2017

Blow fly (Diptera: Calliphoridae) Responses to Different Colors of Baited Traps

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Blow fly (Diptera: Calliphoridae) Responses to Different Colors of Baited Traps

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Department of Biology.

By

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ABSTRACT

Blow flies (Diptera: Calliphoridae) are usually the first insects to colonize human remains. By determining the time of colonization, a postmortem interval (PMI), or "time of death", can be estimated. To develop more accurate PMI estimates, it is important for forensic entomologists to understand the cues that Blow flies use to locate vertebrate remains. The purpose of this study was to determine whether Blow flies use visual cues, in addition to olfactory cues, to locate carrion. Two colors of fly traps, clear and green, were constructed and chicken gizzard used as bait. Three Blow fly species exhibited a significant preference for clear traps over green traps. Although these results were unexpected, it provides clear evidence that multiple Blow fly species use visual cues to locate vertebrate remains.

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April 2017
Department of Biology
University Honors Program
Georgia Southern University

Acknowledgements

I would like to thank the Biology Department at Georgia Southern and the University Honors Program for providing me with an opportunity like this and providing the needed funds.

I would like to thank Terry Whitworth for suggesting the fly trap designs.

To Dr. Mondor, thank you for your supervision throughout this project. You taught me how to pay attention to little details, and look in between the lines to find the big picture. I really appreciate your patience and help.

To Erin Martins, thank you for always keeping me on track with my honors requirements and exploring all available options with me.

To Laura Hawkins, thank you for making sure I did not quit after coming so far.

To Alexis Davis, thank you for helping me through the trial experiments.

To Kaeleigh Jackson, thank you for helping me identify flies in the laboratory.

To Hyelsinta Ali, thank you for helping me count flies in the laboratory, even though it was your spring break.

To my lab peers, thank you for proofreading this thesis.

To my pastor and church friends, thank you for your prayers and encouraging words through the process of writing this thesis.

Lastly to my parents, thank you for your prayers and being there for me through all my tears and breakdowns and for encouraging me all the way through this journey. I love you both.

INTRODUCTION

Upon discovering a human decedent, the circumstances surrounding the death are frequently unknown (Centers for Disease Control and Prevention 2003). As a result, it is crucial to answer key questions after human remains have been found. Information regarding cause of death, time of death, events preceding death, events succeeding death, and movement or storage of remains are all needed (Sharma et al. 2015), hence, the need for forensic science; the scientific analysis and investigation of events having legal importance (Fraser 2010).

Forensic Entomology is the use of insects in the scientific analysis and investigation of events having legal importance (Catts & Goff 1992). This branch of forensic science is broken into three categories: urban, stored-product pests, and medicolegal. The objective of this research focuses on medicolegal forensic entomology (Catts & Goff 1992). This branch of entomology is the study of insects associated with human remains in the scope of a civil or criminal investigation, such as natural deaths, suicide, or murder (Anderson 2009; Catts & Goff 1992).

The insects most commonly analyzed in medicolegal entomology are in the Order Diptera; the true flies. Calliphoridae (Blow flies), Sarcophagidae (Flesh flies), and Muscidae (House flies) are all key species of forensic importance found in this Order (Joseph et al. 2011). Because they are usually the first to colonize a corpse, Blow flies, are of key importance in medicolegal forensic entomology (Clark et al. 2006, Gallagher et al. 2010). These flies preferentially lay their eggs on or in the natural openings of the deceased, such as in human orifices or open wounds (Açikgöz 2016). The developmental stage of insects obtained from carrion can be used to estimate the time of colonization

(Weidner et al. 2014). This time interval is known as the Post Mortem Interval (PMI) (Anderson 2009).

Using insect evidence to determine the PMI involves the correct identification of the insect species and their developmental stages, found on the body (Joseph et al. 2011). After approximately 72 hours postmortem, Blow fly development is the most accurate, and usually the only, method that can be used to determine the PMI (Anderson 2009). Because insects are ectothermic, their rate of development is dependent on ambient temperatures (Higley et al. 2014). Each species has different predictable development times under particular environmental conditions. Obtaining the age of the oldest Blow fly larvae on the decedent, and correlating it with the temperature conditions at the death scene, permits a PMI estimate calculation (Clark et al. 2006; Joseph et al. 2011).

To calculate an accurate PMI estimate, the flies first have to lay their eggs on the remains. Knowing the cues that Blow flies use to locate a body is an important factor in forensic entomology. Studies have examined the various cues that Blow flies respond to, such as olfactory cues (Gomes et al. 2007). Many studies have shown that Blow flies are attracted to bacterial odors produced by the corpse in the early stages of decomposition (Clark et al. 2006; Chaudhury et al. 2002). Nitrogen and/or sulfur containing compounds, acids, and small alcohols are attractive to Blow flies depending on the stage of decomposition (Brodie et al. 2014). *Lucilia sericata* responds to a multi-modal cue complex consisting of floral odor and specific floral colors (Brodie et al. 2015). This species was attracted to protein and nectar in the plants, which in some cases, served as an alternate to carrion protein (Brodie et al. 2015). Dimethyl trisulfide (DMTS), an organic compound, has also been shown to lead to enhanced attractiveness if coupled with dark colors (Brodie et al. 2014).

Various experiments have examined the visual cues used by Blow flies. Most of these studies examined the reaction of Blow flies to color. These studies, however, have been equivocal. One study found that *L. cuprina* showed significant color preferences to different colored papers (Fukushi 1989). Another study, however, found no significant color preference in Blow flies to different colors of painted traps (Mello et al. 2009). Yet another study, when testing attractiveness of *Lucilia sericata* to Norway rats, found significant color preferences in *L. sericata*, and suggested color was part of a bimodal cue complex used by these Blow flies (Brodie et al. 2014).

The objective of this study was to examine Blow fly responses to different colors of baited traps, in South Georgia. It was hypothesized that color would be an important factor in attracting Blow flies, with increased attraction to the green traps. The green color would simulate the color of the early stages of decomposing flesh. This study will allow forensic entomologists to construct more accurate PMI estimates for human decedents.

MATERIALS AND METHODS

I. Creating Fly Traps

Fly traps were constructed based on a previous trap design (T. Whitworth, personal communication, December 6, 2010). Fly traps were created using Coke (clear) and Mountain Dew (green) soda bottles. Each trap consisted of a 2 L and 500 mL bottle. Bottles were rinsed with mild dish soap and water and had their labels removed, prior to trap construction. To create a trap, a RoadPro soldering iron (Palmyra, PA 17078) was used to melt two rows of three, 3 cm by 1 cm slots, creating a total of six rectangular slots in the 2 L bottle. The soldering iron was also used to melt a rectangular flap 6.5 cm by 5 cm in the side of the 2 L bottle, and a hole 7 mm I.D in the bottom of the 2L bottle.

Cheesecloth was cut into 11 cm x 11 cm squares, and 85 g of chicken gizzard (the bait) placed into it. A SecureLine diamond braid poly rope, 1 m in height was used to tie the cheesecloth, containing the gizzard, into a small pouch. The 1 m rope was tied to allow a length of approximately 0.6 m hang off the pouch. The pouch was passed through the rectangular flap into the 2 L bottle. Using the rope hanging off the pouch, a knot was tied 10.5 cm away from the cheesecloth, to ensure the chicken gizzard hung in the middle of the 2 L bottle. (Fig 1). The residual rope was passed through the 7 mm I.D hole at the bottom of the 2 L bottle.

Two hundred and fifty milliliters of 75 % ethanol was poured into the 500 mL soda bottle. This alcohol served to kill and preserve the flies collected in the trap. The 2 L bottle containing the chicken gizzard was inverted, with the mouth of the bottle at the bottom. The 500 mL bottle containing alcohol was directly connected to the 2L bottle using Parafilm ‘M’ laboratory film 10 cm in length. The film was stretched around the sides of the mouth of the two bottles for a firm hold. For additional structural support, 30 cm of Miracle-Gro garden twist tie was twisted around the 2 L bottle and then around 500 ml bottle to ensure the bottles held together. After this, the traps were ready to be set outside (Fig. 1).

II. Setting Fly Traps

The traps were placed at three different locations. The coordinates of each trap location were obtained using the Compass application on an iOS device (Apple Inc. 2016-2017). Traps were all located on the grounds of Georgia Southern University: Location A was a small thicket of trees by the roadside of Lot 42 (32°25’18 N 81°47’20”W), Location B was a small thicket of trees by the roadside, in front of the Biological Sciences building (32°25’18 N 81°47’24”W), and Location C was a bigger thicket of trees behind the Biological Sciences building (32°25’14 N 81°47’25”W). A map of trap locations was created using the Lat/Long Map Plotting Tool (Ward, n.d.) (Fig. 2).

Overall, there were four traps at three different locations, making twelve traps in total for this experiment. Each location had one clear and one green colored trap each with chicken gizzard as bait, and one clear and one green colored trap without chicken gizzard. The residual rope hanging off the bottom of the 2 L bottle, now at the top of the inverted 2 L bottle, was tied to a tree branch at each location. The traps were hung approximately 1 m above the ground, on Friday, November 11, 2016.

III. Collecting Flies from Fly Traps

Fly traps were left at each location for seven days (Fig. 3; Fig. 4). On Thursday, November 17, 2016 the traps were collected and brought into the laboratory. The daily temperatures during this experiment averaged 20°C for a high and 6°C for a low (Table 1).

The 500 mL bottles containing the flies were detached from the 2 L bottles and placed in a fumehood. The chicken gizzard pouches were removed from the 2 L bottles and disposed of appropriately. The flies were transferred from the 500 mL bottles into 20 ml glass scintillation vials containing 75% alcohol. The scintillation vials with flies were labeled by treatment (with coordinates).

IV. Identifying Flies

Flies were identified using a *Zeiss Stemi DV4* stereo microscope and an online dichotomous key (Marshall et al. 2011).

Statistical analyses

Statistical analyses for this experiment was performed via JMP 12.1.0 (SAS Institute Inc. 2015). All data were analyzed with Generalized Linear Models with a "Poisson" distribution and an "Identity" link function. The data was simultaneously tested for overdispersion during the analyses. A separate analysis was conducted for the five most common fly species caught in the traps: *Calliphora livida*, *Calliphora vicina*, *Chrysomya rufifacies*, *Lucilia coeruleiviridis*, and *Phormia regina*. The independent variable for each analysis was trap color. Location was included in the analysis as a covariate. Trap color*Location was included to determine if there were any significant first-order interactions.

More than five Blow fly species were caught during the experiment, but these additional species were in very low numbers. Therefore, no statistical analysis could be completed on these species. An analysis was performed on a species if the mean number of individuals of a species per trap was more than or equal to 5. The lowest count of species included in analysis had a mean of 7.6 individuals per trap.

RESULTS

The purpose of the control traps was to determine if alcohol, by itself, attracted flies. Flies were not attracted to the alcohol; if there was only alcohol in the trap, without any chicken gizzard, no flies were caught (Table 2; Fig. 4).

For *C. livida*, there was no significant preference between clear and green colored traps, across the three sites (Trap color χ^2_1= 1.06, p = 0.30). There was no significant difference in the response at different locations (Location χ^2_1 = 0.02, p = 0.87), and the response to clear and green colored traps did not differ by location (Trap location*Location χ^2_1= 0.66, p = 0.42) (Fig. 5).

For *C. vicina*, there was a significant preference for clear traps across the three sites, compared to green traps (Trap color χ^2_1= 4.74, p = 0.029). There was no significant difference in the response at different locations (Locationx12= 0.71, p = 0.40), and the response to clear and green colored traps did not differ by location (Trap color*Location χ^2_1= 0.27, p = 0.61) (Fig. 6).

For *C. rufifacies*, there was no significant preference between clear and green colored traps, across the three sites (Trap color χ^2_1= 0.50, p = 0.48). There was no significant difference in the response at different locations (Location χ^2_1= 1.38, p = 0.24), and the response to clear and green colored traps did not differ by location (Trap color*Location χ^2_1= 0.89, p = 0.35) (Fig. 7).

For *L. coeruleiviridis*, there was a significant preference for clear traps across the three sites, compared to green traps (Trap color χ^2_1 = 11.23, p = 0.0008). There was a significant difference in the response at different locations (Location χ^2_1 = 3.91, p =

0.048), and the response to clear and green colored traps differed by location (Trap color*Location χ_1^2= 7.26, p = 0.0071) (Fig. 8).

For *P. regina*, there was a significant preference for clear traps across the three sites, compared to green traps (Trap color χ_1^2= 12.42, p = 0.0004). There was a significant difference in the response at different locations (Location χ_1^2= 4.16, p = 0.041), and the response to clear and green colored traps did differ by location (Trap color*Location χ_1^2= 10.87, p = 0.0010) (Fig. 9).

DISCUSSION

The objective of this study was to examine whether color acts as a visual cue in Blow fly attraction. Contrary to what was hypothesized, there was an increased attraction to the clear traps, compared to the green traps, for three of five Blow fly species. Unexpected as the results were, this study suggests that color acts as a visual cue in Blow fly attraction.

Exactly why Blow flies were found in higher numbers in the clear traps is not easily explained. Perhaps it is easier for the Blow flies to see the bait in the clear traps than in the green traps. Likewise, the green color may block the flies from seeing the bait. To my knowledge, no studies have tested the attraction of Blow flies to clear traps. As such, further experimentation is required to explain these results.

The chemicals used in manufacturing the Coke and Mountain Dew bottles may also be an important factor to consider. Most plastic bottles, including large soda bottles, are made of polyethylene terephthalate (PET) (Carvalho et al. 2007). There is the possibility that different companies use different mixtures in the manufacture of their plastic bottles. Different chemicals in the plastic may impact the cues used by Blow flies when locating baits (Brodie et al. 2014).

Chemicals like small alcohols and acids have been previously found to act as an olfactory cue for flies. By putting only ethanol, without any chicken gizzard as bait, in the control traps it was clearly demonstrated that Blow flies were not attracted to ethanol. Zero flies in the control traps (Fig. 4) after seven days confirms that ethanol did not influence the results of the experiment. The traps captured insects other than Blow flies but they were not analyzed because they were not a part of the experiment. Two insects captured in relatively large numbers were wasps and ants.

A study with similar objectives, but a different Blow fly community, obtained opposite results. After setting out green, red, black, and white traps for 48 h, the calliphorids *Laneela nigripes*, *Hemilucilia semidiaphana*, and *Mesembrinella sp.*, were all captured, but there was no significant difference in color preferences (Mello et al. 2009). Since they did not find any significant role of color in attracting the Blow flies, color was suggested to be only a secondary factor in calliphorid attraction. They concluded that the Blow flies were attracted primarily by substrate odor (Mello et al. 2009).

Even though odor is a well-known cue for Blow flies (Brodie et al. 2014; Clark et al. 2006; Chaudhury et al. 2002), its effect appears to be strengthened when coupled with color (Brodie et al 2015). *Lucilia sericata* in the presence of floral scent responded more strongly to yellow than to green, white, black, blue, and red colors (Brodie et al. 2015). The flies were primarily attracted to the visual cues from yellow and white flowers, suggesting a significant difference in Blow fly color preference (Brodie et al. 2015). Fukushi (1989) found that *Lucilia cuprina* visited green colored paper less frequently than other colors tested such as green, blue, red, orange, and white. This study also found that *L. cuprina* visited green colors least frequently. Our data showing Blow fly species have an increased attraction to clear over green traps corroborate findings from these previous studies; Blow flies do exhibit color preferences.

In conclusion, this study clearly shows that Blow flies (Diptera: Calliphoridae) do not locate vertebrate remains solely on the basis of odor. The data from this study strongly suggests that color is a visual cue used by multiple Blow fly species. By having a better understanding of what attracts Blow flies to vertebrate remains, entomologists can develop more accurate PMI estimates.

Tables

Table 1. Temperature for each day the traps were in the field (traps were set out November 11, 2016 and collected November 17, 2016)*

Day	Maximum Temperature (°C)	Minimum Temperature (°C)
Friday November 11	22	4
Saturday November 12	17	9
Sunday November 13	13	8
Monday November 14	18	7
Tuesday November 15	22	5
Wednesday November 16	22	4
Thursday November 17	24	5

* Data retrieved from http://www.weather.com

Table 2. Number of Blow flies identified in each treatment

Location	Trap Color	Gizzard	*L. coeruleiviridis*	*C. livida*	*C. rufifacies*	*C. vicina*	*P. regina*
1	Clear	Gizzard	47	29	67	10	29
	Clear	No Gizzard	0	0	0	0	0
	Green	Gizzard	16	43	49	9	2
	Green	No Gizzard	0	0	0	0	0
2	Clear	Gizzard	31	23	56	13	14
	Clear	No Gizzard	0	0	0	0	0
	Green	Gizzard	10	12	3	2	1
	Green	No Gizzard	0	0	0	0	0
3	Clear	Gizzard	19	12	17	9	6
	Clear	No Gizzard	0	0	0	0	0
	Green	Gizzard	21	59	40	3	9
	Green	No Gizzard	0	0	0	0	0

Figures

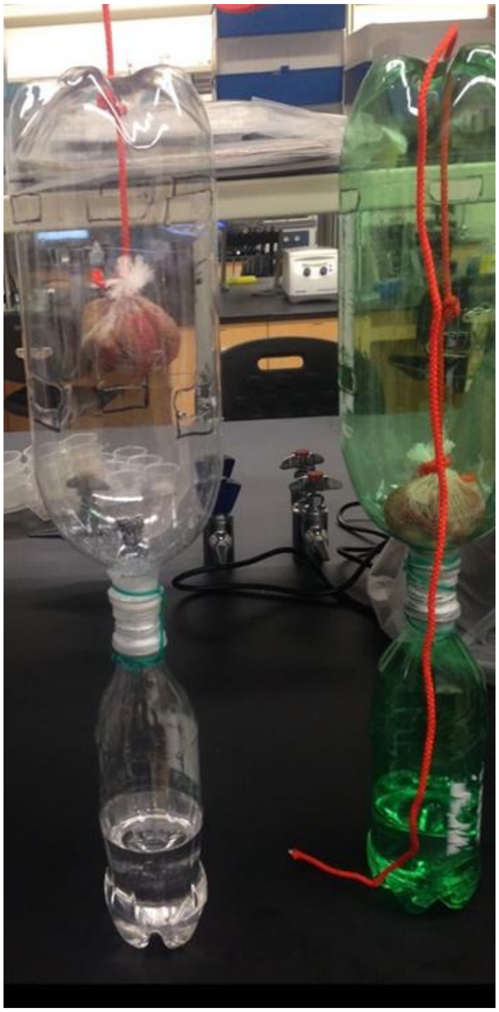

Fig 1. Clear and green fly traps, with chicken gizzard bait

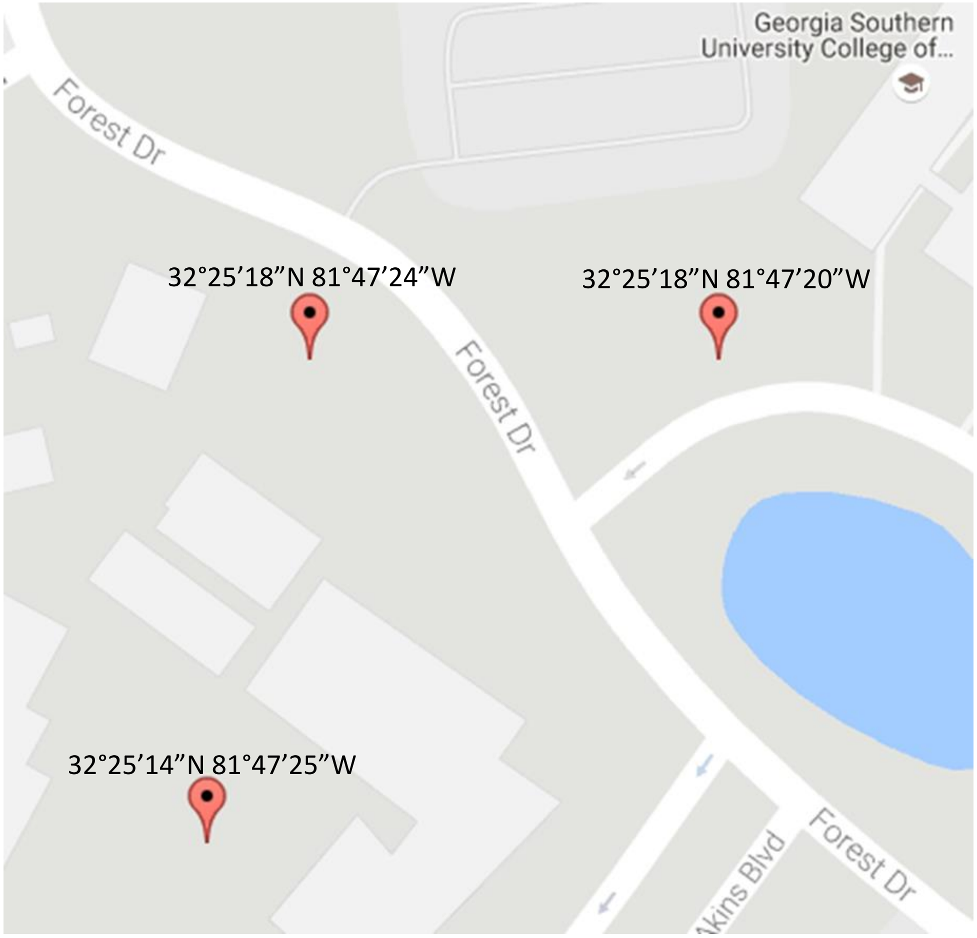

Fig. 2. Map of trap locations and their latitude and longitude (created using the *Lat/Long Map Plotting Tool* by Darrin J. Ward)

Fig. 3. After seven days, a clear trap with bait (note the large number of flies caught in the 500 mL bottle)

Fig 4. After seven days, a clear trap with no bait (note the complete absence of flies in the 500 mL bottle)

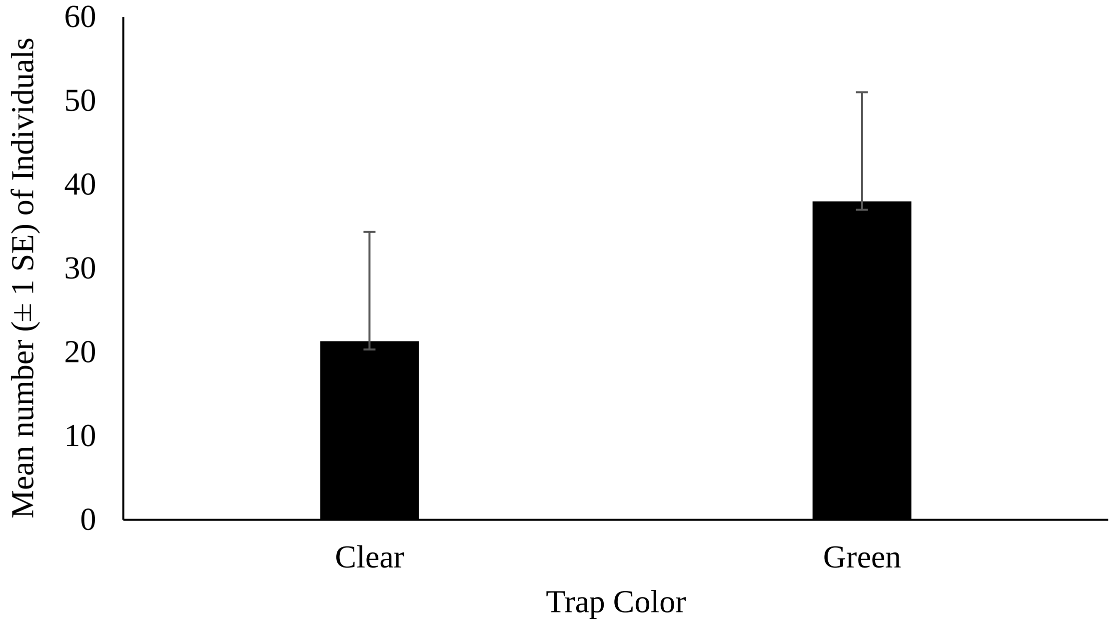

Fig 5. Mean number of *C. livida* in each color of trap, averaged across the three sites

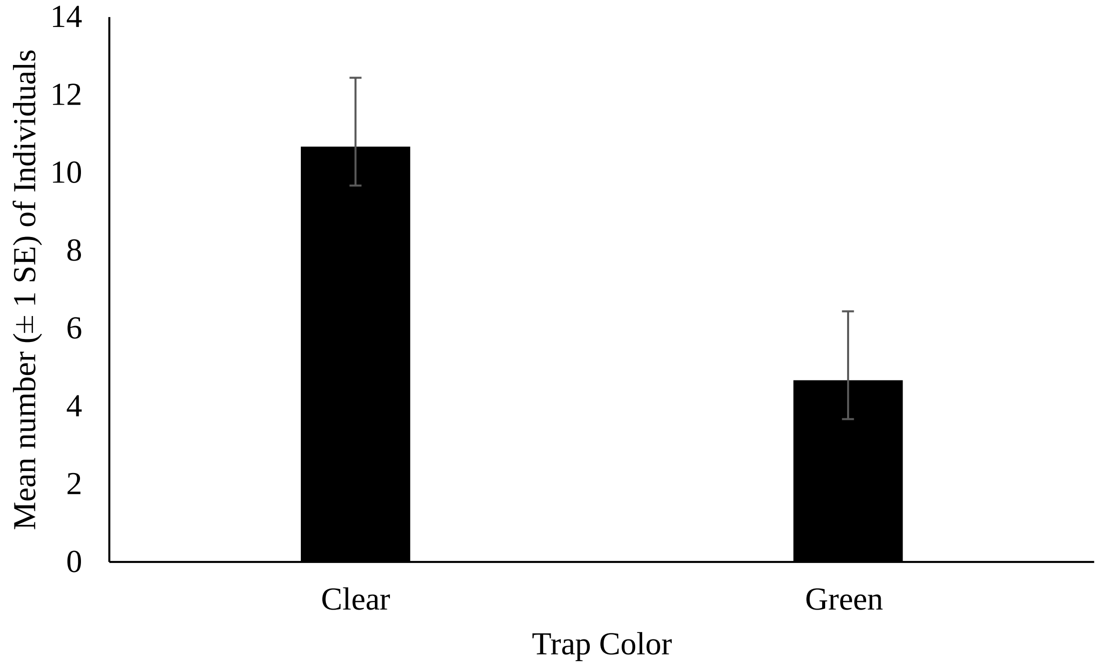

Fig 6. Mean number of *C. vicina* in each color of trap, averaged across the three sites

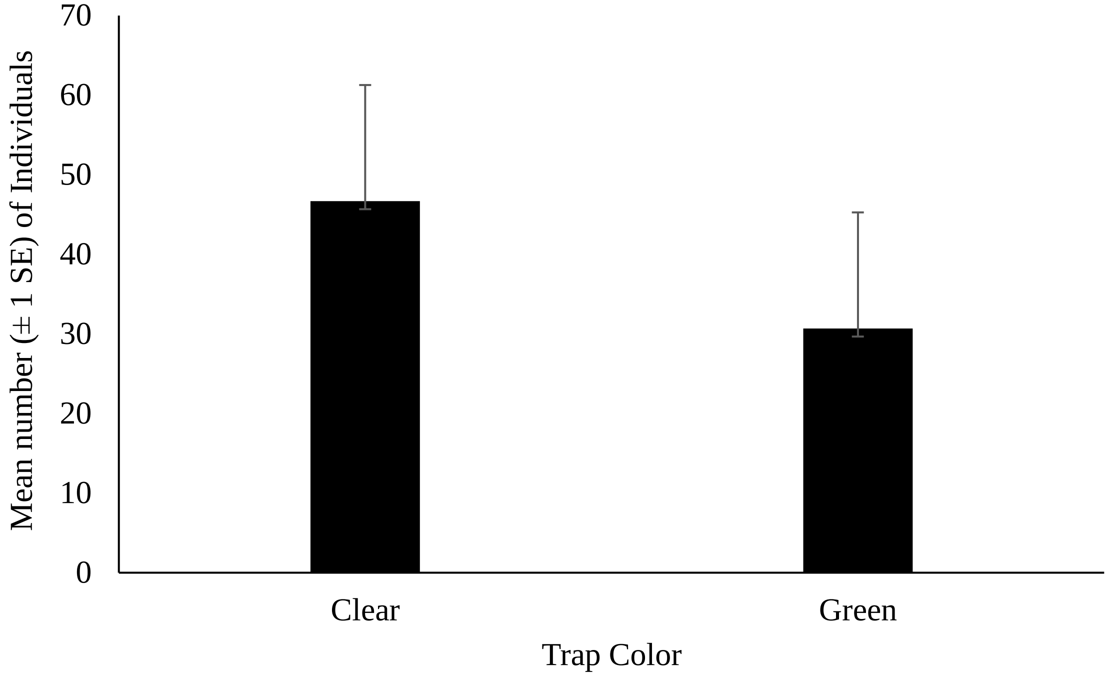

Fig 7. Mean number of *C. rufifacies* in each color of trap, averaged across the three sites

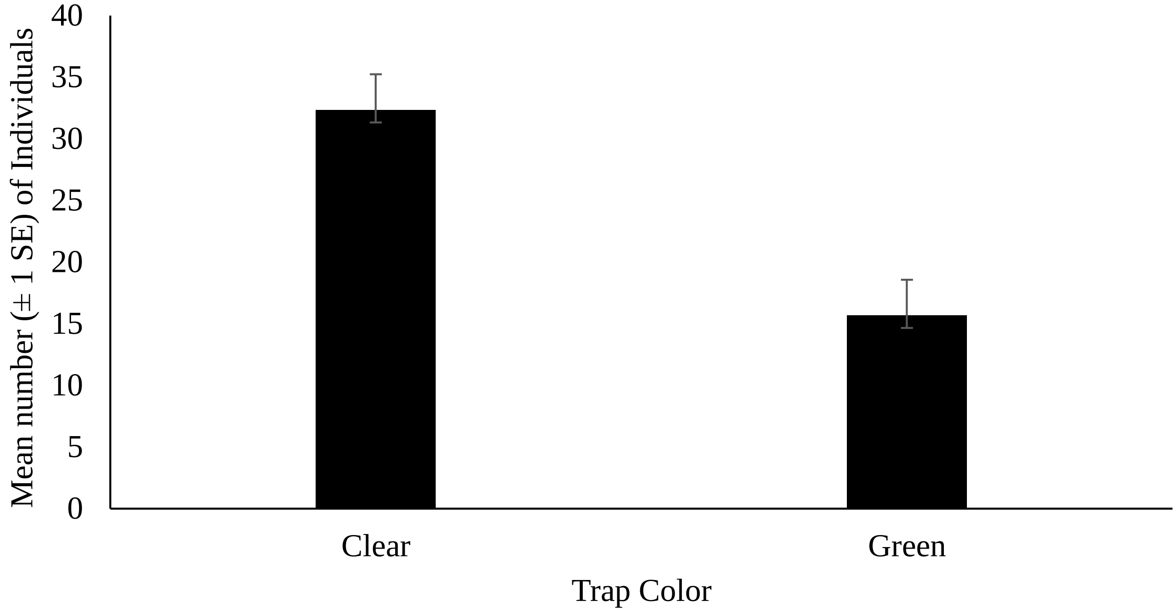

Fig 8. Mean number of *L. coeruleiviridis* in each color of trap, averaged across the three sites

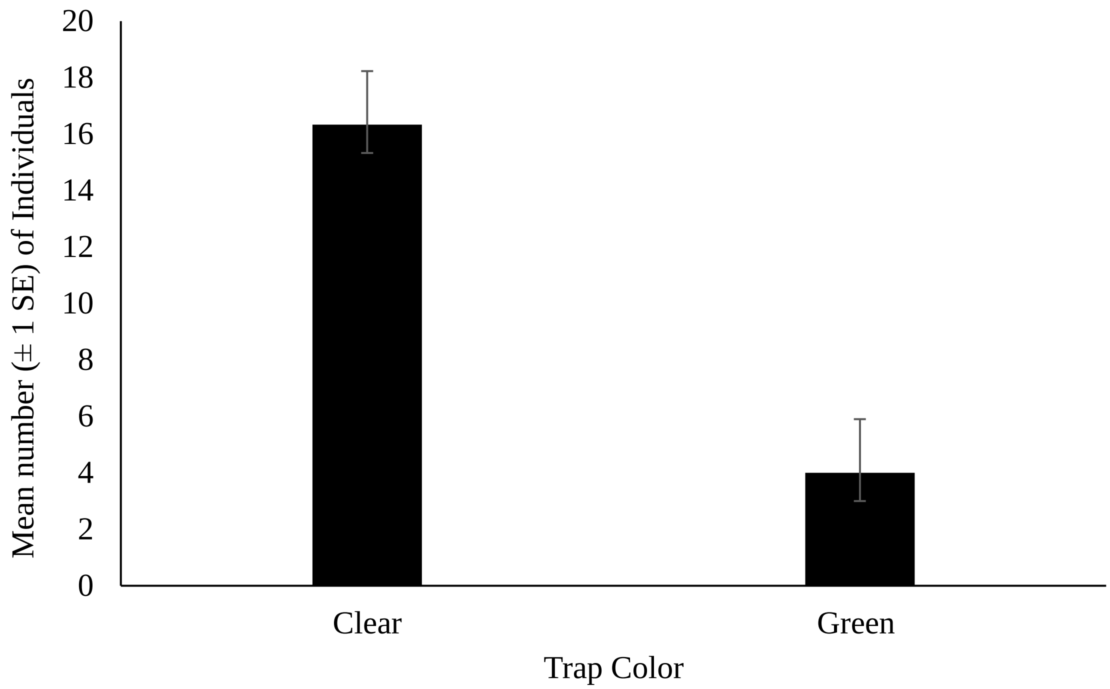

Fig 9. Mean number of *P. regina* in each color of trap, averaged across the three sites

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Appendix A: Blow flies not included in the statistical analyses due to low numbers

Location	Trap Color	Gizzard	*Cochliomyia macellaria*	*Lucilia cuprina*	*Lucilia illustris*
1	Clear	Gizzard	0	6	0
	Clear	No Gizzard	0	0	0
	Green	Gizzard	0	3	1
	Green	No Gizzard	0	0	0
2	Clear	Gizzard	1	0	8
	Clear	No Gizzard	0	0	0
	Green	Gizzard	0	0	2
	Green	No Gizzard	0	0	0
3	Clear	Gizzard	0	0	0
	Clear	No Gizzard	0	0	0
	Green	Gizzard	0	0	1
	Green	No Gizzard	0	0	0