

Determining basis for pit size variation in *Myrmeleon immaculatus* between two lakeshores of Northern Michigan

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Abstract

Environmental factors influencing pit-building behavior of the antlion *Myrmeleon immaculatus* are fairly well studied. The purpose of this study was to identify and differentiate environmental, genetic, and possible organizational or early learning factors influencing different behavioral phenotypes of two populations at Douglas Lake and Sturgeon Bay in northern Michigan, USA. We collected 60 antlions from each site and measured pit diameters in a common garden design. We distinguished treatments by feeding frequency, site, and day for a total of 22 days under a controlled laboratory setting with a regular disturbance regime. Sturgeon Bay antlions built larger pits than Douglas Lake antlions, animals that were subjected to a higher feeding frequency built smaller pits than a lower feeding frequency, and no apparent trend was gleaned from the day effect due to a presumed temperature effect. As a follow-up experiment we measured the effects of temperature on pit-building using light-bulb warming stations on 64 of our animals. Antlion pits that were given supplemental heat grew at a faster rate than controls. We also compared weight and pit diameters of at least 15 animals from seven sites between Sturgeon Bay and Pine Point. No significant difference was found between any site or lakeshore, though our data suggested that the original Sturgeon Bay study site displayed a larger pit size than any other site. These results indicate that genetic effects on these animals are unlikely, due to the close proximity of all populations. We suggest that organizational or early learning effects are significant determinants of pit-building behavior in this species.

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Introduction

The sit-and-wait strategy of predation is found as a foraging technique in several animal phyla. Species adapt this strategy as a way to balance the energetic costs of foraging with the energetic gain and frequency of prey items. Species within the same genus often exhibit either sit and wait or active foraging strategies despite shared environments, depending on differential daily energy requirements (Anderson and Karasov, 1981). Antlion larvae (family: Myrmeleontidae) provide a good model of this tradeoff because their behavior can be categorized into two foraging strategies: sit and wait, and sit and pursue predation (Scharf & Ovadia, 2006).

The family Myrmeleontidae consists of over 2000 different species, but few build pit traps to capture their prey in a sit-and-wait fashion (Devetak et al., 2013). *Myrmeleon immaculatus* is one such species that sits at the bottom of a conical pit in sandy soils during their larval stage and waits until a prey item falls in. Upon encountering a prey item, the larva will fling sand off of its head, causing mini-avalanches and impeding escape, thus increasing the amount of time available for capture by the larva's venomous jaws (Deventak, 2005).

During its larval stage, *M. immaculatus* must capture ants and other arthropods to grow and, consequently, increase fecundity in adulthood (Burgess, 2009). The evolution of pit-digging has allowed relatively immobile antlion larvae to reduce the amount of energy spent actively searching for prey items (Ruxton and Hansell, 2009). Creating a large pit allows the animal to maintain a low metabolic cost in foraging, while increasing the area of prey trajectories they can intercept, resulting in a higher intake rate simultaneous to a decreasing amount of time necessary between meals (Ruxton and Hansell, 2009; Arnett and Gotelli, 2001).

A study by Griffiths (1986) modeled the cost-benefits of increasing pit size. By increasing the pit volume, an antlion can increase the probability of encountering prey items (increasing pit diameter), or increase the capture rate of prey (increasing slope). Optimal pit size should provide the greatest energy gain by balancing these two parameters. However, in response to varying disturbance and feeding levels, antlions often respond by altering the size of their pit, the amount of energy spent on pit building, or by relocating their pit to a more desirable area (Eltz, 1997).

One possible determinant of success in prey capture is the construction of pits large enough to produce a high capture success rate. Animal size has repeatedly been correlated with pit size in many studies, indicating that larger antlions build larger pits (Swenson et al., 2007). However, external constraints, such as disturbance frequency, conspecific density and environmental variability can decrease capture rate and may require a more flexible strategy of antlion pit structure (Klokocovnik et al., 2012).

Several studies examined environmental effects on pit construction of *M. immaculatus* in laboratory and field contexts. In experiments simulating a high disturbance frequency, researchers have observed smaller pit sizes in response to increased disturbance (Barkae et al., 2010; Eltz, 1997). This indicates that differing environments could cause conspecifics to demonstrate varying pit construction outside of a predicted optimum. However, more in depth work on pit size due to disturbance regime is needed to fully understand this relationship.

Frequency of feeding has produced varying results when tested experimentally. An early study indicated that decreased feeding frequency leads to decreased pit size (Youthed & Moran, 1969). However, Scharf et al. (2010) found that starving antlions who were exposed to prey items, but not allowed to eat increased their pit size at a constant rate, whereas those who were not exposed to prey items increased their pit size at a decreasing rate. This suggests that exposure to prey items can be a trigger for increased pit digging investment. Similarly, another study found lower feeding frequency to correspond with an increase in pit-building activity (Arnett and Gotelli, 2001). These two results indicate that a risk-taking strategy may be involved in pit-building behavior when feeding frequency is inconsistent.

Pit construction has also been linked to soil type. Antlions tend to build pits with larger diameters and depth at low substrate densities resulting in a higher prey capture rate within those environments

(Devetak et. al, 2012). Research also indicates that antlions exhibit a preference toward medium-sized sand particles (0.25-0.5 mm in diameter), and live at a higher density in this substrate type (Matsura et.al, 2005).

Though these external constraints are fairly well studied, few researchers have examined factors of pit-building behavior among conspecific antlion populations outside of environmental condition. Arnett and Gotelli (2001) addressed this question by examining pit-building activity under different feeding and temperature regimes among conspecific populations from two northern and two southern-state populations. Under controlled conditions, southern-state individuals built and maintained pits more often than northern-state individuals, indicating behavioral influences independent of environmental effects. Because of the geographic differences, Arnett and Gotelli attributed these behavioral differences to genetic changes along a latitudinal gradient. However, researchers in Northern Michigan have isolated differences among populations in close geographic areas, indicating effects outside both genetic and environmental effects (Wiers, 2009; Roberts, 2007).

Additional factors that may account for conspecific variation in similar geographic regions are organizational effects and early-experience learning. In general, organizational effects are defined as permanent effects on the behavior of an organism when exposed to a specific environment or hormone at a critical moment of development (Elekonich and Robinson, 2000). Unfortunately, this phenomenon is relatively understudied in insects as compared to vertebrates (Creel et al., 2013; Holekamp et al., 2013), though their endocrine systems could facilitate similar mechanisms (Elekonich and Robinson, 2000). The presence or absence of Juvenile Hormone (JH), an important insect chemical for development, has been shown to determine developmental trajectories during one or more critical periods of pre-reproductive growth of insects (Nijhout and Wheeler, 1982). The effect of JH in development and adult behavior has been researched primarily in caste system insects, such as bees and ants, where JH is intentionally up-regulated in larvae through diet to determine future behavior and role within the colony (Hartfelder and Engels, 1998; Wheeler and Nijhout, 1984). Some studies have indicated that steroids in conjunction with environmental effects can also act as organizational effects to influence insect behavior in locusts, such as tolerance for conspecific density (Elekonich and Robinson, 2000). This **implicates that** variation in similar environments, even in close geographic proximity, may have organizational effects on insects that create variation in behavior later in life.

Similar, early experience could create variation in behavior between populations of insect species. Though this influence has not been analyzed in *M. immaculatus*, Hirsch and Tompkins (1994) demonstrated that early experiences in *Drosophila melanogaster* affected different behavioral strategies later in life. In other words, they showed that the formation of intrinsic responses can be influenced by the animal's environment during development. For example, insects have been shown to change preference of feeding or oviposition sites based on early manipulation and increased exposure to those sites during larval stages (Beach and Jaynes, 1954). These characteristics indicate a development plasticity effecting behavior in later life.

For our study we examined two populations of *M. immaculate* in the upper lower peninsula of Michigan. We sought to replicate and extend a previous study done by the University of Michigan Biological Station's Behavioral Ecology class in 2009 (Wiers, 2009). A difference in *M. immaculate* pit size has been consistently reported between these two populations by previous surveys. The study in 2009 sought to determine if an intrinsic difference existed separating the behavior of the two populations by testing their reaction to frequent disturbances in a controlled, laboratory environment. If the antlions did not produce significantly different pit sizes, it could be inferred that the observed behavior in the field is due to environmental effects. However, if the antlions retained a clear difference between populations for a large portion of the study, the results would support the presence of an inherent quality, such as genetics, organizational effects or early learning. Because the two populations are inferred to be in range of each other with probable gene flow, the nature of this quality may not be satisfied by a genetic explanation. Explanation for differences amongst populations' behavior should accommodate consideration of organizational or early-experiential effects on behavioral variation.

The results of the study were ultimately inconclusive, due to the short 10-day study period. Antlions from the two sites showed a similar pit construction throughout the length of the experiment, indicating that environmental conditions were determinant of pit-building behavior. However, on the last day of the experiment, the two populations appeared to separate and reflect the pit size differences previously observed in the field (Figure 1). Whether or not this trend continues further into a disturbance regime was left unknown.

Our study sought to lengthen this previous study to observe whether these two populations of *M. immaculatus* consistently display behavioral differences beyond 10 days of a disturbance regime. However, the previous study limited the antlion diet to an ant every three days. Because the feeding regime of antlions in natural conditions is unknown, we included an additional feeding regime variable to the original study to identify any effects of food limitation.

In a field setting the 2009 study found the two populations exhibit different pit sizes even in the context of the same habitat and sand type (Wiers, 2009). Based on these results, we hypothesized that the differences in behavior consistently observed between the two is due to some other intrinsic factor effecting the populations differently. We predicted that in an extended study where environmental factors were controlled, the animals would display this intrinsic difference in pit-building activity, regardless of feeding frequency. Support for our hypothesis would indicate an important role of genetics, organizational effects or early-experiential factors on development of behavior.

If we did not see a difference in pit sizes between the two populations under a controlled laboratory setting, we would find evidence for an alternative hypothesis, indicating that immediate environmental conditions are the main driver in differentiating the two populations' pit-building behavior. Either result provides a better understanding of behavior in this insect species, and contributes to previous understandings of antlion behavior.

If food availability is the major factor differentiating behavior between the two populations, we would expect pit diameters to be separated by feeding regime, and the site of origin to be irrelevant. We predicted that individuals exposed to a higher feeding-frequency would have the energetic ability to construct larger pit. However, the opposite result would suggest that animals fed at lower frequency adopt a risk-sensitive foraging strategy.

One condition that we were able to control in our laboratory set-up was ambient temperature. Because antlions are poikilotherms, we expect them to have a lower metabolic rate at lower temperatures, resulting in slower or less activity (Tribe and Bowler, 1967). We hypothesized that temperature had a significant effect on antlion pit-building behavior. We sought to test this hypothesis in a supplemental study to understand how uncontrolled temperature might affect the results over time.

As an additional follow-up to our principle study, we also sought to determine the degree of variation between different sites at the two lakeshores of Douglas Lake and Sturgeon Bay. This would inform on the importance of genetic versus environmental effects on antlions within these two regions, and test our assumption of non-limited gene flow between the two lakes. If we found genetic difference separating the two geographic regions, we would predict Douglas Lake and Sturgeon Bay populations to be more similar in pit-building behavior to populations at their own lake than each other. If the difference separating the two lakes is based mainly on micro-environmental factors, we would predict pit diameter to be characteristic of site, and an equal amount of variation between populations at each lake shore.

Methods

Time Course Experiment

All research animals were collected on July 11th, 2013. We collected 60 antlions from the coastline of Lake Michigan's South Sturgeon Bay (N45.68117 W84.97897), and 60 from Douglas Lake's Pine Point (N45.56607 W84.66029), both located in Emmet County, Michigan, USA. Only animals within a 13 to 40 milligram weight range were collected from either site. A total of 120 animals were labeled, weighed, and kept in capped vials for approximately 28 hours before being transferred into a

controlled laboratory environment. Laboratory temperatures were not controlled and fluctuated based on weather conditions.

Deli tubs were filled with the second smallest fraction of sand from a beach on Douglas Lake and labeled according to treatment types. The treatments were arranged in a checkerboard pattern so as to reduce the influence of micro-environmental variation in the lab on the results (Figure 2).

In all four treatments, a designated researcher measured pit diameter daily at 5:00 PM using digital calipers. We averaged the longest diameter with its perpendicular measurement to obtain an individual average pit diameter for each animal. Pits that were disrupted by proximity to the edge of the container, we only recorded the longest diameter measurement. We categorized the presence of pits based on whether the antlion had finished creating the pit and was not displaying any activity. We did not measure doodling, or pits under construction. Pits were measured from July 15th, 2013 to August 5th, 2013 for a total of 22 days.

Following measurements, antlions were fed medium non-formic acid containing ants at a frequency according to their treatment condition (Table 1). All ants were collected from one colony on the campus of the University of Michigan Biological Station and stored in a refrigerator for up to 24 hours. Before feeding the study animal, ants were exposed to a refrigerated environment for a minimum of five minutes in order to facilitate handling.

We disturbed all treatment types every three days by filling in the pits and leveling off the sand. The disturbance regime allowed us to continually renew pits to measure, and reflected recent behavior of the animals. We disturbed pits after measurements were taken and the antlions had finished eating their ant meals and had expelled the empty carcass from the pit.

Because weights from the two sites were not normally distributed (Shapiro-Wilk test of normality $p < 0.001$), we used a nonparametric Mann-Whitney test to test our assumption that our experimental animal's weights were comparably similar.

We ran a factorial ANOVA using each animal's average pit diameter as the dependent variable and day, site, and feeding frequency as our categorical factors. We included Days 1-22, for a total of 22 days, two sites, and two feeding regimes. We evaluated each of the categorical factors as well as the interaction terms between all combinations of factors. We calculated the least squares means of the dependent variable to calculate site, feeding, and day effects.

Temperature Variation Experiment

In order to understand how the varying temperature affected our results, we followed up the time course experiment by setting up a warming station comprised of four iridescent light bulbs to warm 64 of the time-course-animals from 9:40 AM to 5:00PM daily. This procedure was repeated for three days from August 6th-8th, 2013. The remaining 56 animals were treated as a control and did not receive supplemental warmth. Feeding, disturbance regimes, and measurements remained consistent as per the methodology of the previous experiment.

For analysis, the least squares mean pit diameters of each of the three days of the warming experiment were compared with the corresponding diameters of each of the three days preceding the experiment. We ran a repeated-measures ANOVA, omitting individuals who did not build pits for more than one consecutive day during the six-day sampling. For individuals missing one day, averages were calculated from the preceding and subsequent day diameters. If the 1st or 6th pit diameter was missing from any individual, we repeated the adjacent value.

Population Variation Study

On August 5th, 2013, we measured four locations on the shoreline of Douglas Lake (DLsite1: N45.56140 W84.667948, DLsite2: N45.56607 W84.66029, DLsite3 N45.57241 W84.66008, DLsite4: N45.58540 W84.65416) and three along the shoreline of Sturgeon Bay (SBsite1: N45.68117 W84.97897, SBsite2: N45.68465 W84.97179, SBsite3: N45.70500 W84.95224). Distances between sites at each of the lakeshores were calculated using GPS coordinates.

At each site, we measured pit diameter and animal weight. At each pit, we took two perpendicular measurements of pit diameter, and a measure of pit depth. To take each of these measurements, the same designated measurer from the time course experiment measured the longest diameter and its perpendicular using digital calipers and calculated an average pit diameter for each pit. The animal was then weighed using a digital scale to obtain the weight in milligrams as a measure of relative antlion size. This process was repeated until we collected at least 15 individuals from each site within a 13-40 milligram weight range.

For analysis we grouped the antlions into weight classes of 20-29mg and 30-39mg, which were represented at all sites, to control the weight effect. Exploratory data analysis showed that within 10g weight classes, pit diameter did not depend on weight and average weight within weight class did not differ by lake-site. We ran a two-way ANOVA to test the assumption that no lake*site effect existed ($p=0.538$) and that we could categorize pit diameters by weight class for further analysis.

To test variation within and among lakesites, we ran a nested ANOVA with the dependent variable being an individual's average pit diameter, and the categorical factors being site (nested within lake), lake, and weight class to analyze effects of categorical factors on pit diameters.

Results

Time Course Experiment

For our sample populations, a T-test determined that the two populations were not statistically different ($t=-0.287$, $df=116.61$, $p=0.775$). Based on the calculated least square means, the day, site and feeding effects were all significant, and none of the interaction terms were significant (Table 2). Over the course of the experiment, Sturgeon Bay animals built larger pits than Pine Point animals ($F=11.184$, $df=1,1533$, $p=0.00084$) (Figure 3). Animals that were fed once every three days built larger pits than animals fed daily ($F=4.8337$, $df=1, 1533$, $p=0.2806$) (Figure 4). The day had a large effect on the average pit diameter across all animals, with fluctuating effects over time ($F=12.304$, $df=21, 1533$, $p<0.0001$) (Figure 5). Figure 6 displays all main effects.

Temperature Variation Micro-experiment

Within the 6 day experiment period, when supplemental heat was added to 64 of the animals, average and maximum ambient temperatures of the heated animals increased more than in animals that were not exposed to supplemental heat (Figure 7). In addition, whether the animals were heated or not, the day of measurement, as well as the interaction of day and whether they were heated had a significant effect on average pit diameter (Table 3). Animals that were given supplemental heat increased pit size at a faster rate during the three-day course of heating than did animals that did not receive supplemental heat ($F=4.7665$, $df=5, 375$, $p=0.0003$) (Figure 8).

Population Variation Study

The results in testing the effectors of pit diameter, lake, site, and weight class all had insignificant effects on pit diameter (Table 4). The sites all had statistically similar average diameters when weight was controlled, although Sturgeon Bay Site 1 gave a suggestion of higher diameter ($F=0.65602$, $df=5, 107$, $p=0.65755$) (Figure 9). Any effect indicated from lakeshore site is due to data from Sturgeon Bay site 1 (Figure 10). Distances between sites at each of the lakeshores are shown in Table 5.

Discussion

Our time course experiment revealed a significant behavior difference between our sample populations of Douglas Lake and Sturgeon Bay. Consistent with observations from previous years, Sturgeon Bay animals generally built larger pits than Pine Point animals, even when subjected to regular disturbance events over a period of 22 days (Figure 3). This finding supports our intrinsic quality

hypothesis. The behavioral differences antlions of similar weights persisted between the two populations despite shared environmental conditions and consistent stress (disturbances).

The remaining explanations for consistent differences in behavior are genetic, early learning or organizational effect that conditioned these populations to behave differently throughout their larval stages. A genetic explanation seems unlikely because the two lake shores are within the same geographic area, and no restriction to gene flow is apparent in these researchers. However, genetic differences could not be ruled out without a supplemental study. The remaining explanation available is that some type of early experience, whether learning or organizational effect, separates these two populations and results in diverging behavior patterns later in life. The two sites of study differ. Animals located on a Lake Michigan, with a different soil type and degree of exposure are likely to experience different environmental inputs throughout the year, including but not limited to wind, ground temperature, species composition and others. If antlion larvae are sensitive to micro-environmental conditions early in life, they may be set on a permanently different developmental pathway.

Feeding also had a significant effect on pit size within our study. Because pit-building incurs energetic costs, food intake is expected to affect pit size. In fact, some studies have associated starvation with smaller pit size (Youthed & Moran, 1969). However, in contrast to our prediction, antlions who were fed less often tended to build larger pits (Figure 4). This behavior may reflect a risk-sensitive foraging strategy, in which food-stressed individuals increase their pit sizes to raise the probability of prey capture (Abrams, 1991). Animals who are fed regularly, however, should avoid the extra cost of building a larger pit when they are consistently receiving nutrition (Abrams, 1991). High-risk foraging strategies have been found as explanation of spider and bird foraging strategies when subjects are experiencing negative energy budgets, and demonstrate energetically costly behavior to increase probability of a meal (Caraco et al., 1980). This behavior was observed *M. immaculatus* from both sites, indicating that food availability affects pit-building regardless of site of origin.

The day effect was also significant among all antlions, but did not indicate an overall trend (Figure 5). Previous studies found that high disturbance regimes resulted in construction of smaller pits over an extended time period (Barkae et al., 2010; Eltz, 1997). The final pits in the last few days of this study were smaller than the first few days, but no pattern was made evident. As one of the major uncontrolled conditions in our study, temperature likely had a large effect on metabolic function of the animals. In fact, temperature has been determined as a predictor of pit size (Arnett and Gotelli, 2001). During the course of our experiment, temperature average ranged from 60°-75°F (according to NOAA history for Charlevoix, Michigan). This leads us to believe that the large variation in temperature during our study period majorly confounded the results of our day effect. For this reason, we conducted our temperature variation experiment as a follow up to test this hypothesis.

In our temperature variance experiment, we found temperature to have a large effect on antlion pit-digging behavior, supporting our previous lack of confidence in the day effect on antlion pits. Both average and maximum ambient temperature increased for animals given supplemental heat, leading to a faster rate of pit growth over the heated three-day period as compared to the animals that were not given supplemental heat (Figure 8). The non-heated animals had larger pit sizes than the heated animals in the three days preceding the experiment as well as a 0.5°C greater temperature on average. Though they initially built larger pits after the disturbance between day 3 and 4 (the supplemental heat experiment started on day 4), the pits of animals given supplemental heat grew much faster. If the experiment were to continue without disturbance, we suggest that the heated individuals would have been observed to surpass the non-heated individuals in average pit diameter, as predicted by results of previous temperature studies (Arnett and Gotelli, 2001). These results indicate that temperature plays an important role in determining pit size, and likely had a large effect on the relationship of day to pit-size during our time course experiment. A better design for a time course experiment would control temperature and humidity changes to more clearly identify a day effect.

Our last follow-up experiment measuring variation within and between lakeshores revealed that the two locations of study show fewer differences than we expected based on repeated samplings of our isolated sites of study. When we controlled for weight, neither lake nor site served as predictors for pit

diameter. From this result we cannot identify a genetic or environmental effect because antlions at all sites are behaved similarly.

Our data suggested that animals from Sturgeon Bay site 1, the sample site for the time course experiment, build larger pits than other sites, though the study revealed those differences to be not significant (Figure 9). However, because this site has repeatedly shown larger pit sizes than the Pine Point sample site in the past, a difference likely exists between the animals there and other sample sites. Due to this repeated difference, we suggest that an unidentified factor differentially effects Sturgeon Bay site 1 antlions, which helps us interpret the role of genetic versus environmental factors within our experiment.

Distances of Sturgeon Bay site 1 from the other Sturgeon Bay sites are both fewer than 3 km, which is likely too short of a distance to restrict gene flow between populations (Table 5). Though we cannot dismiss genetic factors to be distinguishing site 1 in pit-building behavior completely, the close proximity gives further confidence to our assumption of open gene flow between the two sites, and likely the two lakes. Furthermore, SB site 1 represents a micro-environment, though great variation in environmental conditions from the other SB sites on a day to day basis likely doesn't exist. This indicates another influencing factor of long term larva behavior beyond environmental and genetic differences. This follow-up study increases our confidence that an early experience or organizational effect is acting on the Sturgeon Bay site 1 antlions differently than antlions at all other sites, causing them to act differently in a controlled laboratory setting.

In summary, our study finds that antlion behavior is affected by factors other than environmental condition. We suggest that genetics can also be ruled out as an explanation, though further studies are needed to increase confidence in this hypothesis. Slight inconsistency in measuring times and human error may have impacted the results of our study. In addition, we did not differentiate antlions based on age, nor had records of condition during the early life of any of our sample animals. In order to better understand the role that organizational effects and early learning may have on antlion behavior, a long-term experiment could be conducted in antlion rearing, where environmental conditions were manipulated during critical developmental periods of larva. Such an experiment could also be used to weigh the role of genetic effects in later instar behavior. Overall, these findings suggest a role of organizational or early learning effects in development of insect behavior, a fairly understudied topic outside of the order Hymenoptera.

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Tables

Table 1: Treatment conditions for the time course experiment. All antlions were exposed to a disturbance every 3 days, on the same day that they were fed.

	Pine Point animals	Sturgeon Bay animals
Fed every three days (low-feeding frequency)	Treatment 1	Treatment 2
Fed daily (high-feeding frequency)	Treatment 3	Treatment 4

Table 2: Day, site, feeding regime, and interaction term effects on pit diameter for time course experiment. Day, site, and feeding regime all have significant effects on average pit diameter.

	SS	Degr. of	MS	F	p
Intercept	3239190	1	3239190	35777.25	0.000000
Day	23393	21	1114	12.30	0.000000
Site	1013	1	1013	11.18	0.000845
Feeding	438	1	438	4.83	0.028057
Day*Site	927	21	44	0.49	0.975236
Day*Feeding	2476	21	118	1.30	0.161844
Site*Feeding	70	1	70	0.78	0.378249
Day*Site*Feeding	951	21	45	0.50	0.971119
Error	138794	1533	91		

Table 3: Presence of heat, site, and interaction term effects on pit diameter for temperature variation experiment. Presence of heat, day(TIME), and presence of heat*day(TIME) interaction terms are statistically significant.

	SS	Degr. of	MS	F	p
Intercept	987502.0	1	987502.0	2321.407	0.000000
heat y or n	7593.0	1	7593.0	17.849	0.000067
site num code	1.2	1	1.2	0.003	0.958140
heat y or n*site num code	620.7	1	620.7	1.459	0.230854
Error	31904.2	75	425.4		
TIME	6620.0	5	1324.0	59.908	0.000000
TIME*heat y or n	526.7	5	105.3	4.766	0.000309
TIME*site num code	40.0	5	8.0	0.362	0.874471
TIME*heat y or n*site num code	158.1	5	31.6	1.431	0.212253
Error	8287.8	375	22.1		

Table 4: Lake, site nested within lake, and interaction term effects on pit diameter for population variation study. No predictor has any significant effect on average pit diameter.

	SS	Degr. of	MS	F	p
Intercept	239677.9	1	239677.9	2910.682	0.000000
Lake	178.2	1	178.2	2.164	0.144180
site(Lake)	270.1	5	54.0	0.656	0.657552
weightclass	206.0	1	206.0	2.502	0.116652
Error	8810.8	107	82.3		

Table 5: Distances between sites in the population variation experiment. Calculated by GPS coordinates

	DL1	DL2	DL3	DL4	SB1	SB2	SB3
DL1		0.79 km					
DL2			0.71 km				
DL3				1.52 km			
DL4							
SB1						0.68 km	
SB2							2.73 km
SB3							

Figure Legends

Figure 1: Average pit diameter results from previous class study (Wiers, 2010). Average pit diameters were significantly different between sites on day 0 ($Z=4.932$, $p<0.001$) and day 10 ($Z=2.428$, $p=0.015$). Days 1-9 were insignificant.

Figure 2: Checkerboard layout of animals in time course experiment. This pattern was repeated twice for a total of 120 animals.

Figure 3: Average pit diameter (mm) separated by site (1=Pine Point, 2=Sturgeon Bay) for time course experiment

Figure 4: Average pit diameters (mm) separated by feeding regimes (1=one ant every three days (low feeding frequency), 3=one ant daily (high feeding frequency)) for time course experiment

Figure 5: Average pit diameters (mm) separated by day of study for time course experiment

Figure 6: Average pit diameter (mm) separated by day, feeding regimes, and site. Vertical lines represent disturbance events every three days.

Figure 7: Maximum and average ambient temperature of antlions given supplemental heat and not given supplemental heat, three days prior (day 1-3), and three days during (day 4-6) the temperature variance experiment.

Figure 8: Time and presence of heat interaction effect on average pit diameter (mm) for temperature variation experiment.

Figure 9: Site effect at each lakeshore (1=Pine Point, 2=Sturgeon Bay) on average pit diameter (mm) for population variation study.

Figure 10: Lakeshore effect (1=Pine Point, 2=Sturgeon Bay) on average pit diameter (mm) for population variation study.

Figures

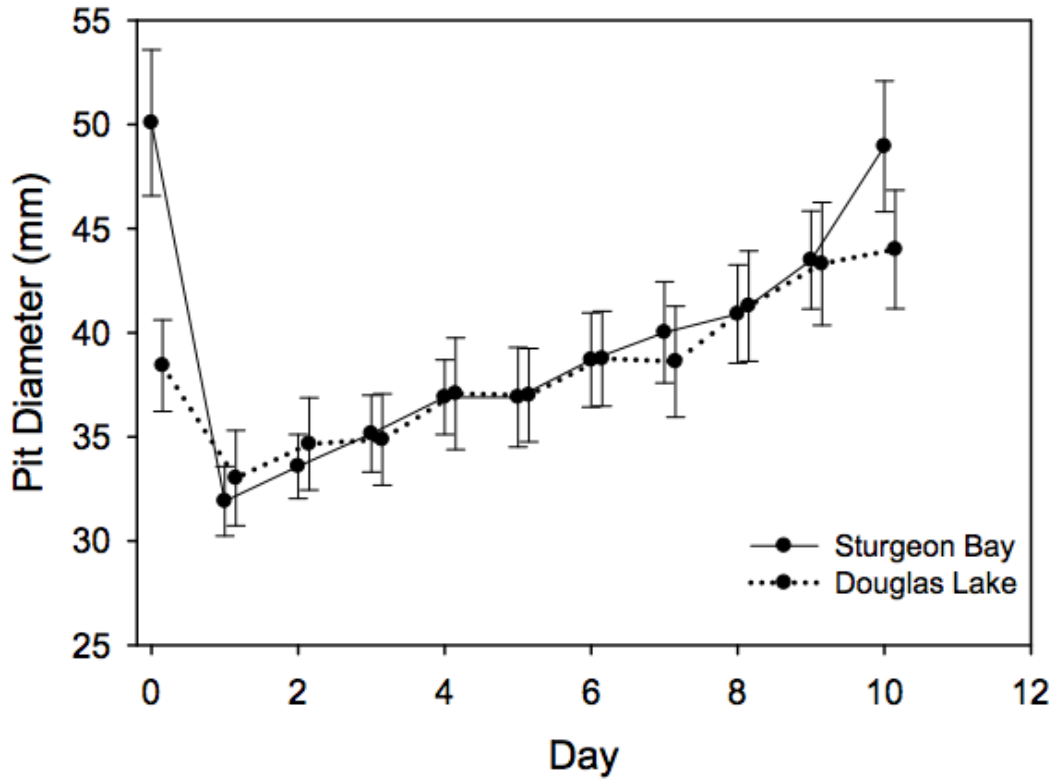
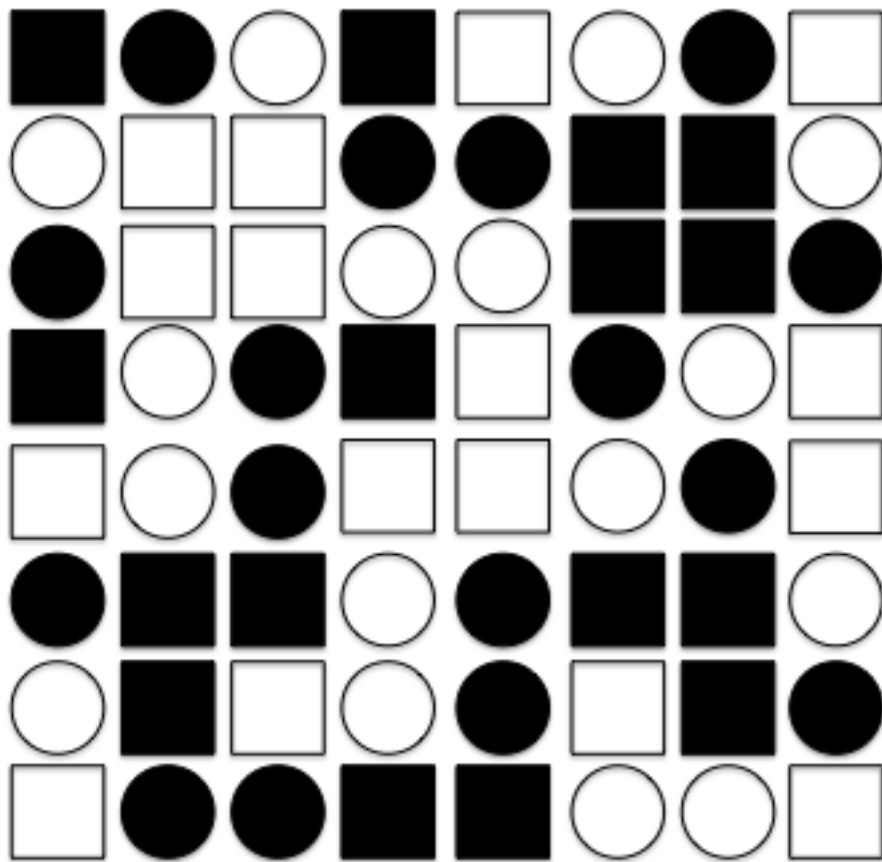


Figure 1



LEGEND





	South Sturgeon Bay Fed Daily
	South Sturgeon Bay Fed Every 3 Days
	Pine Point Fed Daily
	Pine Point Fed Every 3 Days

Figure 2

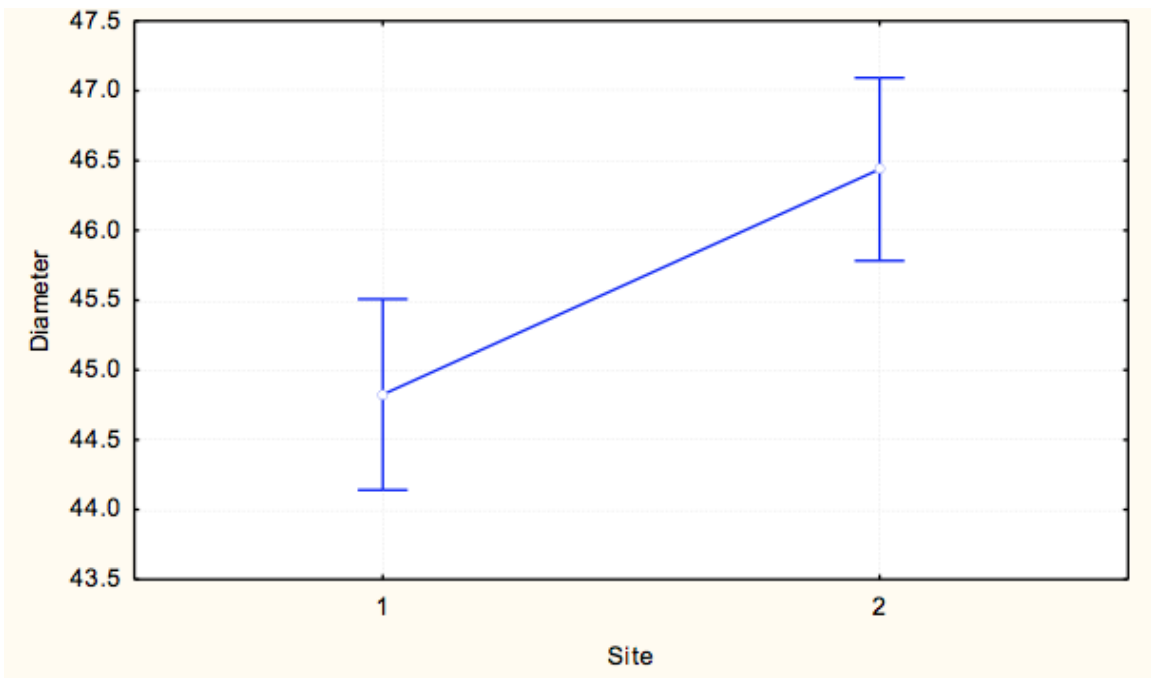


Figure 3

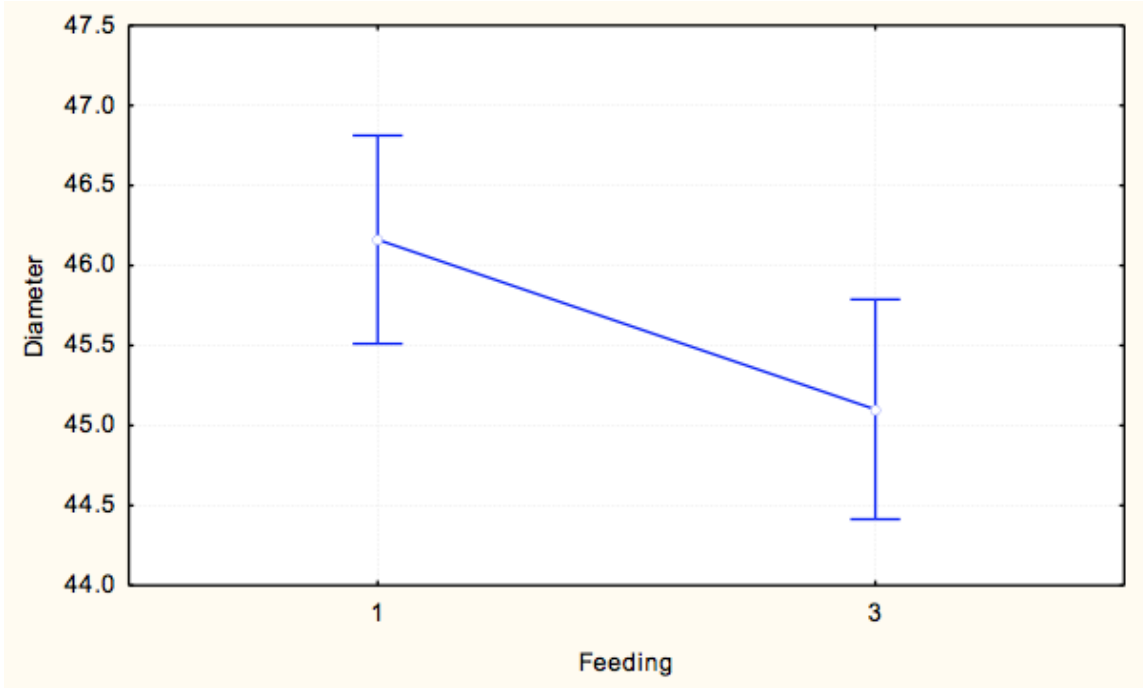


Figure 4

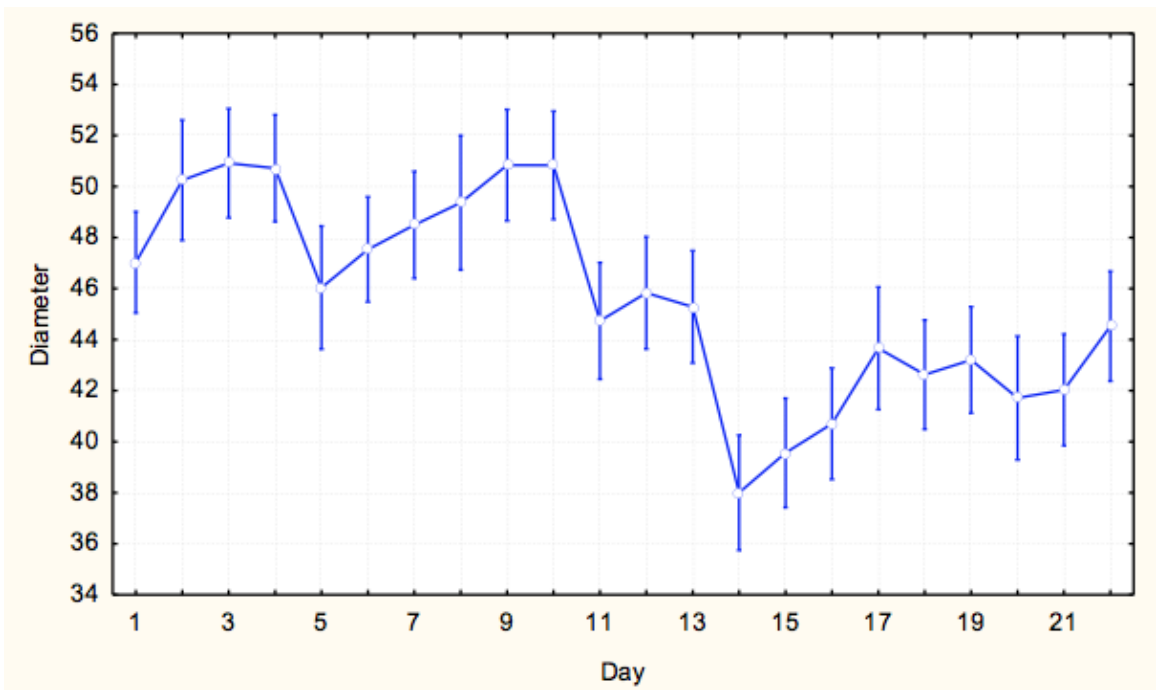


Figure 5

Pit Diameter Across Disturbance Regime

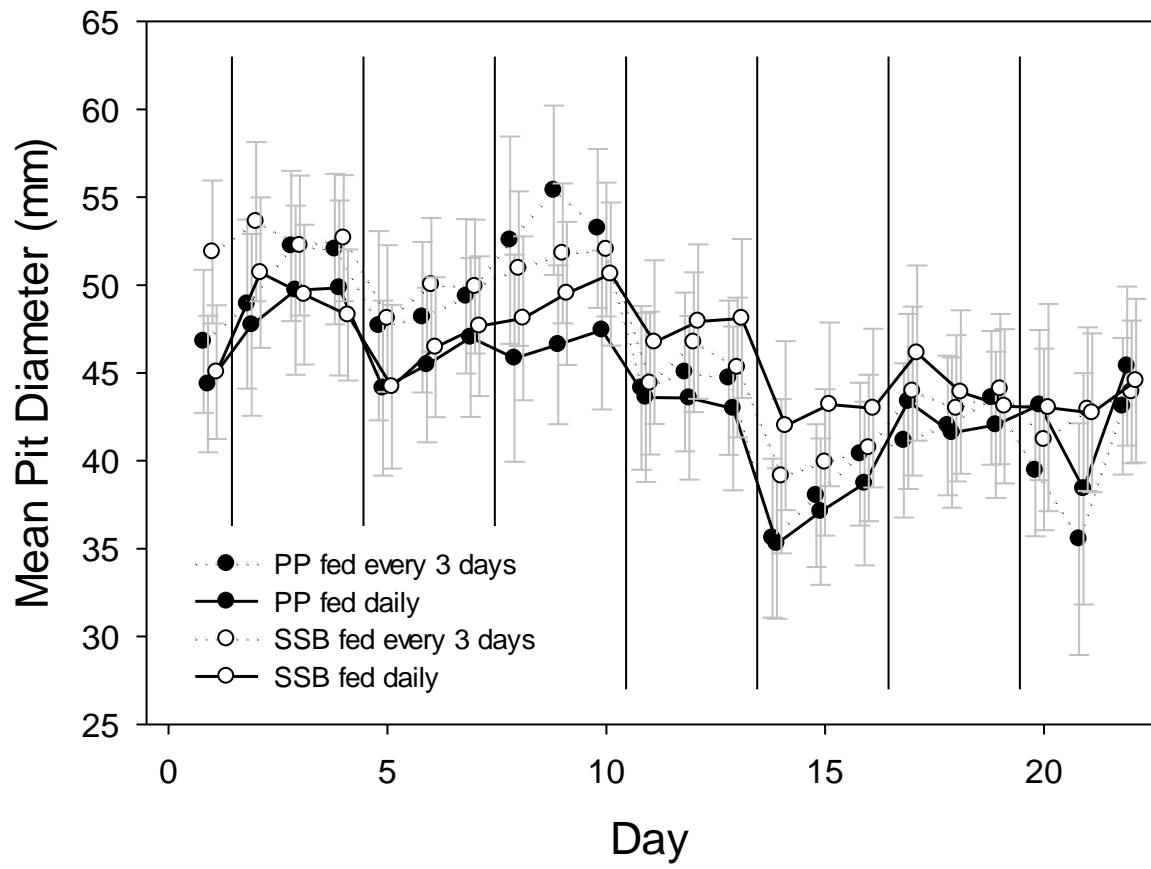


Figure 6

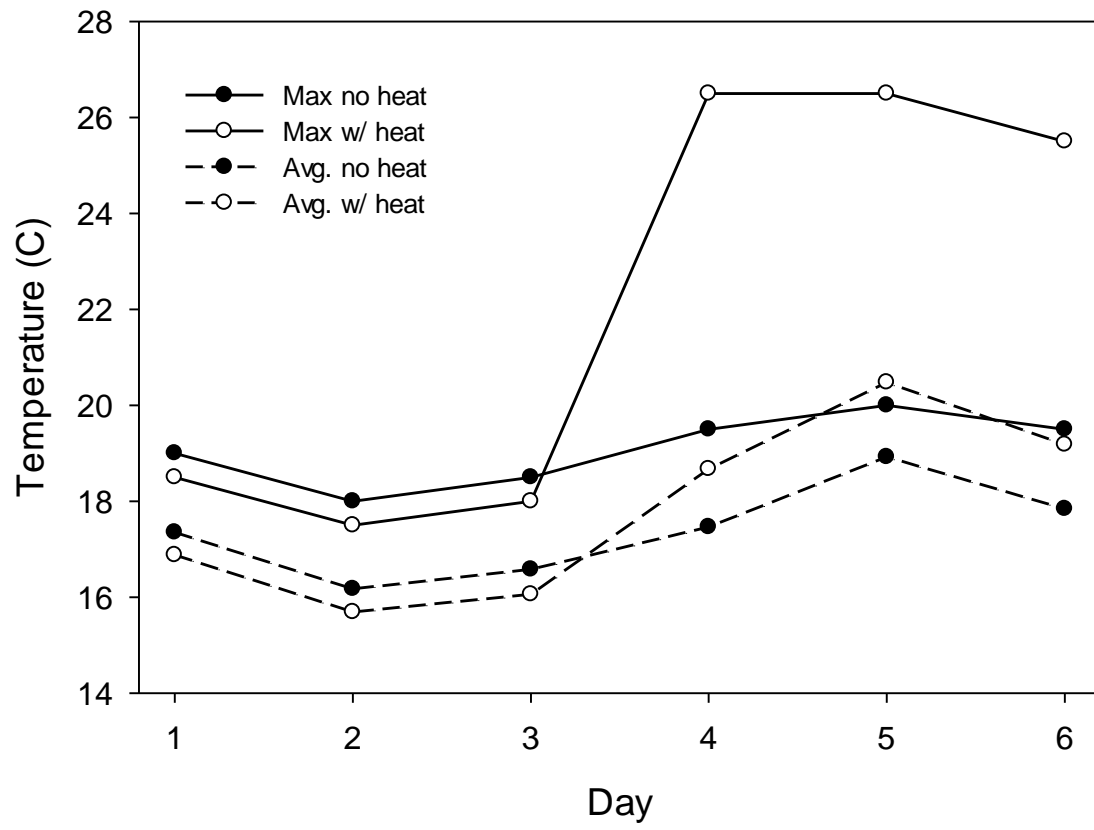


Figure 7

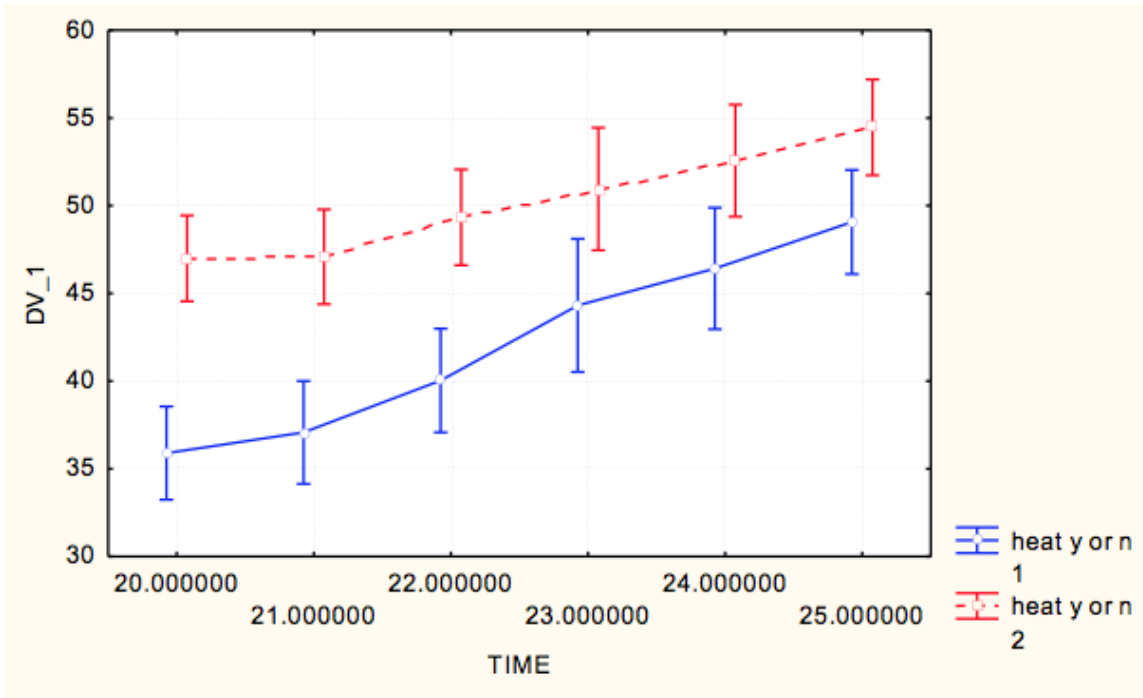


Figure 8

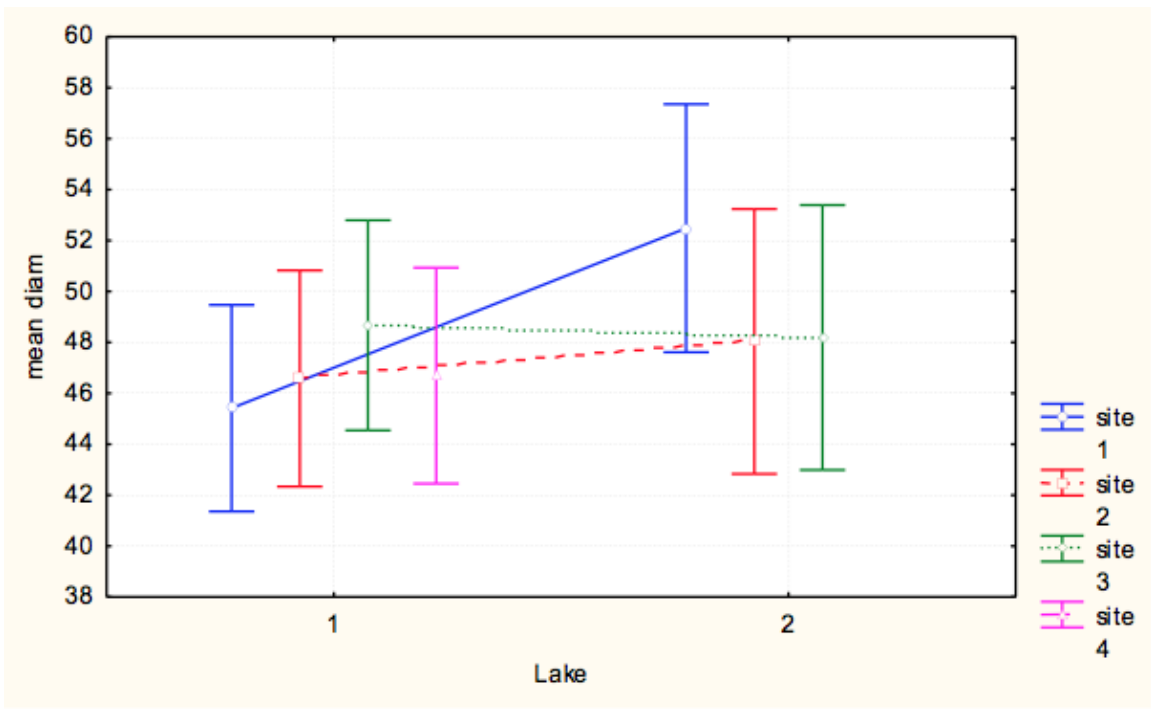


Figure 9

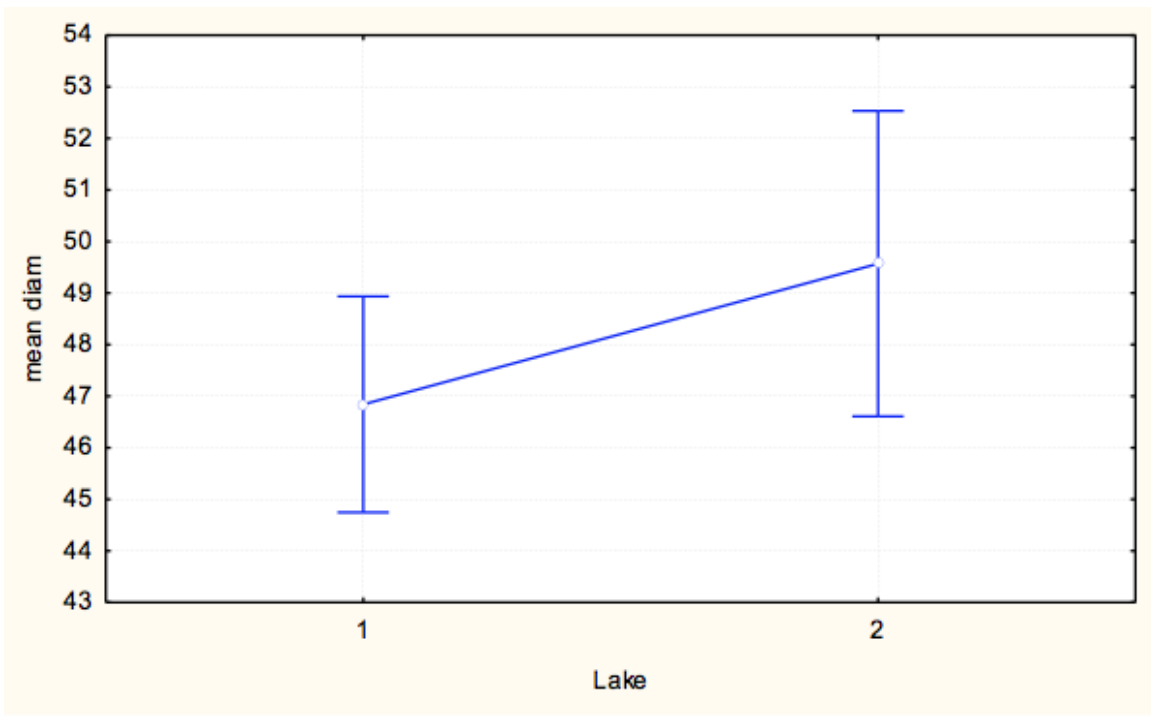


Figure 10