Michelle Rearick

Influence of Light on Tunneling Behavior of Western Harvester Ants, *Pogonomyrex occidentalis*

ABSTRACT

Ants are everywhere and occupy keystone positions in most terrestrial environments, sometimes accounting for up to 25% of the animal biomass. The objective of this study was to examine how varying amounts of artificial light affect western harvester ant colony behavior, specifically, foraging and tunneling. This was conducted by performing observations on three artificial ant habitats over a two week period. One habitat (A) was exposed to artificial light for 24h/day while another (B) was kept in complete darkness for 24h/day. A third habitat (C) was rotated between artificial light and complete darkness every 12h to simulate the light conditions of a typical day. Results show that habitat A had the largest area of tunnel excavation (3280.24 mm^2, 27.3%) and the habitat B had the least (2186.57 mm^2, 18.2%). Habitat C fell in between with 3098.60 mm^2 or 25.8% area excavated. Rates of nest development were non-linear. Results are significant as they demonstrate light contributes to an ant's tunneling behavior and general activity.

INTRODUCTION

Ants are everywhere; they thrive in forests, fields, deserts, and cities all over the earth and are one of the most dominant groups of insects, forming 10-25% of the animal biomass in some terrestrial ecosystems (Schultz, 2000). How are ants so successful? Like humans, ants are social organisms; they live in colonies in which a wingless neuter daughter chaste cooperates and unite toward the common purposes of survival, growth, and to raise subsequent generations of the mother queen's offspring (Schultz, 2000). Today, ants occupy keystone positions in most terrestrial environments, serving as major conduits of energy and organic material. They are, for example, important turners of the soil, matching or exceeding the activity of earthworms in this role. They are among the leading predators of invertebrates in most ecosystems, and in the tropics they are the leading herbivores as well, with leaf-cutter ants taking more than 15% of the fresh vegetation (Schultz, 2000).

One species of ant is the Western Harvester Ant, *Pogonomyrex occidentalis*. Western Harvester Ants are found throughout the Great Plains, southwest, and Rocky Mountain regions of the United States and prefer moderately warm habitats (Halfen and Hasiotis, 2010). Most workers range in size from 6 ½ mm to 8 mm, but can become as large as 11 mm and have a light red body color (Rogers, 1972). Because of nest densities, the longevity of nests, and the amount of seed harvested and soil handled, harvester ants have significant direct and indirect effects on community structure and ecosystem functioning. Harvester ants change plant species composition and diversity near their nests. These changes result from differential seed predation by the ants, their actions as seed dispersers and competitors with other granivores, and the favorable soil conditions they create through their digging. Their nest building creates islands of increased nutrient density. In some areas, the effects of their activities may be so pervasive that plant community structure is strongly influenced (MacMahon *et al*., 2000; Rogers, 1972).

A typical underground nest is composed of a large numbers of chambers interconnected by a network of galleries (Buhl *et al.*, 2006). Nest development occurs in tandem with changes in colony population size and involves the addition of chambers and galleries that increase the depth of the nest and the amount of space occupied through time (Halfen and Hasiotis, 2010). Nests show large variations in their branching pattern, whether they are composed of only one vertical shaft connecting one or few chambers, several branched shafts, or a mesh-like pattern of interconnected galleries (Cassill et al., 2002; Mikheyev and Tschinkel, 2004; Tschinkel, 2003). It seems these variations may be correlated with colony size: the greater the number of individuals in a colony, the greater the amount of branching and the complexity of the nest. Generally speaking, nests with 700 workers or less are less complex and comprise a single main chamber connected by a single gallery (Cassill et al., 2002; Lavigne, 1969). Despite the functional importance of these structures, few quantitative studies of subterranean networks built in natural conditions have been conducted, and subsurface behaviors and nest construction behaviors are largely unknown (Buhl *et al.*, 2006; Halfen and Hasiotis, 2010).

Previous investigations of the Western Harvester Ants' responses to environmental factors indicates influence in establishing colony operating parameters and periods of daily and seasonal activities (Rogers, 1972).

For example, colonies often contain an assymetrical cone shape in order to catch and store the rising sun's rays as well as warming chambers near the earth's surface to aid in thermoregulation. This warming would lead to possible fitness benefits such as workers leaving the mound earlier in the morning to forage and more rapid maturation of larvae inside the warmer mound (Cole, 1994; Halfen and Hasiotis, 2010; Romey, 2002). Other studies suggest that these ants can "sense" inclement weather and that they build nest entrances on the southeast face of mounds to avoid rain or floodwater (Romey, 2002).

Foraging behavior is also influenced by abiotic conditions. Seasonal variation in foraging activity is coupled with surface temperatures, seed availability, or the distribution of rainfall and occurs from April to October (MacMahon *et al*., 2000). Daily variation is influenced by soil surface temperature and potential water loss. Most foraging activity occurs at surface temperatures of 20° to 50°C, and peak activity occurs from 30° to 45°C (MacMahon *et al*., 2000; Rogers, 1972). Like most foraging species, the Western Harvester Ant is diurnal and activity takes place mostly in the morning and afternoon. Studies conflict in results regarding whether ants forage at night or whether all activity outside of the nest ceases in darkness (McCluskey, 1963; Rogers, 1972). It is likely, however, that temperature is the driving force behind the ants' diurnal activity, not amount of light present.

In nature, the effect of light on tunneling behavior alone has not been investigated as tunneling occurs beneath the earth's surface and it is not easy to track the rate of nest development. Often ant foraging success is the only measurable indication of colony fitness in nature as it is the only easily observable behavior (Carroll and Janzen, 1973). Gordon (1986) describes a reciprocal relationship between nest maintenance and foraging. She elaborates that an increase in one behavior is accompanied by a decrease in the other, and when both activities are interfered with, the colony chooses foraging instead of nest development.

It is likely that multiple factors, both biotic and abiotic, influence ant behaviors, however, the purpose of this study was to examine how varying amounts of artificial light alone affect Western Harvester Ant colony behavior, specifically, tunneling. It was hypothesized that when temperature is held constant, the amount of light exposure to an artificial ant habitat would disrupt daily rhythmic behaviors of the ants. It was predicted that the ants within a habitat exposed to artificial light 24h per day would become "hyperactive" and exhibit the most tunneling behavior. Conversely, it was predicted that a habitat housed in total darkness for 24h per day would exhibit very little or no tunneling behavior due as a result of confusion and lack of foraging.

MATERIALS AND METHODS

Assembling ant farms

Three commercially available ant habitat kits (Uncle Milton's, amazon.com), with observation windows measuring 8 ½" x 5 ½" x ¼" (WxHxD), were purchased and prepared per instruction. Each of the habitats were filled with sand (provided); while filling, sand was gently compacted by tapping the unit on a hard surface. Sand was made damp by adding 2oz. tap water. Units sat for several hours in order to allow water to properly diffuse through the sand. A tunnel measuring 1cm was started by inserting a plastic stick (supplied) through the opening in the plastic and into the tunneling sand. Dark paper was taped onto the habitats to cover the area containing sand in order to mimic being underground.

Inoculation and maintenance of the ant farm habitat

Western Harvester Ants (*Pogonomyrex occidentalis*) were obtained commercially (insectkits.com). All ants received were female, nonbreeding, worker ants. In preparation for inoculation, ants were kept in a refrigerator for 5 minutes in order to reduce ant activity. Twenty-five ants were transferred into each ant habitat through an opening in the top frame of the enclosure. The cap was then replaced and ant farms were moved to their respective experimental location. Location 1 consisted of exposure to artificial white light, and location 2, the inside of a cabinet, was completely free from light. Both locations were within the same room, therefore holding the same temperature. Ants were fed a piece of apple, approximately the size of a popcorn kernel, once a week and given two drops of water every three days.

Observations, data collection, and interpretations

Observations were collected over a 2 week period. Observational data points varied, and ranged from 4hr to 24hrs apart depending on the rate of nest development. Nest development progress was tracked by taking photographs using a digital camera. Only one side of the habitat was photographed; apparatus was thin enough that the network appeared the same on both sides. Images were uploaded on to a computer, and prepared for analysis by cropping to include the tunneling section of the habitat only.

Ant tunnels were artificially-colored green using GIMP (GNU image manipulation program) painting software (Sup.Figs. 1A, 2A, 3A). This program classified each of the images in order to calculate the area in pixels that was colored in green (tunnels), as well as the area that wasn't colored in green (no tunnel). Pixel value was then converted into the area measurement unit of millimeters squared. Using this process, a comparison of total nest area was made and the rate of nest development per unit area was calculated.

RESULTS

As predicted, light exposure and ant behavior are directly related. Results show that the habitat exposed to constant artificial light (A) had the largest area of tunnel excavation (3280.24 mm^2, 27.3%) and the habitat completely withheld from light (B) had the least (2186.57 mm^2, 18.2%). The control habitat (C), cycled between artificial light and darkness fell in between with 3098.60 mm^2 or 25.8% area excavated (Figure1). Rates of nest development occurred in a non-linear fashion: habitat A ranged from a 4.45% area increase per day to 1% area decrease per day, habitat B ranged from 0.46% area increase per day to 7.87% area increase per day , and habitat C ranged from 6.34% area increase per day to a 1.27% area increase per day (Figure 2).

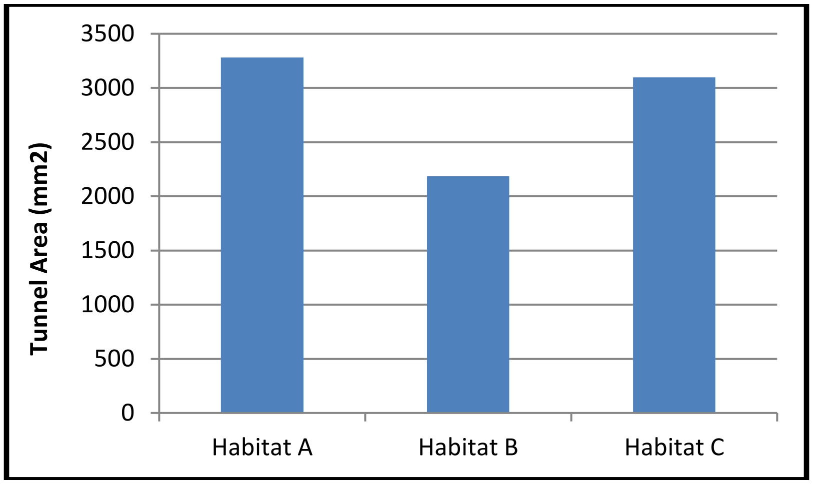

Figure 1 Area of day 14 tunnel excavation. Comparison of experimental endpoint tunnel areas in 100% artificial light (habitat A), 100% darkness (habitat B), and 50% artificial light and 50% darkness (habitat C). Habitat A had the largest area of excavated tunnels (3280.24 mm^2) and habitat B had the least (2186.57 mm^2). Habitat C fell in between with 3098.60 mm^2.

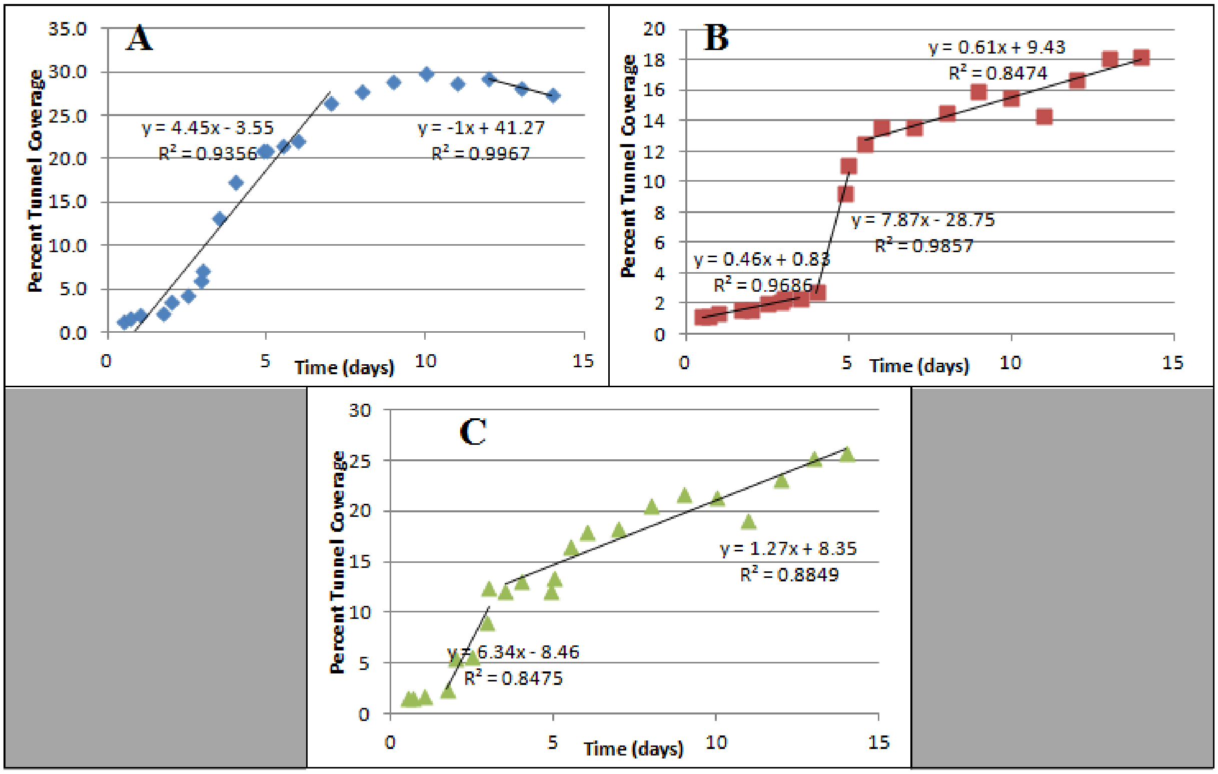

Figure 2 Rate of nest development. Rate of nest progression throughout all three habitats developed in a non-linear fashion. Habitat A, 100% artificial light (A) ranged from a 4.45% area increase per day to 1% area decrease per day, habitat B, 100% darkness (B) ranged from 0.46% area increase per day to 7.87% area increase per day , and habitat C, 50% artificial light, 50% darkness (C) ranged from 6.34% area increase per day to a 1.27% area increase per day.

DISCUSSION

The effect of light, an abiotic variable, on behavior of Western Harvester Ants was examined by conducting observations on tunneling behavior and nest development. Results show that the habitat exposed to constant artificial light (A) had the largest area of tunnel excavation (3280.24 mm^2, 27.3%) and the habitat completely withheld from light (B) had the least (2186.57 mm^2, 18.2%). The control habitat (C), cycled between artificial light and darkness fell in between with 3098.60 mm^2 or 25.8% area excavated. This data supports the original hypothesis that when temperature is held constant, the amount of light exposure would affect daily rhythmic behaviors of the ants. Original predictions were partially supported. Ants within a habitat exposed to artificial light 24h a day did appear to become slightly hyperactive and exhibited the most tunneling behavior. Rate of nest development within this habitat also occurred in the most linear fashion with a rate of 4.45% increase through day 7. The tunneling behavior of ants within the habitat housed in complete darkness was greater than anticipated and the original predication was not supported, though this habitat did have the lowest amount of excavated tunnel area.

Daily rhythms have been observed in a wide variety of animals, plants, and microorganisms. Nuptial flights of various ants occur at species-characteristic times of the day and workers of some species are active during only a certain part of the day. These rhythms can be constant, or shift, and seem to have been analyzed very little under controlled conditions. Often, such rhythms are thought to occur endogenously (McCluskey, 1963) and are influenced based on temperature and exposure to light; the Western Harvester Ant appears to be influenced by both.

When compared to the control habitat, ants within the habitat that was exposed to light 24h/day appeared to be slightly hyperactive. While the initial excavation rate was not as high as the control, maximum tunnel area (29.8%, day 10) was greater than the maximum tunnel area achieved (25.8%, day 14). The rate of excavation was also more linear throughout the course of the experiment; it did not appear step-wise or punctuated as would be anticipated if the tunneling behavior was diurnal. Should this experiment have had the opportunity to progress, it would be predicted that the individuals within this habitat would not live as long due to their constant activity; they would simply "wear out" before the ants in the other habitats.

Due to the complications inherent to nighttime research, relatively little is known about how and when ant species forage at night. The Western Harvester Ant exhibits diurnal behavior, but previous research conflicts regarding their activity in darkness (Despickere *et al.,* 2004; McCluskey, 1963; Rogers, 1972). Despickere and colleagues (2004) state that light is required for foragers to leave the nest for food. In total darkness, the foragers have no task to perform; while initially this allows foragers to decrease their consumption of energy and facilitates their recruitment for other tasks when needed, a poorly developed nest with little food stores could suffer. A weak light source is sufficient to disperse and lead foraging in ants (Despickere *et al.,* 2004; Klotz and Reid, 1993). Experimental results show only an approximately 0.5% increase in tunnel area within the habitat housed in complete darkness over the first five days. While tracking the progress of nest development, it was observed that these ants exhibited very little movement in comparison to their counterparts. Interestingly, the sharp increase in tunneling rate within this habitat coincided with the day in which the habitat was removed from darkness briefly in order to add water. As previously mentioned, decreased temperature is the more likely cause of decreased or lack of foraging at night. A study on rhythmic foraging in a species of leaf cutter ant observed that as temperatures remained well above the threshold for movement on most nights, the ants did not slow down appreciably when foraging in darkness (Lewis *et al.*, 1974).

The slower rate of excavation could be partially explained by differences in the speed of travel in light and dark conditions. Cosens and Toussaint (1985) present that in the light, ants ran on both outward and inward journeys at the same rate, whereas in the dark when denied visual clues, ants leaving the nest ran more slowly. When returning to the nest in the dark, however, they ran at speeds comparable to in the light. This implies that, in contrast to their outward journey, they had some concept of their location and ran with more confidence (Cosens and Toussaint, 1985). Such knowledge of location could be attributed to structural or olfactory clues (Koltz and Reid, 1993). As the experiment progressed, the ants became more accustomed to leaving the nest without visual or spatial recognition and speed of excavation increased.

Multiple potential sources of error were present within the experiment which should be taken into account when interpreting data. First, while habitats were assembled as similarly as possible, it is likely that the tunneling sand was not evenly compacted within all areas of the habitat as well as between habitats. Also of note, the habitats were filled upside down and then returned to their upright position; over time, the tunneling sand could settle towards the bottom due to gravity. It was observed that the area of tunnels excavated did not always appear to correlate with the amount of tunneling sand that was excavated about the “surface”. If the sand was looser in some areas than others, tunnel excavation could be achieved at a faster rate. Such results are believed to have been observed experimentally within habitat B (Appendix Figure B). The sharp increase in tunnel area was observed when a tunnel suddenly appeared across the entire viewing window, yet little sand appeared to have been excavated.

Another source of error arose when it was noted that tunnels could be excavated in areas that were not easily visible by eye and not able to be recorded digitally. In both habitats A and C, tunnels on the far left and right as well as bottom were excavated; they were first observed when ants could be seen running across the bottom of the habitat. These areas could not be included in the tunnel area calculations or subsequent rate of tunnel development calculations. This is especially problematic when calculating rate of tunnel development; in habitat C, it appeared that excavation stalled on multiple occasions. It is more likely that excavation did not stall, merely it occurred in an area in which it could not be recorded.

This study was developed to become integrated into a local 5th grade classroom’s current curriculum. Due to limited resources, artificial habitats could not be assembled in duplicate or triplicate. The resources and results will be handed over to a local teacher and her students will restart the experiment in order to obtain more experimental data. Before conducting this experiment, the educator can lead a short unit introducing the natural habitats, anatomy, and life cycle of ants (worksheets included within Appendix). Both unit and experiment fit into National Standards, including introduction and understanding of structure and function of living systems (science), as well as formulating questions that can be addressed with data and collect, organize, and display relevant data to answer them (math). Besides complying to standards, the experiment would also serve to stimulate students in inquiry-based education. The lesson could also serve to introduce native ant species in Ohio and could tie in conservation importance of ants by stressing their importance in the ecosystem.

Ants are fascinating and highly beneficial insects. Because of their complex colony-level behaviors, ants serve as model organisms for the highly visible disciplines of behavioral ecology and sociobiology, particularly in studies focused on the dynamics of kin selection, within colony conflicts of interest, caste differentiation, and division of labor (Schutlz, 2000). It is key to educate today's young minds on the importance of these small creatures; should they disappear, the big gap they leave within the ecosystem may be difficult to fill.

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SUPPLEMENTAL FIGURES

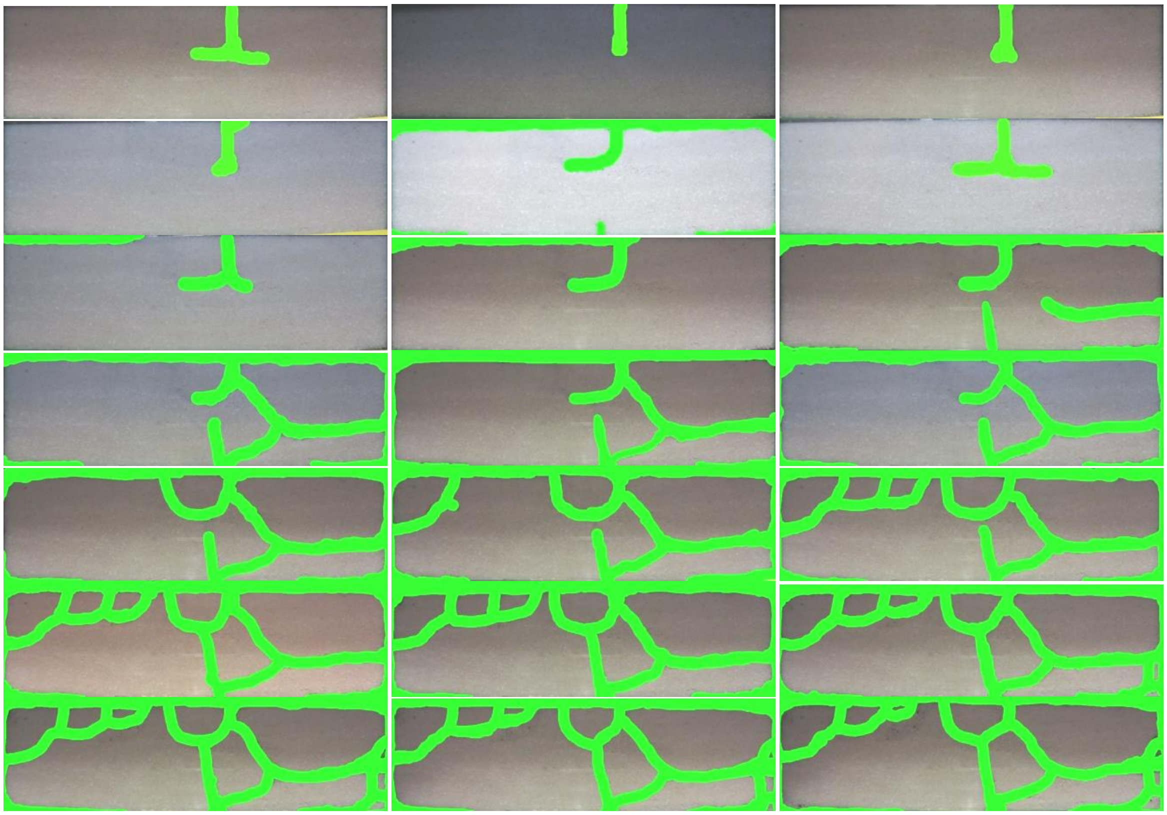


Figure 1 GIMP false coloration of habitat A. Nest development of habitat exposed to artificial light 100% of experimental time. Timepoints are arranged in order, left to right, top to bottom as follows: 16h, 25h, 36h, 48h, 60h, 67h, 72h, 84h, 96h, 107h, 5d, 5.5d, 6d, 7d, 8d, 9d, 10d, 11d, 12d, 13d, 14d.

Figure 2 GIMP false coloration of habitat B. Nest development of habitat exposed to no light (complete darkness) 100% of experimental time. Timepoints are arranged in order, left to right, top to bottom as follows: 16h, 25h, 36h, 48h, 60h, 67h, 72h, 84h, 96h, 107h, 5d, 5.5d, 6d, 7d, 8d, 9d, 10d, 11d, 12d, 13d, 14d.

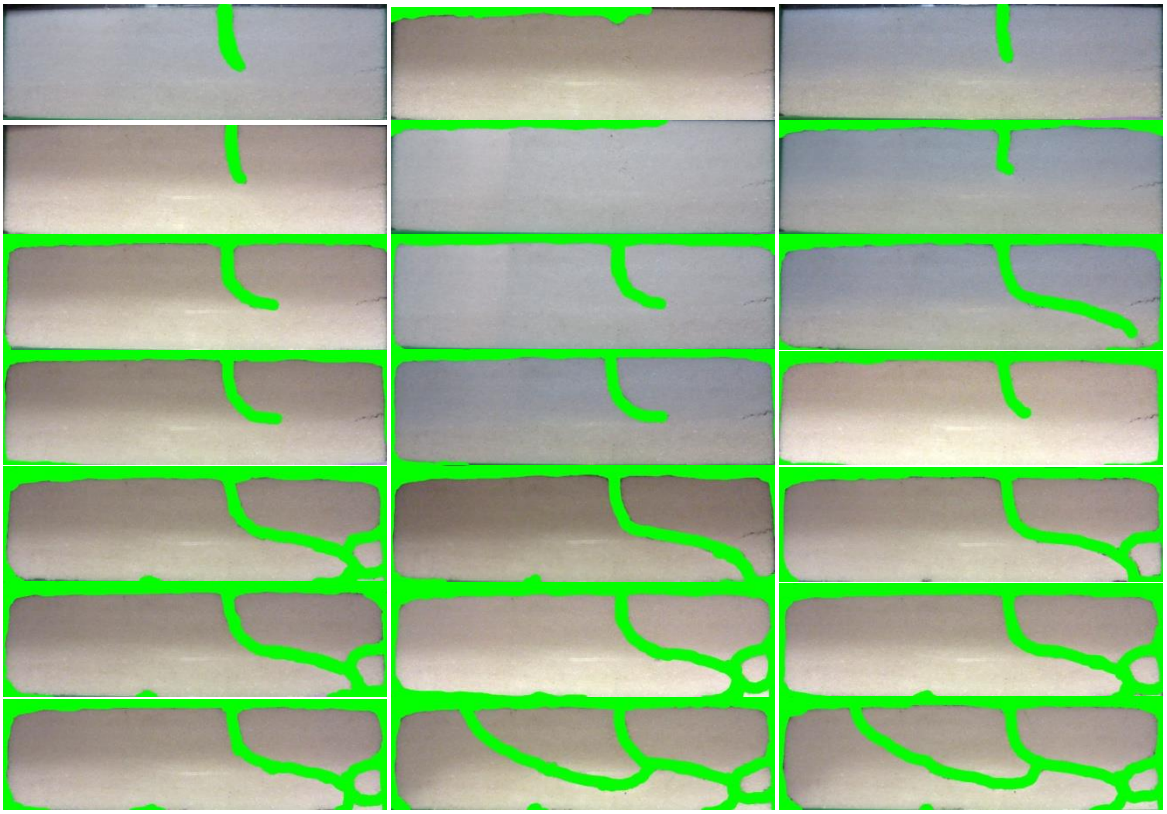
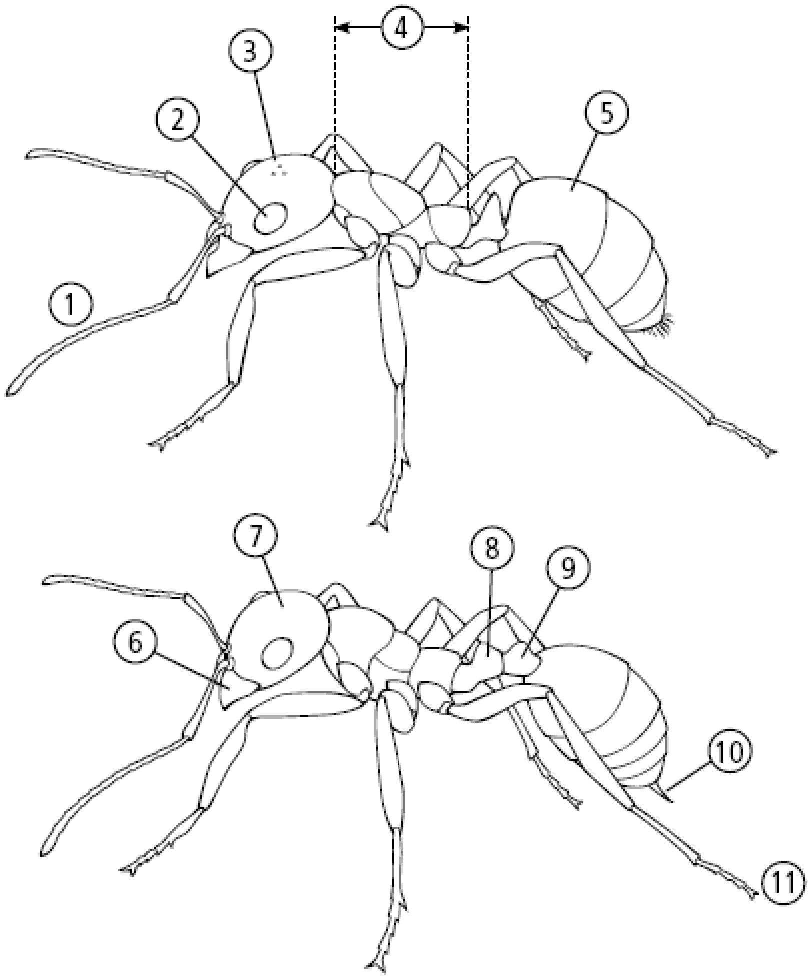


Figure 3 GIMP false coloration of habitat C. Nest development of habitat exposed to artificial light 50% of experimental time and complete darkness 50% of experimental time. Timepoints are arranged in order, left to right, top to bottom as follows: 16h, 25h, 36h, 48h, 60h, 67h, 72h, 84h, 96h, 107h, 5d, 5.5d, 6d, 7d, 8d, 9d, 10d, 11d, 12d, 13d, 14d.

APPENDIX; TEACHING RESOURCES

Ant Anatomy Worksheet

The parts of each ant have been labeled. Your challenge is to write the correct name for each part!



1. ____

2. ____

3. ____

4. ____

5. ____

6. ____

7. ____

8. ____

9. ____

10. _______________________________________

11. _______________________________________

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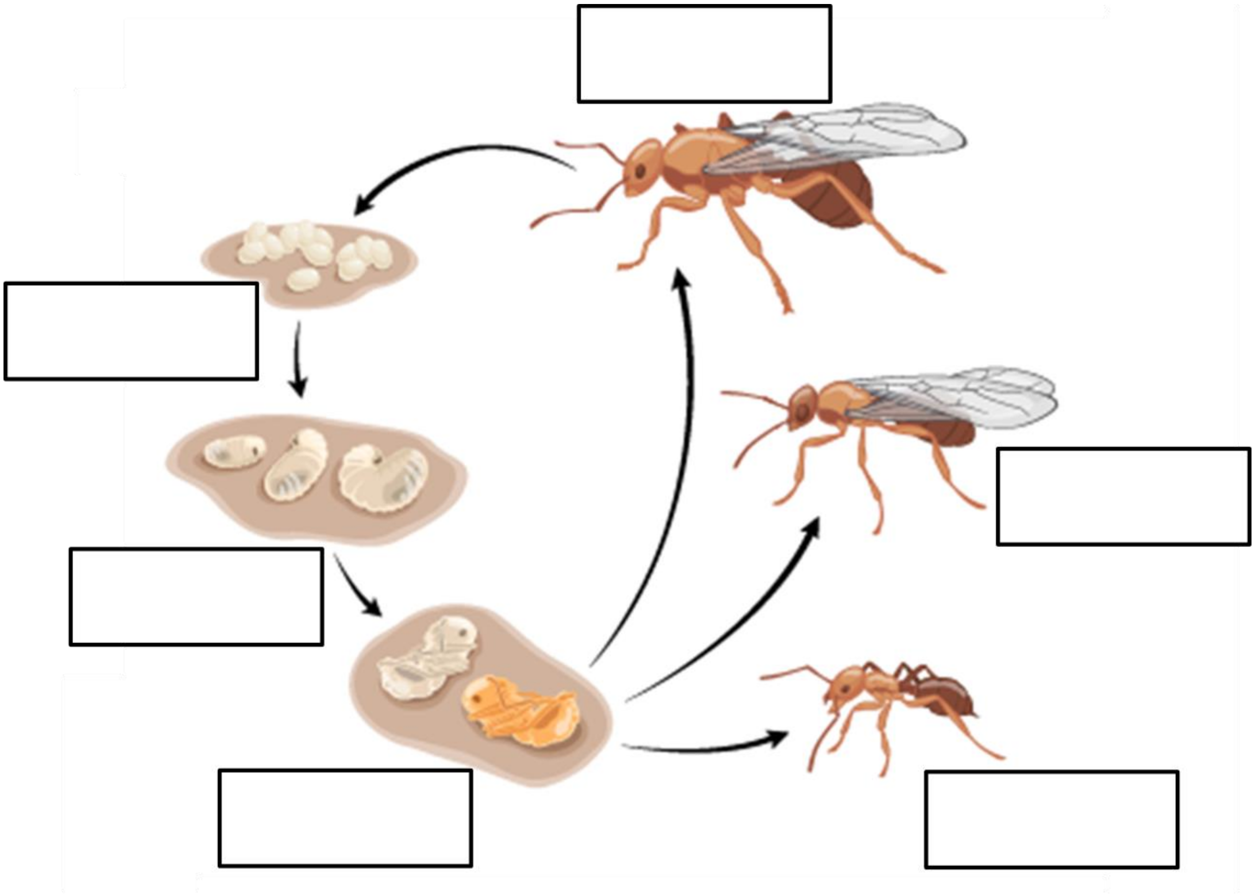
Ant Life Cycle Worksheet

The phases of the ant life cycle are detailed below. Your challenge is to correctly label each stage and member of the colony!



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Ant Worksheet Answer Keys

Ant Anatomy Activity Worksheet:

1. Antennae
2. Compound eyes
3. Ocelli
4. Mesosoma
5. Gaster
6. Mandibles
7. Head
8. Petiole
9. Post-petiole
10. Stinger
11. Leg

Ant Life Cycle Worksheet: