Effect of Sunlight Versus Shade on the Magnitude of Presence of Forensically Important Diptera in Toco, Trinidad

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Abstract: Various environmental factors can affect the duration of dipteran life cycles, a cycle important in forensic entomology when determining the time of colonization, which could be a time of death estimate. The amount of sunlight a carrion receives can influence the magnitude of presence of Diptera. In order to investigate, FTD2 disposable fly traps were used in lieu of carrion and were placed in direct sunlight and under shade for a total of 5 days between approximately 0700 and 1730. Diptera were collected and counted daily. Monitors of Ambient Light Intensity (MALI) sensors were used to determine the amount of sunlight a trap received by counting pulses of light outside a preset lux meter minimum or maximum. Shaded traps collected higher numbers of flies as time progressed while traps placed in direct sunlight collected fewer. A Fisher Exact test was used, and it was determined that there was a statistically significant trend that the flies preferred the shaded traps over the sunlit. Further trials and emphasis on ultraviolet radiation could be beneficial in determining a more statistically significant trend.

Key words: Diptera, sun, shade, north eastern Trinidad, ultraviolet radiation

Forensic entomology involves the use of insects or other arthropods in a criminal investigation such as studying dipteran life cycles and determining a time of colonization (TOC) estimation, which could be used to calculate time of exposure (Weidner *et al.* 2014). Dipteran life cycles are of great importance to this field because much research has been done to determine how long it takes to go from egg to adult for a variety of species, and they are the first to appear on a body making TOC and, possibly, time of death very accurate. The time it takes to complete this part of the life cycle can be used in legal cases involving the death of humans in order to determine the TOC (Amendt et al. 2004). The TOC is calculated by the most advanced stage of the Diptera life cycle present on the cadaver; there are many factors that affect the timeline such as weather or the exposure of a cadaver to the air (Amendt et al. 2004). Insect development can also be accelerated by higher temperatures and decelerated by lower temperatures (Bansode *et al.* 2016). Perceived temperature changes in areas with direct sunlight versus shaded areas, which could be a factor to TOC.

Studies by Sharanowski et al. (2008) and Shean et al. (1993) have investigated the impact of sunlight and shade on decomposition Saskatchewan in and Washington State, respectively. Both studies utilized pig carcasses as their carrion. Sharanowski et al. (2008) determined that carrion more exposed to the sun had a greater variation of fauna than the shaded carrion and Shean et al. (1993) stated that the more exposed carrion also decomposed faster than the shaded ones. This observation is to be expected as carrion fully exposed to sunlight would potentially continue to gain heat as decomposition progresses. In contrast to the previous studies, Castro et al. (2011) performed a similar study in Portugal also utilizing pig carrion but reported that more species were collected from pigs placed in the shade than in the sunlight.

A study of this kind has not been performed in Trinidad. The purpose of this study is to determine if Diptera in Trinidad, specifically northeastern Trinidad (Toco), prefer carrion in direct sunlight or in the shade and if this preference and magnitude of prevalence is statistically significant.

Materials and Methods

FTD2 disposable fly traps were used to collect all specimens (Stearling International INC, Spokane, WA). Four traps were taken approximately 150 m from the Jammev Beach Resort in Toco, Trinidad (10°49'34.1"N 60°56'05.7"W) at the top of an incline (Fig. 1) and hung on flowerpot hangers.



Fig. 1. Satellite photo of research site from Google Maps. Grey pin indicating traps' location.

Active ingredients in the traps were sucrose, putrescent whole egg solids, yeast, trimethylamine, and indole. Trimethylamine produces a fishy smell that, in combination with the other aromatic ingredients, produces a favorable smell for flies that acts as an attractant (PubChem Compound Database). The traps were then activated using water, following the manufacturer's instructions. The yellow caps on the traps allow flies to enter, but not escape. The flies drown in the water used for activation. The two sun traps were placed on top of the flat platform (Figs. 2 and 3) which had minimal shade from surrounding trees of structures.



Fig. 2. Sun trap location with close-up of sun traps placed and with proximity to Monitors of Ambient Light Intensity shown.



Fig. 3. Sun trap location with wide shot of sun traps placed and with proximity to Monitors of Ambient Light Intensity shown.

The two shade traps were placed underneath the bridge to the platform (Figs. 4 and 5). The traps were activated between 0705 and 0735 to ensure full sun at the time of placement. The two shade traps were placed underneath the bridge: one directly under and one slightly more south, but still under the platform overhang and the bridge. The locations of the traps remained stationary throughout the study.



Fig. 4. Shade trap location with close up of shade traps placed and with proximity to Monitors of Ambient Light Intensity shown.



Fig. 5 Shade trap location with wide shot of shade traps placed and with proximity to Monitors of Ambient Light Intensity shown.

To determine the amount of sunlight each trap received during deployment, a Monitor of Ambient Light Intensity (MALI) (Figs. 6 and 7), created specifically for this study to count sunlight as pulses, was placed beside each trap.



Fig. 6. Functional block diagram of the Monitor of Ambient Light Intensity.

The MALI monitors the ambient light surrounding the system and determines if that light exceeds or drops below a predetermined lux intensity. When the ambient light is within the predetermined range (e.g. the MALI for detecting high-intensity sunlight is in direct sunlight), the MALI is in sleep mode to preserve battery life. However, if the ambient light is out of range (e.g. the MALI for detecting high intensity sunlight is in shade), the pulse counter begins to count at a set frequency for the duration the MALI is out of range. Frequency can be increased but was kept at 1.0 Hz. The lux range could be altered by tightening or loosening the screw to the right of the ambient light intensity sensor (Fig. 7). Sunlight was determined to be above 100,000 lux and shade was determined to be below.



Fig. 7. Component diagram of the Monitor of Ambient Light Intensity with labeling for each significant part.

Immediately after the traps were activated, the appropriate MALI was turned on and placed within one foot (~0.3 m) of the base of the trap's hanger. The time of activation of each trap was recorded to the nearest minute. The traps were then collected between 1708 and 1726. Sunset was at approximately 1830, so this maximized the amount of sun the traps could receive without including shade created during the

process of the sun setting. The traps were closed and, immediately after, the MALIs were shut off. The pulse counter remains on while the rest of the system is powered off, so the pulses counted can still be read. The time of collection as well as the pulse count at the time of collection were recorded. The beginning and end times were used to calculate the total time for which each trap was active in minutes. The pulse counts between the two sensors per location were averaged and converted into minutes. The two values were used to create a 'percentage out of range' by taking the pulse count divided by the total time multiplied by 100. The percent out of range is the time the traps were out of the predetermined lux range (e.g. when the traps placed in the sun were covered by shade).

Upon collection, insects were freeze-killed and removed for identification to the lowest taxonomic level using Whitworth (2010) and Carvalho and Mello-Patiu (2008). This protocol was repeated for a total of 5 trials. Total number specimens recorded per day were compiled into a graph to better observe possible trends, and the statistical significance of these trends were tested with a Fisher Exact test.

Results

The traps in the sun on the first day of testing were active for 10 hours and 16 minutes. During this time, the MALI counted 29,571 seconds of shade. This resulted in 80.008% of the time active out of range for the traps. The traps in the shade on the first day of testing were active for 10 hours and 13 minutes. The MALI counted 362 seconds of sunlight resulting in 0.984% of the time active out of range. The traps in the sun on the second day of testing were active for 9 hours and 44 minutes. The MALI counted 5,662 seconds of shade resulting in 16.16% of the time active out of range. The traps in the shade on the second day of testing were active for 9 hours and 39 minutes. The MALI counted 1,644 seconds

of sunlight resulting in 4.73% of the time active out of range. The traps in the sun on the third day of testing were active for 10 hours and 11 minutes. The MALI counted 5,815 seconds of shade resulting in 15.86% of time active out of range. The traps in the shade on the third day of testing were active for 10 hours and 8 minutes. The MALI counted 1,150.5 seconds of sunlight resulting in 3.15% of time active out of range. The traps in the sun on the fourth day of testing were active for 9 hours and 44 minutes. The MALI counted 15.239 seconds of shade resulting in 43.39% of time active out of range. The traps in the shade on the fourth day of testing were active for 9 hours and 42 minutes. The MALI counted 1,204 seconds of sunlight resulting in 3.448% of time active out of range. The traps in the sun on the fifth day of testing were active for 9 hours and 52 minutes. The MALI counted 31,277 seconds of shade resulting in 88.05% of time active out of range. The traps in the shade on the fifth day of testing were active for 9 hours and 47 minutes. The MALI counted 1,727.5 seconds of sunlight resulting in 9.81% of time active out of range. The percentage of sun, in terms of lux minimum and maximum. did not significantly affect the magnitude of presence of Diptera.

Alongside monitoring the amount of sun or shade the traps received during the day, the weather conditions were also recorded. It had rained for most of the time the traps were active on day 1, day 2 was sunny, but with high humidity due to the rain the previous day, day 3 was sunny and with less humidity, day 4 was partly cloudy, and day 5 was cloudy.

A 2x3 Fisher's Exact Test was calculated with the results from Graph 1. The test was calculated with two degrees of freedom and

p was determined to be 0.04655. With p < 0.05 being considered statistically significant, and because most of the results contained under 5 specimens collected, the results from this study may be significant.



Total Number of Specimen Recorded per Day

Graph 1. Bar graph of total number of specimens collected per trap per day separated by sun and shade. The Y-axis is specimen count to 20 units. The X-axis is day/location with days progressing chronologically and further split by sun and shade. Day 1 and 2 both Sun and Shade have zero specimens collected. Day 3 Sun had two specimens collected. Day 3 Shade had zero specimens collected. Day 4 Sun had two specimens collected. Day 4 Shade had nine specimens collected. Day 5 Shade had seventeen specimens collected.

Discussion

The results from this study determined that the shaded traps attracted more Diptera than the traps left in the sun and the amount of specimen collected from the shaded traps each day continued to increase the longer the traps were in use while the traps left in the sun remained at a stagnant low level of specimen.

The lack of Diptera collected on day 1 and 2 could be explained by the rain on day 1 and high humidity on day 2 (Azevedo and Krüger 2013). The odor emitted from each trap also became more potent throughout the trials, which could also explain the absence of specimens in the first two days of the study. Although the traps emitted a stronger odor as time progressed, the number of specimens recorded per day from the sun traps was minimal and approximately the same number were collected each day beginning with day 3 while the number of specimens recorded per day from the shade traps continued to increase and the number collected was noticeably different for both sun and shade (Graph 1). The number of specimens collected from the sun traps remained approximately the same while the number of specimens collected from the shaded traps appeared to continue to increase as time progressed. The specimens from each bag were identified to the lowest taxonomic level possible. The calculated p value of less than 0.05 suggests that the data is statistically significant, however, further testing with more trials should be done to confirm.

One notable finding in this study is that although the shaded traps collected more specimens as the trap became more potent, the sun traps remained relatively stagnant. These results mirror those of Castro *et al.* (2011) who collected more flies from carrion placed in the shade as opposed to the carrion placed in the sun. The results from

this study were opposite as anticipated as Diptera had been shown to prefer the sun in both the Sharanowski et al. (2008) and Shean et al. (1993) studies. It is important to note the location of the three studies, however. The studies run by Sharanowski et al. (2008) and Shean et al. (1993) were performed in the northern hemisphere above the 47° N latitudinal line. The study run by Castro et al. (2011) was also performed in the northern hemisphere, but performed at approximately 39° N, nearly 10° further south. The latitudinal coordinates of Toco, Trinidad are approximately 10.8° N. Using the results from these studies, latitude location and UV radiation, sun intensity effects flies' preference for shaded or sunlit carrion. Flies prefer shaded carrion in locations closer to the equator and with higher average UV indexes and prefer sunlit carrion in locations further from the equator with lower average UV indexes.

The data collected in this study may suggest that forensically important Diptera in Trinidad are more attracted to carrion located within shade rather than carrion that receive full sunlight. One proposed hypothesis could be ultraviolet radiation and its effects on insects. Shortwave radiation, which includes UVA and UVB radiation, is known to be lethal in insects (Hori *et al.* 2014). According to the World Health Organization, UV levels are higher and more dangerous when closer to the equator (0° N) due to the shorter distance required to travel through the atmosphere and less of the harmful radiation being absorbed. Some radiation can, however, be avoided by seeking shade and avoiding direct sunlight (Olsen et al. 2012). Although the sunlit bags received a substantial amount of shade throughout the day, this was shade cast from clouds rather than an object. It could be possible that the negative effects of higher levels of UV are outweighing the desire for higher temperature and driving Diptera to choose carrion located in the shade over carrion located in the sunlight. A study conducted by Trájer et al. (2018) determined that solar radiation was the most important factor for breeding sites of Phlebotomus neglectus (Diptera: Psychodidae) (Tonnoir). Trájer et al. (2018) suggests that the amount of direct heat, solar radiation, and low air humidity are key factors in the selection of breeding sites by female Phlebotomus sp. due to the larvae being sensitive to extremes in these conditions. This study was conducted in Hungary (45.51° N, 18.25° E) in late July. The conditions of the experiment were full sun, no clouds, and in dry conditions with the focus being on the solar radiation throughout the day. The results of this study determined that Phlebotomus neglectus preferred shadier areas and this preference may serve as a means to avoid high levels of solar radiation.

Further research should be conducted to investigate the correlation between a higher UV radiation and dipteran attraction to carrion by recording UV index. A similar study as this one could be executed with emphasis on the UV index rather than solely the amount of sunlight a trap was receiving.

The information gathered from this study could potentially further research in determining if UV radiation has a drastic effect on the presence (in quantity) of Diptera as well as assisting in predictions for potential criminal investigation cases entomology. involving forensic More accurate predictions could be achieved utilizing weather reports to determine if there is a statistically significant correlation between UV index and dipteran preference of directly sunlit or shaded carrion.

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