

I give permission for public access to my thesis and for any copying to be done at the direction of the archives librarian and/or the College librarian.

Meredith M. Baker

The Role of Learning In An Insect Predator-Prey Relationship

by

Meredith M. Baker

A Thesis

Presented to the Faculty of Mount Holyoke College

In partial fulfillment of the requirements

For a degree of Bachelor of Arts

With Honor

Program in Neuroscience and Behavior

Mount Holyoke College

South Hadley, Massachusetts

ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Karen Hollis, for her continuous support throughout the past two years. Thank you for your guidance, encouragement and assurance that I would finish writing, even when I did not believe you. I also would like to thank the members on my Thesis Committee, Gary Gillis and Denise Pope, for their comments and suggestions. Thank you to Janelle Gagnon and Janice Gifford for their statistical help.

The completion of this project would not have been possible without the dedication of Victoria Beja-Glasser, Erin Caine, Joeeta Chowdhury, Julianne Jones, Aja Kane, Alexandra McIe, Jessica Olson, Meredith Pinta, Colleen Regal, Katie Taylor, Allison Visvader and Caitlin Walsh. Thank you for your dedication the project

Thank you to my family, friends and the members of Psychology 411 class for their constant support, willingness to learn about antlions and laughter therapy.

Thank you to Mr. and Mrs. Harap for funding my research.

TABLE OF CONTENTS

	Page
Acknowledgements.....	ii
List of Figures.....	v
List of Tables.....	vi
Abstract.....	vii
Introduction.....	1
Learning in Insects.....	1
Anti-predation behavior.....	2
Reproductive behavior.....	3
Foraging behavior.....	6
Learning in a Sessile Insect.....	13
Sit-and-Wait Predation in Larval Antlions.....	14
Pit construction.....	14
Predatory strategy.....	16
Prey detection.....	18
Pupal Stage.....	19
Adult Stage.....	20
Predator versus Prey.....	20
Present Study.....	23
Method.....	22
Subjects.....	22
Apparatus and Stimulus Materials.....	23

Apparatus.....	27
Sound cues.....	31
Mealworms	31
Procedure	32
Data analysis	36
Results.....	43
Pit Volume	43
Sand-Throwing Behavior.....	46
Effect of type of training day	46
Effect of cue	50
Extraction Measures.....	54
Days to Molt	57
Discussion.....	58
Changes in Predatory Strategy.....	59
Changes in Feeding Behavior	66
Changes in Growth Rate	68
Limitations	70
Conclusions.....	71
References.....	73
Appendix.....	78

LIST OF FIGURES

<i>Figure 1.</i> Apparatus filled with sand, positioned inside sound attenuating chamber with MP3 player and speaker	29
<i>Figure 2.</i> Diagram of procedure	34
<i>Figure 3.</i> Pit volume (mean cm ³ ± SEM) across the first block segment and last three blocks for all LRN (<i>n</i> = 16) and CTL (<i>n</i> = 16) subjects	44
<i>Figure 4.</i> Rate of sand-throwing (number of sand-throws/s ± SEM) for the first block and last three blocks of training (a) before the sound cue and (b) during the sound cue in prey rescue (P _R) and prey availability (P _A) trials for LRN (<i>n</i> = 16) and CTL (<i>n</i> = 16) subjects	48
<i>Figure 5.</i> Rate of sand-throwing (Mean number of sand-throws/ s ± SEM) before and during playing of the sound cue during the (a) P _R trials and (b) P _A trials for LRN (<i>n</i> = 16) and CTL (<i>n</i> = 16) subjects	52
<i>Figure 6.</i> (a) Extraction efficiency (% ± SEM) (b) Extraction rate (g / sec ± SEM) (c) Extraction efficiency rate (% / s ± SEM) for LRN (<i>n</i> = 16) and CTL (<i>n</i> = 16) subjects	55

LIST OF TABLES

<i>Table 1.</i> Computations for Extraction Measures	39
--	----

ABSTRACT

Previous research has shown that larval antlions, *Mrymeleon crudelis* Walker, a type of sit-and-wait predator, are capable of using a learned cue to anticipate the arrival of prey before they fall down the steep sides of the antlions' conical pits. Ants, a common prey item of the antlion, have developed an anti-predator tactic to the antlions' sit-and-wait predatory strategy, namely a highly sophisticated form of rescue behavior. Ants will attempt to rescue nestmates struggling on the of the pit and also may injure antlions in the process.

This experiment investigated whether antlions could anticipate ant rescue by associating a learned vibratory "rescue" cue with removal of a prey item from the pit, and discriminate this cue from another, different, vibratory cue paired with uninterrupted feeding. Thirty-two antlions were assigned to the Learning (LRN) ($n = 16$) or Control (CTL) ($n = 16$) groups. The LRN group was exposed to a vibratory cue paired with an uninterrupted feeding opportunity and a different vibratory cue paired with "rescue," when the antlion received no food. The CTL group experienced randomization of vibratory cues and feeding or "rescue" events. Pit volumes, feeding and sand-throwing behaviors were recorded for the 40 days of the experiment. During "rescue" trials, LRN antlions decreased their rate of sand-throwing during the vibratory cue more than antlions in the control condition, whereas during feeding trials no difference between rates of sand throwing of LRN and CTL

antlions was detected. This finding suggests that antlions used learned cues to anticipate that struggling prey would be rescued and changed their rate of sand-throwing to reflect the anticipated outcome of a prey event.

INTRODUCTION

Studies of insects have illustrated their dynamic and complex abilities to learn and retain information. Learning, a relatively enduring change in behavior that results from experience or practice, has been studied widely in both vertebrates and invertebrates, but was not documented in insects until the mid 20th century. These findings contradict observations from the early 20th century that insect behaviors were simple, fixed reflexes, unlike behaviors of higher-learning vertebrates (reviewed in Wheeler, 1930). Studies of honeybees by von Frisch (1953) suggested that this characterization of insects' learning abilities was incorrect. Honeybees learned to associate a color with food and they returned to this learned color more frequently than other colors, even after several days of no exposure to the learned color (von Frisch, 1953). Studies of the learning abilities of a broad range of insects have continued to illustrate their cognitive capacities.

Learning in Insects

A type of learning that has been studied extensively in insects is associative learning – the novel temporal pairing between two stimuli, or events, as a result of an experience (Alloway, 1972a; Papaj & Prokopy, 1989). In addition to studying whether insects are capable of associative learning, researchers have investigated the adaptive advantage of learning, demonstrated by an increase in survival and reproduction (Papaj & Prokopy, 1989). Associative learning has the potential to positively affect an animal's

fitness if learned associations improve the organism's reproductive success (Dukas and Duan, 2000). The animal also must possess sensory, learning and memory mechanisms capable of forming and remembering such associations (Dukas and Duan, 2000). It is important to understand learning in the context of its adaptive significance because researchers must understand how learning improves the survival of the insect and its genes (Dukas, 2007).

Associative learning has been observed in every major insect order and in eusocial, social and solitary insects that actively search for mates and food (reviewed in Guillette & Hollis, 2010), including bees (Menzel, Erber & Masuhr, 1974; Couvillon & Bitterman, 1980; Blackawton et al., 2010), cockroaches (Alloway, 1972b; Watanabe et al., 2003), grasshoppers (Dukas & Bernays, 2000), fruit flies (Quinn, Harris & Benzer, 1974), and parasitoid wasps (Dukas & Duan, 2000; Gutiérrez-Ibáñez, Villagra & Niemeyer 2007; Rains, Utley & Lewis 2006). Major benefits of learning, such as improvement of an insect's anti-predation, reproduction, and feeding behaviors, result in increased survival and reproduction and have been observed in many species of insects (Guillette & Hollis, 2010). In the next sections, I will provide a brief survey of how learning affects different types of behaviors to illustrate that the role of learning to improve fitness spans many insect orders.

Anti-predation behavior. Learning can help develop and improve anti-predation behaviors in insects. For example, damselfly larvae, *Enallagma boreale* (Order: Odonata), were able to modify their foraging and movement

behavior in the presence of pike, a common predator, after a single exposure of a pike paired with a nearby injured damselfly larva (Wisenden, Chivers & Smith, 1997). Damselfly larvae also were able to associate the presence of pike with the injured conspecific. Damselfly larvae decreased their feeding and movement behavior in response to a chemical pike stimulus after only one exposure to the pike stimulus paired with the injured larva. These observations suggest that previous exposure to pike acted as a cue to damselfly larvae and caused them to modify their behavior; however, the anti-predation response was not innate because it did not exist in the damselfly larvae previously unexposed to pike (Wisenden, Chivers & Smith, 1997). Learning modified feeding and movement responses in damselfly larvae and improved anti-predation behavior, critically important to survival.

Reproductive behavior. Another important aspect of an insect's life is reproduction (Dukas and Duan, 2000). Reproduction is critical to fitness and changes in reproductive behavior due to learning can demonstrate its adaptive advantage for the insect. The effect of learning on oviposition site choice has been studied in the parasitoid wasp, *Biosteres arisanus* (Order: Hymenoptera), the mosquito, *Aedes aegypti* (Order: Diptera), and cabbage butterfly, *Pieris rapae* (Order: Lepidoptera). The environment that female mosquitoes were raised in affected the oviposition site choice of adult female mosquitoes (Kaur, Lai and Ginger, 2003). Females that were reared in water containing a mosquito repellent chose oviposition sites containing the mosquito repellent

significantly more than female mosquitoes that were reared in clean water. This finding suggests that larval exposure to a particular chemical decreased adult avoidance of that chemical, as the female mosquitoes associated the chemical with a suitable oviposition site choice (Kaur, Lai & Ginger, 2003).

Cabbage butterflies also used chemical stimuli to determine favorable oviposition sites. In a study by Traynier (1984), the butterflies were briefly exposed to paper disks of one color wetted with sinigrin, a naturally occurring oviposition releaser found in plants of the Cruciferae family, and a paper of a different color wetted with water. In the preference test given after training, butterflies were allowed to choose between disks of two different colors that had been wetted with water instead of sinigrin. This procedure tested the strength of the association between the color of the disk and preference of the oviposition site created by the presence of sinigrin in training. Significantly more butterflies chose to land and oviposit eggs on paper disks of the color paired with sinigrin in training. Butterflies preferred to land on oviposition sites that resembled the favorable conditions to which they were exposed during training, suggesting the learned association between the presence of sinigrin and a visual stimulus guided butterflies' choices. Sinigrin can be detected from a distance. Therefore, females that selected only oviposition sites containing sinigrin would decrease the amount of time spent searching for favorable oviposition sites (Traynier, 1984).

In addition to the use of chemical cues to determine favorable oviposition sites, exposure to cues indicating more oviposition opportunities also influences site choice. Female parasitoid wasps oviposit their eggs in the eggs of tephritid fruit flies that reside on different types of fruit (Dukas & Duan, 2000). During experimental trials, parasitoid wasps were exposed to one type of fruit that contained host eggs and another type of fruit that did not contain host eggs and then were given a choice test to determine their fruit preference. Compared to a control group in which wasps were not given the opportunity to learn which type of fruit contained host eggs versus which type of fruit did not, wasps in the learning group parasitized significantly more eggs and produced more offspring than the parasitoid wasps in the control group. Thus, parasitoid wasps that learned were able to increase their fitness over the parasitoid wasps that did not learn (Dukas & Duan, 2000).

The previous examples illustrate how learned information was retained during a single life stage and affected reproductive behavior. While retention of learned information during one life stage is important for insects, retention across multiple life stages is adaptive if situations requiring learned information are encountered repeatedly. Parasitoid wasps, *Aphidius ervi* (Order: Hymenoptera), retained learned olfactory information through metamorphosis, suggesting benefits of learning can span more than one life stage. Larval wasps were exposed to their environment for approximately 24 hours during the third instar stage via the ventral opening, an opening in the

cocoon wall (Gutiérrez-Ibáñez, Villagra & Niemeyer, 2007). Researchers exposed third instar larval wasps to either vanilla extract or water while the ventral opening was present and then conducted a preference test after the wasps had pupated into adults (Gutiérrez-Ibáñez, Villagra & Niemeyer, 2007). Adult wasps exposed to vanilla during the third instar larval stage spent significantly more time at the location with vanilla scented air than the location with unscented air, compared to adult wasps exposed to water (Gutiérrez-Ibáñez, Villagra & Niemeyer, 2007).

These results not only suggest that wasps retain learned information prior to pupation, but also that the retention of this information may be adaptive. The authors hypothesized that the olfactory cues to which third instar larval wasps are exposed before pupation may have affected where the females chose to oviposit her eggs. That is, she was attracted to a similar environment to what she was exposed to as a larva, suggesting a fitness benefit from this learning (Gutiérrez-Ibáñez, Villagra & Niemeyer, 2007). Insects are capable of associating different types of cues with favorable oviposition sites and they use these cues to influence their oviposition site choices.

Foraging behavior. The role of learning in foraging behavior has only been studied in insects that actively seek out food. The use of environmental cues to guide this behavior has been investigated in many species. Codling moth larvae, *Cydia pomonella* (Order: Lepidoptera), were exposed to ginkgo

leaves treated with saccharine (Pszczolkowski & Brown, 2005). Gingko leaves are noxious to codling moth larvae and consumption of limited amounts of gingko leaves results in unfavorable regurgitation of the gingko leaf matter. The larvae were given a choice test and exposed to both saccharine-treated apples and inert solvent-treated apples. Larvae exposed to saccharine-treated gingko leaves avoided saccharine-treated apples and chose to burrow in solvent-treated apples, compared to larvae exposed to untreated gingko leaves that did not show a preference for either solvent-treated or saccharine-treated apples (Pszczolkowski & Brown, 2005). This aversion also developed after only one exposure to the saccharine and noxious plant pairing. This aversion also was resistant to extinction and had a long retention time; that is, 70% of the larvae trained to avoid saccharine still showed the aversion to saccharin after molting into the next instar, approximately five days after training (Pszczolkowski & Brown, 2005). This fast learning ability, as well as its persistence, suggests that the ability of the larvae to learn an aversion quickly was adaptive, so that codling moths did not repeatedly ingest deleterious food and avoided other food containing the noxious chemical.

Learning also can be used in locating favorable food, in addition to avoiding unfavorable food. Honeybees, *Apis mellifera* (Order: Hymenoptera), are capable of using visual stimuli to learn the location of food. Honeybees learned an association between an illuminated disk and food availability and were capable of learning this association when the disk was illuminated at

different wavelengths of light, producing different colors (Menzel, Erber & Masuhr, 1974). Honeybees also were able to discriminate between disks illuminated with two different wavelengths, where a disk illuminated with one color was paired with food availability and a disk illuminated with a second color was paired with no food. However, the rate of learning was affected by the color of the illuminated disk. That is, they learned the association fastest when food was paired with violet light and slowest when food was paired with blue-green light. These findings suggest that honeybees have evolved to learn information more rapidly about flower colors encountered frequently in nature, as purple flowers are most frequently visited by the honeybees and this flower color is common in nature (Menzel, Erber & Masuhr, 1974). Color preference also influenced retention time of the association, as colors learned faster, such as purple, were retained for five days, while colors learned more slowly, like blue-green, were retained for only two days. This finding further supports that the honeybee's memory storage has evolved to preferentially store information about more relevant flower colors.

Fruit flies, *Drosophila melanogaster* (Order: Diptera), are capable of using both chemical and visual stimuli to avoid noxious food (Quinn, Harris & Benzer, 1974). When exposed to one olfactory stimulus with an electric shock and a different olfactory stimulus with no electric shock, fruit flies developed an aversion to the olfactory stimulus paired with the electric shock. The fruit flies retained the learned information for a short amount of time and

retention time was dependent on the amount of training; that is, as the number of training sessions increased, retention time of information increased as well (Quinn, Harris & Benzer, 1974).

The fruit flies' associative learning ability also was tested with visual stimuli. Fruit flies were exposed to a maze with two arms; one arm was illuminated with red light and the other arm illuminated with blue light. The surface of one arm also was coated with quinine sulfate powder, a substance that fruit flies naturally avoid (Quinn, Harris & Benzer, 1974). After exposure to both arms and the quinine powder, fruit flies were exposed to both colors without quinine powder and avoided the color paired with the quinine. These findings suggest that fruit flies are capable of using multiple types of learned environmental cues, both chemical and visual, instead of being limited to particular types of stimuli.

The ability to use learned cues not only improves foraging behavior, but also has secondary benefits resulting from the improved ability to secure food. Learning improved foraging behavior and increased growth rate in grasshoppers, *Schistocerca americana* (Order: Orthoptera), resulting in fitness advantages (Dukas & Bernays, 2000). Grasshoppers in the learning group received one dish of nutrient-rich food and another dish of nutrient-deficient food. Each food dish was paired with a specific color and location combination. Grasshoppers in the control group were exposed to nutrient-rich and deficient food in both locations and in food dishes of both colors, which

prevented grasshoppers in the control group from learning any associations between food type, location and dish color (Dukas & Bernays, 2000).

Compared to grasshoppers in the control group, grasshoppers in the learning group chose the balanced diet a greater percentage of the time which allowed them to minimize energy expenditure, avoid predation danger associated with foraging and grow faster than grasshoppers in the control group (Dukas & Bernays, 2000).

African migratory locusts, *Locusta migratoria* (Order: Orthoptera), also used learned cues to locate nutritious food and discriminate between foods fortified with proteins or carbohydrates (Raubenheimer and Tucker, 1997). Locusts were trained to associate a visual cue, either a green or yellow tinted arm of a cylindrical apparatus, with protein or carbohydrate fortified food. After training, the locusts were fed either protein or carbohydrate-deficient diets and given a preference test. Locusts deprived of carbohydrates entered the arm illuminated with the color previously paired with carbohydrate-enriched food significantly more than the arm illuminated with the color for protein-enriched food. The opposite finding was observed for protein-deprived locusts, as they entered the arm tinted with the color paired with protein-enriched food significantly more than the arm tinted with the color previously paired with carbohydrate-enriched food (Raubenheimer & Tucker, 1997). The locusts were able to use learned cues to choose the food that provided a balanced diet. This finding demonstrates an adaptive

advantage of learning for food selection and agrees with findings in grasshoppers that insects can use learned cues to achieve an optimal diet.

Complex learning abilities, including the ability to learn spatial relationships, have been observed in bumblebees, *Bombus terrestris* (Order: Hymenoptera), and silverfish, *Lepisma saccharina* (Order: Zygentoma). Spatial learning abilities can aid insects in foraging by improving their ability to find food sources, resulting in decrease time spent foraging. Bumblebees are capable of learning spatial relationships between the location of food and the surrounding environment (Blackawton et al., 2010). Bumblebees learned the position of flowers paired with sugar water in relation to the position of flowers paired with salt water (Blackawton et al., 2010). The researchers combined these flowers in a pattern so that 12 flowers containing salt water always surrounded four flowers containing sugar water. However, the colors of the flowers with salt water and sugar water were changed in half the trials so that the bumblebees were forced to learn the spatial pattern, instead of only learning the colors of the flowers. The bumblebees were able to learn the pattern and visited the flowers containing sugar water significantly more than the flowers containing salt water (Blackawton et al., 2010). This study suggests that bumblebees are capable of learning associations between specific stimuli, such as the location of flowers containing sugar water, while ignoring other stimuli, such as the colors of the flower. This ability helps

bumblebees to find flowers containing nectar, because remembering locations of food will improve the forager's success (Blackawton et al., 2010).

Silverfish also are able to use spatial learning to improve their foraging behavior. Spatial learning in silverfish was investigated using a maze task. While in the maze, silverfish were exposed to fluorescent light, an aversive stimulus, and only upon reaching the end of the maze were they allowed to enter a more favorable darkened chamber. Silverfish learned to associate the end of the maze with the favorable darkness and significantly decreased the amount of time spent inside the maze by navigating the quickest route to the end of the maze (Punzo, 1980). The ability to learn how to escape the aversive light source demonstrates that silverfish are capable of learning spatial relationships. This ability would aid in finding favorable feeding locations and improve their fitness by decreasing the amount of time they are exposed to predators and decreases the amount of energy spent on foraging (Punzo, 1980).

The previous section provides numerous examples of how learning improves the daily lives of many different orders or insects. All of these insects actively move around their environments, searching for food and mates. However, not all insects are active in their environments, and instead some are sedentary and have adopted a unique predatory strategy influenced that may be affected by learning in different ways from active insects' predatory strategy.

Learning in a Sessile Insect

In contrast to insects that actively interact with their environment, sessile insects are sedentary and exhibit a sit-and-wait predatory strategy, in which they wait for prey to approach them and only expend energy during prey capture (Guillette & Hollis, 2010). Therefore, sessile insects are affected by a different set of constraints than insects that are active in their environments because of the differences in energy expenditure (Guillette & Hollis, 2010). Larval antlions are a good model organism to use in research of sessile insects because they are easy to care for and exhibit a sit-and-wait predatory strategy (Wheeler, 1930; Griffiths, 1980; Guillette & Hollis, 2010). It is important to determine how learning can affect prey capture, feeding behaviors and, ultimately, fitness in sit-and-wait predators. Changes in predatory and feeding behaviors provide good indicators of how learning could benefit the antlion's predatory strategy.

There are over 2,000 species of antlions (Neuroptera: Myrmeleontidae) distributed around the world (Scharf & Ovadia, 2006). Many of these species are sit-and-wait predators; however, only a few species are pit-building antlions, the most sedentary of sit-and-wait predators (Devetak, 2005). This predatory strategy distinction is only relevant during the antlions' larval stage, because it is the only stage in which feeding occurs (Scharf, Golan & Ovadia, 2009). The success of antlions' predation during the larval stage affects the rest of their life cycle (Scharf, Golan & Ovadia, 2009).

The antlion life cycle consists of four stages: egg, larva, pupa, and adult, with a complete metamorphosis occurring during the pupa stage (Scharf & Ovadia, 2006).

Sit-and-Wait Predation in Larval Antlions

Pit construction. Pit-digging antlions build conical pitfall traps in sandy substrates that allow them to trap prey (Scharf & Ovadia, 2006). These pitfall traps have steep walls that function to hinder prey's ability to escape from the pit (Fertin & Casas, 2006). Antlions construct the pit by moving in a circular path backwards in sand, using their mandibles to throw sand grains out of the pit while they spiral downward into the sand (Fertin & Casas, 2006).

Pit building techniques differ between antlions at different instars. Antlions in the second and third instar construct their pits in the manner described above. However, first instar antlions are so small that they pivot around their own bodies to construct a much smaller pit than those constructed by second and third instar antlions (Griffiths, 1980). Across all instars, antlions cease pit building when the circumference of the circle they are navigating becomes too small for them to continue; this position becomes the vertex of the pit (Griffiths, 1980). The area of the pitfall trap increases as a function of antlion size, as bigger pits can accommodate bigger prey that larger antlions require for feeding (Griffiths, 1980).

Regardless of pit size, the walls of the trap are constructed so that they are the steepest angle possible without collapsing, known as the angle of repose (Fertin & Casas, 2006). Sand particle size is important, as it influences the angle of repose. Antlions prefer to construct pits in finer sand not only because the walls can be steeper without collapsing, but sand grains are also easier to dislodge and allow the pit walls to collapse, thwarting a prey item's escape attempts (Botz et al., 2003; Devetak et al., 2007). Use of sand particle size is affected by the antlion's age. Antlions in the first instar favor very fine-grained sand, probably because their small size limits the size of sand particles that they are capable of throwing. Antlions in the second and third instars are bigger and therefore are capable digging pits in coarser grain sand (Devetak et al., 2005). However, because finer grain sand is more favorable for pit construction, antlions of all ages will construct pits in finer-grained sand, given the option.

Pit construction is not only an integral part of antlions' predatory strategy, but also a costly behavior. Antlions exhibit a 10-fold increase in metabolic rate during pit construction in relation their resting metabolic rate (Lucas, 1985). Metabolic rate also increases with pit diameter (Lucas, 1985). This large metabolic requirement of pit construction is produced by the sand-throwing behavior in which the antlions engage. The high metabolic rate demonstrates an energetic constraint on antlions' predatory strategy, as energy

input into pit construction is limited by the amount of energy antlions receive from food.

Predatory strategy. Antlions' larval stage -- the life stage during which they obtain nearly all of their food -- can last between one to two years (Scharf & Ovadia, 2006). During the larval stage, antlions spend all available energy on feeding and maintaining their pitfall traps (Heinrich & Heinrich, 1984). Because larger antlions are more successful as adults, obtaining enough food and growing is critical to the antlions' success (Scharf, Golan & Ovadia, 2009). Antlions feed on small arthropods that fall in their pitfall traps (Devetak, 2005). Once prey falls down the side of an antlion's pit, the antlion captures the prey, pierces the prey with its mandibles and injects a poison that kills the prey (Griffiths, 1980). If the prey attempts to escape, the antlion will throw sand in the direction of the prey to try to trigger a collapse of the sidewall, which funnels the prey into the center of the pit (Napolitano, 1998). Capture of prey by antlions depends on the amount of sand-throwing in which the antlion engages (Heinrich & Heinrich, 1984). Antlions also show selectivity of prey through their sand-throwing response, as the proportion of prey capture increases with an increased number of sand-throws by the antlion (Heinrich & Heinrich, 1984).

Once the antlion has successfully captured the prey, it will either immediately kill the prey and begin feeding or subdue struggling prey (Napolitano, 1998). To subdue struggling prey, the antlion may engage in prey

beating behavior, the rapid flicking of the prey, or may submerge itself and the prey under the sand. However, these behaviors are not always observed, especially if the prey is not struggling (Napolitano, 1998).

After the prey is dead, antlions inject enzymes into the prey to externally digest the preys' inner contents and then ingest these nutrients through their mandibles (Griffiths, 1980). Once feeding is complete, antlions discard the preys' exoskeletons by throwing them out of pits with their mandibles (Napolitano, 1998). Lastly, antlions perform pit maintenance to return pits to their conical shapes, which may have been disturbed during the latest prey encounter (Napolitano, 1998). If the last prey encounter was unsuccessful and prey escaped, antlions will enlarge the diameter of their pits to help prevent another escape (Scharf, Golan & Ovadia, 2009).

Unsuccessful prey encounters incur high metabolic costs for antlions, as pit diameter increases after each prey escape and metabolic rate increases with pit diameter (Scharf, Golan & Ovadia, 2009). Antlions also would expend energy sand-throwing at the struggling prey and reconstructing their pits after successful escapes (Scharf, Golan & Ovadia, 2009). These actions result in large expenditures of energy that is not recouped through feeding, as antlions sand-throw both in an attempt to capture prey and in reconstruction and enlargement of their pits. Therefore, prey escape is not in the antlions' best interests and should be avoided when possible.

Prey detection. Antlions are able to detect the arrival of prey through vibrations produced by the prey as they travel through the sand (Mencinger-Vracko & Devetak, 2008). Antlions can detect prey that is in close proximity to them and rely heavily on vibratory cues from potential prey to sense its presence (Devetak et al., 2007). Previous research has shown that antlions are able to use these vibrations to determine the direction of approaching prey. That is, even when their sight is hindered and they cannot use visual information to assist them, antlions can still determine direction of origin accurately (Mencinger-Vracko & Devetak, 2008). Antlions use a combination of Rayleigh waves, a type of sound wave capable of traveling through a solid material like sand, produced by small arthropods, and low frequency signals produced by prey items walking across the top of sand to detect prey (Devetak et al., 2007). Signals of typical prey moving across sand range in frequency from 0.1 to 4.5 kHz (Devetak et al., 2007).

Low frequency sound waves travel farthest in the sand and with the least amount of attenuation, or loss in intensity, as compared to higher frequency sound waves (Devetak et al., 2007). The distance through which sound waves can travel without attenuation is affected by sand grain size. This characteristic of sound waves is another reason why antlions prefer to live in medium-sized sand grains, as sound waves travel farther in medium-grained sand than in fine-grained sand. However, pit construction is easier and more favorable in fine-grained sand. Previous research has found that ants escape

the greater percentage of the time in course-grain sand, compared to fine-grained sand (Devetak et al., 2007). Thus, when choosing the site where they will construct a pit, antlions face a trade-off between the distances at which prey are detectable and the costs of maintaining an efficient pit (Devetak et al., 2007).

Pupal Stage

Antlion larvae molt between instars one and two and between instars two and three. They retreat into the sand below their pits and stop eating for approximately eight days, while they shed their current exoskeletons for new ones (Guillette & Hollis, 2010). Antlions flatten the vertexes of their pits, a characteristic sign of molting, possibly to prevent wandering prey from becoming trapped in the pits while the antlions are unable to catch them. When antlions have finished molting, they throw their old exoskeletons out of their pits and continue feeding in the same pits (Guillette & Hollis, 2010).

After the third instar, antlions cease eating and encase themselves in sandy cocoons underneath the sand and pupate for approximately 28 days, emerging as winged, but feeble-flying insects (Arnett & Gotelli, 2001). The weight of the larval antlion at the time of pupation affects the size of the reproductive organs and the average size and fat content of the female's eggs (Griffiths, 1985). The amount of fat stored by the larval antlion determines when pupation will occur, as enough fat must be stored so that the antlion will

survive during pupation and ideally still have fat stores available for use as an adult (Griffiths, 1985).

Adult Stage

After emerging from the cocoon, adults live only for approximately one month (Scharf & Ovadia, 2006). During this stage, females breed and oviposit in the sand (Arnett and Gotelli, 2001). Because of their limited mobility, the location of breeding and ovipositing by the female is limited to a close vicinity to where the female resided during its larval stage (Devetak et al., 2005).

Predator versus Prey

Antlions' diets are comprised primarily of small arthropods and, thus, ants are a common prey item (Godzinska, 2004). Ants can be challenging prey for the antlion to capture, as some species of ants have been observed attempting to free nestmates that are struggling on the walls of antlion pits (Czechowski, Godzinska & Kozlowski, 2002). For example, one species of ant, *Formica fusca*, pulled at the limbs of struggling conspecifics, dug sand around the struggling ants' legs, and attacked antlions in an attempt to free conspecifics (Czechowski, Godzinska & Kozlowski, 2002). Nowbahari, Scohier, Durand & Hollis (2009) found that another species of ant, *Cataglyphis cursor*, responded to entrapped nestmates and attempted to free them by biting at snares in which the nestmates were placed by the researchers. This biting behavior has also been observed in ant rescue from

antlion pits (Nowbahari et al., 2009). If struggling ants are rescued by other ants, antlions lose a valuable feeding opportunity. Due to the possibility of a lost feeding opportunity and potential harm to the antlion by the rescuing ants, antlions could benefit from the ability to anticipate the arrival of rescuers. In this way they could modify their response to ants that have fallen into their pits to prevent prey rescue, thus maximizing their fitness.

Although previous research has not yet demonstrated that antlions can anticipate rescuers, antlions are capable of associative learning. Guillette, Hollis and Markarian (2009) and Hollis, Cogswell, Snyder, Guillette and Nowbahari (2011) found that associative learning in antlions led to significant fitness advantages. Antlions in the learning group molted to the next instar faster than in the control condition (Guillette, Hollis & Markarian, 2009). Hollis et al. (2011) also found that antlions pupated faster when presented with a vibratory cue paired with food. In both experiments, antlions in a control group received food and a vibratory cue at separate times. Antlions in the learning groups of both experiments that exhibited faster molting or pupation time spent less time in the larval stage resulting in decreased exposure to predation, compared to antlions in the control groups (Guillette, Hollis & Markarian, 2009; Hollis et al., 2011).

Antlions in the learning condition had both faster extraction rate, the rate at which antlions extracted from prey, and extraction efficiency rate, percentage of mass extracted from prey per unit time, than antlions in the

control group. This suggests that these antlions were able to better prepare themselves for the arrival of prey than those antlions that did not learn (Guillette, Hollis & Markarian, 2009).

This acceleration of time between molts occurred because antlions in the learning group were able to receive more nutrients from their prey, observed by improved extraction rate and extraction efficiency rate, and faster growth than those antlions in the control group (Guillette, Hollis & Markarian, 2009). While Hollis et al. (2011) did not report extraction measures, decreased time to pupation for antlions in the learning condition probably resulted from differences in extraction measures between the learning and control group as well. Antlions must reach a critical mass before pupation in order to have enough fat stores for pupation (Griffiths, 1985). Faster movement through the larval instar stages means that antlions reached this critical mass faster.

In addition to fitness benefits, antlions used the learned vibratory cue to modify their predatory behavior. Antlions developed a sand-throwing behavior in response to the vibratory cue (Hollis et al., 2011). This response was absent in antlions in both the learning and control groups at the beginning of the experiment. Antlions in the learning group exhibited increased occurrences of sand-throwing during the middle of the experiment, but then stopped sand-throwing all together. Hollis et al. (2011) hypothesized that this marked increase followed by a decrease in occurrences of sand-throwing reflected the effect of a learned response, as the presence of the sand-throwing

behavior did not affect the outcome of the trial, because antlions always received food. Therefore, antlions ceased the sand-throwing behavior, as it was not essential to securing food and continuation of the behavior was not energetically favorable (Hollis et al., 2011).

Although these experiments were conducted in a laboratory setting using vibratory cues that mimicked the vibration produced by prey as they approach antlion pits (Guillette, Hollis & Markarian, 2009; Hollis et al., 2011), these results suggest that learning potentially could play a beneficial role in the antlions' predatory strategy in its natural environment.

Present Study

The current experiment aimed to determine whether antlions could use associative learning to anticipate ant rescue attempts by discriminating between a cue that signals an imminent rescue attempt and a cue that signals an opportunity to eat undisturbed. This experiment also aimed to demonstrate the importance of learning for the antlions' predation strategy and reproductive fitness by evaluating how antlions' modify their predatory and feeding behaviors using learned cues. A vibrational cue of a specific frequency and intensity was paired with the arrival of prey and the opportunity for antlions to eat the prey undisturbed. Another vibrational cue of a different frequency, but the same intensity as the first cue, was paired with a simulated rescue attempt, namely the subsequent removal of prey, as would occur in nature if the prey's nestmates were successful in removing it from the pit.

I predicted differences in sand-throwing behavior would exist between antlions in the learning and control groups. That is, because antlions can learn to associate a cue and its location with feeding or prey rescue, they would be able to change their predatory strategy to anticipate a specific outcome. I also expected that antlions would decrease or cease sand-throwing when exposed to the cue leading to prey rescue. This decrease in sand-throwing would occur because of the high energetic costs of sand-throwing that are not recouped during a lost feeding opportunity resulting from a successful rescue of prey. Sand-throwing also disrupts pit construction and antlions must expend additional energy in pit maintenance. The total energetic costs of sand-throwing are higher than the benefits if the antlion is unable to capture prey. Unlike antlions in the learning group, antlions in the control group would not learn the association between the cue and the resulting rescue attempt and would not decrease or cease sand-throwing when the cue is played.

I predicted antlions in the learning group would digest food more efficiently because they can prepare for feeding in advance of prey arrival. Thus, I predicted that a difference in the extraction efficiency rate and extraction rate would be present between the antlions in the learning group and the control group. Antlions in the learning and control conditions would not differ in extraction efficiency, because antlions in both groups would extract the same amount of food irrespective of how fast the extraction occurs, similar to findings by Guillette, Hollis & Markarian (2009). The learned

anticipatory response present in the antlions in the learning group allowed these antlions to prepare for prey before its arrival and to begin extracting mass sooner than antlions in the control condition, which had to prepare for feeding after the prey had been delivered and increasing their feeding time.

Due to the improved ability to extract food from its prey, I expected antlions in the learning group to molt or pupate faster than antlions in the control group. This would occur because the antlions in the learning group would grow faster.

METHOD

Subjects

One-hundred and twenty larval antlions (*Myrmelon crudelis* Walker) were purchased from Antlionfarms.com in Pensacola, Florida. Upon arrival, each antlion was placed in a 355 mL plastic bowl filled with 300 mL of Estes Marine Sand. The antlions were left unfed and undisturbed for 48 hours to construct pits. After 48 hours, or when the antlions constructed pits, whichever occurred sooner, antlions were fed, each day, between two and six wingless fruit flies, *Drosophila melanogaster*, obtained from Connecticut Valley Biological Supply in Southampton, MA. The fruit flies were delivered to the center of each antlion pit and feeding behavior was monitored. Each fruit fly was fed individually so that the antlions had time to digest the contents of each fruit fly before being given another one. All antlions were housed at room temperature (20-23°C) and had a 14/10-hr light/dark cycle (lights on at 0900, off at 2300).

During the pre-experiment time period, all antlion pits were observed before feedings to check for pits that appeared to be flat-bottomed instead of the normal conical shape, as this appearance was a characteristic sign that the antlion was either molting or pupating. Antlions with flat-bottomed pits, or antlions without pits, were excluded from feeding that day. Antlions also were monitored for the presence of exoskeletons outside their pits, as a confirmation that molting had occurred.

The experiment consisted of two different replications. The first replicate was run between October 2010 and December 2010; seven antlions received the learning treatment and seven antlions received the control treatment. The second replicate was completed between January 2011 and March 2011; nine antlions received the learning treatment and nine antlions received the control treatment. An antlion was admitted into the experiment after it molted, emerged, and constructed a new pit, a procedure that ensured that all antlions entered the experiment in either the second or third instar and exhibited normal pit-building behavior. After each antlion emerged from molting, it was weighed to the nearest 0.1 mg and the depth and width of its pit was measured to the nearest 1.0 mm. Antlions were paired based on weight and pit volume and each member of the pair was randomly assigned to one of two groups, learning or control.

Apparatus and Stimulus Materials

Apparatus. Once antlions were entered into the experiment, they were moved to individual plastic boxes (28 cm long x 18 cm wide x 11.5 cm deep) filled with fine, filtered Estes Marine Sand to a depth of nine cm and placed on one of four shelves, each shelf holding six antlions. Two shelves housed antlions in the control group and two shelves housed antlions in the learning group. The identities of the groups housed on these shelves switched from replicate one to replicate two to ensure that location was not an experimental confound. Each plastic box rested upon 2 in. of regular density urethane foam,

surrounded by an additional 2 in. of regular density urethane foam and was placed in a sound-attenuating chamber constructed from three pieces of 2.54 cm thick extruded polystyrene insulation, which surrounded the box on three sides (Fig. 1). Temperature and light settings were kept constant in the pre-experiment and experiment.

Antlions were placed in the center of the plastic box and plastic barriers constructed from an 18 oz. Solo Grip® cup (9.0 cm diameter, 3.0 cm tall and 0.05 cm thick) were inserted into the sand around the antlions. The plastic barriers were removed at least two hours before training and were reinserted after training was complete. If an antlion moved and constructed a new pit outside of the center of the box and it was still possible for training to occur given the location of the antlion, training proceeded normally and the experimenter relocated the antlion to the center of the box after training was completed and the plastic barrier was replaced around the antlion to encourage it to construct a new pit in the center of the box. If the antlion was in a location where it was not possible to perform the training, the antlion was moved back to the center of the box and given at least two hours to construct a pit before undergoing training. After the training session finished, the plastic rim was placed around the antlion to ensure it dug a pit within the required area for the experiment.

Figure 1. Apparatus filled with sand, positioned inside sound attenuating chamber with MP3 player and speaker.



Sound cues. Because antlions are capable of detecting vibrations through the sand (Devetak et al. 2007), two different vibratory cues were used in this experiment. The sound cues were generated using Finale 2010, music editing software, and saved as audio MPEG-1 (mp3) files. The high frequency cue was created at a frequency of 2793.8 Hertz (Hz) and the low frequency cue was created at a frequency of 659.3 Hz. Both cues had the same intensity of 8 decibels (dB). The frequency and intensity values were chosen using measurements of *Formica* walking across sand published in previous work (Devetak et al. 2007), to ensure that they were detectable by the antlion. The sound cues were downloaded to an MP3 music player and a pair of earphones was attached. One earpiece of the earphone was placed on the sand, with the speaker facing down in the sand, 8 cm from the center of the antlion pit. The earphone not being used was covered in tape to reduce extraneous noise during the training and placed outside the apparatus so that noise would not influence training. The same MP3 player and earphone was used for all antlions throughout the entire experiment.

Mealworms. *Tenebrio larvae* (mealworms), obtained from Connecticut Valley Biological Supply, were used as the food source for the antlions in the experiment. During trials in which antlions were fed, they received one-quarter of a live mealworm, weighing between 0.005 g and 0.010 g. Mealworms were cut behind their third set of legs and the portion of the mealworms containing the head and legs were delivered to the antlions, so

that mealworms continued moving and the antlions detected its presence.

Antlions were fed a quarter of a mealworm, instead of a whole mealworm, because the whole mealworms are bigger than the size of the antlions' natural prey and are capable of injuring the antlions.

Procedure

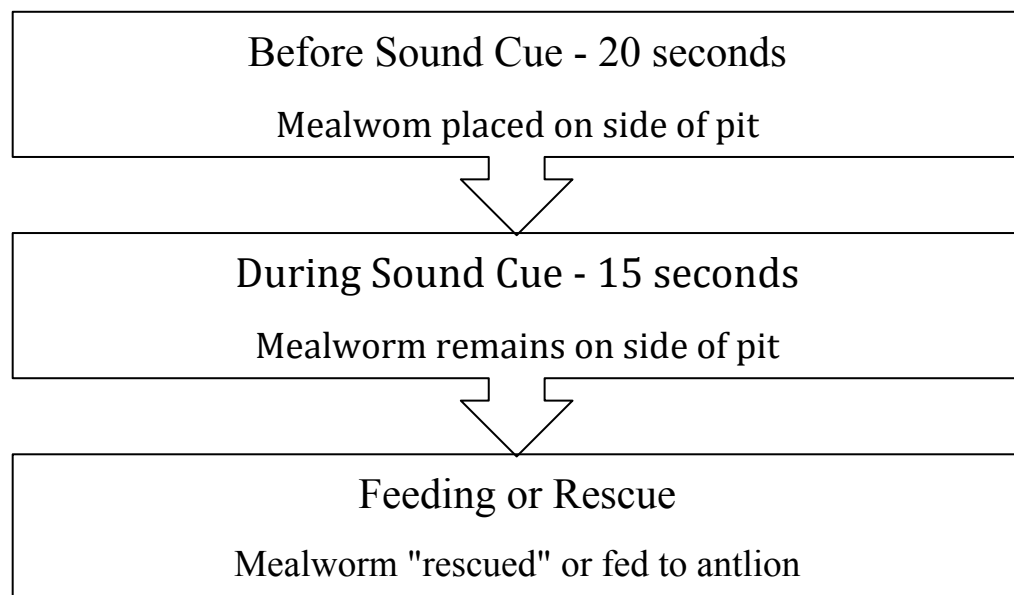
Antlions were trained for four days followed by a one-day rest, which together constitutes one block segment. Training occurred between 1200 hours and 2300 hours. Each block segment included two trials in which prey were made available and two trials in which prey were removed, simulating escape. The order of these four trials was rearranged within each block segment to ensure that trial order was not an inadvertent cue (Appendix A). All antlions were fed the same number of times per block segment and all six possible combinations of four trials were used. As these combinations were only enough for the first six block segments, the order of prey availability and rescue days in the last two block segments were a repeat of block segments one and two. For data analysis, two block segments were combined to create a block. Each block consisted of eight training days and two rest days. There were four trials in which prey were made available and four trials in which prey were removed. The experiment lasted for a total of 40 days and was comprised of eight block segments and four blocks.

Each trial began by exposing the antlion to a live, whole mealworm approximately halfway down one sidewall of its pit for 35 s. A string was

threaded through the posterior part of the mealworm body for easier and more precise placement on the side of the pit. After the mealworm was placed on the side of the pit for 20 s, a sound cue, lasting 15 s, was played on the same side of the pit as the mealworm. On trials in which prey were made available, prey availability (P_A) trials, a quarter of a live mealworm was delivered to the center of the pit simultaneously with the removal of the whole mealworm from the side of the pit and the end of the sound cue. The quarter of the live mealworm that was fed to the antlion was weighed before delivery to the pit and after the antlion threw the carcass out of its pit. Feeding was considered complete after the antlion threw the mealworm carcass out of its pit. In cases in which the antlion did not throw the mealworm carcass out the pit, mealworm carcasses were removed once antlions did not have contact with the mealworm for 10 s. The elapsed time for feeding was recorded. For trials in which prey was not made available, simulating rescue (P_R), the mealworm was removed from the side of the pit simultaneously with the ending of the sound cue, and no food was delivered to the center of the pit (Fig. 2).

For P_A trials, subjects in the learning group (LRN) experienced one of the two sound cues paired with the opportunity to eat prey uninterrupted on the left side of the pit. In P_R trials, the learning subjects were exposed to the other cue paired with the presence and the subsequent removal of prey on the right side of the pit. The cues were counterbalanced to ensure learning was not specific to one cue. However, to help avoid experimental error, the position

Figure 2. Diagram of procedure.



cue, left versus right, was not counterbalanced. Both location and sound acted as stimuli that the antlions used to create associations with the different feeding events, availability or rescue. Subjects in the control group (CTL) received each of the four possible cue-prey treatment combinations to prevent them from learning associations between cues and prey treatments.

Each training trial was video recorded. Each subject was filmed for 15 s before the mealworm exposure, for the duration of the sound cue and the mealworm exposure (35 s in total) and for 5 min after the mealworm exposure during P_A trials or 10 s during P_R trials.

All subjects were recorded using Panasonic PV-GS31 and PV-GS39 Mini DV Digital Camcorders and 90 miniature (LP) Panasonic Mini DV tapes. Cameras were positioned 8.13 in. away from the antlion habitat and angled 45° downward. The light located on the front of the camera was set to the lowest setting to provide additional light in the pit and decrease the shadow from the camera. The video recordings of each subject were analyzed for behaviors and feeding time.

Data Analysis

Data were collected throughout the experiment; measures included the number of days to molt or pupate, pit volume, latency to grab the prey, latency to throw the mealworm carcass, and extraction measures. These measures provided information about subjects' growth, feeding and predatory behavior.

These data were analyzed in PASW Statistics 18.0 to determine if differences existed between the learning and control groups.

Antlions that molted during the experiment were not present during the last block (Block 4) of the experiment. In order to include these antlions in the analysis, only the last three blocks of training were analyzed for all antlions. Thus, Blocks 1 – 3 were used for antlions that molted and Blocks 2 - 4 were used for antlions that did not molt. This set-up allowed me to compare all antlions at a similar stage in training, namely the last 30 days before they left the experiment.

Pit volume was calculated using the equation for the volume of a cone, $\text{Volume (cm}^3) = 1/3 \pi x r^2 x h$. Pit depth and diameter were measured daily by the experimenter for the duration of the experiment and used to calculate pit volume. Pit depth, the height of the pit measured from the vertex to the top of the pit walls, and diameter, the width of the pit at its widest point, were measured using a ruler and estimated to the nearest millimeter to ensure as much accuracy as possible in the measurements without disturbing the antlions or the pits. The same experimenter performed pit measurements throughout the entire experiment to maintain consistency of measurements. Pit volumes for each day of the block, a total of ten days, were used to calculate the average pit volume for each block of the experiment.

Pit volume increases with antlion size; therefore, this measure provided information on how antlions in the LRN and CTL groups grew in

relation to each other. Differences in pit volume between subjects in the LRN and CTL group were analyzed using a 2 x 3 (Group x Block) mixed ANOVA. Group served as the between-subjects variable and Block served as the within-subject variable.

Three extraction measures, which have been used in previous research, were used to detect differences between feeding behavior for LRN and CTL subjects. Extraction *rate*, which is the rate at which antlions extract mass from prey, was calculated by subtracting the final weight of the mealworm carcass (W_f) from the initial weight of the mealworm (W_i) divided by the feeding time (t), the time antlions initially grasp the mealworm subtracted from the time the mealworm carcass was thrown out of the pit. Extraction *efficiency*, which is the percentage of mass antlions extract from the prey, was calculated using the equation $(W_i - W_f) / W_i$. Extraction *efficiency rate*, which is the amount of mass antlions extract from the prey per unit time, was calculated using the equation $((W_i - W_f)/W_i)/t$ (Table 1). These three measures were collected during P_A trials, as these were the days that antlions were allowed to feed uninterrupted.

Extraction measures were analyzed using a mixed analysis of variance (ANOVA) with a 2x3 (group x blocks) factorial design. Group served as the between subjects factor and Block served as the within-subjects factor. The within-subjects factor had three levels, the third-to-last block, second-to-last block, and last block.

Table 1. Computations for Extraction Measures

Extraction Measure	Definition	Equation
Latency to Grab (GrabLat)	Amount of time taken to initially grasp prey after delivery	Time Taken to Grab Prey – Prey Delivery Time
Feeding time (t)	Amount of time taken to feed	(Prey Carcass Throw Time – Prey Delivery Time) - GrabLat
Extraction Rate	Rate of mass extraction from prey	$(W_i - W_f) / t$
Extraction Efficiency	Percentage of mass extracted from prey	$(W_i - W_f) / W_i$
Extraction Efficiency Rate	Amount of mass extracted from prey per unit time	$((W_i - W_f) / W_i) / t$

The number of days to pupate or molt were recorded for each antlion and analyzed using a Chi-Square Goodness-of-Fit test to determine if more antlions molted in the LRN group molted than in the CTL group and if this difference is greater than chance. The Chi-Square Goodness-of-Fit test was appropriate, as it determines whether a sampling distribution differs from the theoretical distribution for a given sample size. Then, a Mann-Whitney U test was conducted to determine whether days to molt for the LRN and CTL subjects were different. The Mann-Whitney U test is a nonparametric test that is used when data does not follow a normal distribution and cannot be analyzed following assumptions of a normal distribution regarding the distribution of around the mean. This test was appropriate, as these data did not follow a normal distribution, groups were independent of each other and measurements were ordinal. The days to molt or pupate provided information about growth rate of antlions.

Sand-throwing, a behavioral response by antlions to prey moving on a pit wall, was observed both before the sound cue was presented and while the cue was played. The number of sand-throws performed by each antlion before the sound cue was played (10 s) and during the sound cue (15 s) was recorded and rates of sand-throwing for before the cue and during the cue were calculated, so that sand-throwing behavior of before and during the sound cue could be compared to each other.

Differences in sand-throwing behavior were analyzed to measure how learning affects predatory strategy. Sand-throwing behavior was analyzed using a mixed ANOVA with a 2 x 2 x 3 (group x cue x blocks) factorial design. The between-subjects variable, group, was comprised of two levels, LRN and CTL. Cue, a within subjects variable, was comprised of two levels, before cue and during cue. The before cue level was defined as the 10 s before the cue began and the during cue level was defined as the 15 s during which the cue played. A second within-subjects variable, blocks, had three levels, the third-to-last block, second-to-last block and last block of training.

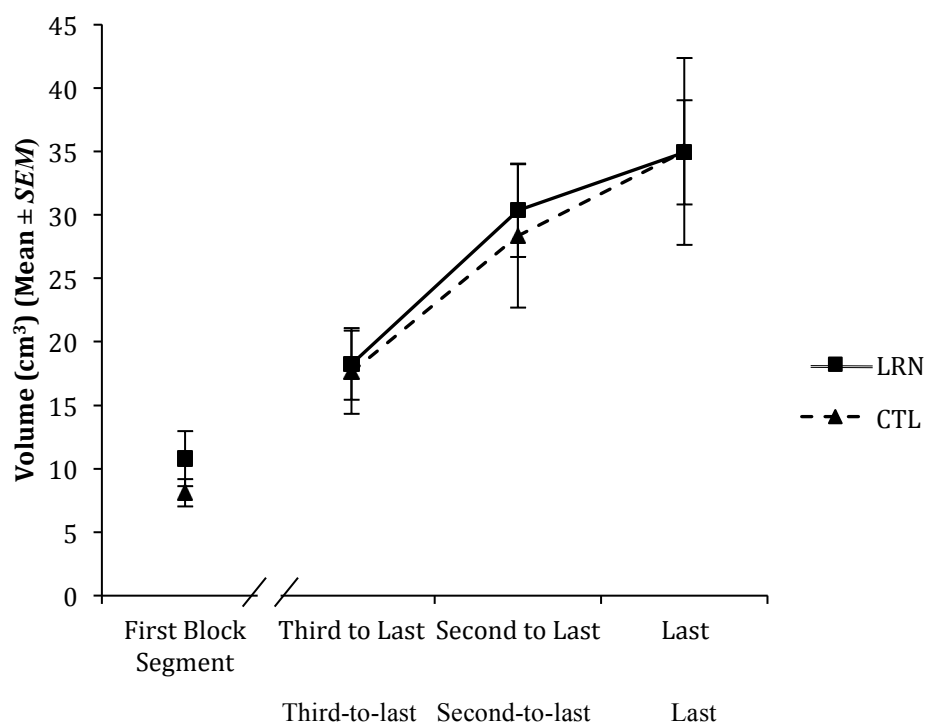
RESULTS

Antlions were entered into the experiment in pairs, matched by mass (g) and pit volume (cm³). An independent sample *t*-test was performed on initial mass and no significant difference was found between the LRN group ($M = .01$, $SEM = .002$) and CTL group ($M = .01$, $SEM = .002$), $t(30) = -.22$, $p > .05$. Another independent sample *t*-test was performed comparing pit volumes on day one of the experiment and no significant difference was found between LRN ($M = 7.28$, $SEM = 2.0$) and CTL ($M = 5.72$, $SEM = 1.1$) subjects, $t(30) = .68$, $p > .05$.

Pit Volume

Pit volumes for the LRN ($n = 16$) and CTL ($n = 16$) subjects were analyzed for the first block segment of training for all subjects and last three training blocks for each subject (Fig. 3). The first block segment was included in Figure 2 to show that pit volumes were the same for all subjects at the start of the experiment. Pit volumes for LRN subjects and CTL subjects were the same during the first block segment (first four days of training), $t(30) = 1.11$, $p > .05$. Pit volumes for all subjects significantly increased from the third-to-last block to the last block, $F(2,60) = 10.13$, $p < .05$. However, pit volumes did not increase significantly more for LRN subjects than CTL subjects from the third-to-last block to the last block of the experiment, $F(2, 60) = 1.01$, $p > .05$.

Figure 3. Pit volume (mean $\text{cm}^3 \pm \text{SEM}$) across the first block segment and last three blocks for all LRN ($n = 16$) and CTL ($n = 16$) subjects.



Sand-Throwing Behavior

Independent *t*-tests were performed on rates of sand-throwing before and during the sound cue in Block 1 to determine whether LRN subjects ($n = 16$) and CTL subjects ($n = 16$) threw sand at the same rate during the first four P_R trials and first four P_A trials of the experiment. Rates of sand-throwing between LRN subjects and CTL subjects before the sound cue played during P_R trials were not significantly different, $t(30) = .70, p > .05$. Another independent *t*-test showed no significant difference between rate of sand-throwing for LRN subjects and CTL subjects during the sound cue during P_R trials, $t(30) = .64, p > .05$. No significant difference was found between rate of sand-throwing for LRN subjects and CTL subjects before the sound cue played during P_A trials, $t(30) = .227, p > .05$. Rates of sand-throwing during the sound cue in P_A trials did not differ significantly between LRN subjects and CTL subjects, $t(30) = -.39, p > .05$.

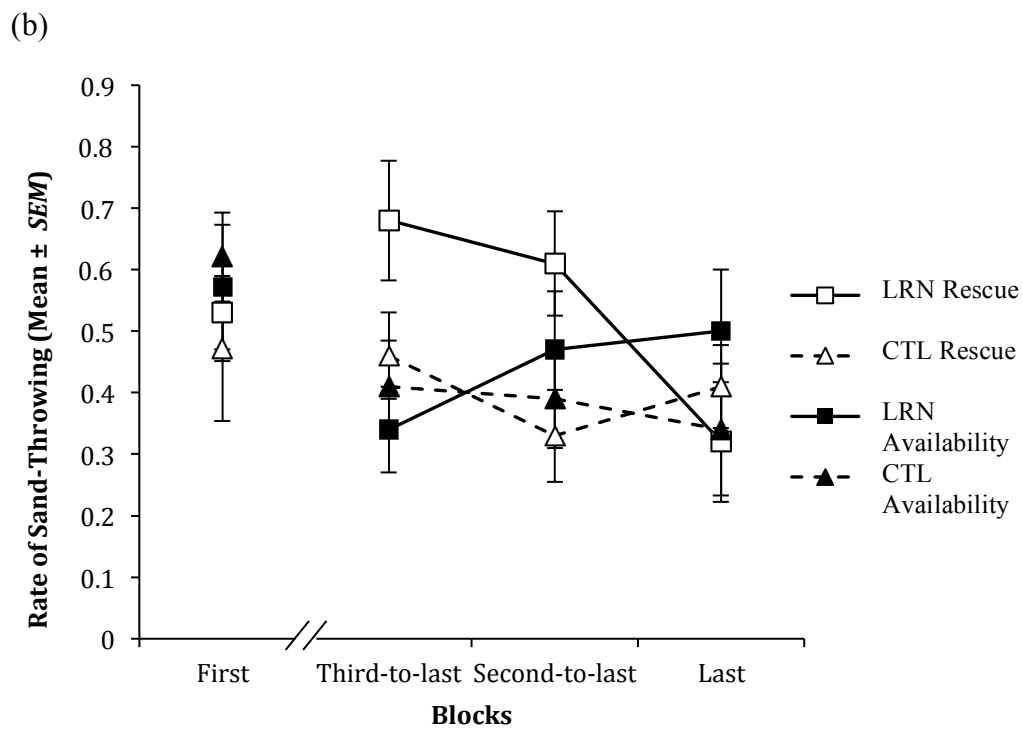
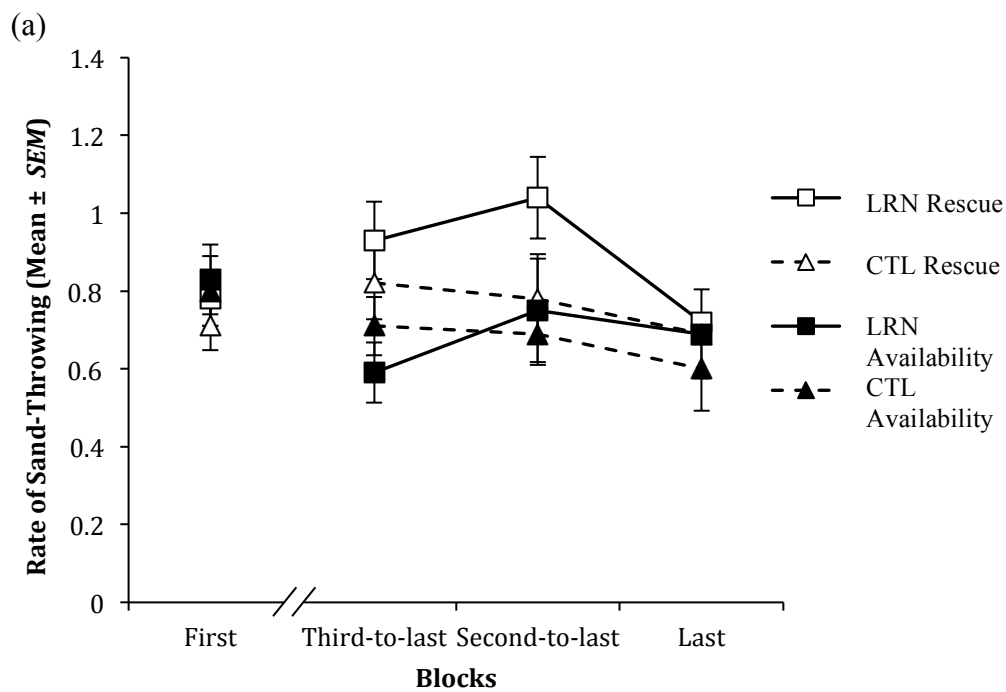
Effect of training day. Rates of sand-throwing for the first, third-to-last, second-to-last, and last blocks are presented in Figure 4. Block 1 was included in the figure to demonstrate that all subjects exhibited similar rates of sand-throwing at the beginning of the experiment. Rates of sand-throwing for subjects that molted are the same value for Block 1 and the third-to-last training block. Therefore, Block 1 was not included in the analysis to prevent repetition of the data.

Rate of sand-throwing before the sound cue differed between P_R and P_A trials. All antlions exhibited higher rates of sand-throwing in P_R trials before the cue than in P_A trials, indicated by a main effect for day $F(1, 30) = 12.03, p < .05$. No differences in rate of sand-throwing before the cue were found between LRN and CTL antlions across training days, $F(2, 60) = 1.34, p > .05$ (Fig. 4a), indicating that the type of training day did not influence rate of sand-throwing before the sound cue played.

An analysis of rate of sand-throwing during the sound cue only in P_R trials showed differences between LRN and CTL subjects (Fig. 4b). All subjects decreased their rates of sand-throwing from the third-to-last block to the last block, $F(2, 60) = 4.13, p < .05$. However, LRN subjects significantly decreased their rates of sand-throwing across blocks, while CTL subjects did not significantly decrease their rates of sand-throwing across blocks, as a significant interaction between block and group was present, $F(2, 60) = 3.68, p < .05$. This interaction indicates that the decrease of all subjects from the third-to-last block to last block was biased by the decrease of the LRN subjects. Analysis of rates of sand-throwing during the cue in P_A trials showed no significant interaction between block and group on rate of sand-throwing across blocks for LRN and CTL subjects, $F(2, 60) = 1.35, p > .05$. No differences were observed between LRN and CTL groups during P_A trials.

An analysis of the last three blocks for each subject during the sound cue revealed an interaction between block, day, and group, $F(2, 60) = 3.86,$

Figure 4. Rate of sand-throwing (number of sand-throws/s \pm SEM) for the first block and last three blocks of training (a) before the sound cue and (b) during the sound cue in prey rescue (P_R) and prey availability (P_A) trials for LRN ($n = 16$) and CTL ($n = 16$) subjects.



$p < .05$. Because this interaction only indicates that at least two data points are different, post-hoc Bonferroni t -tests were performed to determine where differences occurred. These tests confirmed that LRN subjects sand-threw at a higher rate than CTL subjects during the third-to-last block in P_R trials, $t(60) = 2.20, p < .05$, and in P_A trials, $t(60) = 2.62, p < .05$ (Myers, 1979). LRN subjects also threw sand at a higher rate during the third-to-last block in P_R trials than in P_A trials, $t(60) = 3.33, p < .05$ (Myers, 1979). The rate of sand-throwing decreased for LRN subjects from the third-to-last block to the last block in P_R trials, $t(60) = 3.52, p < .05$ (Myers, 1979). No differences were found between rates of sand-throwing of CTL subjects for any of the blocks in P_A or P_R trials, $t(60) \leq .27, p > .05$ (Myers, 1979). These post-hoc tests confirm that LRN subjects decreased their rate of sand-throwing from the third-to-last block to the last block during the sound cue in P_R trials, while rate of sand-throwing for CTL subjects during the cue did not change. Subjects in the LRN group also changed their rate of sand-throwing during the experiment depending on the type of training day, while the CTL group maintained the same rate of sand-throwing throughout the last three training blocks of the experiment regardless of the type of training day.

Effect of cue. Rate of sand-throwing for the first, third-to-last, second-to-last and last blocks for before and during the sound cue are displayed in Figure 5. Block 1 of training was included in the figure to show that LRN and CTL subjects had similar rates of sand-throwing at the beginning of the

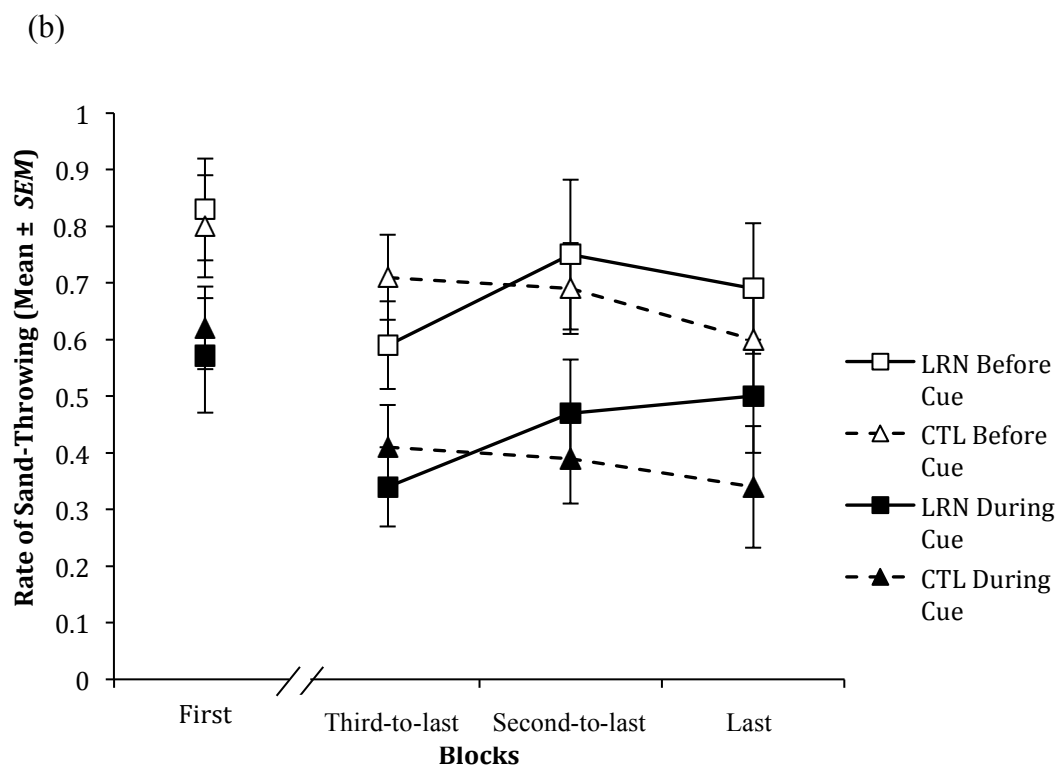
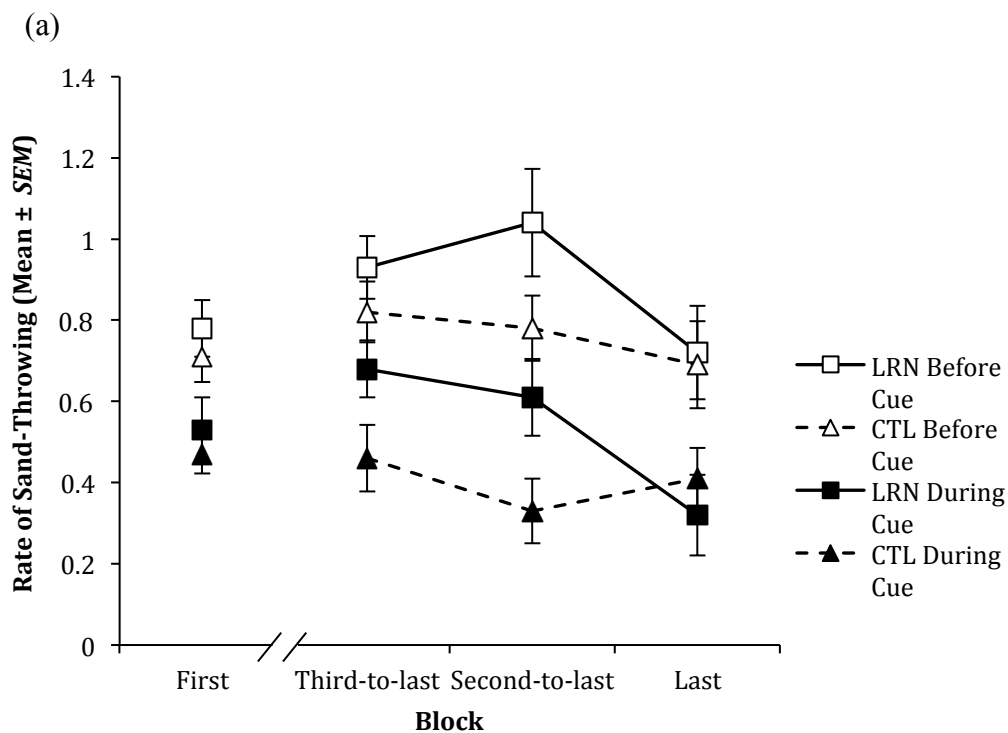
experiment. Only the final three blocks of training for each antlion were included in the analysis to avoid redundancy in the data, as the values for rate of sand-throwing in the first block and the third-to-last block are the same for antlions that molted during the experiment.

In P_R trials, rates of sand-throwing for all subjects decreased during the cue, as compared to rates of sand-throwing before the sound cue, $F(1, 30) = 173.09, p < .05$. LRN and CTL subjects decreased their rates of sand-throwing the same amount when the sound cue played, $F(2, 60) = .00, p < .05$, and this decrease did not change from the third-to-last to last block, $F(2, 60) = 1.88, p < .05$, and (Fig. 5a).

During P_A trials, rates of sand-throwing were higher before the sound cue than during the sound cue, $F(1, 30) = 65.96, p < .05$, for all subjects. No differences existed from the third-to-last block to last block and between before the cue was played and during the cue was played for LRN and CTL subjects in P_A trials, $F(2, 60) = .02, p < .05$ (Fig. 5b).

Differences in rates of sand-throwing were present between LRN and CTL subjects during the cue in P_R trials. Subjects in the LRN group decreased their rates of sand-throwing over time, while CTL subjects did not change their rate of sand throwing. This difference was not present before the sound cue played. During P_A trials, LRN and CTL subjects exhibited the same rate of sand-throwing before the sound cue and all subjects decreased their rates of

Figure 5. Rate of sand-throwing (Mean number of sand-throws/ s \pm SEM) before and during playing of the sound cue during the (a) P_R trials and (b) P_A trials for LRN ($n = 16$) and CTL ($n = 16$) subjects.



sand-throwing when the sound cue was played. Subjects also exhibited the same rates of sand-throwing during the sound cue.

Extraction measures

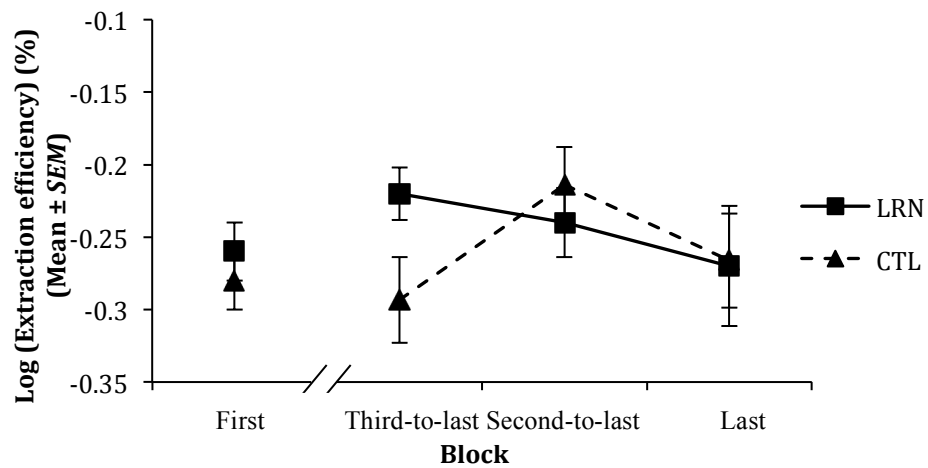
Extraction measures for the first, third-to-last block, second-to-last block and last block were included in Figure 6 to show similarities in feeding behavior at the beginning of the experiment and differences between groups that arose by the end of the experiment. Similar to analyses of pit volume and sand-throwing, only the last three blocks of training were analyzed for differences because extraction measures for Block 1 and the Third-to-Last block were the same values for antlions that molted.

Independent sample *t*-tests were performed on feeding measures for Block 1 to determine if differences in feeding behavior were present at the start of the experiment. Independent sample *t*-tests revealed no significant differences between LRN and CTL subjects for extraction rate, $t(29) = .18, p > .05$, extraction efficiency, $t(29) = .65, p > .05$, and extraction efficiency rate, $t(29) = .54, p > .05$. One CTL subject was not included in the *t*-tests, because it did not begin feeding until Block 2.

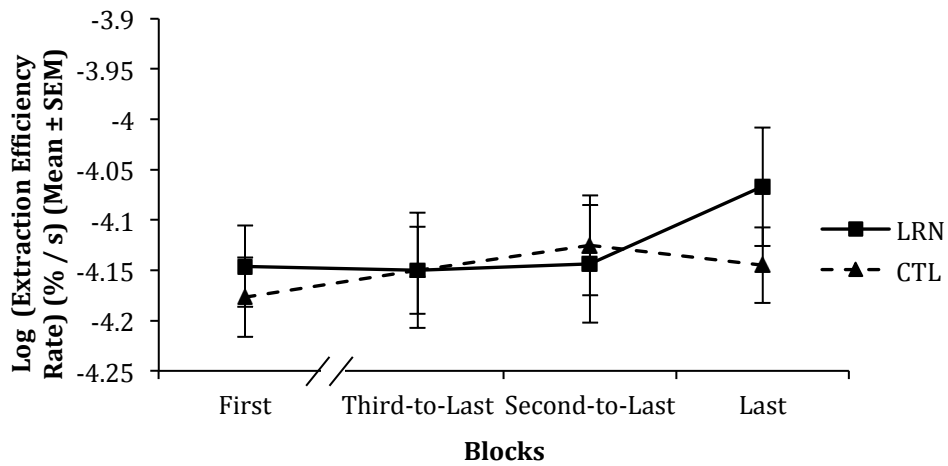
Analysis of feeding measures during the last three blocks of training confirmed that all subjects exhibited similar feeding behavior throughout the experiment. No significant differences were found between LRN and CTL subjects from the third-to-last block to last block for extraction efficiency,

Figure 6. (a) Extraction efficiency ($\% \pm SEM$) (b) Extraction rate ($g / sec \pm SEM$) (c) Extraction efficiency rate ($\% / s \pm SEM$) for LRN ($n = 16$) and CTL ($n = 16$) subjects.

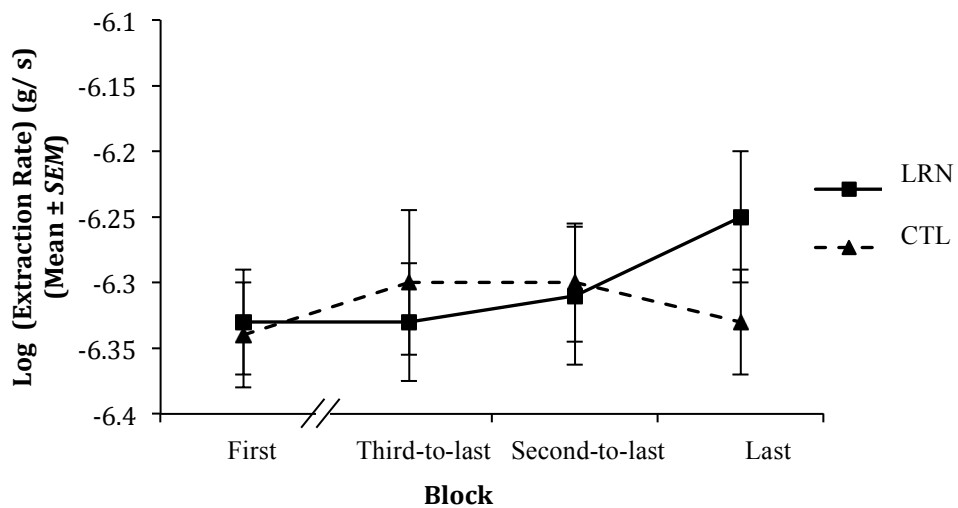
a)



(b)



(c)



$F(2, 60) = 2.09, p > .05$ (Fig. 6a), extraction rate, $F(2, 60) = 1.73, p > .05$ (Fig. 6b), and extraction efficiency rate, $F(2, 60) = 1.01, p > .05$ (Figure 6c).

Days To Molt

Although two LRN subjects were the first to molt, only seven subjects molted during the 40 days of the experiment (5 LRN subjects and 2 CTL subjects). The difference between the number of LRN and CTL subjects that molted was not statistically significant, $\chi^2(1) = 1.65, p = 0.20$, indicating that the difference between the number of antlions that molted in each group could be attributed to chance. Likewise, a Mann-Whitney U test revealed that LRN subjects did not differ from CTL subjects in the number of days to molt, $U = 151, N_1 = N_2 = 16, p > 0.05$, indicating that LRN subjects did not molt faster than CTL subjects.

DISCUSSION

Sit-and-wait predators have evolved a different predatory strategy from their active foraging counterparts. Instead of actively searching for food, sit-and-wait predators wait for prey to fall into their traps (Guillette & Hollis, 2010). Ants, a common prey item for antlions, have been observed rescuing their nestmates from antlions' pits (Czechowski, Godzinska & Kozlowski, 2002). Successful ant rescue would result in a lost feeding opportunity for antlions (Heinrich & Heinrich, 1984). For antlions to improve their ability to capture prey when it falls into their pits, learning to anticipate both the arrival of prey and the subsequent arrival of rescuers could possibly help antlions develop efficient predatory and feeding strategies, such as modifying their sand-throwing response to prey and preparing to feed when they sense that prey is approaching.

This experiment investigated whether antlions were capable of discriminating between a cue that results in uninterrupted feeding and a cue that results in the successful "rescue" of the prey and no feeding. Pit volumes did not differ during the first block segment of the experiment, the first four days of training. Rates of sand-throwing and feeding measures also did not differ between subjects in the learning and control conditions during the first block, the first eight days of training, indicating that both groups had similar behaviors, which meant that differences between the groups during the

experiment were a result of the experiment and not influenced by differences that were present between the groups before the experiment started.

I hypothesized that antlions in the learning and control groups would differ in feeding and sand-throwing behaviors, as well as pit volume, demonstrating that antlions in the learning condition are capable of associative learning and changed their behavior predatory and feeding behavior as a result of discriminating between two cues. Antlions in the learning and control groups differed with respect to sand-throwing behavior, but did not differ respect to feeding measures, pit volumes and days to molt.

Changes in Predatory Strategy

Sand-throwing is a behavioral response used by antlions to aid in prey capture (Napolitano, 1998). Sand-throwing represents an energy investment for antlions, because not only is the behavior costly, it also requires antlions to return their pits to the original shape (Griffiths, 1985). Therefore, use of this behavior would only be advantageous when feeding opportunities were guaranteed, as the lost energy would be replaced. Antlions in the learning group decreased their rate of sand-throwing in P_R trials across the last three blocks of training, while the rate of sand-throwing of antlions in the control group did not change. This finding suggests that antlions given the opportunity to learn were able to associate the sound cue with prey rescue and discriminate this sound cue from the sound cue meaning prey availability, as the same trend was not observed when prey was made available to the

antlions. This discrimination developed over time because differences between learning and control groups were not present in Block 1, but were present in the last block of training for each antlion. This change demonstrates that antlions in the learning condition were able to associate the sound cue paired with prey rescue after repeated exposures to the cue and prey treatment pairing.

During trials where prey was made available, antlions in both learning and control groups responded with similar rates of sand-throwing. This rate of sand-throwing may represent the normal rate of sand-throwing used by antlions to respond to struggling prey. Antlions in the control condition exhibited this rate of sand-throwing because they responded to the arrival of all prey as potential feeding opportunities, as sound cues were not paired with either prey rescue or feeding and the antlions responded with the same rate of sand-throwing to all trials the same. Antlions in the learning condition also responded to the sound cue as a feeding opportunity because they learned to associate this sound cue with the opportunity to feed. Information from the sound cue paired with feeding opportunities influenced antlions' rates of sand-throwing, as antlions should be able to replace the energy lost in the sand-throwing behavior. When antlions were able to feed, the replacement of energy was accomplished and therefore the sand-throwing was a favorable response to struggling prey. This finding shows how antlions were able to use

learning to influence their sand-throwing behavior in relation to the energy expenditure and replacement of predatory behavior.

In prey rescue trials, antlions in the learning group exhibited higher rates of sand-throwing in the third-to-last block than control antlions. Antlions in the learning group also exhibited higher rates of sand-throwing than all antlions during prey availability trials. The rate of sand-throwing for learning antlions during the third-to-last block is greater than the maximum rate of sand-throwing normally exhibited by antlions in response to struggling prey. This finding suggests that antlions responded to the information that prey would be rescued by increasing their rates of sand-throwing. The subsequent decrease in sand-throwing indicates that antlions decreased their sand-throwing response to struggling prey after increasing their rate of sand-throwing did not result in the opportunity to feed. However, the rates of sand-throwing for antlions in the learning group was not different from antlions in the control group or all antlions in prey availability trials during the last block of training.

By the last block of training, all antlions threw sand at the same rate during the cue regardless of the day, indicating that this rate of sand-throwing is a normal response to prey. The change in response by learning antlions during prey rescue trials illustrates a situation where learning was adaptive, as continuing an elevated rate of sand-throwing would be energetically costly without yielding any benefits, such as a feeding opportunity. By decreasing

the rate of sand-throwing, antlions conserved their energy in a situation where sand-throwing was not beneficial to them.

Modification of the sand-throwing response also was reported Hollis et al. (2011) during training trials when antlions were given feeding opportunities. When antlions received a vibratory cue of falling sand paired with the arrival of food, they first increased the proportion of trials in which they threw sand in the middle of the experiment, and then decreased the proportion of sand in which they threw sand at the end of the experiment (Hollis et al., 2011). The vibratory cue led to feeding in 100% of the trials for the learning group, therefore it was not necessary to throw sand, as food was always delivered regardless of the antlions' responses (Hollis et al., 2011). While the study by Hollis et al. (2011) investigated the presence of the sand-throwing behavior only during a vibratory cue paired with feeding, antlions modified their behavior in a situation where sand-throwing did not change the number of feeding opportunities and the sand-throwing behavior was not energetically favorable.

In the current experiment, antlions in the learning condition responded to prey rescue similarly as antlions did for feeding opportunities in the experiment by Hollis et al. (2011). The similarity between the two experiments in the response to a vibratory cue indicates that antlions in the learning condition of the present study may have used information from the vibratory cue similarly to antlions given the opportunity to learn in experiment

by Hollis et al. (2011). However, antlions in the current experiment experienced lost feeding opportunities in addition to feeding opportunities. Energetic consequences of sand-throwing during lost feeding opportunities are larger than during prey availability (Lucas, 1985), which may explain why the same trend for sand-throwing behavior was observed during prey rescue trials and not prey availability trials.

The discrimination ability between a cue that led to prey rescue and a cue that led to a feeding opportunity is adaptive, because antlions modified their rates of sand-throwing depending on which cue was played and exerted different amounts of energy based on information gained from vibrations produced by the sound cues. The information from the cues gave antlions that learned an advantage over antlions that did not learn, as antlions in the control condition exhibited the same rate of sand-throwing during all trials and learning antlions exhibited different rates of sand-throwing between prey rescue and prey availability trials. This change in behavior for antlions in the learning condition not only indicates that these antlions learned to associate the presence of the sound cue the arrival of food, but also that they used this learned information to develop the most efficient predatory strategy.

In the current experiment, antlions experienced two cues during each training session. Prey moving on one side of the pit produced a location cue and this cue was followed by a vibratory sound cue. Antlions first experienced the location cue by itself, and then experienced the location and vibratory

sound cues together. The sound cues, one to signal prey availability and another to signal prey removal, exploited differences in frequency to provide antlions with distinguishable vibrations produced by the different frequencies. During the last three blocks of training, all antlions reacted to the vibratory cue by decreasing their rates of sand-throwing during the playing of the sound cue, as compared to when only the location cue was present. All antlions decreased their rates of sand-throwing similar amounts, as there were no differences between the learning and control groups. This decrease in the rate of sand-throwing suggests that the onset of the sound cue influenced the rate of sand-throwing for all antlions. One explanation for this finding is that antlions received information from the sound cue at its onset and decreased their rates of sand-throwing to process this information.

Antlions used information from the vibratory cue more than from the location cue. Differences in rates of sand-throwing between antlions in the learning and control conditions were observed only in prey rescue trials during the cue and not before the vibratory cue was played, indicating that antlions in the learning condition did not form associations between only the location cue and prey rescue. Instead, antlions formed associations between the location cue, the vibratory cue and prey rescue. Because the vibratory cue was not presented to the antlions by itself, there is no way to know if antlions only used the vibratory cue to form associations with the outcome of the

training trial or if location combined with the vibratory cue was necessary for antlions to form associations with feeding outcomes.

Location cues have been used in studies of learning in multiple insects (Dukas and Bernays, 2000; Menzel, Erber & Masuhr, 1974; Raubenheimer and Tucker, 1997). The design of the present study was similar to that used in the study of grasshoppers by Dukas and Bernays (2000), because it paired location with a secondary cue as well. Grasshoppers used both cues to learn the location of nutritious food, while antlions primarily used the sound cue to form a learned association. This difference in stimulus utilization may be explained by the fact that grasshoppers are active foragers and location is an important aspect of their foraging strategy. Antlions rely heavily on vibrations through sand since they are sessile and are unable to see their surrounding environment from the bottom of their pits.

Modification of predatory behavior, namely the rate of sand-throwing, of learning and control subjects between prey rescue trials and prey availability trials suggests that antlions are able to discriminate between different vibrational cues and change their predatory strategy based on the information associated with a learned cue. This discrimination ability would be adaptive in ant rescue situations, as the arrival of multiple nestmates would produce a different vibratory signal than the approach of a single ant. Antlions that can anticipate the arrival of nestmates based on the vibratory signal they produce will be able to alter their predatory strategies in a similar fashion to

the changes observed during this study. Antlions also are capable of discriminating between favorable and unfavorable prey encounters by their rate of sand-throwing and by allowing unfavorable prey to escape (Heinrich & Heinrich, 1984). Antlions may stop actively trying to capture a struggling ant on the side of the pit because it is unfavorable prey as nestmates are arriving to rescue the ant and this rescue could result in a lost feeding opportunity, injury and wasted energy, despite sand-throwing efforts by the antlion. Similarly, maintaining a constant rate of sand-throwing when prey will be available and rescue does not occur, like during prey availability trials, increases the likelihood that prey will be dislodged from the side of the pit and will result in a feeding opportunity. In these instances, sand-throwing behavior is advantageous as antlions will be able to feed and replace the lost energy. These modifications of predatory behavior illustrate how antlions can use information from learned cues to create more energy efficient predatory strategies depending on the situation.

Changes in Feeding Strategy

Extraction measures were used to determine whether or not learning to anticipate feeding opportunities affected feeding behavior. No differences between learning and control subjects were found with respect to extraction rate, extraction efficiency and extraction efficiency rate. All three of these measures determined how antlions extracted mass from their prey. Previous research found that extraction rates and extraction efficiency rates increased

for antlions given the opportunity to learn over antlions not given the opportunity to learn and concluded that antlions given the opportunity to learn prepared sooner for the arrival of prey than the subjects in the control after exposure to learned cues (Guillette, Hollis & Markarian, 2009).

In the present experiment, extraction rate increased from the third-to-last block to the last block for antlions in the learning group, while it decreased slightly for the antlions in the control group (Fig. 5b). Though none of these differences are statistically significant, they suggest that differences may have started developing between the learning and control subjects and, if the experiment had continued, statistically significant differences in extraction rate may have been observed. Extraction efficiency rate followed a similar trend and mean extraction efficiency rates for learning and control subjects during the third-to-last and second-to-last blocks were very similar (Fig. 5c). Antlions in the learning and control groups began to diverge from each other by the last block. While these differences are not statistically different either, they do suggest a possible difference that was beginning to develop between antlions in the learning and control groups during the last ten days of the experiment. No obvious differences appeared in the data for extraction efficiency, as there was no divergence of the means between LRN and CTL.

The lack of differences in extraction measures between LRN and CTL subjects in the present study could be attributed to the short duration of the experiment. Differences in extraction rate and extraction efficiency rate did

not appear in a previous study until antlions were given 30 feeding opportunities (Guillette, Hollis & Markarian, 2009). In the current experiment, antlions were given only a total of 16 feeding opportunities. There may not have been enough time in the experiment for differences in feeding measures to develop.

Changes in Growth Rate

Pit volume, a measure of antlion size, increased for all antlions during the last three training blocks of the experiment. This finding was expected, as pit size increases with antlion size (Heinrich & Heinrich, 1984), and has been observed in previous antlions studies (Guillette, Hollis & Markarian, 2009; Scharf, Barkae & Ovadia, 2010). However, antlions in the learning and control conditions did not differ in their pit volumes. In previous work, differences in pit volume between antlions given the opportunity to learn and antlions not given the opportunity to learn were observed (Guillette, Hollis & Markarian, 2009). Antlions given the opportunity to learn had larger pit volumes than antlions not given the opportunity to learn, though this study also observed differences in extraction measures. However, these differences were not statistically significant until day 90 of the experiment (Guillette, Hollis & Markarian, 2009). This length of time was much longer than the duration of the present study and the experiment may have ended before differences in pit volume became statistically significant. Given the lack of differences in extraction measures between learning and control subjects in

the current experiment, it is not surprising that no difference in pit volumes between groups was observed. Antlions grew at the same rate and increased their pit volumes at the same rate, because their food intake was similar.

Another reason for differences in extraction measures of this experiment and previous work was differences in procedure between the two studies. In the previous study, antlions fed during every training session and in the current study antlions were only fed during half the training sessions. The lost feeding opportunities in the current experiment may have affected the rate of pit volume increase, as antlions respond to lost feeding opportunities by increasing the circumference of their pits to increase the number of prey encounters (Scharf, Barkae & Ovadia, 2010). All antlions experienced equal amounts of lost feeding opportunities during the experiment; however, the learning and control groups reacted differently to prey rescue and may have increased their pit volumes differently in response to the lost feeding opportunities, though the experiment was not long enough to fully understand what these differences were.

Seven antlions molted during the course of the experiment and, although a greater number of LRN antlions molted than CTL antlions, neither the number of antlions nor the number of days to molt were different between antlions in the learning and control conditions. Given the lack of difference in extraction measures and pit volumes, this result is not unexpected based on previous research. Previous studies showed that antlions given the opportunity

to learn molted faster than antlions not given the opportunity to learn due to better extraction of nutrients from prey. Faster molting confers a fitness advantage for antlions, as spending less time in each instar decreases amount of time spent in larval stage, which decreases generation time and mortality risk of larval antlions that have limited mobility (Heinrich & Heinrich, 1984; Griffiths, 1985). Lack of difference in molting times between antlions in the learning and control conditions could be attributed to the short duration of the experiment. Differences in feeding measures were not able to develop and, because training days are split between feeding and non-feeding training days, molting may take longer in all antlions because they are not receiving as much food as in the study by Guillette, Hollis & Markarian (2009).

Limitations

As described above, the duration of this study, 40 days, was very short compared to the 90-day study by Guillette, Hollis & Markarian (2009). Antlions were given enough time to learn the association between sound cues and prey treatments and change their sand-throwing behavior, but the duration of the experiment may not have been enough time for learned information to affect pit volume and extraction measures, resulting in a failure to find differences between the learning and control conditions.

In addition to short experiment duration, antlions were not always visible during training trials. These days could not be analyzed for sand-throwing behavior, as antlions were not visible and it was not possible for

them to throw sand. Missing data made data analysis of block segments impossible and I had to increase the number of days in each block to insure that each antlion had at least one day where it was visible and had the opportunity to throw sand. The use of blocks, eight days of training, instead of block segments, four days of training, decreased the number of data points and made differences between learning and control groups more difficult to see. A total of four blocks in the experiment also meant that only three blocks included all antlions, due to the molting of some antlions. Because antlions that molted were only present in the experiment for Blocks 1 – 3, the third-to-last block of training was the same as Block 1 for these antlions and these two blocks could not be compared for the learning and control conditions, as some of the values were repeated.

Conclusions

Associative learning has been observed in a wide range of active foragers. Recent studies have shown that sit-and-wait predators also are capable of associating a single stimulus with an event (Guillette, Hollis & Markarian, 2009); Hollis et al., 2011). This study demonstrated that sit-and-wait predators not only can learn a single cue paired with a stimulus, but also can discriminate between two cues paired with two different stimuli, representing a complex predatory strategy similar to that of some active insects, including grasshoppers and honeybees. This ability benefits antlions, as they can use learned cues to respond to different stimuli in their

environments and develop a most energy efficient predatory strategy, demonstrating that learning is adaptive for sessile insects. Not only have I provided more evidence that sessile insects are capable of associative learning, but I also have demonstrated that sit-and-wait predators are capable of complex predatory strategies that are influenced by learned cues in their environment.

REFERENCES

- Alloway, T. M. (1972a). Learning and memory in insects. *Annual Review of Entomology*, 17(1), 43-56.
- Alloway, T. M. (1972b). Retention of learning through metamorphosis in the grain beetle (*Tenebrio molitor*). *Integrative and Comparative Biology*, 12(3), 471.
- Arnett, A. E., & Gotelli, N. J. (2001). Pit-building decisions of larval ant lions: Effects of larval age, temperature, food, and population source. *Journal of Insect Behavior*, 14(1), 89-97.
- Blackawton, P. S., Airzee, S., Allen, A., Baker, S., Berrow, A., Blair, C., Churchill, M., Coles, J., Cumming, R. F. -J, Fraquelli, L. Hackford, A., Hinton Mellor, M., Hutchcroft, B., Ireland, D., Jewsbury, Al, Littlejohns, G. M., Littlejohns, M., Lotto, M., McKeown, J., O'Toole, A., Richards, H., Robbins-Davey, L., Roblyn, S., Rodwell-Lynn, H., Schenck, D., Springer, J., Wishy, A., Rodwell-Lyn, T., Strudwick, D., & Lotto, R. B. (2010). Blackawton bees. *Biology Letters*, 7, 168-172.
- Botz, J. T., Loudon, C., Barger, J. B., Olafsen, J. S., & Steeples, D. W. (2003). Effects of slope and particle size on ant locomotion: Implications for choice of substrate by antlions. *Journal of the Kansas Entomological Society*, 76(3), 426-435.
- Brownell, P. H. (1977). Compressional and surface waves in sand: Used by desert scorpions to locate prey. *Science*, 197 (4302), 479.
- Couvillon, P., & Bitterman, M. (1980). Some phenomena of associative learning in honeybees. *Journal of Comparative and Physiological Psychology*, 94(5), 878-885.
- Czechowski, W. (2002). Rescue behaviour shown by workers of *Formica sanguinea* latr., *F. fusca* l. And *F. cinerea* Mayr (Hymenoptera: Formicidae) in response to their nestmates caught by an ant lion larva. *Annales Zoologici*, 52(3) 423-431.
- Czechowski, W., Godzińska, E. J., & Kozłowski, M. W. (2002). Rescue behaviour shown by workers of *Formica Sanguinea*, 52, 423-431.
- Devetak, D., Spornjak, A., & Janzekovic, F. (2005). Substrate particle size affects pit building decision and pit size in the antlion larvae *Euroleon*

nostras (Neuroptera: Myrmeleontidae). *Physiological Entomology*, 30(2), 158-163.

Devetak, D. (2005). Effects of larval antlions *Euroleon nostras* (Neuroptera, Myrmeleontidae) and their pits on the escape-time of ants. *Physiological Entomology*, 30(1), 82-86. doi:10.1111/j.0307-6962.2005.00433.x

Devetak, D., Mencinger-Vračko, B., Devetak, M., Marhl, M., & Špernjak, A. (2007). Sand as a medium for transmission of vibratory signals of prey in antlions *Euroleon nostras* (Neuroptera: Myrmeleontidae). *Physiological Entomology*, 32(3), 268-274.

Dukas, R. (2007). Evolutionary biology of insect learning.

Dukas, R., & Bernays, E. A. (2000). Learning improves growth rate in grasshoppers. *Proceedings of the National Academy of Sciences of the United States of America*, 97(6), 2637.

Dukas, R., & Duan, J. J. (2000). Potential fitness consequences of associative learning in a parasitoid wasp. *Behavioral Ecology*, 11(5), 536.

Fertin, A., & Casas, J. (2006). Efficiency of antlion trap construction. *Journal of Experimental Biology*, 209(18), 3510-3515.

Finale 2010 [Computer software]. Seattle, WA: eMedia

Godzinska, E. W. (2004). Earth: Planet of the ants. *Social Animals*, 3(3), 10-13.

Griffiths, D. (1980). The feeding biology of ant-lion larvae: Prey capture, handling and utilization. *The Journal of Animal Ecology*, 49(1), 99-125.

Griffiths, D. (1985). Phenology and larval-adult size relations in the ant-lion *Macroleon quinquemaculatus*. *The Journal of Animal Ecology*, 54(2), 573-581.

Guillette, L. M., & Hollis, K. L. (2010). Learning in insects, with special emphasis on pit-digging larval antlions (Neuroptera: Myrmeleontidae). *Proceedings of the Tenth International Symposium on Neuropterology*, Maribor, Slovenia. 1-12.

Guillette, L. M., Hollis, K. L., & Markarian, A. (2009). Learning in a sedentary insect predator: Antlions (Neuroptera: Myrmeleontidae) anticipate a long wait. *Behavioural Processes*, 80(3), 224-232.

- Gutiérrez-Ibáñez, C., Villagra, C. A., & Niemeyer, H. M. (2007). Pre-pupation behaviour of the aphid parasitoid *Aphidius ervi* (haliday) and its consequences for pre-imaginal learning. *Naturwissenschaften*, *94*(7), 595-600.
- Hollis, K. L., Cogswell, H., Snyder, K., Guillette, L. M. & Nowbahari, E. (2011). Specialized learning in antlions (Neuroptera: Myrmeleontidae), pit-digging predators, shortens vulnerable larval stage. *PLoS One*, *6*(3), e17958.
- Heinrich, B., & Heinrich, M. J. E. (1984). The pit-trapping foraging strategy of the ant lion, *Myrmeleon immaculatus* DeGeer (Neuroptera: Myrmeleontidae). *Behavioral Ecology and Sociobiology*, *14*(2), 151-160.
- Lucas, J. R. (1985). Metabolic rates and pit-construction costs of two antlion species. *Journal of Animal Ecology*, *54*, 295 – 309.
- Kaur, J. S., Lai, Y. L., & Giger, A. D. (2003). Learning and memory in the mosquito *Aedes aegypti* shown by conditioning against oviposition deterrence. *Medical and Veterinary Entomology*, *17*(4), 457-460.
- Mencinger-Vracko, B., & Devetak, D. (2008). Orientation of the pit-building antlion larva *Euroleon* (Neuroptera, myrmeleontidae) to the direction of substrate vibrations caused by prey. *Zoology*, *111*(1), 2-8.
- Menzel, R., Erber, J., & Masuhr, T. (1974). Learning and memory in the honeybee. *Experimental Analysis of Insect Behaviour*, *195*, 217.
- Myers, J. L. (1979). *Fundamentals of experimental design*. Boston: Allyn and Bacon, Inc.
- Napolitano, J. F. (1998). Predatory behavior of a pit-making antlion, *Myrmeleon mobilis* (Neuroptera: Myrmeleontidae). *The Florida Entomologist*, *81*(4), 562-566.
- Nowbahari, E., Scohier, A., Durand, J. L., Hollis, K. L., & Adler, F. R. (2009). Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. *PLoS One*, *4*(8), 126-128.
- Papaj, D. R., & Prokopy, R. J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, *34*(1), 315-350.

- Pszczolkowski, M. A., & Brown, J. J. (2005). Single experience learning of host fruit selection by Lepidopteran larvae. *Physiology & Behavior*, 86(1-2), 168-175.
- Punzo, F. (1980). Analysis of maze learning in the silverfish, *Lepisma saccharina* (Thysanura: Lepismatidae). *Journal of the Kansas Entomological Society*, 53(3), 653-661.
- Quinn, W. G., Harris, W. A., & Benzer, S. (1974). Conditioned behavior in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences*, 71(3), 708.
- Rains, G. C., Utley, S. L., & Lewis, W. J. (2006). Behavioral monitoring of trained insects for chemical detection. *Biotechnology Progress*, 22(1), 2-8.
- Raubenheimer, D., & Tucker, D. (1997). Associative learning by locusts: Pairing of visual cues with consumption of protein and carbohydrate. *Animal Behaviour*, 54(6), 1449-1459.
- Scharf, I., Barkae, E. D., & Ovadia, O. (2010). Response of pit-building antlions to repeated unsuccessful encounters with prey. *Animal Behaviour*, 79(1), 153-158.
- Scharf, I., Golan, B., & Ovadia, O. (2009). The effect of sand depth, feeding regime, density, and body mass on the foraging behaviour of a pit-building antlion. *Ecological Entomology*, 34(1), 26-33.
- Scharf, I., & Ovadia, O. (2006). Factors influencing site abandonment and site selection in a sit-and-wait predator: A review of pit-building antlion larvae. *Journal of Insect Behavior*, 19(2), 197-218.
- Traynier, R. M. M. (1984). Associative learning in the ovipositional behaviour of the cabbage butterfly, *Pieris rapae*. *Physiological Entomology*, 9(4), 465-472.
- Von Frisch, K. (1953). *The dancing bees (D. ilse, trans.)*. San Diego: Harcourt, Brace, Jovanovich.
- Watanabe, H., Kobayashi, Y., Sakura, M., Matsumoto, Y., & Mizunami, M. (2003). Classical olfactory conditioning in the cockroach *Periplaneta americana*. *Zoological Science*, 20(12), 1447-1454.

Wheeler, W. M. (1930). *Demons of the dust*. New York: W. W Norton & Company.

Wisenden, B. D., Chivers, D. P., & Smith, R. J. F. (1997). Learned recognition of predation risk by *Enallagma* damselfly larvae (Odonata: Zygoptera) on the basis of chemical cues. *Journal of Chemical Ecology*, 23(1), 137-151.

Appendix

Training Schedule for Frequency Learning Group 1 (High)

Block	Day	Cue Pattern	Cue	Treatment Condition
1	1	A	High Frequency on Left	Prey Available
	2	R	Low Frequency on Right	Prey Removal
	3	R	Low Frequency on Right	Prey Removal
	4	A	High Frequency on Left	Prey Available
	5	OFF		
2	6	R	Low Frequency on Right	Prey Removal
	7	A	High Frequency on Left	Prey Available
	8	A	High Frequency on Left	Prey Available
	9	R	Low Frequency on Right	Prey Removal
	10	OFF		
3	11	A	High Frequency on Left	Prey Available
	12	A	High Frequency on Left	Prey Available
	13	R	Low Frequency on Right	Prey Removal
	14	R	Low Frequency on Right	Prey Removal
	15	OFF		
4	16	A	High Frequency on Left	Prey Available
	17	R	Low Frequency on Right	Prey Removal
	18	A	High Frequency on Left	Prey Available
	19	R	Low Frequency on Right	Prey Removal
	20	OFF		
5	21	R	Low Frequency on Right	Prey Removal
	22	A	High Frequency on Left	Prey Available
	23	R	Low Frequency on Right	Prey Removal
	24	A	High Frequency on Left	Prey Available
	25	OFF		
6	26	R	Low Frequency on Right	Prey Removal
	27	R	Low Frequency on Right	Prey Removal
	28	A	High Frequency on Left	Prey Available
	29	A	High Frequency on Left	Prey Available
	30	OFF		
7	31	A	High Frequency on Left	Prey Available
	32	R	Low Frequency on Right	Prey Removal
	33	R	Low Frequency on Right	Prey Removal
	34	A	High Frequency on Left	Prey Available
	35	OFF		
8	36	R	High Frequency on Left	Prey Available
	37	A	High Frequency on Left	Prey Available
	38	A	Low Frequency on Right	Prey Removal
	39	R	Low Frequency on Right	Prey Removal
	40	END		

Training Schedule for Frequency Learning Group 2 (Low)

Block	Day	Cue Pattern	Cue	Treatment Condition
1	1	A	Low Frequency on Left	Prey Available
	2	R	High Frequency on Right	Prey Removal
	3	R	High Frequency on Right	Prey Removal
	4	A	Low Frequency on Left	Prey Available
	5	OFF		
2	6	R	High Frequency on Right	Prey Removal
	7	A	Low Frequency on Left	Prey Available
	8	A	Low Frequency on Left	Prey Available
	9	R	High Frequency on Right	Prey Removal
	10	OFF		
3	11	A	Low Frequency on Left	Prey Available
	12	A	Low Frequency on Left	Prey Available
	13	R	High Frequency on Right	Prey Removal
	14	R	High Frequency on Right	Prey Removal
	15	OFF		
4	16	A	Low Frequency on Left	Prey Available
	17	R	High Frequency on Right	Prey Removal
	18	A	Low Frequency on Left	Prey Available
	19	R	High Frequency on Right	Prey Removal
	20	OFF		
5	21	R	High Frequency on Right	Prey Removal
	22	A	Low Frequency on Left	Prey Available
	23	R	High Frequency on Right	Prey Removal
	24	A	Low Frequency on Left	Prey Available
	25	OFF		
6	26	R	High Frequency on Right	Prey Removal
	27	R	High Frequency on Right	Prey Removal
	28	A	Low Frequency on Left	Prey Available
	29	A	Low Frequency on Left	Prey Available
	30	OFF		
7	31	A	Low Frequency on Left	Prey Available
	32	R	High Frequency on Right	Prey Removal
	33	R	High Frequency on Right	Prey Removal
	34	A	Low Frequency on Left	Prey Available
	35	OFF		
8	36	A	Low Frequency on Left	Prey Available
	37	R	High Frequency on Right	Prey Removal
	38	A	Low Frequency on Left	Prey Available
	39	R	High Frequency on Right	Prey Removal
		END		

Training Schedule for Frequency Control Group

Block	Day	Cue	Treatment Condition
1	1	Low Frequency on Left	Prey Available
	2	Low Frequency on Right	Prey Removal
	3	High Frequency on Left	Prey Removal
	4	High Frequency on Right	Prey Available
	5		
2	6	High Frequency on Left	Prey Removal
	7	High Frequency on Right	Prey Available
	8	Low Frequency on Left	Prey Available
	9	Low Frequency on Right	Prey Removal
	10		
3	11	Low Frequency on Left	Prey Available
	12	High Frequency on Right	Prey Available
	13	Low Frequency on Right	Prey Removal
	14	High Frequency on Left	Prey Removal
	15		
4	16	Low Frequency on Left	Prey Available
	17	High Frequency on Left	Prey Removal
	18	High Frequency on Right	Prey Available
	19	Low Frequency on Right	Prey Removal
	20		
5	21	High Frequency on Right	Prey Removal
	22	Low Frequency on Right	Prey Available
	23	Low Frequency on Left	Prey Removal
	24	High Frequency on Left	Prey Available
	25		
6	26	High Frequency on Right	Prey Removal
	27	Low Frequency on Left	Prey Removal
	28	High Frequency on Left	Prey Available
	29	Low Frequency on Right	Prey Available
	30		
7	31	Low Frequency on Left	Prey Available
	32	Low Frequency on Right	Prey Removal
	33	High Frequency on Left	Prey Removal
	34	High Frequency on Right	Prey Available
	35		
8	36	Low Frequency on Left	Prey Removal
	37	High Frequency on Left	Prey Available
	38	High Frequency on Right	Prey Available
	39	Low Frequency on Right	Prey Removal
	END		