Learned Behavior in *Myrmeleon immaculatus* Populations from Two Distinct Sites

Jessica J. Fletcher

University of Michigan Biological Station

EEB 492: Behavioral Ecology
8/13/13

Niemisto, Meeth

ABSTRACT

The sit-and-wait model of predation is a fascinating response to the costs of active foraging. Antlions (*Myrmeleon immaculatus*) in the larval stage are an extremely well studied example of a sit-and-wait predator. Antlion populations from two different sites in northern Michigan (South Sturgeon Bay and Pine Point) were collected and studied to examine if the animals would retain their routines following removal from site of origin and acclimation to laboratory conditions. Antlion pits were destroyed once every 3 days and animals were fed on two separate feeding regimes to examine effects of resource abundance on routine retention. Antlions that originated from South Sturgeon Bay continued to build larger pits than those from Pine Point. Antlions that were fed daily built smaller pits, on average, than antlions that were fed once every 3 days suggesting risk-prone behavior. Average pit diameter varied significantly with day, potentially as a result of temperature. Heat lamps were added later in the experiment, and showed that antlions significantly increased their pit diameter with the added stimulus. Genetic variation between the two populations is unlikely due to similarity of pit diameter with regards to antlion weight in several sites along Douglas Lake (Pine Point) and Sturgeon Bay with the exception of South Sturgeon Bay, where the antlions used for this experiment were found. Antlions from South Sturgeon Bay and Pine Point seemed to retain learned behavior from environmental stimuli present in sites of origin after acclimation to laboratory settings.

INTRODUCTION

The antlion (*Myrmeleon immaculatus*) is a model "sit-and-wait predator", an animal defined by Huey and Pianka (1981) as a "sedentary ambush predator". However, unlike many sit-and-wait predators, antlions create "traps" or "pits" with which to capture their prey. According to Turner (1915), while antlions often dig their pits in sandy, protected areas, they can also build traps in any "loose friable soil protected from rain". An antlion will begin building a pit by using its abdomen as a plow to scrape out a circle, and its head as a shovel to remove unwanted substrate (Turner 1915; Griffiths 1980). Once the pit is built, the antlion will sit at the bottom with its mandibles open and wait for prey to tumble in. After the prey is caught, the antlion uses its front mandibles to inject poison that kills the prey (Griffiths 1980). Excluding the exoskeleton, the food item is then digested and its contents extracted before the carcass is flung from the pit.

Antlion larvae transition into adulthood through three instars, which may last as long as two years in total (Arnett & Gotelli 2000). During this larval period, antlions forage solely through the use of their pits. The success of the antlion relies heavily on the construction of its pit and antlion weight is positively correlated with pit size (Swenson et al. 2007). Antlion pit size establishes the types and quantities of food captured (Heinrich and Heinrich 1983). Larger antlion pits catch both large and small prey, while small pits only capture small prey (Heinrich and Heinrich 1983). Under this assumption, antlions should strive to build the largest pit possible to expand the range of ant sizes it can entrap. However, antlion pits are extremely costly to create and energy budgeting becomes a problem for the animal (Lucas 1985). Other factors affecting antlion pit size and pit relocation include density of prey, level of disturbance, sand temperature, and competition with other antlions in the area (Matsura and Takano 1989). Antlions may benefit greatly from retaining information about these factors within their site of origin and designing routines based around this organizational learning.

Organizational learning is defined by Levitt and March (1988) as "learning encoded by inferences from history into routines that guide behavior". Studies of organizational learning in insects have suggested that some orders, such as Hymenoptera, have the ability to learn and retain routines (Kaiser et al. 2003; Greggers & Menzel 1993). Through organizational learning, early experiences could encourage different behaviors among individuals in a population. Organizational learning and the acquisition of new learned skills are well studied in mammals, particularly at the neurological level (Asanuma & Keller 1991; Bronson 1991), but not as well studied in insects. However, environmental effects such as temperature have been studied in insects extensively. In a common garden experiment, Arnett and Gotelli (2000) found data that suggested that a latitudinal gradient between their two antlion populations of study contributed strongly to differences in mean pit size. We strive to understand whether these environmental effects that modify insect behavior create routines in behavior that are retained in the animal regardless of new environmental stimuli.

This experiment was designed to replicate a previous experiment performed by the Behavioral Ecology class at the University of Michigan Biological Station in 2009. This class designed a common garden experiment that would show if antlion populations from two distinct collection sites in Michigan would retain any learned behavior from their site of origin when moved to a laboratory. A collection of antlion pit diameters and heights from these two sites in the northern part of the lower peninsula of Michigan (Pine Point, MI and South Sturgeon Bay, MI) showed a significant difference in antlion pit size between these two sites (D. Anderson, pers. comm.). The common garden experiment was ended after only ten days and the results were ambiguous. The mean antlion pit sizes from both sites began distinct, converged, and then diverged suddenly on day ten (Fig. 1). Based on these results, the previous students excluded any genetic influences in determining pit size and hypothesized that animals from the two populations took current environment into consideration and changed their pits to match accordingly (Weirs 2009).

The experiment we designed follows this previous experiment with an added variable. Feeding times were changed from the previous study to see how overfeeding or starvation would affect pit size. We hypothesized that antlion populations from these two sites will continue to show differences in pit size once acclimated to this disturbance regime as suggested by the data from 2009. This would indicate that much of the difference in antlion pit size is due to learned routine behaviors that are retained in the laboratory. We also hypothesized that antlions fed at a higher rate will build a larger pit due to surplus energy and the correlation between antlion success and pit size (Heinrich and Heinrich 1983).

MATERIALS AND METHODS

Intrinsic Behavior Experiment

In July 2013, 120 antlion (*Myrmeleon immaculatus*) specimens were collected from two distinct sites: South Sturgeon Bay in Wilderness State Park, MI (N45º 40’ 9.08”, W84º 58’ 6.40”) on the shore of Lake Michigan and Pine Point at the University of Michigan Biological Station in Pellston, MI (N45º 34’ 6.53”, W84º 41’ 9.16”) on the shore of Douglas Lake. Antlions were randomly collected using sand sifters to locate the animals. Age of the antlion larvae does not affect pit building ability and was therefore ignored for this experiment (Arnett & Gotelli 2000). During collection, antlions were weighed using a digital scale and only those with a weight between 12 milligrams and 45 milligrams were taken back to the University of Michigan Biological Station for experimentation. The animals were kept in glass vials for approximately 24 hours before being placed in plastic tubs with equal amounts of Douglas Lake sand. Douglas Lake sand was sifted for 500-micrometer grain size homogenous sand to prevent any effect that substrate may have on pit size. Four experimental groups of 30 antlions each were created. Antlions were separated by site and divided into two experiment types: one group from each site was fed daily and the other group was fed once every three days. All antlions were disturbed every three days to make sure that the animals continued to build new pits so that new data could be collecting on a regular basis. Disturbance was defined as shaking the tubs until the sand inside was level and no evidence of the former pit could be seen. Antlions were all fed with the same non-formic acid ant species collected on site at the University of Michigan Biological Station. The sizes of the ants are negligible, as ant species have similar nutritional value regardless of individual size (Griffiths 1977). Following placement in and numbering of tubs, antlions were arranged in a checkerboard pattern based on group assignment (by site and feeding regime) to negate any edges effects (Fig. 2).

The experiment was run for 25 days in total. During days 1-22, antlion pits were measured daily using calipers by a single group member to maintain consistency. If both diameters of the pit could be reached, measurements were taken starting with the longer diameter and the two diameter measurements were averaged. If only one diameter could be reached due to the constraints of the tub, the diameter perpendicular to the edge of the tub was measured. Antlion doodles and pits under construction were not measured and were recorded as “no pit”. Following measurement, animals were fed based on their feeding regime placement. Half of the antlions were fed daily, while the other half were fed once every three days. Small temperature loggers were placed on the table that held the antlions and recorded room temperature every hour. Weight data for the common garden experiment were compiled by site and analyzed using SPSS to test for normality. Data collected during the course of the experiment were analyzed using Statistica. Antlions that had died or had never built pits were removed from the data.

Temperature Effects

On the 23rd day of the experiment, a temperature stimulus was added to 64 of the 120 antlions. A large divider separated the two groups and four heat lamps were suspended over the 64 animals receiving stimulus. Heat lamps were turned on at 9:40 AM and turned off at 5:00 PM for days 23 through 25. Additional thermometers were placed on either side of the table and temperature measurements were recorded at 5:00 PM daily. Antlion pit diameters were measured and animals were fed according to intrinsic behavior methods.

Site Differences

To further investigate differences in antlion pit diameter between Douglas Lake and Sturgeon Bay populations, measurements were taken from four sites along the shores of Douglas Lake and three sites along Sturgeon Bay. Two diameters were measured for each antlion pit using calipers. A dry spaghetti noodle was placed over the rim of the antlion pit to correctly identify the edge of the pit for accurate measurement. One student performed all of the measurements for each site to maintain consistency between measurements. Following pit measurement, the animal was removed from its pit and its weight was measured using a digital scale and recorded. Data from antlions with a weight between 13 and 40 milligrams were used during analysis. Animals were released back in their site of origin following weight measurement. Weight and diameter data from the seven sites along Douglas Lake and Sturgeon Bay were analyzed using Statistica.

RESULTS

Intrinsic Behavior Experiment

Antlion weights within and between the two sites (Pine Point and South Sturgeon Bay) were analyzed using the Sharpiro-Wilks test and found to not be normally distributed (Sharpiro-Wilk, Pine Point, $T = 0.917$, $df = 60$, $P = 0.001$; Sharpiro-Wilk, South Sturgeon Bay, $T = 0.939$, $df = 60$, $P = 0.005$; Sharpiro Wilk, Both Sites, $T = 0.938$, $df = 120$, $P < 0.0001$). An independent samples t-test showed that antlion weights between South Sturgeon Bay and Pine Point for the animals used in this experiment were not significantly different ($T = -0.287$, $df = 118$, $P = 0.775$). All 120 antlions were separated into four groups based on site of origin and assigned feeding regime. A multiple comparisons ANOVA test showed that antlion weights between the four groups were not significantly different (Tukey Post Hoc, $F = 0.088$, $P = 0.966$).

Data were then analyzed using a Factorial ANOVA test, which suggested a strong day (1-22), site (South Sturgeon Bay or Pine Point), and feeding regime (daily or once every three days) effect on antlion pit diameter. No significant interactions between any of the three variables existed. Days 23 through 25 were excluded from this test due to added environmental stimulus of temperature. Antlions from South Sturgeon Bay built larger pits than antlions from Pine Point ($F_{1,1533}$ = 11.184, P = 0.0008; Fig. 3). Animals that were fed daily built, on average, significantly smaller pits than those fed once every three days ($F_{1,1533}$ = 4.8337, P = 0.0281; Fig. 4). Antlion pit diameter varied significantly for both sites with changes in day ($F_{1,1533}$ = 12.304, P < 0.0001; Fig. 5).

Temperature Effects

Temperature and pit diameter data from days 20-22 were compared to data from days 23-25 where day 23 marks the beginning of heat stimulus. A repeated measures ANOVA test showed that the presence or absence of heat had a significant effect on antlion pit size (F = 17.849, df = 1, P < 0.0001). Antlions that received additional heating increased average pit diameter at a faster rate than animals not receiving additional heat (Fig. 6).

Site Differences

The seven sites surveyed were grouped into two pairs based on distance to Douglas Lake and Sturgeon Bay. Sites were labeled as follows: DL1, DL2, DL3, DL4, SB1, SB2, and SB3 where DL1 corresponds to Pine Point and SB1 corresponds to South Sturgeon Bay. Site distances and coordinates are shown in Fig. 7. A univariate test of significance showed that mean antlion pit diameter between all seven sites were not significant ($F_{5,107}$ = 0.656, P = 0.1441). However, when the result was graphed, mean pit diameter for antlions in South Sturgeon Bay (SB 1) was larger than for the other six sites (Fig. 8).

DISCUSSION

In some ways, we are surprised that antlions that are fed frequently built smaller pits than those fed infrequently due to energy constraints. We originally hypothesized that antlions that were being fed more frequently would have more energy to expend on building and maintaining a larger pit than antlions fed infrequently. However, this can be explained by starvation effects leading to risk-prone behavior in animals. Animals that require more resources (in this case, food) are more willing to undergo risky behavior to obtain that resource item (Caraco et al. 1980). For antlions, building a larger pit expends more energy than building a smaller pit (Lucas 1985). However, the animals benefit from building larger pits because larger pits are able to capture a wider variety of prey and are more likely to encounter a prey item due to larger surface area (Heinrich and Heinrich 1983).

Despite our hypothesis that antlions would lose learned behavior from site of origin after acclimation to laboratory settings, antlions from South Sturgeon Bay continued to build larger pits than Pine Point antlions, indicating that adaptation to laboratory settings was not occurring. This phenomenon could be due to learned environmental factors or genetic variability between the two populations. While genetic variation cannot be completely excluded, analysis of the seven sites in Sturgeon Bay and Douglas Lake suggest that genetic variation cannot explain the dichotomy seen in average pit size between the two populations studied. Six of the seven sites along Douglas Lake and Sturgeon Bay had statistically similar average pit size on the day of collection: DL1, DL2, DL3, DL4, SB2, and SB3. The only site to have a statistically significantly difference in average pit size was SB1, or South Sturgeon Bay. If gene flow were not occurring between Sturgeon Bay and Douglas Lake populations, the two lakes would have statistically different pit sizes, which they do not. Because South Sturgeon Bay is the only site to have a significantly different pit size even when compared to a population less than a kilometer away, we assume that something other than an absence of gene flow is occurring.

The two sites where we collected antlion data are very different in the size of available antlion habitat and surrounding flora and fauna. South Sturgeon Bay, MI is characterized by small dunes, sandy soil, and borders Lake Michigan on its north side. Pine Point, MI lies on the North Fishtail Bay of Douglas Lake and is characterized by a very small sandy beach leading into a wooded area. Some of Douglas Lake, including Pine Point, is owned and protected by the University of Michigan Biological Station so fauna are able to roam undisturbed by humans. We originally hypothesized that antlion pit size differences between the two sites could be environmentally determined by a variety of factors, including disturbance rate, prey availability, or intraspecific competition.

Disturbances from other animals in the area could cause the antlion population to build smaller pit sizes. The wooded area behind the small sandy beach at Pine Point is home to a variety of fauna including deer, chipmunks, and birds that are allowed to roam undisturbed by humans. The antlion colony found on Pine Point also rested very closely to Douglas Lake and may be impacted by birds coming out of the lake, or intense wave action disturbing the beach. Past experiments have showed that antlion growth rate decreases with increasing levels of sand disturbance, a phenomenon that would help explain the size disparity between the two sites. (Barkae et al. 2010). Antlion size has been positively correlated with pit size. Disturbance could therefore be an adequate explanation for smaller pit sizes at Pine Point as compared to South Sturgeon Bay by decreasing antlion growth. However, Pine Point antlions did not build significantly smaller pits than antlions from the other six sites around Sturgeon Bay and Douglas Lake. This led us to believe that if disturbance does have an effect on antlion pit size, then South Sturgeon Bay (SB1) antlions should be either the most affected or least affected by disturbances.

The area available to Pine Point antlions for colonization was significantly smaller than for antlions in Sturgeon Bay. Prey availability in Pine Point may be significantly reduced compared to prey availability in South Sturgeon Bay due to decreased area. The area available for colonization by antlions in South Sturgeon Bay was the largest out of the seven sites surveyed and had the most suitable substrate. This lack of suitable substrate and potential resource competition could explain why the other six sites had significantly smaller pit sizes as compared to South Sturgeon Bay. The relatively smaller areas in the other six sites may also force antlions to undergo increased intraspecific competition with one another, which causes reduced growth rate (Barkae et al. 2010; Matsura and Takano 1989). Intraspecific competition manifests in areas of high antlion density when individuals fling sand into others pits, compete to capture prey, and even cannibalize neighbors (Matsura 1989). While all antlions around Douglas Lake and antlions in SB2 and SB3 have a smaller area to colonize, South Sturgeon Bay antlions have a large area available, allowing them to spread out and avoid potential intraspecific competition more easily.

Based on the temperature data collected during days 23 through 25 of the common garden experiment, we can conclude that temperature has a positive effect on average pit diameter for antlions from both South Sturgeon Bay and Pine Point. Poikilotherms rely on the surrounding environment for internal temperature and often have to compensate for this reliance through behaviors such as basking to increase internal temperature (Bullock 1954). In the laboratory, temperature had a significant effect on antlion pit size. Antlions were kept in a poorly insulated cabin that often closely reflected outside air. July and August of 2013 at the University of Michigan Biological Station was consistently lower than 25 ºC with frequent rain and wind. Windows in the cabin that the antlions were housed in were often kept open, further lowering the internal temperature. Temperature as a factor of pit diameter was suggested after observations of infrequent pit building and small pit sizes in the experimental populations regardless of site of origin. We also attribute the strong affect that day had upon average antlion pit diameter to changes in temperature. After heat lamps were added, the temperature for the area around the animals receiving heat was absolutely and relatively higher than for those not receiving stimulus

(Fig. 7). Following the addition of the heat lamps, antlion pit sizes increased at a faster rate for all 64 of the animals receiving stimulus than for antlions not receiving additional heat (Fig. 8). For this reason, temperature may have been a limiting factor in our experiment. For further evidence of the intrinsic learned behavior in these two antlion populations, further experimentation should be conducted that reduces environmental effects in the laboratory by using an environment that maintains a regular temperature cycle that would mimic temperature in northern Michigan during a typical summer period.

REFERENCES

Arnett, A.E., Gotelli, N.J. 2000. Pit-building decisions of larval ant lions: effects of larval age, temperature, food, and population source. Journal of Insect Behavior 14: 89-97.

Asanuma, H., Keller, A. 1991. Neuronal mechanisms of motor learning mammals. Neurological Report 2:217224.

Bronson, G. 1991. The hierarchical organization of the central nervous system: implications for learning processes and critical periods in development. Behavioral Science 10: 7-25.

Bullock, T.H. 1954. Compensation for temperature in the metabolism and activity of poikilotherms. Biological Reviews 30: 311-342.

Caraco, T., Martindale, S., Whittam, T.S. 1980. An empirical demonstration of risk-sensitive foraging preferences. Animal Behavior 28: 820-830.

Greggers, U., Menzel, R. 1993. Memory dynamics and foraging strategies of honey bees. Behavioral Ecology and Sociobiology 32: 17-29.

Griffiths, D. 1977. Caloric variation in Crustacea and other animals. Journal of Ecology 46: 593-605.

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

Heinrich, B., Heinrich, M.J.E. 1983. The pit-trapping foraging strategy of the antlion, *Myrmeleon immaculatus* DeGeer (Nueroptera: Myrmeleotidae). Behavioral Ecology and Sociobiology 14: 151-160.

Huey, R.B., Pianka, E.R. 1981. Ecological consequences of foraging mode. Ecology 62: 991-999.

Kaiser, L., Pérez-Maluf, R., Sandoz, J.C., Pham-Delégue, M.H. 2003. Dynamics of odour leaning in *Leptopilia boulardi* a hymenopterous parasitoid. Animal Behaviour 66: 1077-1084.

Levitt, B., March, J.G. 1988. Organizational learning. Annual Review of Sociology 14: 319-340.

Lucas, J.R. Metabolic rates and pit-construction costs of two antlion species. Journal of Animal Ecology 54: 295-309.

Matsura, T., Takano, H. 1989. Pit-relocation of antlion larvae in relation to their density. Researches on Population Ecology 31: 225-234.

Swenson, N.G., Mahler, D. L., Ferro, M., Ritchie, A. 2007. The energetic determination, spatial dispersion and density dependence of *Myrmeleon* ant lion pits in Las Cruces, Costa Rica. Biotropica 39: 774-777.

Turner, C.H. 1915. Notes on the behavior of the ant-lion with emphasis on the feeding activities and letisimulation. Biological Bulletin 29: 277-307.

Wiers, A. E. 2009. The effects of disturbance and energy budget, intrinsic and environmental factors, and density on antlion pit size. Term paper in Behavioral Ecology, University of Michigan Biological Station.

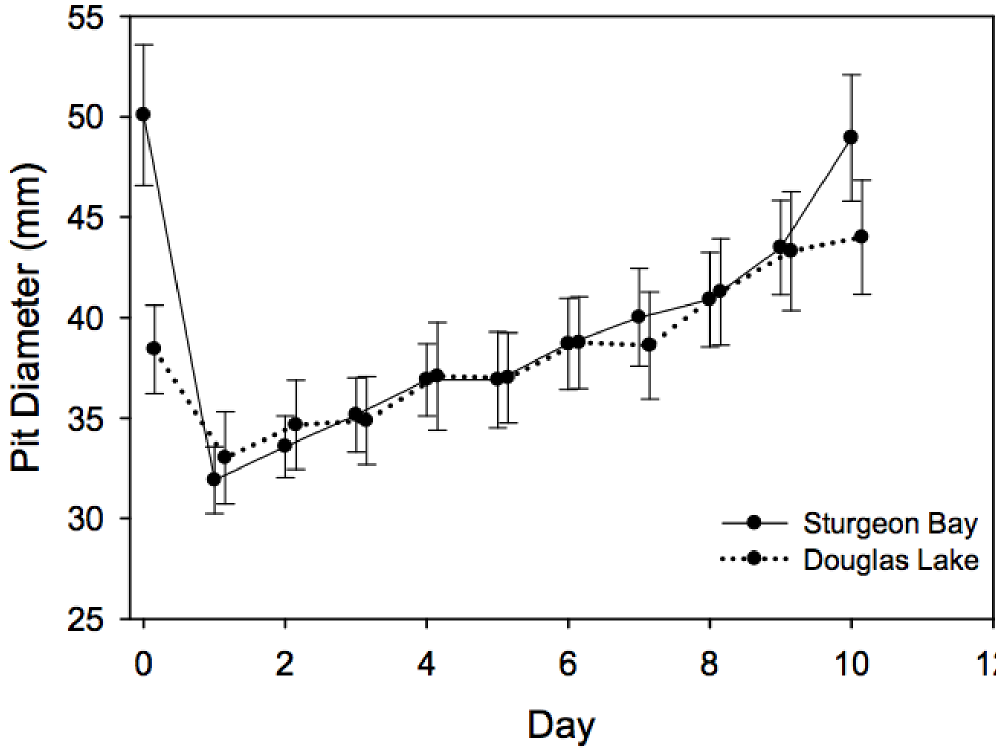

Fig. 1 The average pit diameter for antlions from South Sturgeon Bay and Pine Point during the common garden experiment conducted in 2009 by Weirs. Antlion pits were disturbed on days 0, 3, 6, and 9 with pit diameters statistically different only on days 0 and 10.

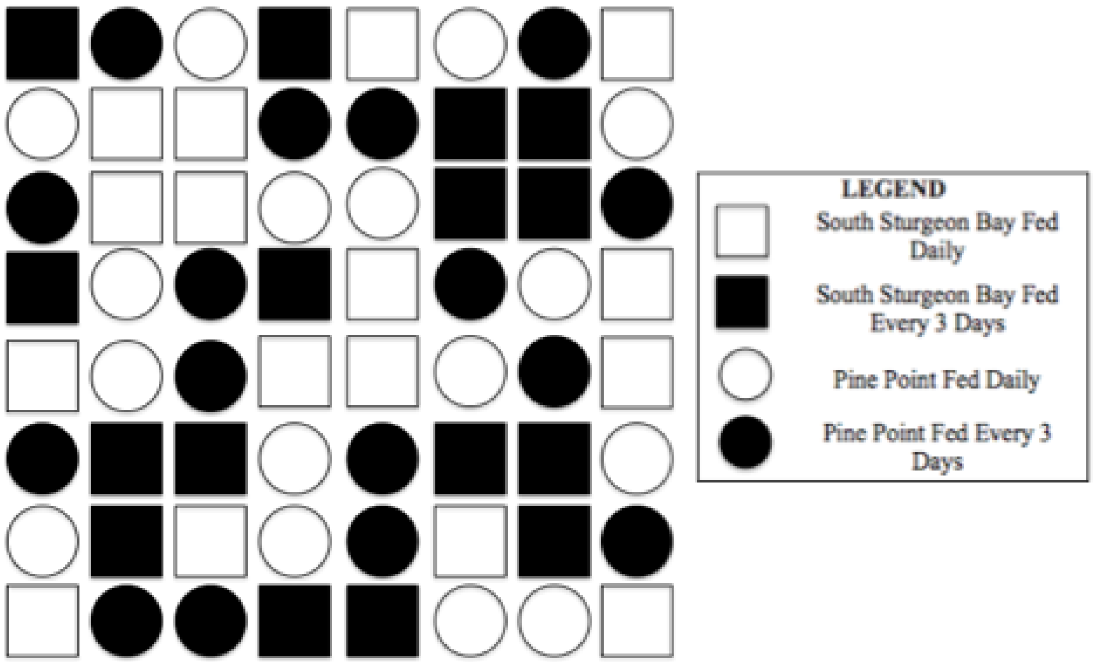

Fig. 2 The checkerboard pattern instituted for this experiment to negate any potential edge effects between groups of antlions. Of the 60 antlions from South Sturgeon Bay, 30 were fed daily and 30 were fed once every 3 days. Of the 60 antlions from Pine Point, 30 were fed daily and 30 were fed once every 3 days. 64 of the antlions were set up in the checkerboard pattern shown above while the other 56 were set up in the same pattern excluding the last row and positioned directly next to the group of 64.

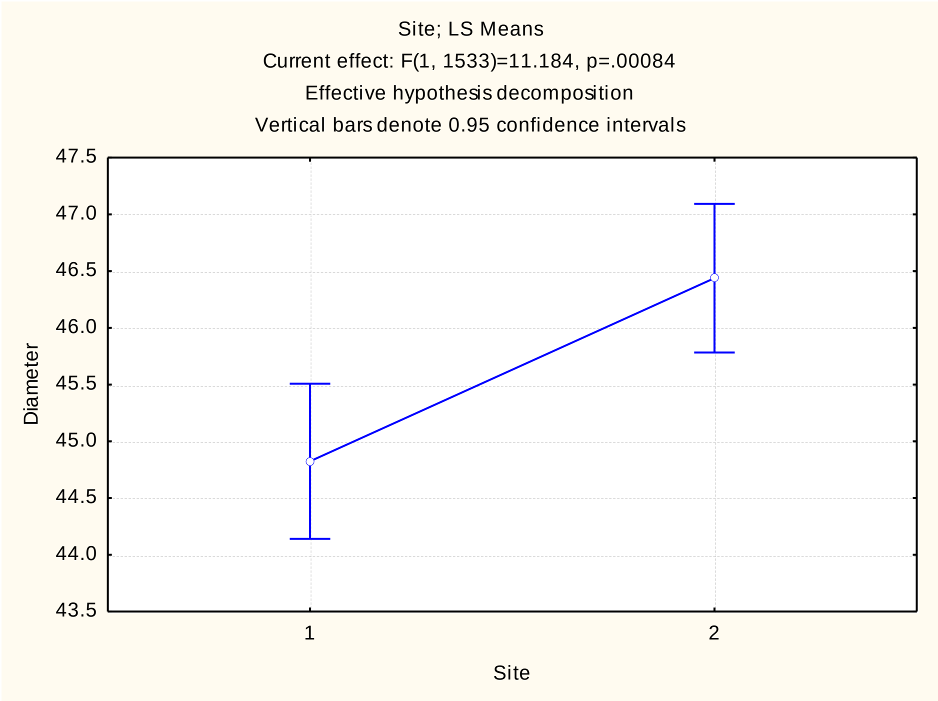

Fig 3. A significant site effect between South Sturgeon Bay and Pine Point antlions exists where South Sturgeon Bay antlions build larger pits ($F_{1,1533}$ = 11.184, P = 0.0008). On this graph, site 1 corresponds to Pine Point while site 2 corresponds to South Sturgeon Bay. Average pit diameter is expressed in millimeters.

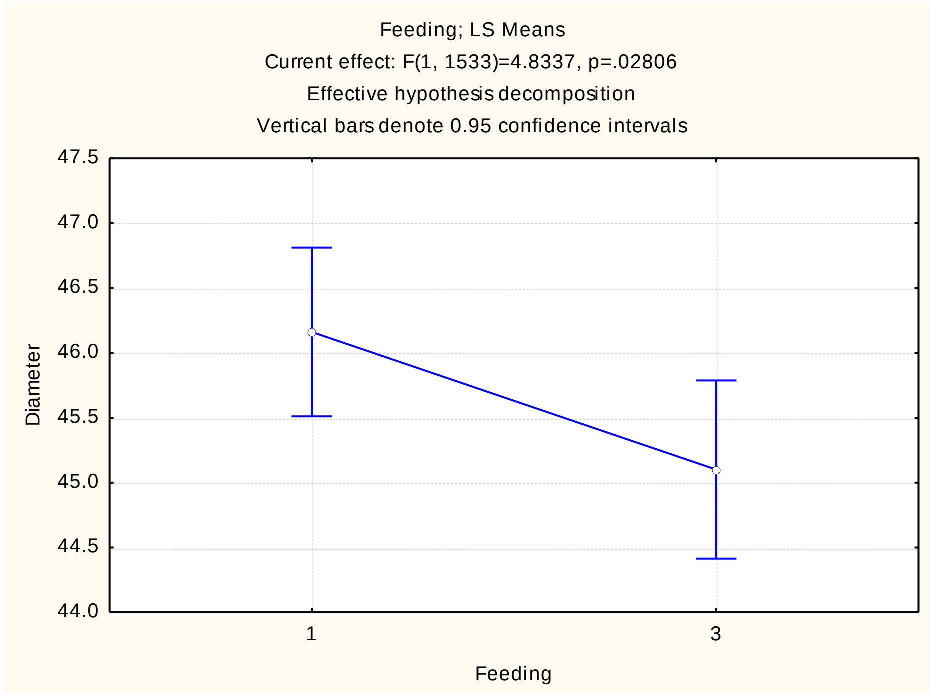

Fig 4. A significant feeding effect between antlions fed frequently and antlions fed infrequently exists where antlions fed infrequently build larger pits ($F_{1,1533}$ = 4.834, P = 0.028). On this graph, feeding 1 corresponds to antlions that were fed once every 3 days while feeding 2 corresponds to antlions fed daily. Average pit diameter is expressed in millimeters.

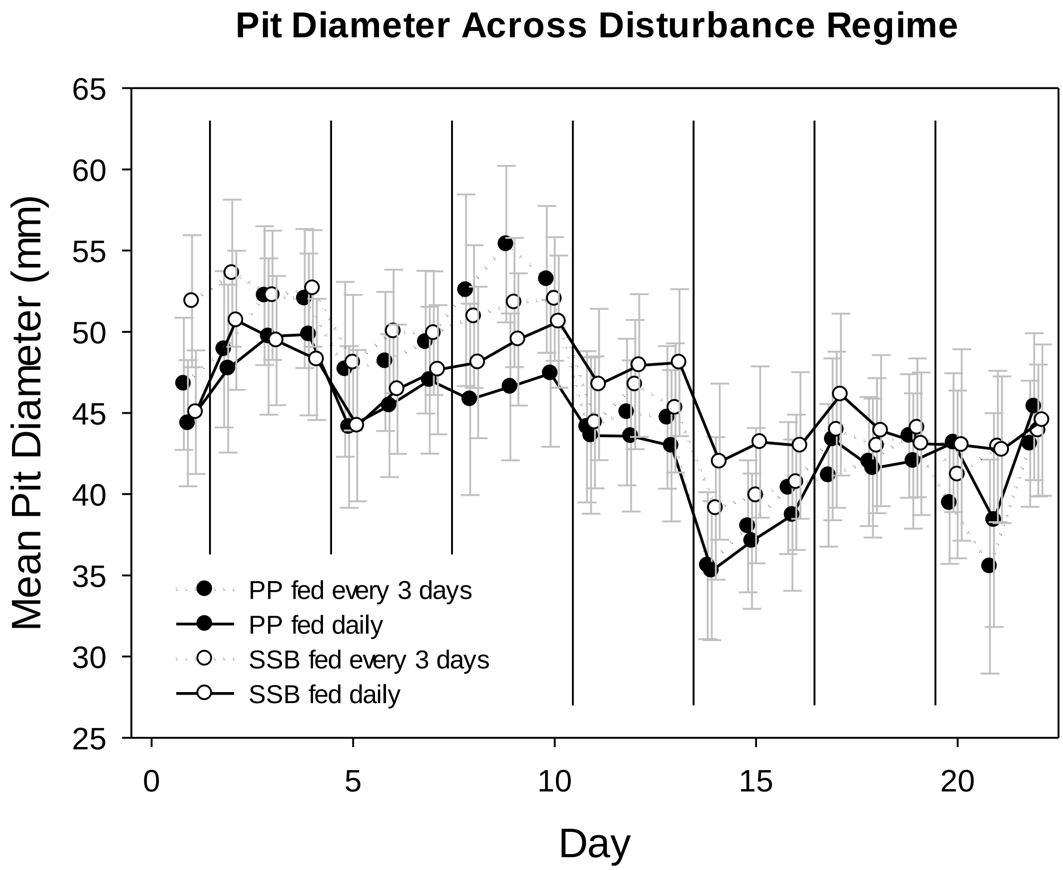

Fig. 5 The average antlion pit diameters during days 1-22 for all antlion treatment groups. On average, South Sturgeon Bay animals built larger pits than Pine Point animals. Until day 11, antlions fed once every 3 days built consistently larger pits than those fed daily.

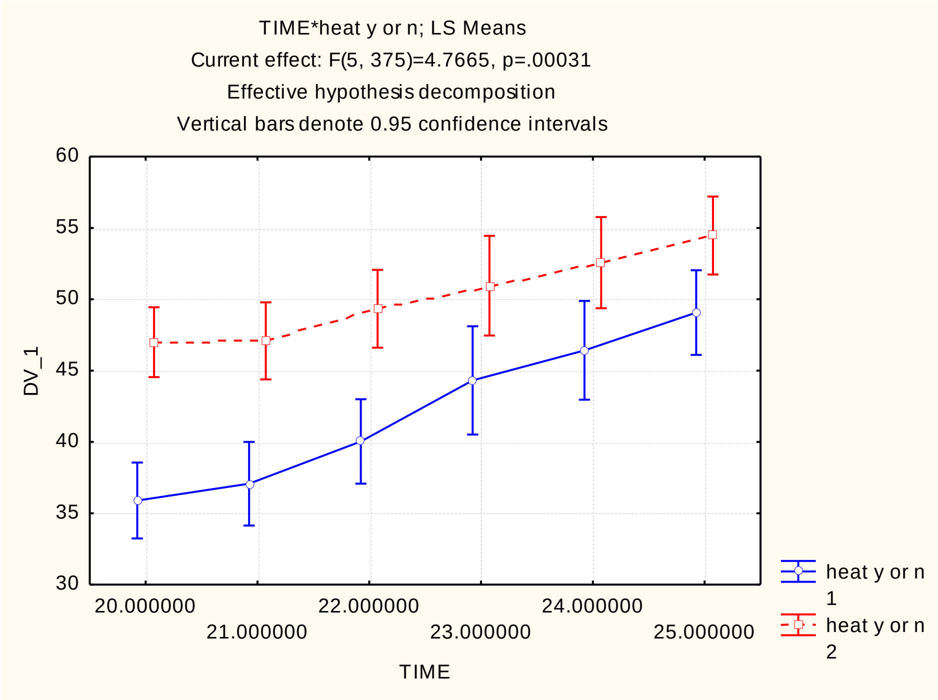

Fig 6. Increase in average pit diameter between antlions receiving temperature stimulus and antlions not receiving additional heat. Heat Y or N 1 shows antlions who were under a heating lamp for days 23-25. Heat Y or N 2 shows antlions who were not under a heating lamp for days 23-25. Time is expressed in days, beginning at day 20 and ending at day 25. DV_1 shows least squares mean pit diameter.

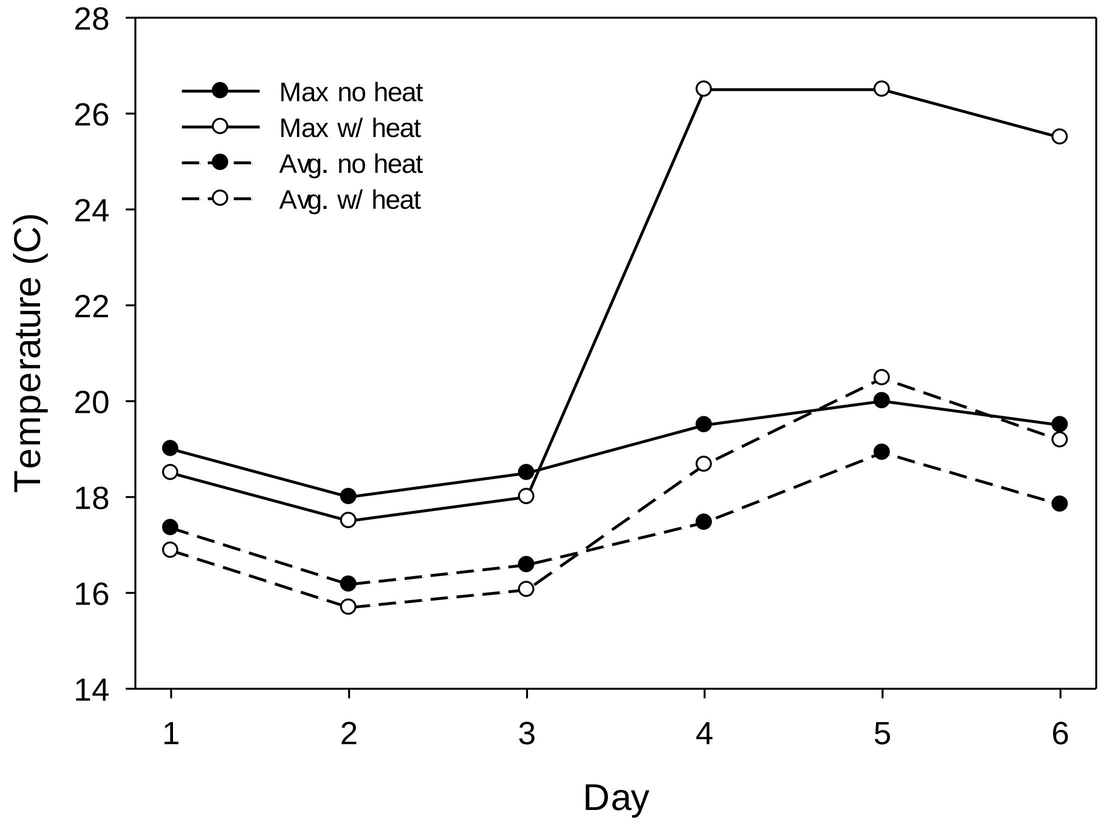

Fig. 7 Temperature readings from days 20-25 showing an absolute and relative increase in temperature due to the addition of heat lamps to the experimental set-up.

	Coordinates	DL1	DL2	DL3	DL4	SB1	SB2	SB3
DL1	N45.56140, W84.667948		0.79 km					
DL2	N45.56607, W84.66029			0.71 km				
DL3	N45.57241, W84.66008				1.52 km			
DL4	N45.58540, W84.65416							
SB1	N45.68117, W84.97897						0.68 km	
SB2	N45.68465, W84.97179							2.73 km
SB3	N45.70500, W84.95224							

Fig. 8 Longitudinal and latitudinal coordinates for each of the seven sites measured. Distances show distance between the site in question and the previously recorded site within either Douglas Lake or Sturgeon Bay (e.g. DL3 is 0.71 km away from DL2).

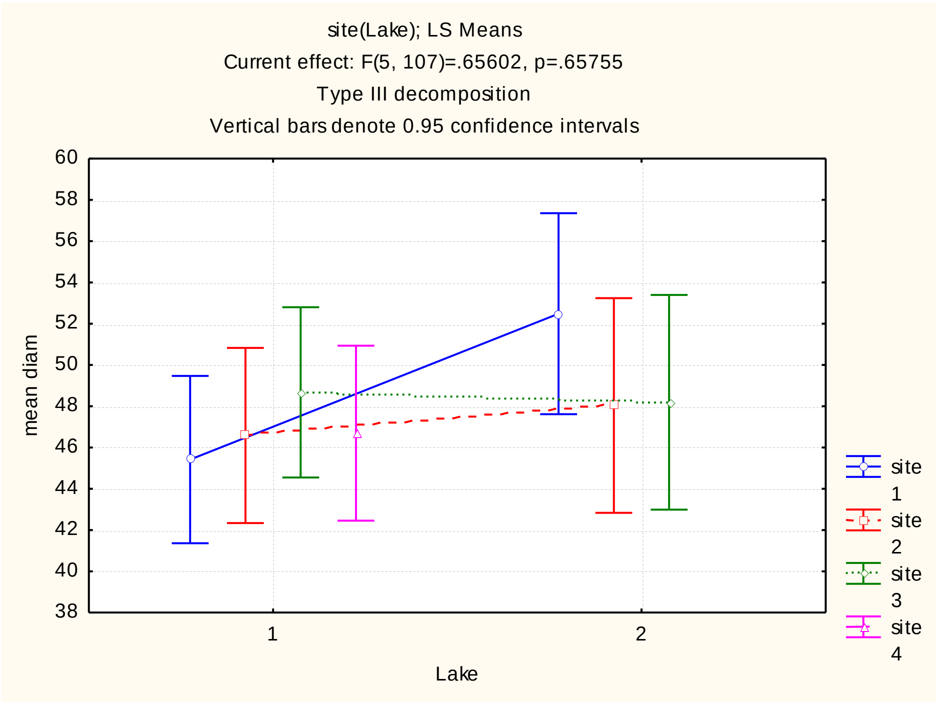

Fig. 9 Average pit diameter of antlions at seven sites in northern Michigan. Lake 1 corresponds to Douglas Lake while Lake 2 corresponds to Sturgeon Bay. Sites are arranged from left to right as DL1, DL2, DL3, DL4, SB1, SB2, and SB3. All sites have similar mean pit diameters with the exception of South Sturgeon Bay (SB1).