more LRN antlions threw sand as compared with the CON antlions suggests that learning did occur. The LRN subjects developed a sand-throwing behavior, which in nature is indicative of prey capture, in response to the learned association of a vibratory cue and forthcoming food.

While no significant difference was found for experimental condition and sand-throwing responses in second instar subjects, a significant association between experimental condition and sand-throwing response was found for third instar subjects. A significantly greater number of third instar subjects in the LRN condition threw sand as compared with subjects in the CON condition.

The data show that the more developmentally advanced third instar antlions threw sand but the second instar antlions did not throw sand. Differences in sand-throwing behavior could be the result of differences in pit construction across second and third instars. That is, differences in the pit morphology and prey capture strategies have been documented between the three instars of antlion larvae. For example, Griffiths (1986) found that first and second instar larvae construct steeper walled pits than third instar larvae. As a result, capture success in the first two instars increases rapidly with pit size but in the third instar the relationship is weaker. For a given pit volume, an antlion can maximize either capture success or prey encounters but not both since an increase in pit circumference necessitates a decrease in pit slope, and *vice versa*. Third instar larvae construct pits with larger diameters, and less steep walls to maximize encounters with larger prey. This may necessitate a more active prey capture response – if it is easier for prey to escape from third instar pits, then it would be advantageous for third instar subjects to throw sand in order to aid in prey capture. On the other hand, if second instar antlions have steeper pits that aid in capture success, it would be detrimental to expend energy on sand-throwing behavior when the construction of the pit itself will ensure that the prey can't escape.

Yet another difference between second and third instar antlions that could have contributed to differences in sand-throwing behavior is their metabolic demands. Antlion growth rate appears to be strongly food limited (Crowley & Linton, 1999). Third instar antlions are larger than second instar antlions and, presumably, require more food consumption to meet their metabolic demands. In the current experiment, second and third instar subjects received the same amount of food, which corresponds to a smaller proportion of food-to-body weight in third instar subjects. Perhaps the amount of food that was presented to the third instar antlions in the experiment was not sufficient, resulting in hungry third instar antlions. If third instar antlions didn't receive sufficient food, then it would make sense that third instar LRN subjects that could predict forthcoming food based on associative learning would exhibit sand-throwing behavior to maximize effectiveness of prey capture. In the current experiment, learning may have been more beneficial for third instar larvae because maximizing the consumption of the limited food that they received was crucial to development. To test this hypothesis, different groups of third instar larvae could be fed with prey items of different masses. One would predict that third instar larvae that receive the smallest prey mass would exhibit more sand-throwing behavior than those that receive the largest prey mass.

Survival Analysis

I hypothesized that antlions in the LRN condition would demonstrate greater fitness as measured by faster development and molting/pupation. The survival analysis data support my hypothesis. Overall, LRN animals molted/pupated faster than CON animals. When the survival analysis was split by instar, I3 LRN animals were found to pupate in significantly less time than I3 CON animals, while I2 LRN animals did not molt in significantly less time than I2 CON animals.

Faster pupation is indicative of learning because subjects are able to predict and prepare for forthcoming food and maximize its nutritional benefits to develop faster. An anomaly exists in the results of the current study with respect to the significant survival analysis for I3 LRN subjects, and the non-significant extraction efficiency rate and pit volume measures.

Pupation is strongly size-dependent, large larvae are more likely to pupate than small larvae (Griffiths, 1980). However, is larval size weight-dependent or length-dependent? Griffiths (1985) describes how the minimum size for pupation appears to be determined by the quantity of fat stored for pupal consumption and, thus, weight-dependent. Large third instar larvae store energy as fat versus protein since they have the non-feeding pupal stage to survive through. This differential allocation of energy could affect both the relative performances of different sized larvae (i.e., small larvae may be more efficient than they appear) and the shape of the growth curves (i.e., large larvae grow slower than small larvae) (Griffiths, 1980). If size is weight-dependent, then instead of growing larger, third instar antlions may be converting more food to fat versus protein in order to prepare for pupation. Hence, antlions may be increasing in weight, but not size. An increase in weight does not necessitate an increase in pit volume. Griffiths (1985) found that initial pit diameter increases with larval size up to 100 mg but declines above this size; > 100 mg larvae often have reduced pit volume. Griffiths (1985) hypothesized that reduced pit volume in larger larvae reduces energy expenditure; the larvae have accumulated sufficient energy (in the form of fat) to successfully reach the adult stage and should not spend much energy on pit maintenance. These findings suggest that pit volume may not be an accurate measure of large third instar antlions that are preparing to pupate.

If as a result of learning, antlions are able to convert more of their food to fat in preparation for pupation then I would not expect pit volume to increase in $I3_P$ LRN subjects. Instead, as a result of learning, I would expect $I3_P$ LRN antlions to convert more of their food to fat and, consequently, to have smaller pits to conserve energy and prepare for the pupal stage.

Future studies should monitor subjects through adulthood to determine if faster development and pupation resulted in the production of a larger number of viable offspring.

Pit Measures

I hypothesized that antlions in the LRN condition would demonstrate greater pit volume than antlions in the CON condition. The data do not support my hypothesis. Pit volume significantly increased across days for all subjects, which is consistent with the fact that as antlions grow, their pits increase in size; thus, pit volume is closely correlated with antlion size (Heinrich & Heinrich, 1984). Consequently, I can presume that all subjects were growing throughout the experiment.

There were no significant differences in pit volume between LRN and CON conditions; while $I2_M$ subjects in the LRN group appear to have larger pit volumes than subjects in the CON group, this difference was not significant. Surprisingly, subjects in the $I3_P$ LRN condition appear to have smaller pit volumes than subjects in the CON condition, however, this difference also was not significant. These data are not consistent with the survival analysis results which show that $I3_P$ LRN subjects pupated faster than $I3_P$ CON subjects. One would expect $I3_P$ LRN subjects to be larger, resulting in faster pupation. Since pit volumes are correlated with antlion size, such that the larger the antlion, the larger the pit (McClure, 1976; Griffiths, 1980; Mansell, 1992; Crowley & Linton, 1999; Day & Zalucki, 2000), it would follow that $I3_P$ LRN subjects would be expected to have larger pits. However, as previously discussed, if $I3_P$ LRN subjects were preparing for pupation, their pit volumes may not have increased as hypothesized.

Larger pit volume results for $I3_P$ CON subjects, while not significant, are consistent with the extraction efficiency rate results, in which $I3_P$ CON subjects tended to have higher extraction efficiency rates than $I3_P$ LRN subjects. However, it is likely that small sample size accounts for this anomalous result; the pit volumes for $I3_P$ CON subjects were based on measurements for only five antlions. That is, these five subjects may not be representative of all third instar CON subjects.

Pit volume should not be used as the only method of determining if learning occurred because pit volume is variable within subjects. The volume of the sand pits constructed by individual larvae can vary considerably from day to day, and some large animals even construct very small pits before molting or pupating (Griffiths, 1986). According to Griffiths' (1986) observation, the final block of training may not be representative of each subject's maximum pit volume. A comparison of the highest pit volume for each subject may produce more significant results.

Limitations and Directions for Future Research

Twenty-three of the 56 antlions moved their pits during training. The majority of these pits were relocated to the edges of the plastic container, suggesting that subjects sought out the container wall. Similar behavior was observed by Boak and Visscher (1984), in which antlions placed in the center of a sandy plot moved towards the edges of the plot. Perhaps this preference for and movement to the edges of a plot is in an effort to find shelter. Gotelli (1996) observed that antlions built pits along cliff edges which provided protection and shelter from wind and rain. These results suggest that the benefits of protection provided by pit walls might outweigh the costs of incomplete pits and possible prey escapes. In future research, a container with sloped walls could be used to prevent antlions from building their pits against a wall.

Future research should investigate associative learning in antlions across all three instars in order to determine if differences in learning exist at different developmental stages. In addition, experiments should investigate whether learning persists across instars.

Conclusions

Associative learning should be the foundation for our understanding of other forms of behavior and cognition in human and non-human animals. By extending insect learning research to encompass sit-and-wait predators, namely antlions, I'm advancing the study of insect learning in new directions. The findings of this study suggest that associative learning does occur in antlions and that it confers the advantage of increased fitness and faster development. The current study also suggests that learning may be dependent on developmental stage.

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