

Running head: THE ROLE OF LEARNING IN THE FEEDING BEHAVIOR

The Role of Learning in the Feeding Behavior of Antlions

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## Abstract

Although previous research has demonstrated that insects are indeed capable of responding to a learned food signal, those studies focused mainly on insects with active methods of predation. The present study investigated the role of learning in the feeding behavior of antlions (*Myrmeleon immaculatus*), a sit-and-wait predator. The study used 24 antlions and consisted of three phases. In the Pre-training Phase (4 days), each antlion was placed in an individual bowl within an enclosure and allowed to dig a pit and feed once daily. During the Training Phase (16 days), antlions received one of two treatment groups: Pavlovian (PAV) or Control (CON). The PAV treatment group received 5 s of sand dropping next to each animal's pit preceding by the presentation of food. The CON treatment received 5 s of sand dropping next to each animal's pit at one time of the day, and presentation of food at another, different time of the day such that these events independent of one another. On the Test Day (Day 17), antlions from both groups were exposed to 5 s of sand dropping followed by the presentation of food. Measures of the pit (depth, diameter and volume) and prey consumption (extraction rate, extraction efficiency and extraction efficiency rate) via extraction, which refers to the amount of prey fluid removed from the carcass, were obtained. A comparison of groups' feeding behaviors during the Training Phase, as well as between the Training Phase and Testing Day, revealed that antlions respond to learned food signals by an increase in extraction efficiency, extraction rate, pit diameter and pit volume.

## Introduction

Previous research has established that vertebrates are capable of learning (Tully, 1983). More recently, research involving honeybees and octopi has revealed that invertebrate and vertebrate perform similarly in learning experiments (Quinn, 1974; Menzel and Erber, 1978). Such findings suggest that some central similarities exist between vertebrate and invertebrate learning (Carew and Sahley, 1986). Therefore, They are both capable of changing behavioral responses by forming associations between novel cues and biologically important events such as obtaining food, water and mates (Sahley, 1995). In fact, work with invertebrates has begun to reveal compelling details regarding biochemical and physiological events associated with learning (Tully, 1983). Insects are a particular invertebrate group that has proven capable of learning. They are capable of learning to respond to a signal with a classically conditioned response (McGuire, 1984). In such a process, an initially neutral stimulus or signal, the conditioned response (CS), is repeatedly paired with an unconditioned stimulus (US), which already elicits a response, the unconditioned response (US) (Hollis, 1982; Hawkins et al. 1983; Domjan, 2005). The classically conditioned response tends to be directed toward the signal and often precedes the biologically important event (Hollis, 1982; Hollis, Dumas, Britton & Field, 1997). Insects are, of course, both physiologically and morphologically equipped to respond to certain critical biological events without learning. However, insects can optimize these interactions by associating particular signals with these events and responding

to signals that announce the appearance of the events. Therefore, learning functions as a biological mechanism for preparation.

The association formed between the CS and the US occurs only if the CS serves as a predictor for the US (Hollis, 1982). Thus, learning can be understood as a process that allows a signal to elicit appropriate preparatory behavior before a biologically significant event (Timberlake, 1983; Brembs, 2000). The CR is often similar in nature to the UR (Hollis, 1982).

Consequently the way in which the insect approaches the signal tends to be characteristic of the forthcoming event. For example, if the UR is of appetitive nature, such as feeding, the CR often reflects a behavior that occurs during the actual presence of the food (Hollis, 1984). For example, normal feeding behavior of moths and honeybees involves extension of the mouthparts, namely, the proboscis, when a distinctive odor (CS) precedes the presentation of a small drop of sucrose solution (US), the CR is an extension of the proboscis. Upon exposure to the same odor after training, extension of the proboscis (CR) will continue to occur even without the sucrose present (Dukas, 1995; Gerber et al. 1996; Daly & Smith, 2000). In this case, the signal itself elicits the feeding behavior.

In addition to eliciting feeding behavior upon the presentation of a particular signal, an insect will also move toward and contact a stimulus that predicts food. For example, when honeybees are repeatedly exposed to a specific flower color (CS), which precedes the presentation of sucrose (US), they will fly to the flower of the color originally paired with sucrose, even if

the flowers of different colors with sucrose are present (Couvillon, Leito and Bitterman, 1999; Dukas, 1993). Also, when cockroaches are exposed repeatedly to a specific odor that precedes the presentation of sucrose (US), they will move toward that odor and remain in the area where that odor is located even without the presence of sucrose (Chen, Aranda and Luco, 1970; Sakura and Mizunami, 2001; Wantabe, 2003). The approach (CR) is elicited by a signal that has been reliably paired with the presence of food.

Insects will also discriminate among certain stimuli in order to select for a nutritionally valuable food source (Behmer, Elias & Bernays, 1999). For example, locusts discriminate among cues based on their nutritional needs. After repeated exposure to colors (CS) paired with food rich in protein or carbohydrates (US), the carbohydrate-deficient will move toward the color previously associated with food rich in carbohydrates. Locusts deficient in protein will move toward the color previously associated with food rich in protein. In other words, locusts employ learning for the selection of nutrient-specific foods (Raubenheimer & Tucker, 1996; Simpson & White, 1990). Similarly, honeybees that are repeatedly exposed to colors paired with a particular concentration of sucrose develop a preference for the color that was originally paired with the highest concentration sucrose (Couvillian et al. 1990; Gould, 1993).

Although these studies primarily demonstrate that an insect learning capacity, several researchers have suggested that the conditioned response may play an important role in nature. The adaptive value of learning is, "...to

enable an animal to optimize interactions with forthcoming biological events...”(Hollis, 1982). Animals able to associate signals in their environment, such as color or odor with a biologically significant event, such as food, are likely to have an advantage of obtaining more resources than animals that do not make those associations. For example, Dukas and Bernays (2000) exposed grasshoppers to an environment that either provided a balanced diet (suitable for growth) or a carbohydrate-deficient diet (unsuitable for growth). Some of the grasshoppers were provided with a learning procedure in which specific colors were paired with nutrient-specific foods while other grasshoppers were not. Grasshoppers who were able to employ such learning for diet choice experienced higher growth rates. While it may seem intuitive that a biological mechanism for preparation should contribute to the success of the insect in terms of health and reproductive potential, the grasshopper study is one of the first to provide experimental data that has directly shown improved fitness from learning in insects.

The subjects were exposed to a learning procedure in which behavioral changes were used to determine the occurrence of learning and the subsequent improvement of fitness. Although many approaches to examining the learning behavior of insects exist, for this study I chose to utilize antlions as the subjects. I will briefly describe a form of foraging behavior known as sit-and-wait predation and describe some of the factors that influence such a method of predation by providing specific examples of animals (including insects) that exhibit sit-and-wait predation. I also describe feeding behaviors specific to



antlions and factors involved in the success of a feeding episode. Most importantly, I describe how the use of learning could influence the success of sit-and-wait predation in antlions.

#### *Sit-and-Wait Predation*

Certain predators, including some insects, move continuously through their foraging area, while others tend to be inactive and remain in a particular location, such as sit-and-wait predators. Sit-and-wait predation is a method of foraging behavior in which the animal obtains prey passively. In other words, rather than actively pursuing prey, the animal constructs capture devices such as webs, snares or pits and waits for prey to come to it and it then ambushes the prey (Lawrence, 1985; Crowley & Linton, 1999). Therefore, the process of sit-and-wait predation relies on active prey. Animals that exhibit sit-and-wait predation tend to spend months in a single place. They often live in areas of low prey density and extreme environments such as deserts (Griffiths, 1982; Hassel and Southwood, 1978). The sit-and-wait method allows for predators to hide from extreme temperatures and conserve as much moisture as possible. Sit-and-wait predators tend to use digestive enzymes injected by means of biting the prey to subdue their prey, which is generally. Field studies of sit-and-wait predators, mainly ecological, have implicated food as a major factor limiting the survival and maturation of this distinct class of predators (Lawrence, 1985). Therefore, if prey density is too low, the animal must relocate; however, changing locations also has major implications on its survival (Griffiths, 1980; Crowley and Linton, 1999). By changing locations, sit-and-wait predators face important energy losses. For example, the spider, as

a sit-and-wait predator, captures prey by weaving a web. If the web gets destroyed or if prey density becomes too low, then the spider will move its web to a different location. The activity involved in weaving the web and finding a new location is costly in terms of energy (Borror, Triplehorn & Johnson, 1989). Thus, it is likely that spiders rely on factors, such as signals, from the environment as determinants of the web location and further relocation in order to minimize energy losses.

As described above, A sit-and-wait predator interacts with its environment and utilizes signals in its feeding behavior to minimize energy loss. These behaviors could provide insight into how learning as a biological mechanism for preparation influences the survival and the reproductive success of a sit-and-wait predator. Studies have shown that sit-and-wait predators are biologically equipped to detect small changes in their environment, such as vibrations, temperature and sand-moisture content. Studies of scorpions, sand-dwelling sit-and-wait predators, have shown that they use information propagated through compressional and surface waves in the sand to locate their prey and to determine its size and distance (Brownell, 1977).

Dragonflies, another sit-and-wait predators, usually ambush their prey in flight. Studies have found that dragonflies direct their flight path to a point in front of the prey, which suggests that they are somehow able to predict the flight path of their prey. High foraging success rates confirm that such behavior is not the result of dragonflies simply taking off after large, far-away

objects as readily as nearby insects. It appears that such a process of prey capture relies on environmental cues that dragonflies use to determine factors such as angular velocity in order to predict the flight path of their prey (Olberg, Worthington & Venter, 2000). The intimate relations with the environment of the Dragonfly and the scorpion suggest that these animals may not only establish associations between signals and biological events, but also depend on them. Therefore, learning might be a key mechanism in the success of sit-and-wait predation.

Little beyond the influence of prey density is known about factors that govern the sit-and-wait predation (Lawrence, 1985; Gotelli, 1996). However, these previous studies allude to a situation in which learning is likely possible and part of the process of foraging. That is, sit-and-wait predators use environmental signals; those signals may be associated with a particular event (such as prey distance or size). The establishment of such an association may result in a subsequent learned response to that signal. Learning has been demonstrated to optimize active predation by the strong associations created between signals and biologically important events. This system could result in improved sit-and-wait predation as well, by providing these animals with the capability to become more involved with their environment.

### *Antlions*

As sand-dwelling insects, antlions are a classic example of a sit-and-wait predator. They are found all over the world, primarily in warm regions, including North America. There are over 2,000 species of antlions in the

world. The common pit-building antlions found in the eastern United States build virtually identical pits and the geographic ranges of several of these species overlap (Lucas, 1989). A majority of the research and information presented in this paper refer to antlions of North America. The behaviors described are generalized to this population of antlions.

At 1.5 cm, these sand-gray insects are circular in shape with a majority of their body mass in the bristle-covered abdomen. These bristles allow for antlions to sense vibrations caused by prey, predators or changes in the environment (Farji-Brener, 2003). Unlike other sand-dwelling insects that remain in the sand for months at a time, antlions remain in the sand for only a few weeks allowing for the study of sit-and-wait predation during shorter periods of time.

Antlions have two major stages in their life cycle, the larval and the adult stage. During the larval stage, the primary activity is feeding. Once antlions achieve a certain energy intake they cocoon and change into adults. As adults, antlions are nocturnal, short-lived, feeble flyers, their primary activity is reproduction (Wheeler, 1930; Swanson, 1996). The duration of the larval stage is highly variable. Studies have shown that the transition into the adult state and the subsequent reproduction are dependent on the success of the larval stage in terms of health (growth and development) (Griffiths, 1980). Therefore, the feeding behavior of an antlion in larval form directly affects its reproductive potential as an adult.

Antlions appear to be useful subjects for observing feeding behavior. They are easily kept and fed in the laboratory. Their foraging area in their natural environment is small. Most importantly, as sit-and-wait predators, they are sedentary, thus eliminating one of the most complex features of foraging behavior, namely searching (Griffiths, 1986). Antlions are quite responsive to changes in their environment. For example, they are able to perceive the direction of light and to some degree the position of moving objects (Lucas, 1989). Although antlions tend to remain beneath the substratum to conserve moisture, they respond to prey entering the pit, even during the hottest periods of the day (Griffiths, 1980). Also, they seem to track sources of shade or cover as strongly as sources of food (Crowley and Linton, 1999). Antlions are sensitive to vibrations caused by the pit-digging activities of neighboring antlions and the movement of forthcoming prey and predators. For example, antlions throw substrate a distance up to 0.5m; the distances of sand thrown reflects the spatial distribution of the population (McClure, 1976). Thus, while antlion pit-trap use requires that they remain in the sand at the bottom of their pit, antlions are still likely to be responsive to the environment above the substrate surface.

Antlions have primarily been the subject of ecological studies investigating pit use, spatial patterns and prey-capture behavior. However, the feeding behavior of antlions should be made the subject of more studies investigating all aspects of foraging behavior. These creatures tend to live in sparse environments under harsh conditions, yet they have been able to

survive and thrive under the employment of this rather unique form of feeding behavior. Clearly, there is more to antlion foraging behavior than pit construction and prey capture. There are a number of underlying mechanisms within this strategy of feeding behavior that exploit the harsh environments and allows for the success of this creature.

### *Antlion Feeding Behavior*

*Pit construction.* The sit-and-wait predatory behavior of an antlion involves the construction of a conical pit. To construct a pit, an antlion moves backwards, forming a circle on the soil surface. Then, digging deeper, it spirals toward the center, loosening soil particles, which are thrown out of the pit with a flicking motion of its head. Finally, it buries itself at the bottom of the pit with only its head and mandibles visible (Farji-Brener, 2003). The pit not only provides a tool for the capture of their prey, but it also removes antlions from extreme temperatures and predators (Wheeler, 1930; Swanson, 1996). The features of an antlions' pit are not determined solely by the physical properties of the substratum, but rather changes in the pit parameters such as pit depth, slope and diameter, have been found to be of biological significance. That is, the energy an antlion invests into changing the parameters of the pit determines capture success and ultimately the net gain of energy.

Construction of a pit is energetically costly for antlions. Although they can dig pits in coarse-grained soil, antlions always select fine-grained soil patches to dig their pits. For example, antlions that are housed in containers

with only coarse-grained soils do not dig pits, but rather bury themselves in the sand. Such a finding is likely due to the fact that digging a pit in coarse-grained soil requires a greater investment of energy than in fine-grained soil (Farji-Brener, 2003). This behavior of habitat selection reflects the cost-benefit relationship of digging a pit. Cost reflects the energy expended to dig the pit, and benefit refers to the energy gained from prey capture (Griffiths, 1986; Krebs and Kacelnik, 1991). By constructing their pits in a fine-grained medium, antlions selectively dig for higher benefits and lower costs than in the coarse-grained soil. Pits dug in fine-grained soil are 50% wider, 50% deeper and 100% more efficient in capturing prey than pits dug in coarse-grained soil (Farji-Brener, 2003).

Such a balance of costs and benefits in pit construction has been found to influence the fitness of antlions. That is, the energy spent constructing a pit (metabolic expenditures) is higher than energy spent at rest (resting metabolic rates) (Lucas, 1985; Farji-Brener, 2003). The more time spent on pit construction, the more energy is used. If the energy lost in construction is greater than the final gain of energy from prey capture, the net energy loss is detrimental to the health of the antlions. For example, the growth rate and mean weight of antlions whose pits are disturbed are 50% lower compared to undisturbed antlions of similar body size (Griffiths, 1980).

Several features of the pit, such as depth, diameter and slope (angle) influence the efficiency of prey capture (Wilson, 1974; Griffiths, 1980; Farji-Brener, 2003). For example, prey density affects both pit size and probability

of movement (relocation of pit) (Heinrich & Heinrich, 1984). The pit diameter and depth influence capture success by affecting the probability of prey encounter and prey capture (Farji-Brener, 2003). For example, a 2 mm increase in diameter results in a 10% increase of capture success, regardless of prey size and species (Griffiths, 1986). Other benefits of constructing a larger pit exist, such as producing a wider variety of prey captured, and decreased time between feedings. However, a wider pit means a shallower slope and thus an increase in the probability of prey escape.

As the slope of the pit walls increases (steeper) capture success also increases (Griffiths, 1986). The depth of a pit and the angle of its cone shape determine the distance from the vertex of the pit to the soil surface. The depth determines how long a prey item will remain in the pit and consequently how likely the prey will be captured (Lucas, 1982). If the slope of the pit is shallow, the prey can more readily escape. Thus the prey encounter will require more energy investment in capturing the prey. If the slope is steeper, the prey is much less likely to escape, because it is much more difficult to climb out of the pit. Thus, less energy is required in a single prey encounter. Feeding behavior clearly involves a balancing act of obtaining the right dimensions for the pit. For a given pit volume an antlion can maximize either capture success or prey encounters by altering the slope and diameter of the pit (Griffiths, 1986).

For many years, researchers believed that spatial distribution of an antlion population had a strong influence on pit construction and feeding



behavior of the antlion (Crowley and Linton, 1999). For example, antlions do respond to population density by constructing pits at a maximal distance from neighboring pits, but the diameter and size do not reflect population density (McClure, 1976). Therefore, it appears that antlions do not construct their pits at the parameters of a given population, but rather in terms of their feeding needs. However, when antlions densities are high, cannibalism will occur, especially if prey density is low and pit relocation is common (Lucas, 1989). Also, as previously discussed, pit size has been shown to increase with an increase in disturbance to the pit (Heinrich & Heinrich, 1984). If disturbance occurs due to high population density then the pit will likely be affected. For example, in regions of high population density, disturbances, such as sand throwing from neighboring antlions, tend to be high. Such disturbances result in antlions of smaller sizes (body mass) and smaller pits (Lucas, 1989). Therefore, population density does not influence pit size unless it directly disturbs the construction or maintenance of the pit.

Although antlions, as sedentary predators, are unable to track prey abundance, they can modify the parameters of their pit to improve capture efficiency under given environmental conditions, such as low prey density or high population density (Griffiths, 1980). Clearly, pit construction behavior serves as a potential measure for the feeding behavior of antlions.

*Feeding.* Antlions lie buried at the vertex of their pit before feeding. Generally, antlions will remain at the bottom of the pit unless they relocate their pit, which occurs only every couple of weeks. While waiting at the

bottom of the pit, antlions tend to remain out of the visibility range of any encountering prey or predator (Farji-Brener, 2003). Antlions feed by capturing prey that falls into their pit. Antlions are insectivorous; their prey includes beetles, flies, mites and wasps (Griffiths, 1980). Once the prey has fallen into the pit, antlions drag it under the sediment surface. Antlions digest the prey externally by injecting digestive fluids into the abdomen of the prey. The digested prey fluids are then extracted from the prey and consumed through the mandibles, which directs fluid to antlions' mouth (Crowley & Linton, 1999). Prey encounters are infrequent and antlions generally accept all prey items provided that they result in a net energy gain (Griffiths, 1980). If an antlion fails to grab prey, it tosses sand with violent flips of its head, creating landslides that carry the prey back into the mandibles (Wilson, 1974). Such behavior is called sand throwing. The factors that determine antlion feeding behavior have been the subject of many ecological studies of which the primary determinants are prey encounter and capture success (Griffiths, 1980, 1986; Crowley & Linton, 1999).

*Extraction.* Although the parameters of the pit are critical to the success of a feeding episode, there are other factors involved. The actual consumption of the prey represents the ultimate determinant of energy gain for a given feeding event. As sit-and-wait predators, antlions depend on active prey to fall into their pit. Unfortunately, the environments in which antlions live tend to be low in prey density (Gotelli, 1996). The success of consumption of a prey item is reflected in the food (prey mass) extracted from

the prey and the time spent in that extraction (Cook and Cockwell, 1978). That is, the more prey consumed in a given encounter, the longer an antlion will go before the next feeding. The less time spent on extracting body mass from the prey item, the less energy spent on consumption. Essentially, measurements in extraction and time in feeding reflects the net gain in energy, namely calories gained minus calories spent.

Extraction refers to the amount of mass (fluid) removed from the carcass of a prey item. Measures previously used in other studies quantify the net gain of energy upon a single prey encounter combine extraction with the time it takes to consume and throw a carcass from the pit. Such measures include extraction efficiency and extraction rate. Extraction efficiency refers to the amount of prey mass ingested for a given prey item upon a single prey encounter calculated as the initial weight minus the final weight of the prey item, divided by the initial weight of the prey item. This essentially provides the percent of prey mass consumed by the antlion. Several ecological studies have used this measure as a means of determining the net gain of energy in a single prey encounter (Griffiths, 1980; Paul and Roces, 2003). For a given prey mass, extraction efficiency will not change under differing hunger levels.. An increase in extraction efficiency would signify that for a given prey mass, more mass was consumed. For example, antlions feeding on a prey item of a given mass under extreme starvation conditions, mild starvation conditions or normal (i.e. regular feeding) conditions do not differ in extraction efficiency (Griffiths, 1982).

The extraction rate refers to the total mass of the prey item consumed per unit time. That is, it serves as a measure of the rate of energy intake (Roces, 2003). An increase in extraction rate would signify that for a given feeding episode, an antlion would consume more fluid per unit of time. The actual act of consumption requires energy, thus if high quantities of prey mass are consumed at lower intervals of time, less energy is expended and more energy is gained. Ultimately, this results in a higher net gain of energy.

It is possible to bring the extraction efficiency and extraction rate into one measure, which for this study will be called extraction efficiency rate. Such a measure refers to the extraction efficiency of a given prey item per unit time. For a given unit of time, an antlion feeds at a particular efficiency. An increase in extraction efficiency rate would indicate that an antlion is consuming a prey item more efficiently by consuming more fluid of a given mass per unit time. Such a measure has not been used in previous studies, but as a combination of two previously used measures it could potentially provide data that measures the overall feeding behavior of an antlion. Therefore, upon the introduction of a reliable signal for feeding, extraction efficiency, extraction rate and extraction efficiency rate could serve as measures in potential changes in antlion feeding behavior.

*Transition to the adult stage of life.* In order to demonstrate that learning positively influences antlion fitness, it must be assumed that antlions are capable of changing feeding strategies to maximize net energy gain (Griffiths, 1980). Such assumptions can be demonstrated through measures

involved in the feeding behavior of antlions such as pit parameters (depth, diameter and volume) and extraction measures (extraction efficiency, extraction rate and extraction efficiency rate). However, in order to demonstrate that such changes positively influence fitness, a physiological measure of health must be paired with the feeding measures. Such physiological measures must be connected to the ultimate determinate of fitness, reproductive potential. Previous research has quantified fitness by measuring changes in growth rate. For example, grasshoppers that were able to employ learning displayed a positive change in fitness by obtaining higher growth rates than grasshoppers that were not able to learn (Dukas and Bernays, 2000).

Growth rate is a factor involved in determining the length of a given stage in the life cycle of several insects, including antlions. That is, the time at which an insect transitions from the larval stage to the adult stage can change depending on the growth rate of the insect. For example, antlions that are fed well grow more rapidly than those that are fed sporadically, which ultimately results in a shorter time in the larval form (Griffiths, 1986; Crowley and Linton, 1999). For antlions to metamorphose, they must meet a particular energy quota (Crowley and Linton, 1999). If feeding results in maximal net gain in energy, then antlions that exercise optimal foraging behavior will spend less time in the larval form. Therefore, a way in which learning might influence fitness is by increasing growth rate through improving feeding behavior. Because antlions are much more vulnerable to predation,

environmental extremes and starvation in the larval form than in the adult form (Crowley & Linton, 1999), faster growth rates increase fitness by reducing generation time. The faster transition into the adult stage of life, in which reproduction occurs, implies an increase in reproductive potential because with less time in the larval form, the larva is less likely to encounter factors that would prevent reproduction from occurring.

The feeding behavior that occurs during the larval stage of an antlion's life cycle clearly sets the stage for reproductive success in antlions. Changes in feeding behavior could potentially influence transition into the adult form. Therefore, in order to quantify the adaptive value of learning, it appears that measuring the transition into the adult stage through observing growth rates would serve as an accurate measure.

#### *Current study*

The current study explores the role of learning in the feeding behavior of antlions. Such a study provides a new perspective in the theory of learning as a biological mechanism for preparation because antlions have never been used as the subject of a learning study before. Previous literature has demonstrated that insects are capable of learning and that learning enables insects to optimize interactions with forthcoming biological events. However, only limited forms of foraging behavior, namely active predation, have been closely explored in insects. By exploring learning in sit-and-wait predators such as antlions, further insight into the changes in behavior that result from the establishment of a reliable signal and an important event can be explored.

I measure learning by analyzing changes in variables critical to antlion feeding behavior. Extraction efficiency, extraction rate and extraction efficiency rate were used as key measures of consumption (Cook & Cockwell, 1978; Griffiths, 1980). Pit depth and diameter were measured in order to quantify the volume of the pit, which served as a measure of energy investment into prey capture (Youth & Moran, 1969; Heinrich & Heinrich, 1984; Griffiths, 1986). Behavioral measures of feeding include sand throwing, jaw position, and pit visibility. With these measures I examined if an antlion could learn to respond to a food signal. Finally, the adaptive value of learning in the feeding behavior of antlions was measured by the differences in the growth rates between treatment groups. In other words, growth rate was determined by how many subjects within each treatment group molted one week following the conclusion of the experiment.

This experimental set-up allowed for three critical questions to be addressed: Are antlions capable of learning? Although previous research has demonstrated that insects indeed are capable of learning, antlions have yet to be subjects of a learning study. The procedure of the present study will allow for the observation in any changes in feeding behavior upon the presentation of a signal that reliably precedes the presentation of food and thus allow for the direct test for learning in antlions. Second, if learning is indeed employed by antlions, does this provide antlion with the ability to improve feeding behavior? Finally, will a change in behavior as a result of the employment of learning provide the means of improving fitness as measured by molting?

## Method

### *Subjects and Apparatus*

Approximately 50 antlions (*Myrmeleon immaculatus*), provided by Ian Skelley of [antlionfarms.com](http://antlionfarms.com) (Pensacola, FL), were used for this experiment. Each antlion was transferred from its container to an individual small plastic bowl filled with 5 cm of aquarium sand. Each antlion was monitored closely for 4 days to ensure that it was healthy. Health was determined by an antlion's ability to dig a pit, to eat live prey (pill bugs) and throw a carcass out of the pit. From a pool of healthy individuals, 24 antlions were chosen. Each antlion was randomly assigned to a separate enclosure on one of four shelves. Each enclosure (See Fig. 1 for illustration of a single enclosure) consisted of a 2.5 cm foam pad on the floor of the shelf and quilting matting on back and sidewalls to insulate for sound. A sand delivery apparatus (see Fig. 1 for illustration of apparatus within an enclosure) was placed next to each antlion's bowl on the foam pad. Each sand delivery apparatus was constructed from wood, wire and a standard plastic dropper. The sand delivery apparatus enabled the sand signal to be delivered for 5 s without the visible presence of the experimenter. Each enclosure was connected to another enclosure by a single wall and a total of 6 enclosures were connected to one another on each of 4 shelves. In addition, a black curtain was hung in front of the shelves from the ground to the ceiling approximately 0.25 m away from the treatment enclosures to minimize the amount of disturbance from experimenters. The curtain contained two slits per enclosure to enable experimenters to film the



antlions and deliver the food and signal. Lights, above the shelf, were on from 0830 hr until 2000 hr daily.

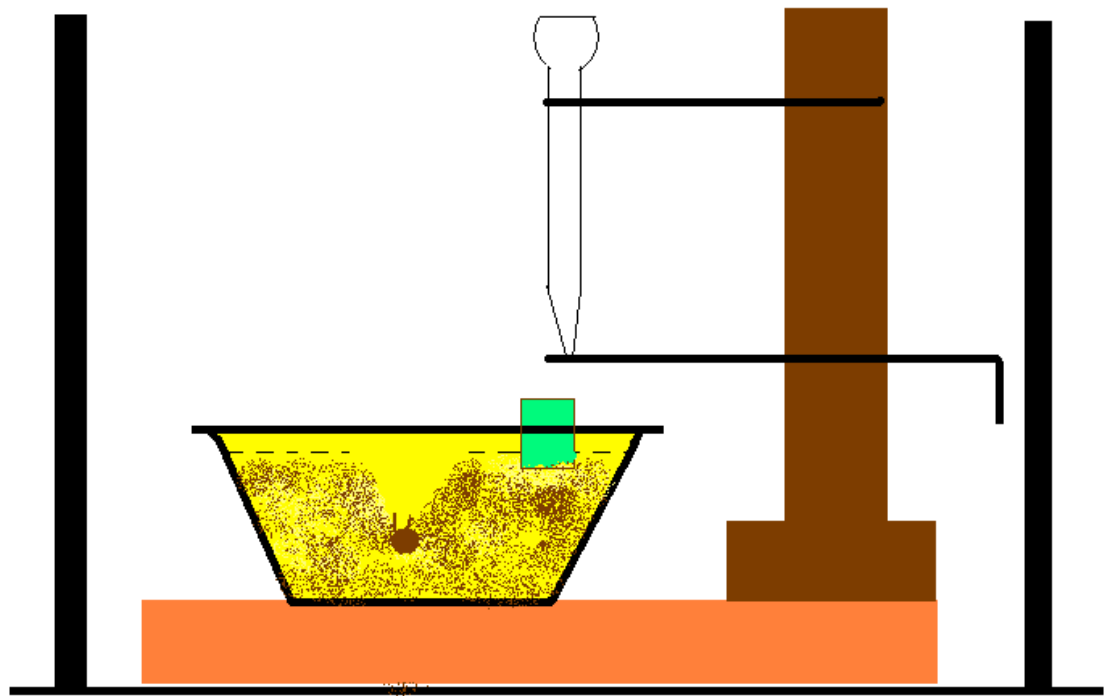
During the Pre-training Phase all antlions were fed pill bugs. The food presented to the antlions of both treatment groups (PAV and CON) during the Training Phase and on Test day was winged fruit flies. In order to prevent the fruit flies from flying away, we removed the wings before they were delivered to the antlions. The fruit flies were de-winged after anesthetizing them with CO<sub>2</sub>. Anesthesia was administered by placing approximately 10 fruit flies into clear tubing, which was attached to a test tube cork. A test tube was filled with ½ teaspoon of sodium bicarbonate. A single dropper of white distilled vinegar was poured into the test tube and the cork with the tube of fruit flies was placed over it (which sealed the tube). The fruit flies remained in the tube for 2-4 minutes in order for the anesthesia to last for at least 5 minutes. The wings were removed with a scalpel and replaced into their holding containers. Fruit flies were anesthetized again, before they were weighed during feeding.

#### *Procedure*

*Training Phase.* Each antlion received one of two treatments, namely Pavlovian or Control. For antlions in the Pavlovian treatment (PAV), a 5s presentation of sand dropping next to the pit (approximately 1 cm away from the rim of the pit) (CS) was preceded by the delivery of food (US) once a day at a randomly assigned time. For antlions in the Control treatment (CON), a 5 s presentation of sand dropping next to the pit occurred at one randomly selected time of the day and the delivery of food at another randomly selected

time of the day. This meant that the signal and food were independent of each other. A schedule was made so that for each day of the Training Phase a time of day was chosen beginning on the hour from 0900 hr to 1900 hr to feed antlions in both groups. The order in which the PAV and CON treatment groups were fed was not fixed. Sometimes the PAV treatment group was fed first, but other times the CON treatment group was fed first. The signal (sand dropping) always preceded food for all antlions in the PAV treatment group. However, the CON treatment group received their signal (sand dropping) at another randomly selected time that was scheduled on the hour from 0900 hr to 1900 hr. This procedure was intended to ensure that the signal was not associated with feeding. For both treatment groups, individuals were videotaped from 10 s prior to the signal presentation, and continued for 10 min after the food delivery. Each antlion pit was checked for a carcass every 2 minutes after the food was delivered until the carcass was thrown from the pit. Once a carcass was thrown, it was weighed to the nearest 0.0001g. Each treatment procedure, PAV and CON, was administered once daily; the Training Phase lasted for 16 consecutive days (see Fig. 2 for illustration of experimental design and procedure).

*Figure 1.* Illustration of a single enclosure (including signal-delivery apparatus)



*Test Phase.* On Day 17, each antlion from both treatment groups (PAV and CON) received the same procedure: for each antlion, a 5 s presentation of sand dropping next to the pit preceded the delivery of food. Each individual was videotaped beginning 10 s prior to the CS and continuing until the antlion threw the carcass from the pit. Each antlion was checked for a carcass every 2 minutes until the carcass was thrown from the pit. Once a carcass was thrown, it was removed from the enclosure and weighed to the nearest 0.0001g (see Fig 2 for experimental design and procedure). The day after the test, all antlions were removed, weighed to the nearest 0.0001g and returned to their individual bowls. Covers were placed over the bowls. Antlions were left to cocoon and metamorphose into adults without any further feeding.

#### *Data Analysis*

The data for this study were collected from one replication that was completed in Spring 2005. Although two experiments were performed, the first experiment had too few subjects at the conclusion of the study and therefore did not have enough power. The data from the replication could not be combined because the Training Phase in each lasted for different lengths of time.

*Training Phase.* During the Training Phase the pit diameter and depth were measured daily. Before food presentation, the food (de-winged fruit flies) was weighed. The time at which the food was delivered and the time at which the carcass was thrown from the pit were recorded and the carcass of

the prey item was weighed after it was thrown from the pit. The extraction was determined by subtracting the final weight of the prey item (food) from the initial weight of the prey item. From these feeding measures the extraction efficiency was determined by the following:

$$\frac{W_{to} - W_{ti}}{W_{ti}}$$

Where  $W_{to}$  is the initial weight of the prey and  $W_{ti}$  is the final weight of the prey item. The extraction efficiency rate was determined by dividing: Extraction efficiency/ time (min) to throw the carcass. Finally, the extraction rate was determined by the following:

$$\frac{W_{to} - W_{ti}}{\text{Time to throw carcass (min)}}$$

Where  $W_{to}$  is the initial weight of the prey item and  $W_{ti}$  is the final weight of the prey item. The time to throw carcass was determined in minutes from the time the prey item was dropped into the pit to the time that the carcass was no longer in the pit (i.e. thrown from pit). The pit volume was calculated by the following:

$$\text{Volume (cm}^3\text{)} = 1/3 \pi r^2 h$$

Where  $r$  is half the diameter of the pit and  $h$  is the depth of the pit.

At the conclusion of the experiment, the videotapes were analyzed for pre-feeding behavior (see Appendix A for operational definitions of pre-feeding behavior) regarding jaw position, pit visibility and sand throwing. A Mann-Whitney U-test was performed to determine differences between blocks. The reason for this analysis is that although treatment groups overall

might be similar, there may be differences between blocks (4 days/block). That is, both groups are likely to be similar at the start of training, but as learning occurs they should become progressively different, which can be determined through a Mann-Whitney U-test.

*Test Day.* The same variables that were measured during the Training Phase were also measured on Test Day, namely, the prey item's initial and final weight of the prey item, pit depth, pit diameter and time to throw carcass. The extraction efficiency, extraction rate, extraction efficiency rate and pit volume were calculated from these measures. To determine if the PAV treatment group responded differently than the CON treatment group to the signal, a Mann-Whitney U-test was used for Test day. To determine if the PAV treatment group responded differently than the CON treatment group to the signal, experimenters scored videotapes from the training phase and on Test Day for feeding behavior in terms of jaw position, pit visibility and sand throwing.

*Figure 2.* Illustration of experimental design and procedure.



Pre-training (3 Days)	Training (16 Days)	Testing (Day 17)
<ul style="list-style-type: none"> <li>• Antlions weighed</li> <li>• Antlions placed in enclosures</li> <li>• Pits measured (depth and diameter)</li> <li>• Feeding: 1 pill bug per day</li> </ul>	<ul style="list-style-type: none"> <li>• Pits measured (depth and diameter)</li> <li>• Each ant lion receives either <b>PAV</b> or <b>CON</b> treatment once per day:</li> </ul> <p style="margin-left: 40px;"><b>PAV: CS → US</b></p> <p style="margin-left: 40px;"><b>CON: CS / US</b></p> <ul style="list-style-type: none"> <li>• Time to throw carcass measured</li> <li>• Extraction measured</li> </ul>	<ul style="list-style-type: none"> <li>• Antlions of both <b>PAV</b> and <b>CON</b> receive same treatment:</li> </ul> <p style="margin-left: 80px;"><b>CS → US</b></p> <ul style="list-style-type: none"> <li>• Time to throw carcass measured</li> <li>• Extraction measured</li> </ul>

## Results

During the experiment a total of ten antlions were eliminated from the data analysis because they did not build a pit or eat. These included 5 PAV and 5 CON subjects. Fourteen subjects remained; these included 7 PAV subjects and 7 CON subjects.

To insure that all antlions were of the same size after assignment to the PAV or CON treatment group, a *t*-test was performed. This *t*-test revealed that there was no significant difference in weight between treatment (PAV and CON) groups  $t(24) = 8.326$ ,  $p = 0.798$ . To also insure that all antlions were at the same level of health another *t*-test was performed. This *t*-test revealed that there was no significant difference in pit diameter and depth between treatment (PAV and CON) groups; for pit diameter:  $t(24) = 10.18$ ,  $p = 0.186$ , for pit depth:  $t(24) = 8.275$ ,  $p = 0.181$ .

### *Training Phase Data*

During the Training Phase, the presentation of a signal elicited a difference between antlions of the Pavlovian (PAV) treatment group and the control (CON) treatment group. Although overall there was no significant difference between the treatment groups, at the end of Training Phase the PAV treatment group exhibited a significantly greater extraction rate than the CON treatment group. However, in other measures of feeding, namely extraction, time to throw carcass, extraction efficiency and extraction efficiency rate, the PAV treatment group was not significantly different from the CON treatment group on any block of the Training Phase or on Test Day.

These observations were supported by statistical analysis via Mann-Whitney U-test comparisons. The extraction between PAV and CON treatment groups did not differ significantly within the Training Phase. Mann-Whitney U-test comparisons indicated that the PAV groups did not differ significantly from one another throughout the blocks of the Training Phase: Block 1  $U(7, 7) = 24, p > 0.05$  and Block 4  $U(7,7) = 12.5, p > 0.05$ . That is, the extraction for each treatment group was not significantly different (did not increase); also the extraction between treatment groups remained the same relative to one another. As shown in figure 3, both treatment groups (PAV and CON) increased in extraction as the Training Phase progressed. By Block 3 the PAV treatment exceeded the CON treatment group, but the difference between the extraction of these two groups was not significant.

For time to throw the carcass, Mann-Whitney U-test comparisons revealed that there was no significant difference between the PAV and CON treatment groups throughout the Training Phase: Block 1  $U(7, 7) = 30.5, p > 0.05$  and Block 4  $U(7,7) = 9, p > 0.05$ . As shown in figure 4, both groups displayed a decrease in the time to throw the carcass of a prey item. The decrease displayed was similar between treatment groups, that is, neither group displayed a particularly higher or lower rate at which they decreased in time to throw the carcass. Both groups displayed a decrease at time to throw the carcass.

After calculating extraction efficiency, extraction efficiency rate and extraction rate, Mann-Whitney U-test comparisons were used to determine differences between treatment (PAV and CON) groups throughout the Training Phase. For extraction efficiency, Mann-Whitney U-test comparisons revealed that there was no difference between the PAV and the CON treatment groups throughout the Training Phase: Block 1  $U(7, 7) = 15$ ,  $p > 0.05$  and Block 4  $U(7, 7) = 10$ ,  $p > 0.05$ . As shown in figure 5, the both treatment groups maintained relatively stable extraction efficiencies. That is, neither the PAV treatment group nor the CON treatment group displayed any sort of change in extraction efficiency throughout the Training Phase. Mann-Whitney U-test comparisons indicate that there was no significant in extraction efficiency rate difference between the PAV and the CON treatment groups throughout the Training Phase: Block 1  $U(7, 7) = 22$ ,  $p > 0.05$  and Block 4  $U(7, 7) = 16$ ,  $p > 0.05$ . As shown in figure 6 the extraction rate efficiency increased in both groups as the Training Phase progressed. Although, the PAV treatment group obtains a higher extraction efficiency rate by Block 3, there is no significant difference.

However, for extraction rate, Mann-Whitney U-test comparisons revealed that there was no difference between the PAV and the CON treatment groups for Block 1, but they were significantly different for Block 4: Block 1  $U(7, 7) = 26$ ,  $p > 0.05$  and Block 4  $U(7, 7) = 4$ ,  $p < 0.05$ . As shown in figure 7, the extraction rate for both groups increased as the Training Phase progressed, however by Block 3 the PAV treatment group displayed a higher

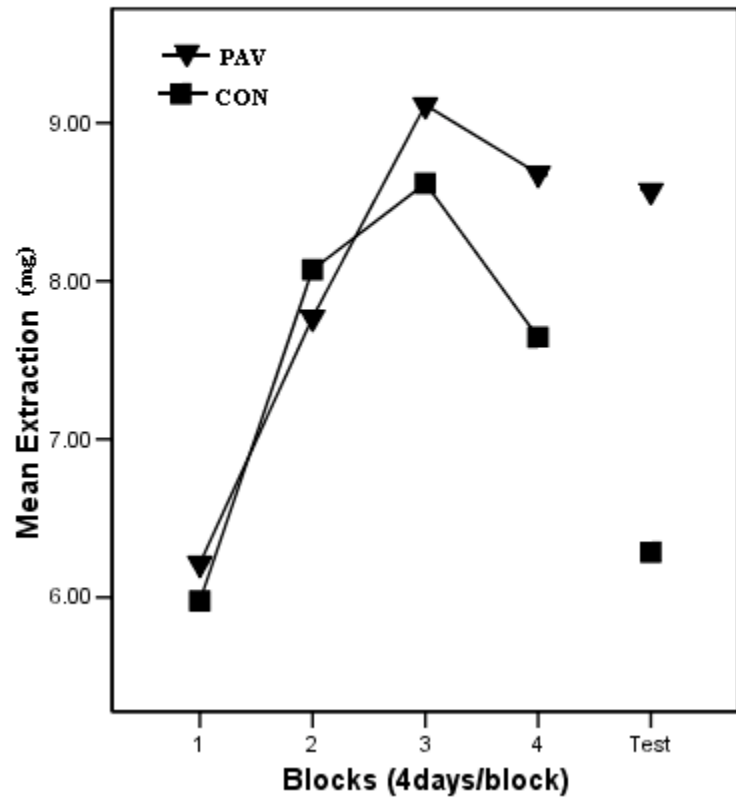
extraction rate, and by Block 4 that PAV treatment group displayed a significantly higher extraction rate. That is, the PAV treatment group extracted more prey mass per unit of time than the CON treatment group. Curiously, after Block 3 the CON treatment group exhibited a decrease in extraction rate.

For pit parameters, Mann-Whitney U-test comparisons were used to determine differences between treatment groups (PAV and CON) in pit diameter, pit depth and pit volume throughout Training Phase. For pit depth, Mann-Whitney U-test comparisons revealed that there was no difference between the PAV and the CON treatment groups throughout the Training Phase: Block 1  $U(7, 7) = 14, p > 0.05$  and Block 4  $U(7,7) = 27 p > 0.05$ . As shown in figure 8 both treatment groups displayed an increase in pit diameter. By Block 3, both groups display a similar decrease in pit diameter. Thus, the pit diameter changes displayed by both treatment groups were not significantly different.

Mann-Whitney U-test comparisons of pit volume revealed that there was no difference between the PAV and the CON treatment groups throughout the Training Phase: Block 1  $U(7, 7) = 24, p > 0.05$  and Block 4  $U(7,7) = 14 p > 0.05$ . As displayed in Figure 10, both groups displayed an increase the pit depth as the Training Phase progresses. There is no significant difference in the rate at which both treatment groups increase in pit depth, thus the treatment groups did not differ pit depth through out the Training Phase.

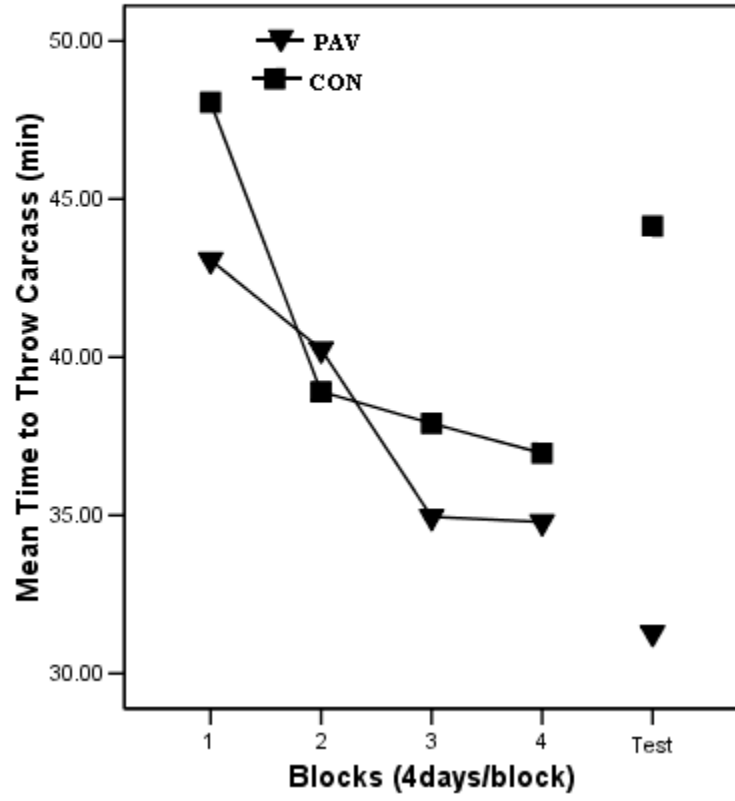
While Mann-Whitney U-test comparisons revealed that there was no significant difference between the PAV and the CON treatment groups for pit volume Block 1, they they were significantly different for Block 4: Block 1  $U(7, 7) = 21, p > 0.05$  and Block 4  $U(7,7) = 1 p < 0.05$ . That is, by the end of the Training Phase the PAV treatment group had constructed pits of a higher volume. Figure 01 indicates that both treatments display an increase in pit volume at the beginning of the Training Phase (Block 1 and Block 2), however by Block 3 the CON treatment group exhibits a decrease in pit volume, while the PAV treatment continues to increase in pit volume. In this manner, the PAV treatment group displayed a significantly higher pit volume by the end of the Training Phase. This decrease in a behavioral measure by the CON treatment group was also demonstrated in extraction rate, which was also significantly different between treatment groups by Block 4.

*Figure 3.* Mean extraction (mg) over blocks of four days during Training Phase and on Test Day.

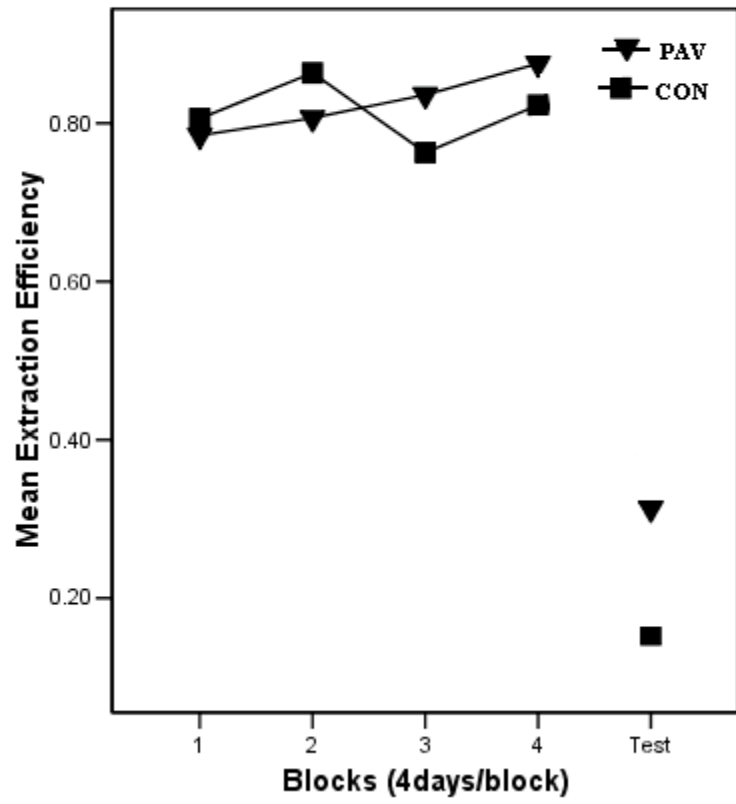




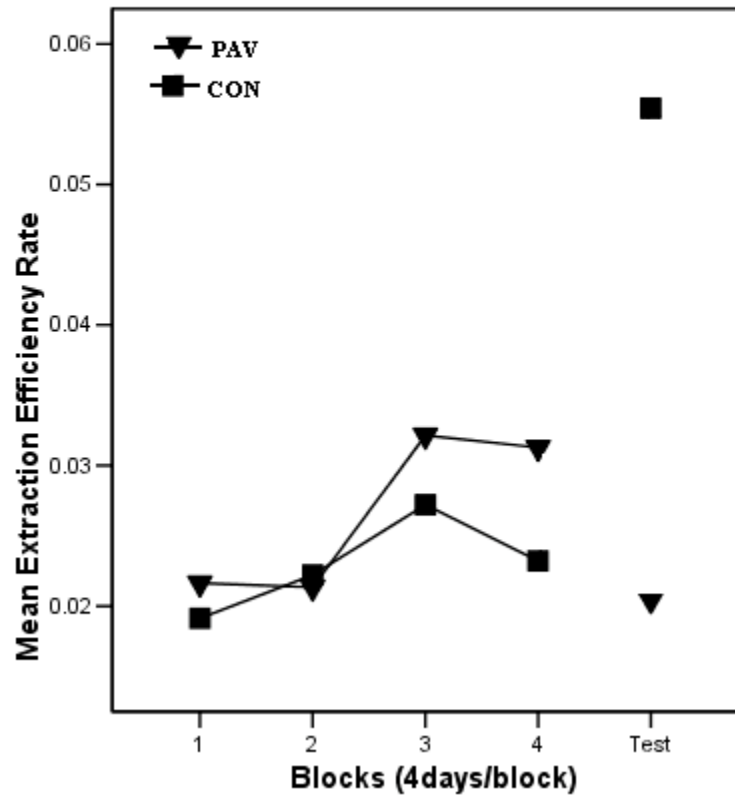
*Figure 4.* Mean time to throw carcass (min) over blocks of four days during Training Phase and on Test Day.



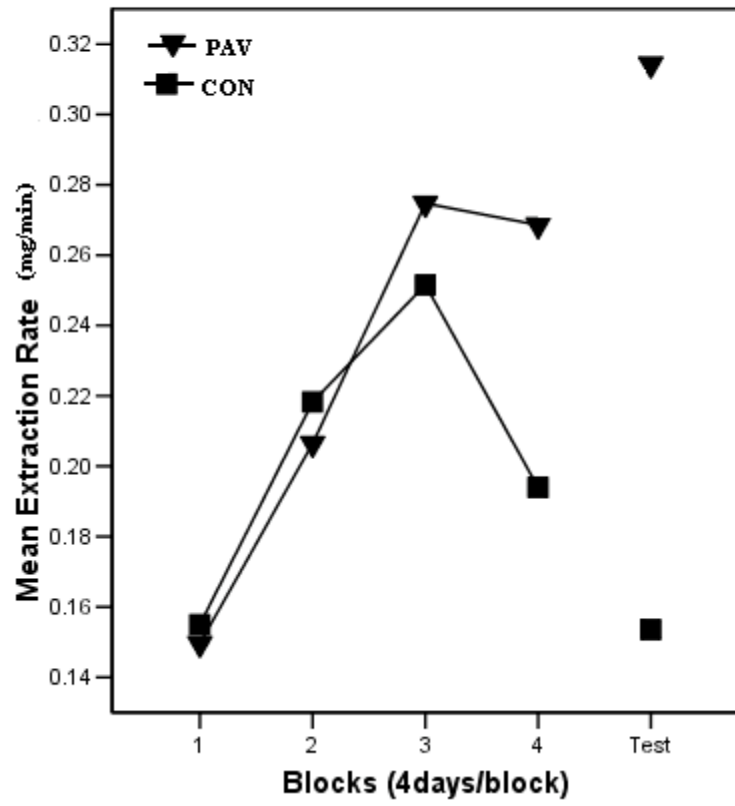
*Figure 5.* Mean extraction efficiency over blocks of four days during Training Phase and on Test Day.



*Figure 6.* Mean extraction efficiency rate over blocks of four days during Training Phase and on Test Day.

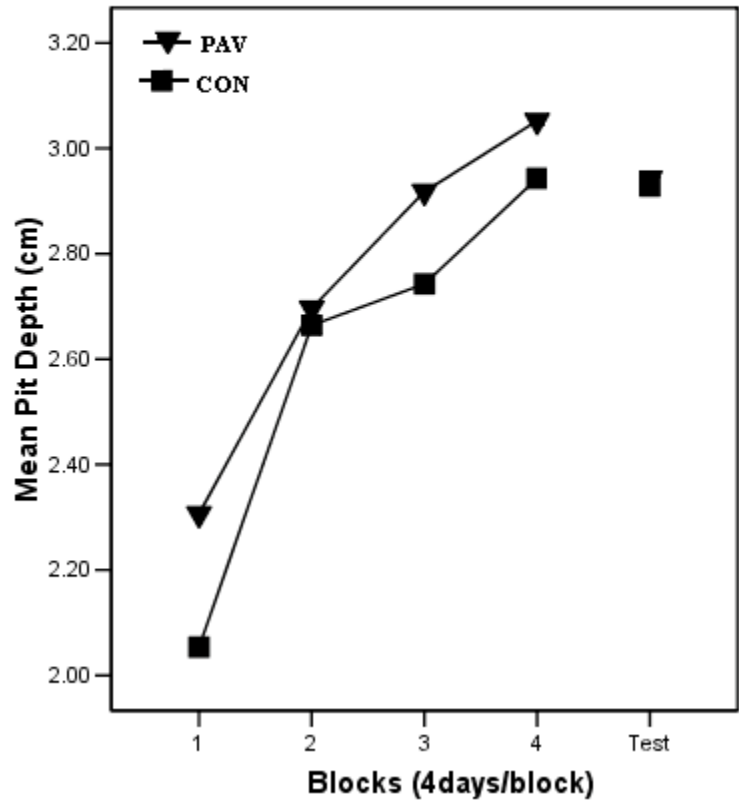


*Figure 7.* Mean extraction rate (mg/min) over blocks of four days during Training Phase and on Test Day.

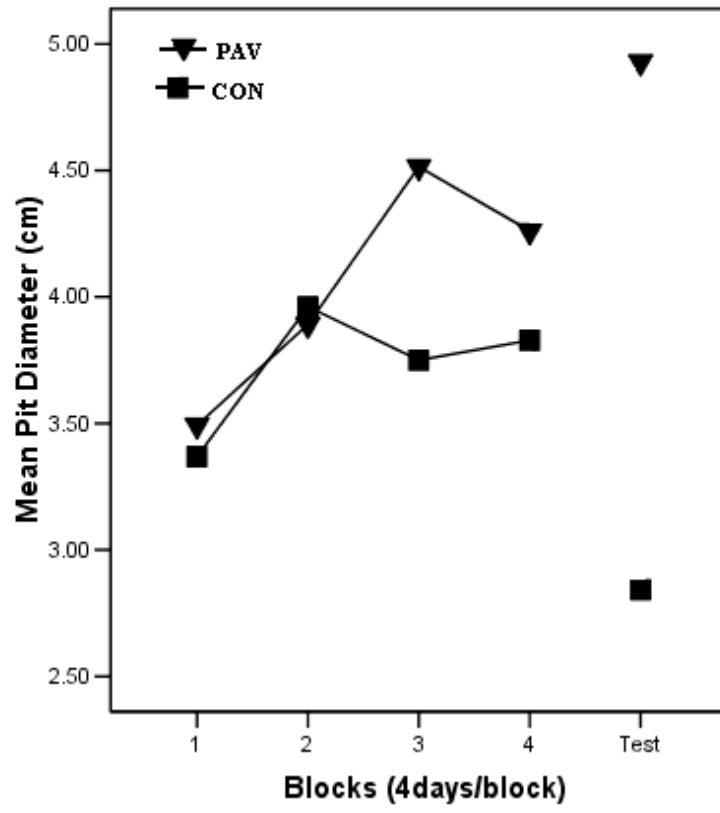




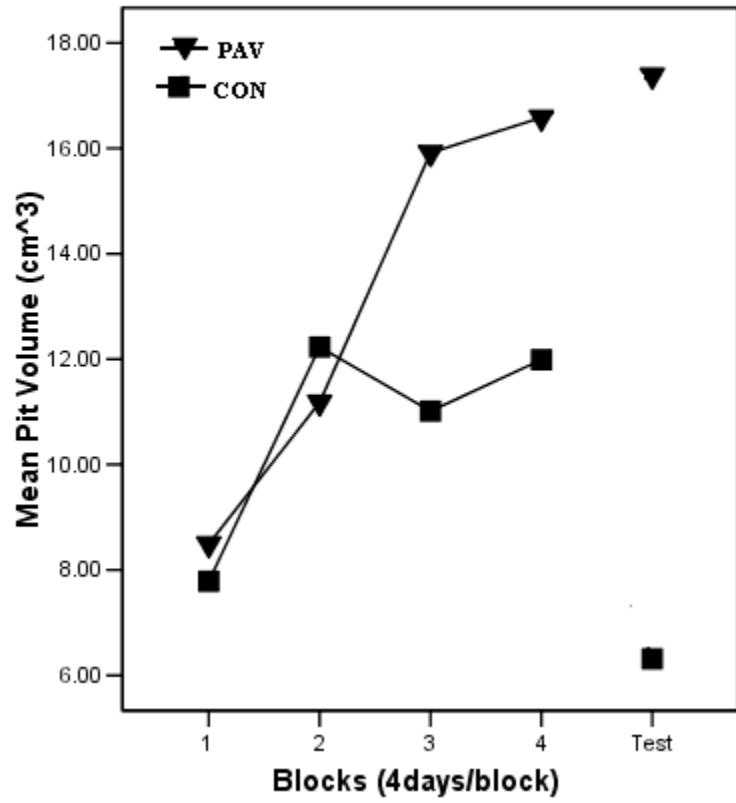
*Figure 8.* Mean pit depth (cm) over blocks of four days during Training Phase and on Test Day.



*Figure 9.* Mean pit diameter (cm) over blocks of four days during Training Phase and on Test Day.



*Figure 10.* Mean pit volume (cm<sup>3</sup>) over blocks of four days during Training Phase and on Test Day.



*Test Day Data*

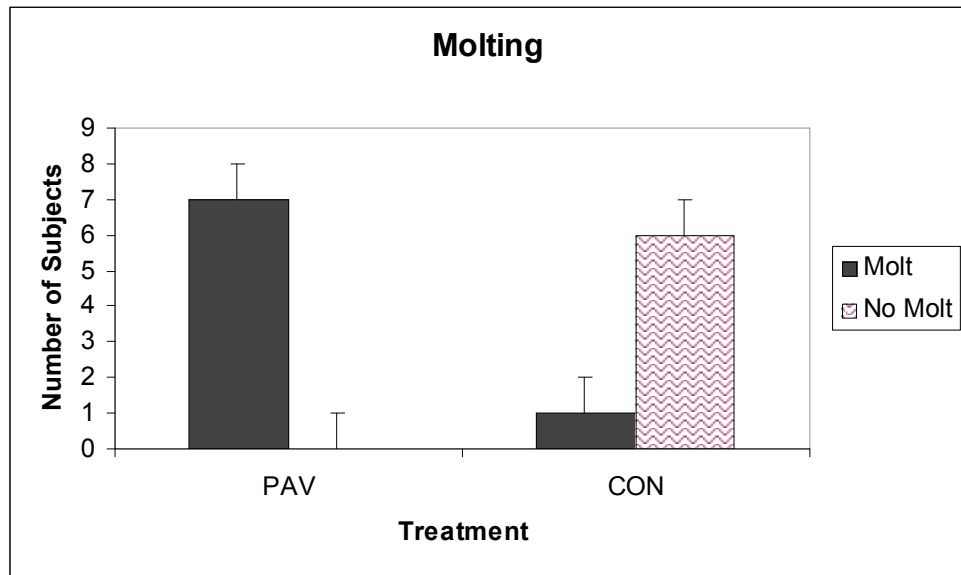
On Test Day the PAV group had significantly greater extraction efficiency (see fig. 7), extraction rate (see fig. 8), pit diameter (see fig. 9) and pit volume (see fig. 11) than the CON group. These observations were supported by Mann-Whitney U-test comparisons. For extraction efficiency the PAV treatment group differed significantly from the CON group:  $U(7,7) = 7.5$   $p < 0.05$ . That is, the PAV treatment group displayed a higher extraction efficiency than the CON treatment group on Test Day. For extraction rate the PAV group differed significantly from the CON group:  $U(7,7) = 4$   $p < 0.05$ . That is, the PAV treatment group extracted prey at a faster rate than the CON treatment group. For pit diameter the PAV group differed significantly from the CON group:  $U(7,7) = 0$   $p < 0.05$ . The PAV treatment group constructed pits of a larger diameter by Test Day. For pit volume the PAV group differed significantly from the CON group:  $U(7,7) = 1.5$   $p < 0.05$ . The PAV treatment group constructed pits of a larger volume by Test Day.

Due to a major malfunction in the camera equipment, our data results regarding pit visibility, jaw position and sand throwing were inconclusive. However, one week after Test Day, all subjects of the PAV group molted while only one CON subject molted. That is, the antlions of the PAV treatment group threw their old exoskeletons from their pit by the 7<sup>th</sup> day following the Test Day (Day 23). These observations were supported by Chi-square analyses. For molting: (df = 1, Chi-square = 10.5) = 0.01,  $p > 0.01$  (to see Chi-square table see Figure 12). As shown in figure 11, the number of

antlions to molt in the PAV treatment group was significantly higher than the CON treatment group.



*Figure 11.* Subjects to molt one week after Test Day.



*Figure 12.* Chi-Square of subjects to molt one week after Test Day

	<b>MOLT</b>	<b>NO MOLT</b>	Total
<b>PAV</b>	7	0	7
<b>CON</b>	1	6	7
Total	8	6	14

**Degrees of freedom: 1**

**Chi-square = 10.5**

***P* is less than or equal to 0.01**

**The distribution is significant  $p < 0.05$**

## Discussion

When antlions are exposed to a situation in which a signal reliably precedes the presentation of food, they respond by displaying changes in feeding behavior that permit them to obtain more resources. The learned signal allows them to prepare, which increases their net gain in energy. More importantly, however, the present study demonstrates for the first time that signaling improves growth rate in antlions. Given the importance of growth and development to the reproductive capabilities of their adult form, the ability to prepare for food through associations with signals could increase reproductive success.

Several changes in feeding behavior were observed in the Pavlovian treatment group that could explain how learning enhances an antlion's ability to obtain more resources and achieve elevated net gains of energy. The antlions of the PAV treatment group responded to the learned food signal by displaying significantly greater extraction efficiency, extraction rate, pit diameter and pit volume than antlions of the CON treatment group. Thus, PAV antlions were able to obtain an increased net gain of energy by learning the signal precedes feeding.

The extraction rate refers to the amount of prey consumed per given unit time, measured in minutes. By exhibiting greater extraction rate upon the presentation of a food item, PAV antlions were able to consume more mass per prey item per minute than CON antlions. In other words, PAV antlions obtained more energy than CON antlions by consuming more prey mass in

less time. By consuming higher amounts of prey per given prey item, the PAV antlions are obtaining more energy. By obtaining more mass per unit time, less locomotory energy is expended in the actual feeding process. Overall this increases the gain in energy for the PAV antlions. The extraction efficiency refers to percentage of mass consumed per given prey item. By exhibiting greater extraction efficiency, PAV antlions are obtaining more mass per given prey item, meaning that they are more effective at consuming prey than CON antlions. Thus, upon the presentation of a food signal, PAV antlions respond with a change in feeding behavior, resulting in a more effective procurement of resources.

Previous ecological studies of antlion feeding behavior suggest that extraction rate and extraction efficiency reflect the net gain in energy and, therefore the success of a particular feeding episode (Griffiths, 1982). In the case of extraction rate, the more food extracted during a given encounter, the longer an antlion can function before the next feeding. The less time spent on consuming the prey, the sooner an antlion can start the next feeding encounter. This advantageous feeding behavior exemplifies how the presentation of a reliable signal enables antlions to optimize interactions with the forthcoming event of food. Thus, learning enables antlions to achieve a higher net gain of energy by obtaining greater amounts of energy at lower energy costs.

Measurements of the conditioned response (feeding) were restricted to a single response system (extraction and pit maintenance). However, the improvement in feeding with learning can also be associated with changes

within a number of digestive and metabolic processes that ultimately allowed antlions to obtain more energy. These processes are referred to as anticipatory responses, which function to maximize interactions with food. In terms of digestion, previous research indicates that conditioned stimuli with food presentations elicit anticipatory autonomic responses such as salivation (dogs) or increased secretion of gastric and pancreatic juices, which contribute to the digestive process in such a way that digestion is improved and more calories are absorbed (humans) (Hollis, 1982). It is likely that the improved feeding behavior observed in the present study is the result of the conditioned stimulus (sand dropping) eliciting anticipatory responses, which in turn permitted PAV antlions to optimize interactions with the forthcoming event of food. Further investigation however, is necessary to confirm the underlying mechanism behind the observed improvement in extraction rate and efficiency. Nonetheless, the current findings provide evidence that learning does, in fact, improve the feeding behavior of antlions.

By exhibiting a greater pit diameter and volume within a situation in which a reliable signal preceded the presentation of food, PAV antlions were able to improve feeding by optimizing prey capture. By investing more energy into pit maintenance, PAV antlions maximized prey capture and thus ultimately obtained more resources. Previous research has emphasized the significance of the parameters of the pit as measures of antlion feeding behavior (Griffiths, 1986). Although pit parameters influence the frequency and success of prey capture, the construction and maintenance of the pit

requires a great expenditure of energy. Therefore, a change in pit parameters will not occur unless it is energetically advantageous.

In terms of specific parts of pit parameters, previous research indicates that pit diameter affects the probability of prey encounter and capture (Farji-Brener, 2003). Also, the volumes of the pit as a function of pit diameter and depth affect the probability of prey encounter, capture and prey escape. If the pit diameter is increased, there is a greater area into which the prey may fall; however, if the depth of the pit is decreased, the prey is more likely to escape. Also if the pit is deeper, then antlions are less able to detect changes on top of the substratum. Increasing pit diameter and depth is energetically costly for antlions and they do not readily change the diameter and depth of their pits. Although both treatment groups displayed an increase in pit diameter and volume over the training period, antlions in the PAV antlions created pits of significantly greater pit diameter and volume ( $p \leq 0.05$ ). This indicates that PAV antlions are displaying energetically costly preparatory behavior as a response to the learned food signal. The adaptive value of such an investment of energy is intimately connected with the learned association between signals and food. Thus, the pit maintenance observed in PAV antlions can be viewed as preparatory behavior that optimizes interactions with the forthcoming food by increasing the possibility of prey capture.

The present study is unable to provide evidence of visible behavior changes when antlions were provided with a signal. Upon review of the Test Day feedings, there were no significant differences in the behaviors: sand



throwing, jaw position and pit visibility. That is, PAV antlions did not appear to respond to the signal by changing actions commonly displayed while feeding. However, previous learning studies have found that insects will respond to a signal that reliably precedes a biological event by displaying behaviors associated with that event. For example, proboscis extension, a behavior commonly displayed during the process of feeding in honeybees, will occur upon the presentation of a color previously associated with sucrose, even without the presence of sucrose (Couvillon et al. 1990).

Based on behaviors previously observed in ecological studies of antlions, it was expected that the PAV treatment group would throw sand, open their jaws or remain visible upon the presentation of a signal. Such behaviors did occur, but at a similar frequency and duration between treatment groups. Several explanations could account for such findings. Firstly, a number of our tapes were damaged due to a technical error in one of the video cameras, decreasing the amount of behavioral data available for analysis. Secondly, other behaviors could account for improved feeding behavior. That is, improved feeding behavior is not likely to be restricted to sand throwing, jaw position and pit visibility. Because this is the first study to use antlions as the subjects of a learning study, the behaviors involved in antlion learning and feeding are not fully explored yet. However, the feeding measures such as extraction efficiency and pit volume in this study do provide enough evidence to suggest that learning enables antlions to feed as optimally as possible. Such improved feeding clearly has adaptive implications. While it may seem

intuitive that superior feeding behavior would result in improved fitness, physical evidence is necessary. The present study provides such evidence via improved growth rates.

Growth rate is generally determined by periodic weighing of the animal throughout an experiment. Given that antlions must remain in their pits at all times during the training period such periodic weighing could not occur. However, growth rate in insects, can also be measured by determining the time between molting. Molting is the process by which an insect sheds and replaces its exoskeleton to allow for growth. Antlions must go through several molts before they reach the necessary size for metamorphosis (Borror et al. 1989). Previous research indicates that given the flexibility of the life stages of antlions, under optimal feeding conditions, antlions will transition into the adult stage of life more quickly (Crowley & Linton, 1999). Other research indicates that both the length of time between molts and the growth that occurs during that time is variable and can be influenced by environmental conditions (Borror et al. 1989). Therefore, under improved feeding conditions, it is possible to accelerate the transition into the adult stage of life through increased growth rates, which may be demonstrated through the higher frequency of molting.

Evidence of improved fitness through increased rate of growth is found in the PAV treatment group. One week following the conclusion of the present experiment, all of the antlions in the PAV treatment group molted, while only one antlion in the CON treatment group molted. Such a finding

suggests that upon the employment of learning, antlions of the PAV treatment group were able to maximize feeding behavior that ultimately allowed for them to obtain higher growth rates. These findings are consistent with the results of a study previously mentioned, in which grasshoppers that responded to a learned food signal through food selection experienced increased growth rates (Dukas & Bernays, 2000). The grasshopper study and the present study are the only experiments to provide evidence both of learning and the subsequent improvement in fitness, namely increased growth rates.

Although these studies suggest that learning can result in conditions in which insects will experience an improvement in fitness, the subsequent reproductive capacity of the adult ultimately determines fitness. Further studies investigating the influence of larval learning on reproduction is useful for determining the adaptive value of learning as a biological mechanism for preparation. In the current study, behavior was not assessed after the larval stage. Further investigation into how increased fitness resulting from learning in the larval stage is manifested in the adult, such as matting efficiency and egg laying, would be useful to provide a complete picture of the reproductive influence of learning.

The results of the current study suggest that learning does play a role in the feeding behavior of antlions. Indeed, the signal of sand dropping used in this experiment could be present in nature, and therefore could exist within an antlion's natural repertoire of signals associated with its environment. For example, previous research has demonstrated that sand thrown by nearby

antlions provide information regarding population density of a given area. Antlions have been found to adjust their pits or relocate to accommodate population densities. Sand dropping or movement within or around the pit could signal a number of other events such as prey presence. However, these events do not always reliably follow a signal such as sand dropping. The present study maintained the reliable presentation of food following a signal, allowing the antlion to establish an association between the signal and food.

A number of other signals for food arrival could exist in nature that facilitates the feeding behavior of antlions. Such signals have yet to be determined, but from the number of ecological studies conducted on antlions, a plethora of signals are likely. Previous research has demonstrated, for example, that antlions are capable of perceiving light and to some degree the position of moving objects (Lucas, 1989). Some cues that come from the external environment include shadow movements caused by prey. Given that antlions are sensitive to vibration signals as compressional waves as a result of prey movement could also serve as a signal for the presentation of food. Other sit-and-wait predators, such as Scorpions respond to prey of varying sizes and distances by changes in compressional waves (McClure, 1976), many sit-and-wait predators could depend on signals of the external environment. However, given that the present study is the first to determine that antlions are indeed capable of learning; further study using other sit-and-wait predators and other signals would be useful.

In conclusion, this study demonstrates that antlions, indeed, are capable of learning. The presentation of a signal facilitates feeding behavior in such a way that more resources are obtained. Learning clearly puts antlions at a selective advantage because it enhances an antlion's ability to grow, which in turn could improve reproduction. Given that antlions are a classic example of a sit-and-wait predator, the implication of these findings is incredible. Sit-and-wait predation, as previously discussed, relies on active prey and much time is spent waiting for that prey. The continuous state of being ready or always prepared for the arrival of prey, such as keeping jaws open, requires a lot of energy and takes away from events such as pit construction. Thus, in order to engage in other biologically significant events and still be able to act upon the presentation of food without energy expenditure, there must be some sort of process that cues the predator to respond to a particular event. Given the performance of antlions in the present study, it is likely that such a process is in fact learning. That is, sit-and-wait predators respond to learned food signals in such a way that less energy is spent "anticipating" the prey and more energy is gained through being ready for the feeding. Clearly, learning enables sit-and-wait predators to optimize interactions with their environment.

A process in which insects respond to a learned food signal has been primarily observed in insects that engage in active foraging behavior. Learned food signals have been demonstrated to result in enhanced interactions with forthcoming food by improved food selection (Wantabe, 2003), food recognition (Couvillon, Leito & Bitterman, 1991; Dukas & Bernays 2000) and

consumptive behavior (Daly & Smith 2000). For all of these cases, the conditioned response results in less energy expended in obtaining resource and more energy gained from that resource. In both active and sit-and-wait predation, the food signal allowed for insects to obtain more overall resources, which gives them a selective advantage. It is clear that insects not only learn, but learning increases fitness with significant implications within this group.

Given the numerical superiority of insects in the animal kingdom and their success in diverse environments, a mechanism, such as learning, that results in increased fitness could have wide-ranging implications on the biological world. Moreover, the power of learning in the insects might reveal the nature their success and reproductive power. Learning has been found to occur not only in insects, but in higher invertebrates and vertebrates as well. The ubiquity of learning in the higher animals indicates that it must provide huge selective advantage; by allowing an animal to prepare for common biological interactions such as obtaining food, water or mates. From antlions to octopi, honeybees to humans, learning functions as a biological mechanism for preparation.

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## Appendix A

Table 1.0 Behavioral Patterns and Operational Definitions

Behavioral Pattern	Operational Definition
Sand Throwing	An antlion tosses sand out of the pit with its head.
Open Jaw	An antlion separates its mandibles so that they are completely open.
Pit Visibility	An antlion is completely visible the bottom of the pit.
Thrown Carcass	An antlion throws the carcass from the pit, so that the carcass is no longer in the pit.
Molting	An antlion throws it's exoskeleton (skin) from the pit.