The Effects Of Nocturnal Light On *Odontotaenius disjunctus* (Coleoptera: Passalidae)

By

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The Effects of Nocturnal Light On *Odontotaenius disjunctus*

(Coleoptera: Passalidae)

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ABSTRACT

THE EFFECTS OF NOCTURNAL LIGHT ON *ODONTOTAENIUS DISJUNCTUS* (COLEOPTERA: PASSALIDAE)

The effects of nocturnal light on the activity of burrowing insects have been rarely investigated. The goal of this study was to examine the effects of nocturnal light intensity on nocturnal activity of Bess beetles (*Odontotaenius disjunctus*). Individuals (n=18) were exposed to three artificial light treatments 25w (low light treatment), 50w (medium light treatment) or 75w (high light treatment) Eco terra Night glo bulbs that were used to simulate the moonlight intensity of new moon, half moon, and full moon, respectively. During each light treatment the activities that were investigated and time spent total activity, walking, feeding and burrowing, resting and under the mulch. These activities of *O. disjunctus* were videotaped for four h under artificial lights on the three nights around each lunar phase. They were then compared to determine if time spent doing each activity was significantly affected by the artificial light. Total activity (walking, feeding and burrowing) differed significantly between the high light treatment and the medium light treatment, but this difference was not due to time spent walking, feeding or burrowing. That difference was due to time spent resting (above the mulch) and time spent under the mulch. These results suggest that nocturnal light intensity may affect the nocturnal activity of *O. disjunctus*. This may be due to the fact that beetles are infesting of wood on the full moon (Zürcher 2001), or creating family living structures or mating during the half moon. Tunnels and chambers were both found after the half moon supporting the

idea that their mating may be affected by the lunar phases, but more studies need to be done.

ACKNOWLEDGMENTS

I would like to thank my committee of advisors for their support. They gave me the freedom to pursue this research even though it had not been performed before at Youngstown State University or on *Odontotaenius disjunctus*. They permitted me the creative freedom that would sometimes make me crazy. I would also like to thank The Department of Biological Sciences at Youngstown State University for the moral and monetary support of my research. Another group I would like to thank is my group of close friends, Becky Rupert, Tammy Diglaw, Amy Schular, Julie Chandlar and Shawn Blohm, who listen to my venting about life, work and my research. Lastly I would like to thank my family, Martha and Mary Cole, which were supportive and understanding through my error and successes. They were there on the nights of videotaping to keep me from going insane, helped me set up traps for my feasibility study, and allowed me solitary time when I need to do work without interruptions.

In loving memory of Mary Cole a loving mother that taught her daughters persistence.

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Introduction

Goals

The goal of this study was to determine whether beetles of the family Passalidae are influenced by lunar cycles. The study focused on one species from the family Passalidae, *Odontotaenius disjunctus* (Illiger) that is native to the eastern United States and southeastern Canada (Evans 2003, Downie & Arnett 1996) and is an important recycler of rotten wood (Romoser & Stoffolano 1998, Borror & White 1970). *Odontotaenius disjunctus* is moderately active at night (Romoser & Stoffolano 1998, Borror & White 1970) and may be affected by the amount of nocturnal light. The objective of this study is to investigate the effect of three nocturnal light intensities on the activity of *O. disjunctus.*

While many diurnal beneficial insects are well studied, less is known about nocturnal beneficial insects (Bedick *et al.* 1999). For example, studies on ground beetles showed that they have effects on pest species in crops (Lang *et al.* 2000, Suenaga & Hamamura 1998), but they did not investigate at what time of day the ground beetles are most effective? Other studies investigated the change in predators' assemblages as a result of human changes (Paarmann *et al.* 2002, Koivula 2002, Varchola & Dunn 1999), but did not investigate the change in predators' assemblages as a result of natural cycles (solar, lunar or seasonal cycles).

The present study was stimulated by the observation that some nocturnal insects, like nocturnal Lepidoptera and some Coleoptera, seem to be attracted to artificial lights. This leads to the following questions:

- If they are influenced by artificial light, are they also influenced by simulated moonlight?
- Does the intensity of simulated moonlight influence insect locomotor activity?
- Can moonlight be simulated to represent the natural environment and would insects be affected by the simulated moonlight?

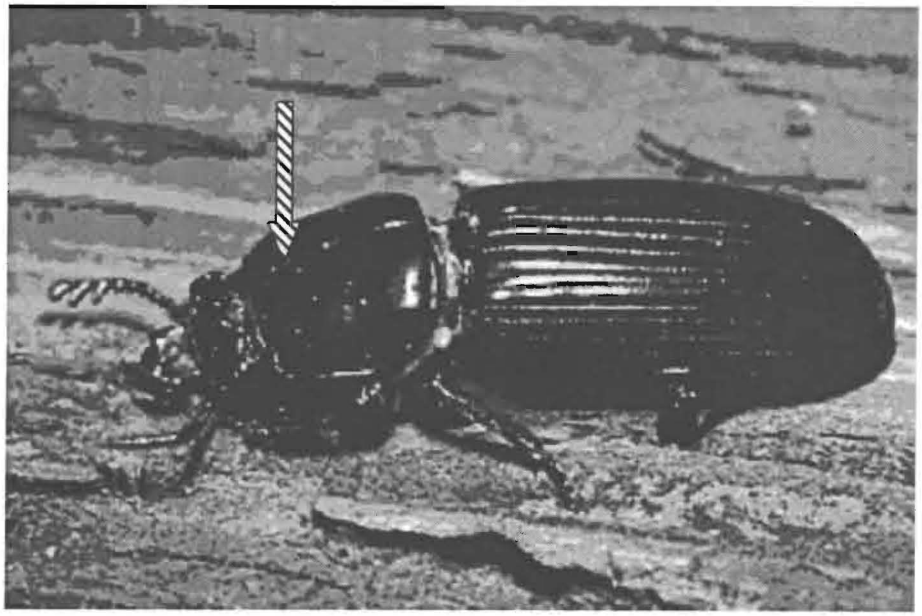
Passalidae background information

There are around 500 species in the passalid family (Evans 2003). Most of them are native to the mainlands in the tropics with three species being native to North America (Evans 2003). Passalids are distinguished by three main characteristics: a deep median groove on the pronotum, which is a dorsal plate of the first thoracic segment (Fig.1 a), deeply grooved elytra, which are hardened forewings that protect the membranous wings (Fig.1 b), a horn on their heads (Fig.1 c).

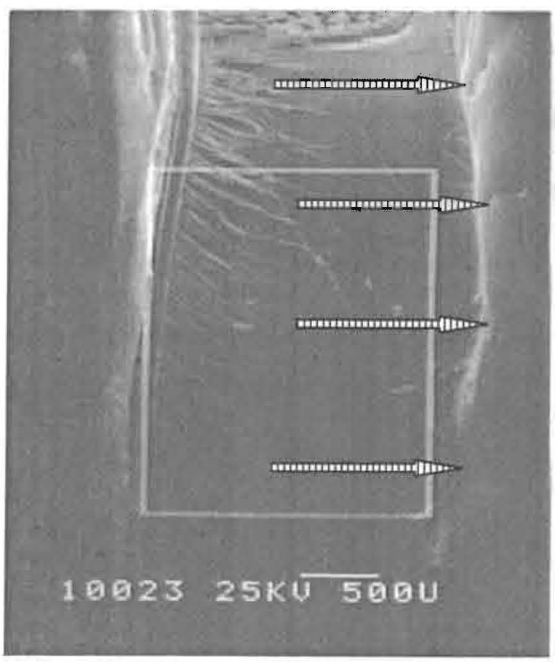
Odontotaenius floridanus and *Odontotaenius disjunctus* are the only two passalid beeltes native to the eastern Unites States. *Odontotaenius floridanus,* was discovered in 1994 in Florida, and identified as a new species (Schuster 1994). It is concentrated in the warmer states in the eastern United States, leaving *O. disjunctus* the only passalid beetle in the temperate northeastern United States.

Odontotaenius disjunctus is commonly known as the patent leather beetle or Bess beetle (Borror & White 1970), and can grow up to 3.5 cm. Although *O. disjunctus* is not listed as a pest, it is not protected either, even though it is known to be beneficial. This leaves *O. disjunctus* vulnerable to human control by insecticides meant for pest species. A whole colony of *O. disjunctus* could be devastated even if

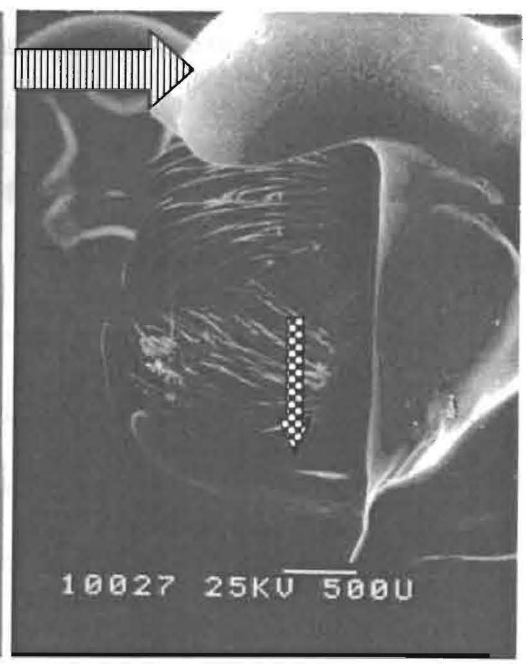
Figure 1. Photos of *O. disjunctus* that show passalid characteristics. A.) A digital photo of *O. disjunctus* showing the deep median groove on the pronotum (lined arrow) (<http:// www.daviessaudubon.org/bess_beetle.htm>) B.) SEM image of the thorax and abdomen of *O. disjunctus* showing the deep longitudinal groves of the elytra (lined arrows). The left side of the picture is the posterior edge of the pronotum while the right side is the anterior edge of the abdomen. C.) An SEM image of the head of *O. disjunctus* that shows the short horn that curves forward on the head (lined arrows) and the mandibles (checked arrows). The bars on the SEM photos represent 500μm.



a



b



c

insecticides kill only the adults. The reason for this is due to the parental care *O. disjunctus* adults give their young (Schuster 1985). The larvae are dependent on the adults for survival. Parenting is needed among insects that have patchy or rapidly decomposing resources (Wilson 1975), due to the resources being hard to find or assimilated by the young and the possibility that the young would perish without the care (Vulinec 1995). *Odontotaenius disjunctus* adults need to chew the mulch and mix it with their feces so their larvae can acquire the cellulose-fermenting bacteria. These bacteria *Leidyomyces attenuatus* are essential for the digestion of wood, and without them the beetles would die (Romoser & Stoffolano 1998).

Odontotaenius disjunctus were chosen for this study because of their nocturnal activity, longevity and hardiness. The beetles would therefore be able to be videotaped for at least one month (one lunar cycle)/ more then just a few days. This allowed them to acclimate to the surroundings and to be affected by the light changes. *Odontotaenius disjunctus* are hardy type of beetle if they are given a moist environment and decomposing hardwood. They lived for about two months or more in the laboratory. *Tenebrio molitor* were considered for study, but they are too short lived (only a few days after receiving them). Carabid beetles were also considered but they would have to be collected from the wild, and are hard to care for. Food species would have to be collected or bought to feed them, and the lids of the tank would have to be left on while taping because the carabid beetles and their prey may escape. The lids on the tanks would have to be left on while taping, cause distortions of the view of the beetles.

Insect activity

There are three types of locomotion used by insects: terrestrial, aquatic, and aerial. Many insects use terrestrial locomotion as their primary source of movement. Examples of terrestrial locomotion are walking, running, jumping, and crawling (Romoser & Stoffolano 1998). *Odontotaenius disjunctus* rarely fly or move their elytra (the hardened forewings) and use walking as their main mode of locomotion (Evans 2003, Downie & Arnett 1996). Thus, terrestrial locomotion was explored in this study.

Abiotic Factors

Abiotic, density independent, factors include soil, water, air, light, nutrients, which effect populations (Andrewartha & Birch 1954, Hale & Margham 1991, Smith & Smith 2001). Light, temperature and moisture are the three most important abiotic factors that influence arthropod activity (Romoser & Stoffolano 1998), although only light was being considered in this study.

Many organisms use light as a cue for natural events, such as the flowering of plants, which tell them when to emerge, mate and rest (Campbell 2002). Many laboratory studies have shown the importance of light to arthropods. The intensity of light affects insects (Campbell 2002, Romoser & Stoffolano 1998, Smith & Smith 2001). Artificial light has been known to affect insects' circadian rhythms (Tomioka *et al.* 1998, Tomioka 1999). Natural light or the absence of it can also affect their spatial distribution (Romoser & Stoffolano 1998). High proportions of Japanese beetles (*Popillia japonica*) are found in shaded areas and are active in the morning

(Kreuger & Potter 2001). Some arthropods do not become active in light, but in the absence of intensive light (Sanchez 1997, Lorenzo & Lazzari 1998).

Moonlight may be an important factor that influences the nocturnal activity of arthropods. The effect of moonlight on the foraging patterns, size and weight of arthropods was clearly demonstrated in a study conducted on scorpions, *Buthus occitanus Israelis*. On dark nights (new moon) adults hunted in open areas, while on moonlit nights (full moon) they hunted under bushes. Juveniles sat and waited on moonlit nights but actively searched on dark nights. Those scorpions that hunted on darker nights were larger and weighed more (Skutelsky 1996).

Some animals are indirectly controlled by the lunar phase because they use the tides as a cue for breeding (Naylor 2001). Polychaetes, African catfish and mysid shrimp, however, are directly controlled by the lunar phase (Naylor 2001, Viherluoto & Viitasalo 2001, Britz & Pienaar 1992). Natural and artificial moonlight effect the activity of Patagonian leaf-eared mice similarly *Phyllotis xanthopygus* (Kramer & Birney 2001).

Temperature and moisture are other abiotic factors that influence the activity of insects, including *O. disjunctus* (Romoser & Stoffolano 1998, Ikeda & Tomioka 1993). The range of temperature survivability for most insects' lies somewhere between 0°C and 50°C, but no one species is likely to survive the entire range (Taylor 1981). Some insects are able to live in hot temperatures (Andrewartha & Birch 1954 & 1973), while other insects, like *O. disjunctus,* can withstand cold temperatures up to around -35°C (Salt 1959, Brennan *et al.* 2000). *Odontotaenius disjunctus* supercool in two parts excavates endogenous nucleating agents from its guts then accumulate cryoprotecting sugars and proteins. Moisture is also crucial to the survival of most life on this planet, which includes insects (Romoser & Stoffolano 1998, Janzen 1971). Most species of passalid beetles are found in moist areas (Evans 2003, Borror & White 1970) because they rely on rotten wood for their survival.

Biotic factors

Biotic, density dependent, factors are influences that occur within an environment as a result of activities of the living, which affect populations (Nicholson 1933, Hale& Margham 1991, Smith & Smith 2001). Food availability and predators are the most important biotic factors that affect arthropod activity patterns.

Food availability, risk of predation and predator activities are important biotic factors that can influence the activity of insects including *O. disjunctus* (Romoser & Stoffolano 1998, Feio & Graca 2000, Suenaga 1998, Lang *et al.* 1999). Their primary food consists of rotten deciduous hardwood stumps (Evans 2003), in which they appear in great numbers (Romoser & Stoffolano 1998). Passalid beetles have an increased risk of predation due to their parental care and communication (Vulinec 1995, Evans 2003). Beetles communicate through squeaks and clicks. The adults guard the tunnels and chambers. Predator activities and numbers can affect the prey species' abundance and activity (Suenaga 1998, Lang *et al.* 1999).

Methods

Study Design

This study was designed to test the hypothesis that the nocturnal activity of *Odontotaenius disjunctus* individuals (n= 18) in nine tanks are influenced by nocturnal light intensity. Individuals were exposed to three nocturnal light treatments (25w, 50w or 75w Eco terra Night glo bulbs) that were used to simulate the new moon (low nocturnal light treatment), half moon (medium nocturnal light treatment), and full moon (high nocturnal light treatment), respectively, at the appropriate time of the lunar phase. The activity of *O. disjunctus* was videotaped for four hours under simulated moonlight intensity on the three consecutive nights around each lunar phase (new moon, half moon, full moon) and compared to determine if time spent doing any activity or inactivity differed between the three light treatments. The time was tested using ANOVA or Kruskal-Wallis and multiple comparisons tests. The beetles were expected to be the most active during the low nocturnal light treatment and the least active during the simulated full moon, due to predator avoidance behavior.

Hypothesis

Ho: There is no effect of the nocturnal light intensity on beetle activity.
Ha: There is an effect of the nocturnal light intensity on beetle activity.

Study Site

The passalid beetles were tested indoors in a room with no external light pollution, so all light (sun and moonlight) had to be simulated artificially. The room was kept at a relative temperature of 25°C. Humidity was kept high in the tanks by keeping the lids on the tanks of all times their activity is not being videotaped.

***Odontotaenius disjunctus* Care**

A male and a female beetle were arbitrarily placed in half-gallon flex tanks because of their sub-social behavior. In their natural environment the beetles live in pairs with their young. The sex of each beetle was determined by the width of the abdomen: "Females tend to have wider abdomens than the males" (Evans 2003). The technique of determining sex by with of abdomen is not unbiased but it ensures survival. The tanks contained approximately 4 cm deep of hardwood mulch on the bottom of the tanks. The beetles were kept alive by feeding them the moistened hardwood mulch. The paper towel was wet and then rung out and placed in the tank. Moist paper towels were dampened once a week to keep moisture levels up. A moist environment was needed to promote microbial growth that increases the beneficial decaying process, which is crucial to the beetles' survival (Evans 2003). The beetles were allowed to acclimate to the light cycle for three days.

The Light Cycles

The light cycle was similar to the natural light cycle. One timer maintained the day light cycle for the passalid beetles from 05:00 to 21:00 Eastern Standard Time. Daylight was simulated using a plant growing light of 15 watts that was placed 1m above all tanks. Another timer maintained the dark light cycle. The dark cycle used two bulbs every night. One light was simulated the lunar phase, while the other was a red light. The lights were kept 1.3m above the tanks. In former studies it was found that red light can not be seen by beetles in the family Carabidae (Frings 1940). *Lasius niger,* ant, aggregation does show a difference but it is not significant when comparing red light and total darkness (Depickere et. al 2004).The red light (60 watt) was used for visual confirmation of the beetles. The lunar phases were simulated by artificial light. The light bulb used to simulate the moonlight was changed at the beginning of the phase (once a week) at the appropriate time of the month to coincide with the natural lunar phase. The full moon, half moon, and new moon were simulated by using a 75-watt, 50-watt, and 25-watt Exo terra™ Night glo® bulbs (black light) respectively. The natural and simulated moonlight was measured with a Gossen Digiflash light meter. All measurements were at the settings '500 expore time/ shutter spped, 5,6 Aperture/F-stop scale, 14 setting window, 115 flash measurement. The natural full moon measurement was 14 and the simulated full moon was measured at 15. The natural half moon measurement was 8 and the simulated half moon was measured at 7. The natural new moon measurement was 1 and the simulated new moon was measured at 2. Many coral breeders recommend the use of a linear light regression to promote normal activities controlled by the moonlight (Tyree 1992).

Table 1: Natural moonlight intensities and simulated moonlight intensities. Natural light intensity is given in lux (MacEvoy 2005). The natural and simulated moonlight was measured with a Gossen Digiflash light meter. All measurements were at the settings '500 expore time/ shutter spped, 5,6 Aperture/F-stop scale, 14 setting window, 115 flash measurement. The readings for simulated moonlight in lux was calculated (R2 =0.0007*EXP(0.3535*x) when full moon is 0.1lux) and (R2 =0.0004*EXP(0.5258*x) when full moon is 1.0lux). The variable x is the light meter reading for natural light.

	Natural light intensity (lux)	Natural light intensity (using light meter)	Simulated light intensity (using light meter)	Simulated light intensity (lux)
Full moon	1-0.1	14	15	1.064977838-0.140587
Half moon	0.01	8	7	0.015868076-0.008313
New moon	0.001	1	2	0.001144891-0.00142

Odontotaenius disjunctus Activity Categories

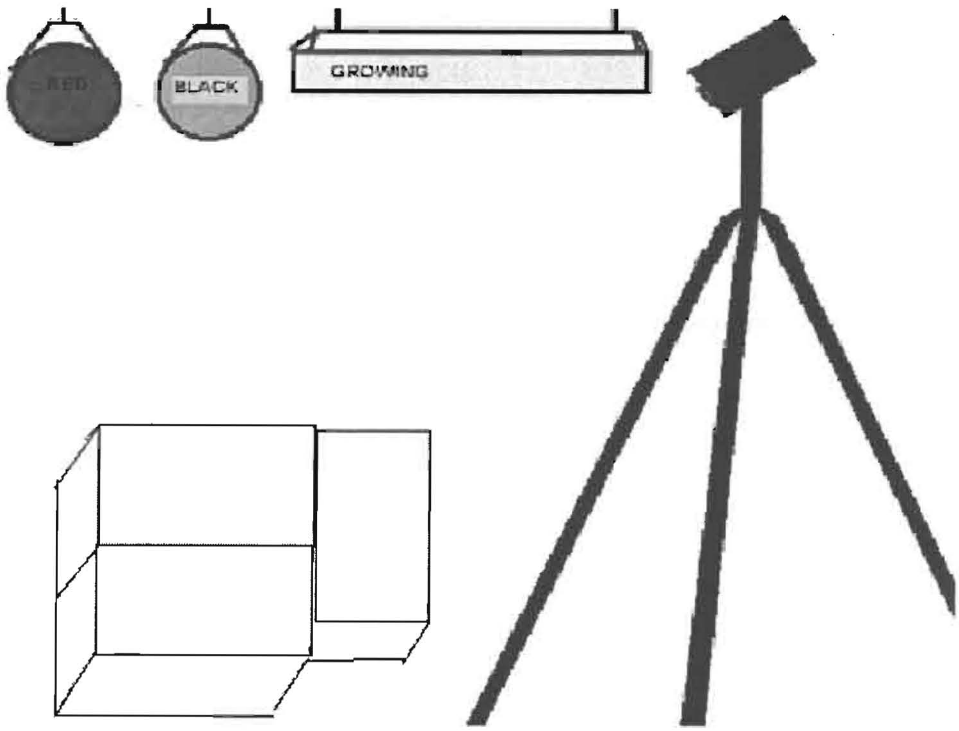
Three active categories were determined as walking, burrowing, and feeding. These three categories were summed up to create the total active time. Time the beetles were not known to be active was divided into two categories resting and the under the mulch time. Two other categories were determined as resting and under much time.

1. Total activity time is the time the beetle spent walking, burrowing and feeding.
2. Walking time is the time the beetle spent moving on top of the mulch.
3. Feeding time is the time the beetle spent moving their heads/pronotum but not their abdomens.
4. Burrowing time is the time the beetle spent coming out or going into the mulch.
5. Under the mulch time is the time the beetle spent under the mulch.
6. Resting time is the time the beetle spent not moving any part of its body while above the mulch.

Videotaping of *Odontotaenius disjunctus*

Beetle activity was videotaped for the three lunar phases during the months of June and July of 2003. All the beetles were watched within the three consecutive days surrounding the climax of the lunar phase. These days around the true lunar phase consisted of the day before, the day of, and the day after the climax of the each phase (Harris Pubulications, Inc. 2003). The phases include new moon, full moon, and half moon, which consist of waxing moon, and waning moon using different black light intensities simulated the lunar phases. Three tanks (six beetles) were videotaped each night for four h during the three phases (full, half and new) (Fig. 2). The beetles were

Figure 2: Camera and light layout for *O. disjunctus* for the experiment in June and July 2003. The beetles' day (05:00 – 21:00 EST) cycle was simulated by plant-growing lights. The red and black lights were on the entire night (21:00- 05:00 EST). Three Night glo bulbs simulated the moonlight (75-watt = high light (full moon), 50-watt = medium light (half moon) and 25-watt = low light (new moon)). A 60-watt red light was used for detection.


RED
BLACK
GROWING

recorded from the beginning of the night light cycle until the tape was finished (approximately 4h). Times spent by beetles in each activity were recorded each night.

Statistical Analysis of Activity

The total amount of time for each beetle in each activity category was recorded from videotapes each night (four hours). The three consecutive days around a phase were combined to create one treatment per phase. The mean was found for each activity for each nocturnal light treatment. The total activity, walking, feeding, burrowing, resting (inactivity), and under mulch time (unknown activity) for each phase were compared to each other. The tanks could have been used a replica but each beetle was used because of their semi-social behaviors (live in pairs).

Normality and Homogeneity

The activity of 18 beetles (9 tanks) was investigated using AVOVA and Kruskal-Wallis (SPSS). Normality was determined for each activity category using both skewness and kurtosis. The appropriate statistical test was performed on the total activity and n the five activity categories after the homogeneity, skewness and kurtosis were calculated and compared to the critical values (Table 1). The activity categories that have at least one of the values significantly different from the critical values were tested using non-parametric tests. ANOVA and Scheffe's multiple comparisons were used if the data are normally distributed. Kruskal-Wallis test and a nonparametric multiple comparisons were used if the data are not normally distributed. Total active time was significant in both skewness and homogeneity.

Table 2. The skewness, kurtosis and homogeneity for beetle activity of *O. disjunctus* for the three nocturnal light treatments collected for June 2003. The critical values for skewness (0.655), kurtosis(1.613) and homogeneity are(0.05). Significant difference (P> 0.05) is indicated with an asterisk (*).

Categories	Skewness (g_1)	Kurtosis (g_2)	Homogeneity (Levene statistic)	Appropriate test
Total activity	-1.260 *	0.710	0.008 *	Kruskal-Wallis
Walking	-0.591	-0.729	0.072	ANOVA
Feeding	0.748 *	-0.578	0.469	Kruskal-Wallis
Burrowing	5.175 *	30.455 *	0.002 *	Kruskal-Wallis
Resting	3.356 *	13.043 *	0.000 *	Kruskal-Wallis
Under mulch	1.372 *	0.918	0.016 *	Kruskal-Wallis

Walking time was not significant in skewness, kurtosis or homogeneity. Feeding time was significant only in skewness. Burrowing time, resting time and under the mulch time were significant in all three (skewness, kurtosis and homogeneity). These results show that walking is normally distributed and homogeneous, so they should be compared using ANOVA and Scheffe's multiple comparisons. Total activity time, feeding time, burrowing time, resting time and under the mulch time are not normally distributed and homogeneous, so they should be compared using both Kruskal-Wallis test and its a nonparametric multiple comparisons.

Results

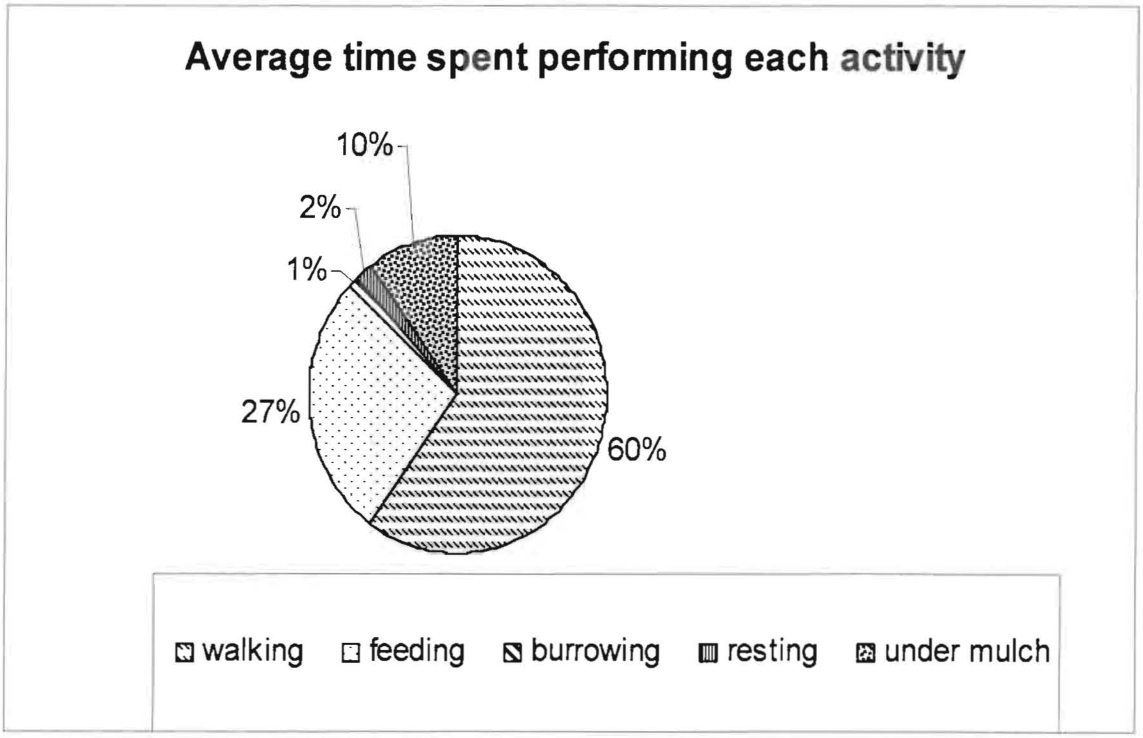
Odontotaenius disjunctus Observations

Prior to the study *Odontotaenius disjunctus* individuals were observed in their tanks during the day and night. This was done to become acquainted with their behavior. Activity was watched and categories (walking, feeding, burrowing, resting, and under the mulch) that can be discerned were used during the study. It was noted that when beetles moved their heads they were manipulating or masticating the hardwood mulch.

Every day the beetles were taped the tanks were non-invasively investigated for any note worthy differences. One of these changes was the creation of tunnels and chambers both in the center of the tank as well as the sides. Another every important change in the tanks was the presence of larvae. Larvae were placed in the chambers that were surrounded by masticated mulch and adult feces by their parents. Larvae were found in a two tanks (I and IV). The larvae were only noted if they could be seen without disturbing the tanks. During the study some beetles were in the mulch when the taping started.

The beetles spent 2% of their time resting and 1% of their time burrowing. The beetles spent 60% of their time walking and 27% of their time feeding, which represented 90.5% of their active time. The beetles spent 10% of their time under the mulch, which is 83.3% of their inactive time. Burrowing time and resting time combined took 2.8% of the beetles' time (Fig. 3).

Figure 3. Average time spent performing each activity, for beetle activity of *O. disjunctus* for the three nocturnal light treatments collected for June 2003, shown using percentages.


Average time spent performing each activity
10%
2%
1%
27%
60%
walking
feeding
burrowing
resting
under mulch

Active Time

The total time beetles spent active, which includes walking, feeding, and burrowing differed significantly with Kruskal-Wallis test ($P = 0.033$). A difference was found using nonparametric multiple comparisons ($P = 0.047$) between the high and medium nocturnal light treatments. Beetles spent more time active during the high nocturnal light treatment ($\mu = 220.83$ min (Table 3))(full moon) when compared to medium nocturnal light treatment ($\mu = 189.70$ min (Table 3)) (1/2 moon) (Figure 5 & Table 2). There was no difference between the nocturnal light treatments using ANOVA for the time spent walking ($P = 0.057$). There was also no difference between the nocturnal light treatments using Kruskal-Wallis test for the time spent feeding ($P= 0.502$) burrowing ($P = 0.111$). Time spent walking, feeding, and burrowing results are shown in (Figure 6 & Table 2), (Figures 7 & Table 2), (Figure 8 & Table 2) respectively.

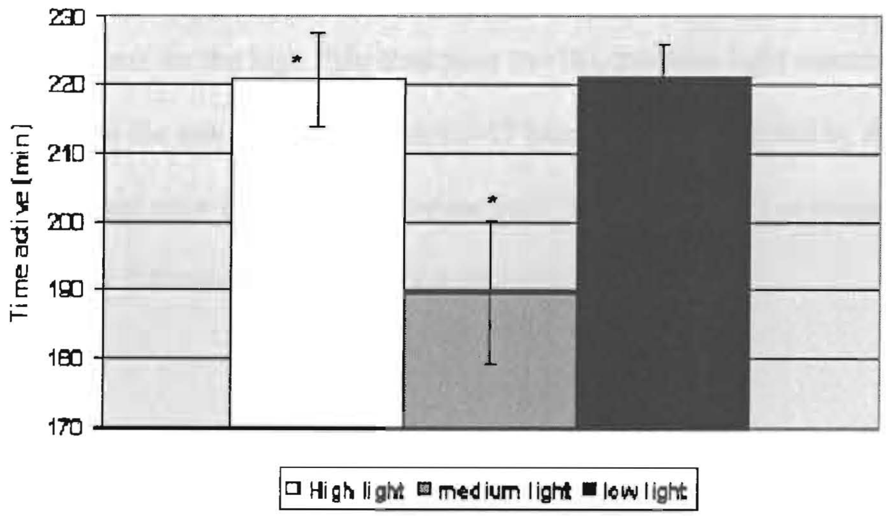
Table 3. P values for the multiple comparisons. Total activity, feeding, burrowing, resting and under mulch time used nonparametric multiple comparisons ($Q_{0.05,3}$).Walking used only multiple comparisons (Scheffe) ($S_{0.05}$).

Categories	High vs. Medium	High vs. Low	Medium vs. Low
Total activity	0.047	>0.05	>0.05
Walking	>0.05	>0.05	>0.05
Feeding	>0.05	>0.05	>0.05
Burrowing	>0.05	>0.05	>0.05
Resting	0.0019	>0.05	0.0047
Under mulch	>0.05	0.049	>0.05

Table 4. Mean time (min) ± SE (min) of the active and non active categories for beetle activity of *O. disjunctus* for the three nocturnal light treatments collected for June 2003.

Categories	High Light	Medium Light	Low Light
Total activity	220.83 ± 6.73	189.70 ± 10.61	221.17 ± 4.87
Walking	161.23 ± 11.87	119.41 ± 15.69	152.15 ± 9.67
Feeding	51.66 ± 7.86	70.22 ± 10.49	70.67 ± 10.50
Burrowing	1.25 ± 0.49	4.51 ± 2.23	0.47 ± 0.16
Resting	0.0 ± 0.0	10.61 ± 3.81	3.26 ± 0.90
Under mulch	19.17 ± 6.73	39.63 ± 9.62	13.45 ± 4.88

Figure 4. Comparison of mean time (min) (± SE) for total active time (walking + feeding + burrowing) of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17). Means are represented by the bar and standard error is represented by the error bars. The asterisks represent significant differences with Kruskal-Wallis (* -P = 0.047).



230
220
210
200
190
180
170
Time active (min)
*
*
High light
medium light
low light

Figure 5. Comparison of mean time (min) (± SE) for walking time of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17 Means are represented by the bar and standard error is represented by the error bars. The letters represent significant differences with ANOVA ($P > 0.05$).

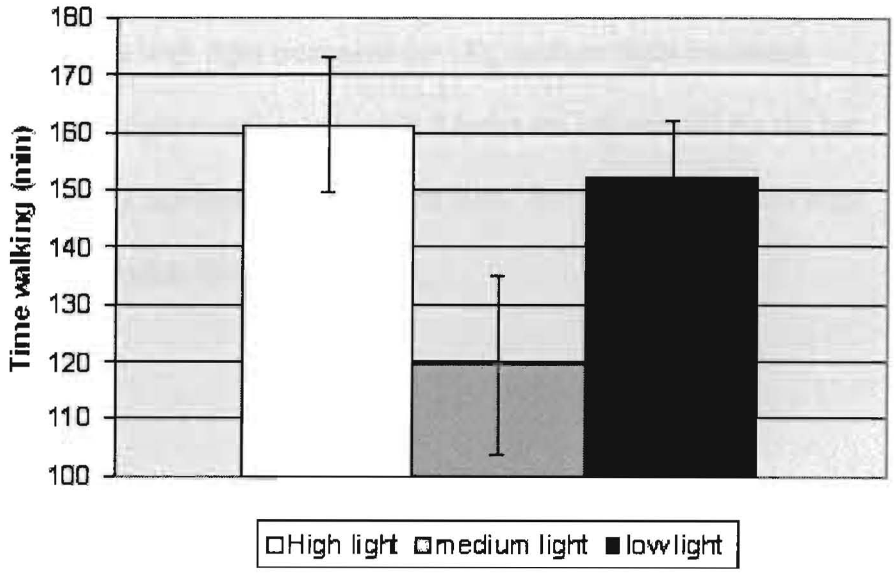

180
170
160
150
140
130
120
110
100
Time walking (min)
High light
medium light
low light

Figure 6. Comparison of mean time (min) (± SE) for feeding time of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17). Means are represented by the bar and standard error is represented by the error bars. Asterisks denote statistical differences with Kruskal-Wallis ($P > 0.05$).

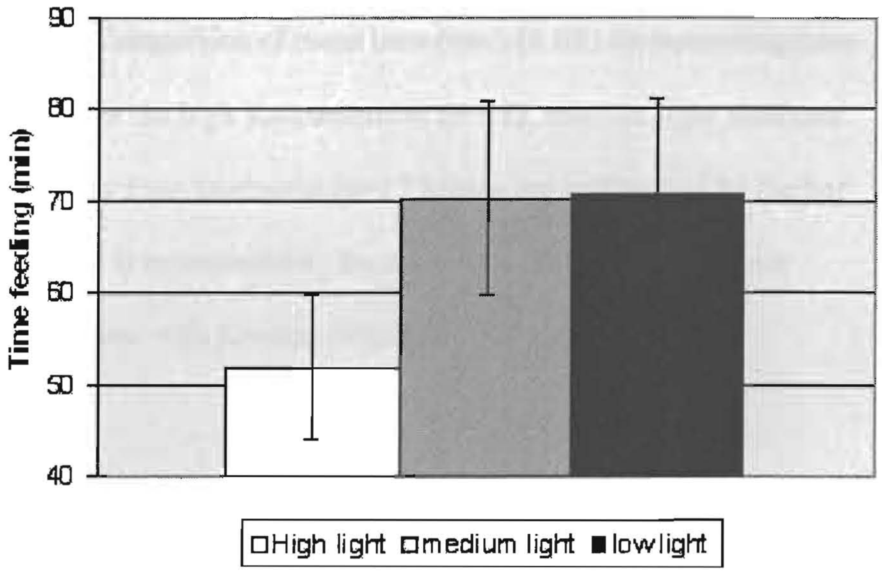
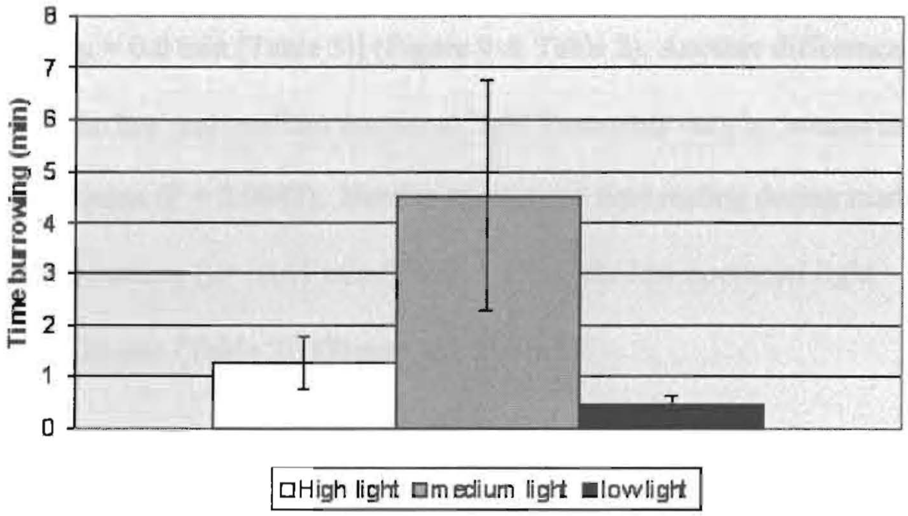

90
80
70
60
50
40
Time feeding (min)
High light
medium light
low light

Figure 7. Comparison of mean time (min) (± SE) for burrowing time of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17 Means are represented by the bar and standard error is represented by the error bars. The asterisks denote statistical differences with Kruskal-Wallis ($P > 0.05$).


Time burrowing (min)
0
1
2
3
4
5
6
7
8
High light
medium light
lowlight

Non-Active Time

Time the beetles were not known to be active was divided into two categories resting and under the mulch time. These categories affected total active time, but not the sub-categories of active time (walking, feeding, and burrowing).

Resting was the only inactive time when beetles could be observed. Resting time was significant using Kruskal-Wallis test (P = 0.000). A difference was found using nonparametric multiple comparisons (P = 0.0019) between the high and medium nocturnal light treatments. Beetles spent more time resting during the medium nocturnal light treatment (μ =10.61 min (Table 3)) than the high nocturnal light treatment (μ = 0.0 min (Table 3)) (Figure 9 & Table 2). Another difference was found between the low and medium nocturnal light treatments only by nonparametric multiple comparisons (P = 0.0047). Beetles spent more time resting during medium nocturnal light treatment (μ=10.61 min (Table 3)) than the low nocturnal light treatment (μ= 3.26 min (Table 3)) (Figure 9 & Table 2).

Under the mulch time was significant using Kruskal-Wallis test (P = 0.043). A difference was found using nonparametric multiple comparisons (P = 0.049) between the medium and low nocturnal light treatments. Beetles also spent more time under the mulch during the medium nocturnal light treatment (μ=39.63 min (Table 3))(1/2 moon) than the low nocturnal light treatment (μ=13.45 min (Table 3))(new moon) (Figure 10 & Table 2).

Figure 8. Comparison of mean time (min) (± SE) for resting time of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17). Means are represented by the bar and standard error is represented by the error bars. The asterisks denote a statistical differences with Kruskal-Wallis (*- P = 0.0019, **- P = 0.0047).

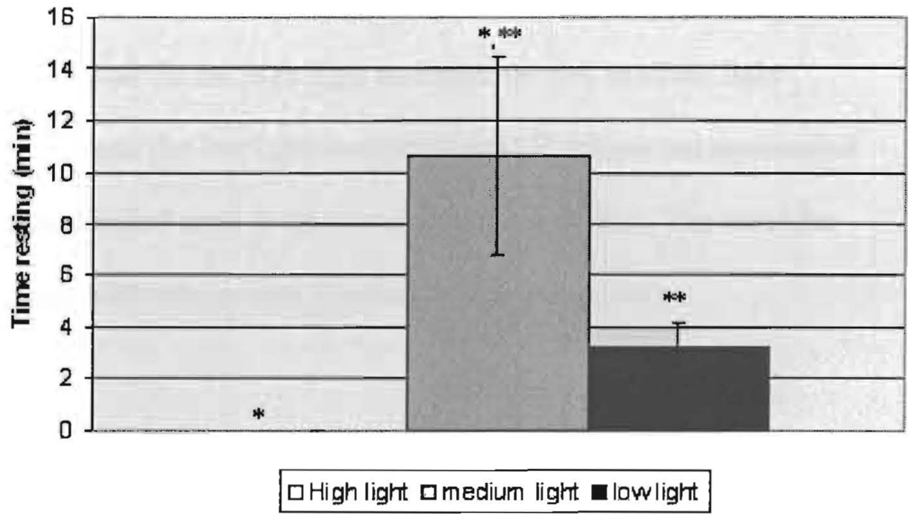
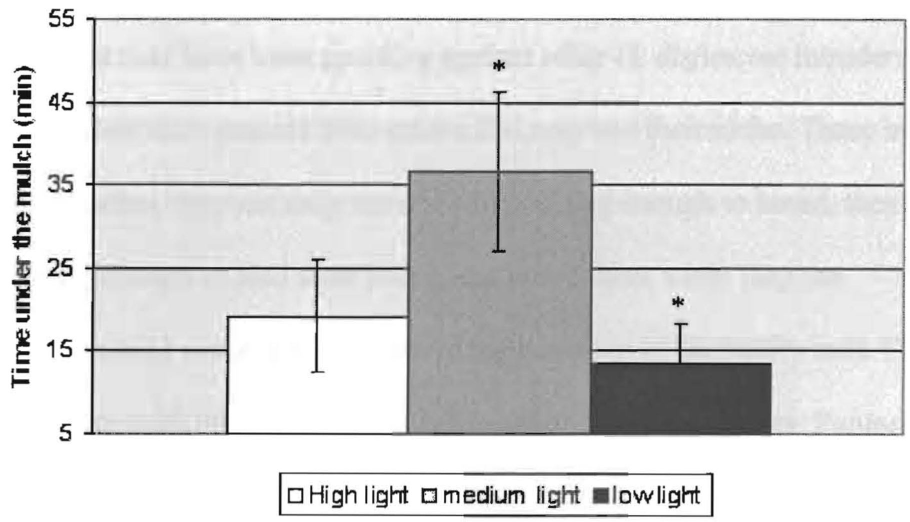

16
14
12
10
8
6
4
2
0
Time resting (min)
* **
**
*
High light
medium light
low light

Figure 9. Comparison of mean time (min) (± SE) for under the mulch time of *O. disjunctus* for the high light treatment (n=18), medium light treatment (n=17) and the low light treatments (n=17). Means are represented by the bar and standard error is represented by the error bars. The asterisks denote statistical differences with Kruskal-Wallis (P = 0.049).



55
45
35
25
15
5
Time under the mulch (min)
*
*
High light
medium light
lowlight

Discussion

These results suggest that nocturnal light intensity may have an effect on the nocturnal activity of *O. disjunctus*. The beetle's total nocturnal activity was significantly (P= 0.047) higher during the high nocturnal light treatment when compared to the medium nocturnal light treatment. The difference in activity is due to the time spent under the mulch and resting not the sub categories of activity.

The time spent walking did not significantly differ, showing that the beetles' main locomotion is not affected by nocturnal light intensity. Although the family units can become large and the beetles do not have to venture away from tunnels to forage, the beetles may have been guarding against other *O. disjunctus* intruders. They need to protect their tunnels from others that may use their niche. These beetles have an added burden, they not only have to survive long enough to breed, they also have to live long enough to feed their young and guard them when they are vulnerable. This would make them vigilant in the guarding of the family unit. The tanks were close to each other and they may have sensed the other pairs. Future studies could separate the tanks to eliminate this possibility.

The beetles may have also been guarding against predators. Their family unit size can put them in danger. Common predators of this kind of burrowing insects would be insectivorous insects, burrowing rodents, raccoons or opossums. Although their walking time did not significantly differ they did spend more time walking during the full moon and new moon times, this could be due to one or more predator activity patterns. Predators would be active at different phases depending upon their foraging types (Kerth 2001, Fournier 2002 & Koivula 2002), habitat disturbances

(Law 2002), moon light levels (Skutelsky 1996) and predator assemblages (Varchola 1999).

The feeding time did not significantly differ between nocturnal light treatments. The beetles seem not sacrifice the time they feed depending on the amount of nocturnal light. This time spent feeding may be due to adults beetles need to masticate the food for both themselves and their young. Their ability to sustain a relative temperature, during the winter that may help their survival, is dependent on the amount they eat during the spring and summer (Brennan 2000). The molecule in their blood that acts like antifreeze is made by the mulch they eat during the spring and summer (Brennan 2000).

Their time burrowing was minimal but they were creating new tunnels and chambers during the two months of study. They could have been doing this during the day as well as the time during the night. Some beetles were in their tunnels when videotaping started. These tunnels and chamber are essential for beetles not just as a home but as protection. Adults block off chambers that house their young; each larvae and pupae are housed in separate chambers to protect them from predators and each other (Evans 2003).

Time spent resting was significantly (P= 0.0019) higher during the medium nocturnal light treatment and the high nocturnal light treatments. Time spent resting was significantly (P = 0.0047) higher during the medium nocturnal light treatment and the low nocturnal light treatments. This may be due to the fact that infestation of wood by insects is more likely to happen during the full moon (Zürcher 2001). The beetles studied showed more activity during the high nocturnal light treatments, which agrees with the observation that during the full moon many young passalid beetles are seen walking and flying in search of new tree stumps (Evans 2003).

Under the mulch time was significantly ($P = 0.049$) higher the medium nocturnal light treatment versus the low nocturnal light treatment. The beetles may be creating family living structures during the medium nocturnal light treatment. More tunnels and chambers were found after the medium nocturnal light treatment supporting the idea that beetles may be affected by the nocturnal kight intensities. Studies that look at the beetles in natural light during the lunar phases would help understand the activities of *O. disjunctus* under moonlight.

Conclusions

Total Active time, which includes walking, feeding, and burrowing differed significantly with Kruskal-Wallis test ($P = 0.033$). Beetles spent more time active ($P = 0.047$) during the high nocturnal light treatment ($\mu = 220.83$ min) when compared to medium nocturnal light treatment ($\mu = 189.70$ min).

Walking, feeding, and burrowing did not differ among nocturnal light treatments. There was no difference in walking time between the nocturnal light treatments ($P = 0.057$). There was also no difference between the nocturnal light treatments for the time spent feeding ($P= 0.502$) burrowing ($P = 0.111$).

Resting time was significant using Kruskal-Wallis test ($P = 0.000$). Beetles spent more time resting ($P = 0.0019$) during the medium nocturnal light treatment ($\mu =10.61$ min) than the high nocturnal light treatment ($\mu = 0.0$ min). Beetles spent more time resting ($P = 0.0047$) during medium nocturnal light treatment ($\mu=10.61$ min) than the low nocturnal light treatment ($\mu= 3.26$ min).

Under the mulch time was significant using Kruskal-Wallis test ($P = 0.043$). Beetles spent more time under the mulch ($P = 0.049$) during the medium nocturnal light treatment ($\mu=39.63$ min) than the low nocturnal light treatment ($\mu=13.45$ min).

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Appendix A

Tables showing the breakdown of activity of beetles

Table 1. Total time (min) beetles spent doing each category for the high light treatment (full moon).

Beetles	Active	Walking	Feeding	Burrowing	Resting	Under mulch
1	240.00	136.667	103.333	0.0	0.0	0.0
2	235.85	98.367	135.467	2.016	0.0	4.15
3	232.483	109.883	121.933	0.667	0.0	7.517
4	240.00	210.383	29.617	0.0	0.0	0.0
5	231.067	195.45	34.35	1.267	0.0	8.933
6	240.00	177.667	62.333	0.0	0.0	0.0
7	220.817	182.967	35.033	2.817	0.0	19.183
8	240.00	211.40	28.60	0.0	0.0	0.0
9	143.683	56.65	85.70	1.333	0.0	96.317
10	213.55	169.267	36.20	8.083	0.0	26.45
11	240.0	209.183	30.817	0.0	0.0	0.0
12	240.0	205.367	34.633	0.0	0.0	0.0
13	240.0	201.783	36.817	1.40	0.0	0.0
14	222.10	205.683	16.333	0.083	0.0	17.90
15	240.0	205.067	34.933	0.0	0.0	0.0
16	179.783	143.667	35.267	0.51	0.0	60.216
17	204.783	122.283	78.233	4.267	0.0	35.216
18	170.833	60.517	110.317	0.0	0.0	69.166
Mean	220.8305	161.2331	51.6571	1.2468	0.0	19.1693
Standard error	6.7287	11.8676	7.8608	0.4905	0.0	6.7287

Table 2. Total time(min) beetles spent doing each category for the medium light treatment (half moon). The letter E represents a beetle that escaped from the tank.

Beetles	Active	Walking	Feeding	Burrowing	Resting	Under mulch
1	193.717	31.25	152.34	9.90	18.65	27.633
2	175.133	27.567	144.166	3.40	54.917	9.95
3	143.50	74.617	64.55	4.333	19.117	77.383
4	E	E	E	E	E	E
5	194.733	55.733	137.333	1.667	35.617	8.65
6	124.417	34.283	90.133	0.0	0.0	115.583
7	223.167	158.333	50.633	14.20	2.167	14.667
8	119.883	147.90	51.317	0.667	0.0	40.117
9	120.417	58.85	59.967	1.60	13.967	105.617
10	157.767	134.65	20.383	2.733	0.0	82.233
11	196.133	139.80	19.583	36.75	0.0	43.867
12	179.033	111.217	67.033	0.0	11.183	49.783
13	207.40	203.25	23.466	0.517	0.0	12.60
14	235.217	210.20	24.217	0.667	0.0	4.783
15	240.00	195.43	44.283	0.0	0.0	0.0
16	234.467	167.667	66.616	0.183	1.667	3.867
17	240.00	175.70	64.30	0.0	0.0	0.0
18	240.00	103.60	113.50	0.0	22.90	0.0
Mean	189.7049	119.4135	70.2247	4.5068	10.6075	39.6314
Standard error	10.6064	15.2216	10.4924	2.2272	3.80951	9.6227

Table 3. Total time (min) beetles spent doing each category for the low light treatment (new moon). The letter X represents a beetle that died.

Beetles	Active	Walking	Feeding	Burrowing	Resting	Under mulch
1	200.75	164.717	34.583	1.45	4.067	35.183
2	228.917	210.483	18.434	0.0	11.083	0.0
3	231.967	95.433	136.534	0.0	8.033	0.0
4	240	133.667	106.333	0.0	0.0	0.0
5	238.583	202.80	35.316	0.467	0.0	1.417
6	240	181.167	58.833	0.0	0.0	0.0
7	237.967	155.817	82.15	0.0	2.033	0.0
8	197.933	189.55	8.383	0.0	0.0	42.067
9	237.233	166.583	70.65	0.0	2.767	0.0
10	238.517	190.517	48.00	0.0	1.483	0.0
11	227.367	184.517	41.983	0.867	6.933	5.70
12	X	X	X	X	X	X
13	215.717	118.90	96.10	0.767	9.933	14.35
14	235.667	84.033	151.633	0.0	4.333	0.0
15	171.50	135.383	35.183	0.933	3.817	64.683
16	205.238	161.10	42.467	1.767	0.85	33.817
17	208.517	91.40	115.40	1.717	0.0	31.483
18	240	120.567	119.433	0.0	0.0	0.0
Mean	221.1743	152.1515	70.6717	0.4687	3.2551	13.4529
Standard error	4.8652	9.6749	10.5045	0.1589	0.8964	4.8847

Table 4. Total time active (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	240.00	193.717	200.75
2	235.85	175.133	228.917
3	232.483	143.50	231.967
4	240.00	E	240
5	231.067	194.733	238.583
6	240.00	124.417	240
7	220.817`	223.167	237.967
8	240.00	119.883	197.933
9	143.683	120.417	237.233
10	213.55	157.767	238.517
11	240.0	196.133	227.367
12	240.0	179.033	X
13	240.0	207.40	215.717
14	222.10	235.217	235.667
15	240.0	240.00	171.50
16	179.783	234.467	205.238
17	204.783	240.00	208.517
18	170.833	240.00	240
Mean	220.8305	189.7049	221.1743
Standard error	6.7287	10.6064	4.8652

Table 5. Total time walking (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	136.667	31.25	164.717
2	98.367	27.567	210.483
3	109.883	74.617	95.433
4	210.383	E	133.667
5	195.45	55.733	202.80
6	177.667	34.283	181.167
7	182.967	158.333	155.817
8	211.40	147.90	189.55
9	56.65	58.85	166.583
10	169.267	134.65	190.517
11	209.183	139.80	184.517
12	205.367	111.217	X
13	201.783	203.25	118.90
14	205.683	210.20	84.033
15	205.067	195.43	135.383
16	143.667	167.667	161.10
17	122.283	175.70	91.40
18	60.517	103.60	120.567
Mean	161.2331	119.4135	152.1515
Standard error	11.8676	15.2216	9.6749

Table 6. Total time feeding (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	103.333	152.34	34.583
2	135.467	144.166	18.434
3	121.933	64.55	136.534
4	29.617	E	106.333
5	34.35	137.333	35.316
6	62.333	90.133	58.833
7	35.033	50.633	82.15
8	28.60	51.317	8.383
9	85.70	59.967	70.65
10	36.20	20.383	48.00
11	30.817	19.583	41.983
12	34.633	67.033	X
13	36.817	23.466	96.10
14	16.333	24.217	151.633
15	34.933	44.283	35.183
16	35.267	66.616	42.467
17	78.233	64.30	115.40
18	110.317	113.50	119.433
Mean	51.6571	70.2247	70.6717
Standard error	7.8608	10.4924	10.5045

Table 7. Total time burrowing (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	0.0	9.90	1.45
2	2.016	3.40	0.0
3	0.667	4.333	0.0
4	0.0	E	0.0
5	1.267	1.667	0.467
6	0.0	0.0	0.0
7	2.817	14.20	0.0
8	0.0	0.667	0.0
9	1.333	1.60	0.0
10	8.083	2.733	0.0
11	0.0	36.75	0.867
12	0.0	0.0	X
13	1.40	0.517	0.767
14	0.083	0.667	0.0
15	0.0	0.0	0.933
16	0.51	0.183	1.767
17	4.267	0.0	1.717
18	0.0	0.0	0.0
Mean	1.2468	4.5068	0.4687
Standard error	0.4905	2.2272	0.1589

Table 8. Total time resting (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	0.0	18.65	4.067
2	0.0	54.917	11.083
3	0.0	19.117	8.033
4	0.0	E	0.0
5	0.0	35.617	0.0
6	0.0	0.0	0.0
7	0.0	2.167	2.033
8	0.0	0.0	0.0
9	0.0	13.967	2.767
10	0.0	0.0	1.483
11	0.0	0.0	6.933
12	0.0	11.183	X
13	0.0	0.0	9.933
14	0.0	0.0	4.333
15	0.0	0.0	3.817
16	0.0	1.667	0.85
17	0.0	0.0	0.0
18	0.0	22.90	0.0
Mean	0.0	10.6075	3.2551
Standard error	0.0	3.8095	0.8964

Table 9. Total time spent under the mulch (min) of each beetle for the three light treatments. The letter E represents a beetle that escape from the tank, while the letter X represents a beetle that died.

Beetles	High light	Medium light	Low light
1	0.0	27.633	35.183
2	4.15	9.95	0.0
3	7.517	77.383	0.0
4	0.0	E	0.0
5	8.933	8.65	1.417
6	0.0	115.583	0.0
7	19.183	14.667	0.0
8	0.0	40.117	42.067
9	96.317	105.617	0.0
10	26.45	82.233	0.0
11	0.0	43.867	5.70
12	0.0	49.783	X
13	0.0	12.60	14.35
14	17.90	4.783	0.0
15	0.0	0.0	64.683
16	60.216	3.867	33.817
17	35.216	0.0	31.483
18	69.166	0.0	0.0
Mean	19.1693	39.6314	13.4529
Standard error	6.7287	9.6227	4.8847