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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA  
RIVERSIDE

Flight Behavior of the House Fly (*Musca domestica*)  
Under Field Conditions in Southern California

A Dissertation submitted in partial satisfaction  
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Levi Keith Zahn

March 2019

Dissertation Committee:

Dr. Alec C. Gerry, Chairperson

Dr. Bradley A. Mullens

Dr. Matthew Daugherty

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2019

The Dissertation of Levi Keith Zahn is approved:

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Committee Chairperson

University of California, Riverside

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ABSTRACT OF THE DISSERTATION

Flight Behavior of the House Fly (*Musca domestica*) Under  
Field Conditions in Southern California

by

Levi Keith Zahn

Doctor of Philosophy, Graduate Program in Entomology  
University of California, Riverside, March 2019  
Dr. Alec C. Gerry, Chairperson

House flies (*Musca domestica* L.) are common synanthropic pest associated with confined animal operations and are known carriers of many disease-causing pathogens affecting humans and animals. House fly production remains a significant problem for producers. Reducing the number of house flies dispersing away from a development site may impact both pathogen transmission and nuisance to humans and animals near fly development sites. This dissertation investigates the flight behaviors of the house fly under field conditions in southern California which may be used in future fly management programs. In chapter 1, a study was conducted to investigate the diel flight activity of house flies on two different active dairies in southern California. Collection period (time of day) was a useful predictor of house fly activity, as time is essentially a proxy for diel changes in temperature, humidity, light intensity, and even wind speed in southern California. Male flight activity peaked before females. Temperature, light intensity, and wind speed had a significant effect on activity throughout the day. In chapter 2, the flying height of house flies was examined using vertical 2.13m-tall sticky traps. House flies were evenly distributed over this trap height, except for flies captured on traps positioned in or

near the edge of an alfalfa field. Temperature, relative humidity, and wind speed were linearly associated with changes in flying height. The even distribution of house flies over the traps indicate house flies are almost certainly flying above top of the traps. In chapter 3, the relationship of house fly flight direction with environmental variables was investigated, using a release-recapture study. House flies are not dispersing randomly, and the short-range flight of male and female flies was toward the southeast. Flight direction was significantly correlated with time of release, solar position, wind speed, and temperature. Dispersing flies may have been using a nearby tree line as a visual cue thus flying in this direction. Chapter 4 investigated the impact a visual target would have on the direction of flight for house flies. The mean direction of flight was altered by the target but did not result in a significant shift in flight direction towards the target. More house flies captured when the target was raised and the aspect of the target facing the release point was sunward (reflecting sunlight). Overall, house fly flight behaviors are complex, and are likely impacted by all observed environmental variables. Changes in temperature and sunlight (time of day) had a wide-ranging impact on house fly flight behavior, affecting flight activity and directionality. It is anticipated that as sampling techniques become more refined the impact of weather on house fly flight behavior will become better characterized, but continued research is necessary.

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## **INTRODUCTION:**

The house fly (*Musca domestica* L.) is a medium sized fly in the family Muscidae (Order: Diptera). These flies are generally less than 6-9 mm in length, light grey in color with four longitudinal black stripes on the thorax, and a yellowing on the sides of the abdomen (Mullen and Durden 2009, Hung et al. 2013). However, their size and exact coloration can vary. House flies are sexually dimorphic with females easily separated from males by their generally larger body and greater interocular distance (West 1951). More complete descriptions of the external morphology of the house fly can be found elsewhere (West 1951, Mullen and Durden 2009).

The house fly is a cosmopolitan pest and is widely distributed throughout the world and can be found on every continent (West 1951), including Antarctica where it is cited as being an invasive species (McKie 2017). The exact origin of the house fly is unknown but is generally thought to originate from the palearctic realm (Marquez and Krafsur 2002) and is likely not native to the western hemisphere, but the exact time of introduction to North and south America is unknown (Legner and McCoy 1966).

House flies are holometabolous and undergo complete metamorphosis. Their lifecycle starts as an egg, proceeds through four larval instars and a pupal stage before emerging as an adult (West 1951). Under optimal conditions development time from egg to adult can be as short as 7 days (Mullen and Durden 2009). Eggs are generally laid in unsanitary conditions ranging from food and green waste to human feces and animal manures (West 1951, Mullen and Durden 2009). House fly development sites are well documented, consisting of rotting organic matter, including animal feces, food waste, sewage, and decaying plant matter (Meyer and Petersen 1983, Keiding 1986, Cook et al. 2011). However, house flies rarely can also infest humans and animals under extreme cases of neglect (Benecke 2010).

House flies are a common pestiferous insect associated with humans, as well as confined animal and agricultural facilities where development sites are numerous (Lysyk and Axtell 1986, Cook et al. 2011). Due to their development and association with unsanitary conditions flies can vector a multitude of human and animal pathogens including *Campylobacter jejuni*, *Entamoeba histolytica*, *Salmonella typhimurium*, *Shigella* spp., *Vibrio cholera*, enterohemorrhagic *Escherichia coli* O157:H7, Newcastle virus, and porcine respiratory virus (Olsen 1998, Kobayashi et al. 1999, Graczyk et al. 2001, Schurrer et al. 2004, Chakrabarti et al. 2007, Khamesipour et al. 2018), and this list is steadily growing (Butler et al. 2010). As residential communities have encroached upon once rural areas, the increased proximity to confined animal and agricultural facilities has amplified the number of nuisance complaints due to house flies. The movement of house flies can also have an impact on the overall health of a herd or a community, as pathogens are more easily spread among confined animals or from animals to humans (Watt and Lindsay 1948, Graczyk et al. 2001). Area-wide control of house flies has been associated with a reduction in human sickness due to enteric pathogens (Watt and Lindsay 1948, Chavasse et al. 1999).

### **House Fly Management**

Multiple control methods have been developed to manage house fly breeding and dispersal from confined animal facilities. These methods can traditionally be broken down into three main categories: 1) cultural control, 2) chemical control, and 3) biological control. However, as technology advance new categories may need to be incorporated to include methods for the use of genetic manipulations using products like RNAi. Management efforts are best selected using the tenets of an Integrated Pest Management (IPM) program, in order to

achieve suitable levels of control with the least cost and environmental impact. Often the most suitable results are obtained from the removal of immature fly development habitat such as moist animal feces (cultural control). This method can and should be supplemented with other control methods including the release of biocontrol agents (natural predators, parasitoids, or entomopathogenic fungi) and the appropriate application of pesticides (Axtell 1970, 1986, Tabashnik 1989, Geden and Hogsette 1994, Malik et al. 2007). Most importantly, but most often overlooked, is the routine monitoring of pest levels on the dairy to establish normal levels and to verify if control measures are effective (Gerry et al. 2011).

Current IPM practices used to control house flies encourage considerable effort in the areas of surveillance and cultural control, particularly in manure management (Lazarus et al. 1989). However, many animal producers still rely heavily on pesticide treatments which may lead to an increase in house fly resistance to many commonly used chemicals (Keiding 1999) and can limit the long-term effectiveness of management strategies that rely on pesticide use. Biological control methods may also be implemented through the encouragement or release of parasitoids, predators, entomopathogenic fungi, or other fly pathogens (Malik et al. 2007), but significant control can be difficult to achieve as many of the released organisms are constrained by specific environmental conditions or are adversely impacted by the application of residual pesticides (Geden et al. 1992, Skovgard and Nachman 2004). Cultural control methods, including removal of animal waste, are another effective method for reducing the prevalence of nuisance flies (Axtell 1986), but can be costly depending on the type of operation, and layout of the facility (Lazarus et al. 1989). Additionally, waste removal has the disadvantage of secondarily removing beneficial insects along with the target species. This can reduce the moderating effects that predators and parasitoids have on the pest populations (Mullens et al. 1996, 2001).

There is a great need to develop alternative control strategies, which can be easily and cheaply implemented by a producer, to supplement current IPM practices. There have been few new methods developed to limit house fly production, with no new methods for mechanically collecting or diverting flies away from sensitive areas such as residential neighborhoods, hospitals, or milking parlors on dairies. One possibility for control that needs further investigation is the use of barriers to reduce house fly emigration from development sites to nearby sensitive areas. Effective methods to contain flies, and perhaps to reduce their numbers, are critically needed to moderate nuisance in residential areas located near animal production facilities, as well as to reduce the movement of human and animal pathogens on and off these animal facilities. A distinct knowledge gap still occurs where house fly dispersal is concerned. Many general house fly behaviors have been well documented, but the specific cues and behaviors house flies use to accomplish these movements are still unknown and a fertile area of research.

### **House Fly Dispersal Behavior**

The movement of house flies away from development sites into unwanted areas is the main issue related to house fly management practices, as most livestock producers and their animals are acclimated to the presence of house flies, while nearby residents may be less tolerant. Additionally, the nuisance threshold for house flies is often far lower than what is necessary to reduce the spread of pathogens, although there is no documented economic threshold for house flies. Therefore, it is important to understand how house flies move within their environment. Understanding natural house fly behaviors could lead to passive

management practices which are low cost and can reduce house fly movements from development sites and into sensitive areas (restaurants, schools, clinics).

The factors effecting dispersal are diverse but can be categorized as either internal and external factors (Hansson 1991). While internal factors are generally not directly observable (*i.e.* hormones, innate behaviors), external factors generally are (*i.e.* temperature, or population density). In general, there are three tenets driving the dispersal of animals: 1) conflict over resources, in which a resource (food, mate availability, breeding sites) becomes limited and individuals will leave to seek more abundant resources, 2) conflict over resources, where lesser fit individuals are forced to leave because they cannot compete with more fit individuals for a particular resource, and 3) inbreeding avoidance, where individuals will leave to avoid inbreeding with close relatives (Hansson 1991). However, it is important to note that these hypotheses came primarily from studies on mammals and birds rather than from insects. Hansson (1991) also suggests that many insects tend to be more affected by weather (temperature, humidity, wind), and are therefore less likely to be density-dependent dispersers. Hansson does not give an exact reason for this line of thought, perhaps since weather fluctuates more rapidly than population size does, the impact of weather appears to have a greater effect on the population fluctuations in the short-term. Since tenets one and two are density dependent the impact they have on dispersal in house flies is likely minimized. However, density dependence as a driver of dispersal cannot be ruled out especially since house fly densities under summertime conditions can become extremely high (personal observation).

These proposed drivers of house fly dispersal are likely codependent, with no single condition solely responsible for house fly dispersal. For example, Pickens et al. (1967) observed that house flies are more likely to disperse away from farms with fewer development sites



(conflict over resources) and observed that young adult house flies (1-d old) are more likely to disperse than older adult flies (inbreeding avoidance). This suggests that both competition and age may drive house flies to disperse.

House flies are highly vagile organisms showing an extraordinary degree of movement throughout their environment. Parker (1916) revealed that flies typically disperse up to 2.1 km in an urban environment and 3.2 km in rural areas. However, as marking and recapture techniques became more refined, greater dispersal distances were recorded; up to 12.2 km in urban (towns) areas and 12.5 km in rural areas (Quarterman, J. W. Kilpatrick, et al. 1954, Quarterman, Mathis, et al. 1954). House flies are strong fliers and are capable of traveling long distances, with maximum dispersal records ranging from 22-32 km from a release point (Bishopp and Laake 1921, Schoof 1959), but it is commonly accepted that the majority of adult house flies are not dispersing to this extent and instead remain within 1-5 km of their development site. However, dispersal distances will vary depending on environment conditions and topography. It is believed that flies will disperse further in an open environment (e.g. open fields) when compared to environments with a high amount habitat complexity, particularly where vertical structures are more numerous (e.g. urban and residential areas) (Nuttall et al. 1914, Parker 1916). Dispersal distances might also be expected to vary by the availability of food and/or the number of development sites encountered by dispersing flies (Schoof and Siverly 1954, Schoof 1959). The size and shape of confined animal facilities may also influence the rate and abundance of dispersing flies. It has been shown that the number of individuals emigrating from a population of insects is not only related to the edge permeability of a habitat but also to the size and shape of the initial habitat. This is commonly expressed as an edge-to-size ratio (ESR) and can be a strong predictor of emigration (Stamps et al. 1987).

Due to the critical role food and development sites play in house fly biology, it is natural to think that flies would consistently orient towards these areas. However, house flies appear to disperse in a haphazard manner with very little predictability in their direction of flight (Schoof 1959). Murvosh and Thaggard (1966) found that flies dispersed away from the release area even though food and development sites were abundant, although their movement did skew towards the food and development sites.

Depending on reproductive and physiological age, the stimuli to which flies respond may change or the fly response to these stimuli may be altered. Flies' behaviors likely change as they age and could be unique to each age class (stage of gonotrophic development for females). For instance, female flies may focus primarily on sugar feeding the first few days after emergence but change to protein feeding in early stages of vitellogenesis, then seek ovipositional sites and ignore food sources when gravid as indicated by the spatial segregation in parous groups of female house flies on an active farm (Krafsur et al. 1985). Females will even display male avoidance during the later stages of vitellogenesis (Krafsur et al. 1985). As a result of these changes, house flies may become unresponsive to cues (odors, food sources, ovipositional sites) that were once attractive. These physiologic changes in responsiveness may have implications when surveillance and control measures are to be implemented. For example, monitoring methods may sample primarily one sex or age class of flies depending upon the monitoring site or methods used (Black and Krafsur 1985, Krafsur et al. 1985). Insecticidal baits, for example, contain attractive odors that may skew catches by fly sex or age responding to these odors, thus leading to incorrect conclusions about the overall population.

## **Direction of Flight**

It has been suggested that house fly dispersal is likely a series of short disjointed circuitous flights culminating in the aggregation of house flies at some more distant attractive site (Schoof and Siverly 1954a, 1954b). Schoof and Silverly (1954a) suggest five possible conditions that may impact house fly dispersal: 1) Population pressure initiates dispersal due to resource competition, 2) Differentially attractive sites concentrate house flies at more attractive sites, 3) Geographic barriers (e.g. rivers, lakes, deserts, and forests) may inhibit or channel house fly dispersal, 4) Preferential movement is linked with differentially attractive sites and would largely depend upon the number and placement of these sites, and 5) Flies exhibit an inherent tendency to disperse from natal sites. Many of these overlap with the more general drivers of dispersal previously mentioned. Nonetheless, conditions two, three, and four likely impact the overall direction of flight for dispersing house flies. A sixth condition, the impact of environmental factors (e.g. wind speed and direction, temperature, precipitation) should also be included in this list as house flies are known to be to react to changes in the environment (Parker 1916, Bishopp and Laake 1921, Holway et al. 1951, West 1951, Reddy 1981, Ngoen-klan et al. 2011, Godwin et al. 2018).

Some studies on house fly dispersal have shown anecdotal evidence of a response to wind (Parker 1916, Bishopp and Laake 1921, Broce et al. 1991). Observations have suggested that house flies will display a certain degree of anemotropism and fly into or across winds of 5.4 m/s or less (Nuttall et al. 1914). These results have been noted in later studies (Pickens et al. 1967). Even though this general trend has been observed, a strong correlation between wind speed, wind direction, and house fly movement has yet to be established. This makes predicting fly movement difficult. There is much uncertainty about how wind speed and wind direction

affect the directional flight of house flies, as many of the conclusions from the following papers are not empirical but generally anecdotal and often contradictory. House flies have been found to disperse against the wind (Nuttall et al. 1914, Hanec 1956, Pickens et al. 1967), at an angle in relation to wind direction (Nuttall et al. 1914), or with no relationship to wind direction at all (Parker 1916, Quarterman, J. W. Kilpatrick, et al. 1954, Quarterman, Mathis, et al. 1954). With one study observing house flies at times moving with, against, and perpendicular to the wind, however, the study was conducted over multiple years and during different months (Bishopp and Laake 1921). Nuttall et al. (1914) found that liberated house flies tended to either fly against or across the wind only after dispersing more than 137-183m, flies which were collected at shorter distances appeared to travel to the nearest shelter.

However, there is no consensus whether house flies show true positive or negative (or other) anemotaxis. In wind tunnel experiments virgin female house flies readily flew upwind in response to the presence of pig volatile odors (Cosse and Baker 1996), but this may be typical only of an appetitive search behavior in a constrained system rather than of more general dispersal flight behavior as a result of other factors present under natural field conditions. However, in the absence of attractive odors will flies move upwind, crosswind or neither? Parker (1916) hypothesized that flies most likely show anemotactic response over short time periods (hours), but over an entire season (months) house fly movement appears to be random since wind direction is constantly changing throughout this time period. So even if flies are moving across or upwind, variable wind direction and speed could cause dispersal to be random. Understanding the movements of animals within small spatial scales can be problematic as it is difficult to isolate individual mechanisms which may drive dispersal behavior, or to even determine if certain behaviors are appetitive or not.

## **Effect of Environment on House Fly Activity**

House fly dispersal (or the dispersal of any insect) is a complex behavior that remains quite enigmatic to entomologists. However, general insect activity is influenced by temperature (Taylor 1963, Schou et al. 2013) and is ostensibly linked to dispersal. The activity level for many species of insect will peak at the insects' optimum temperature, with decreasing activity as temperatures move away from this optimum to one extreme or the other (Schou et al. 2013). For house flies, locomotor activity was shown to increase with increasing temperatures up to 35°C, above which physiologic process in the house fly become compromised and activity levels start to decline. Males were also more active than females at all temperatures tested except for the two extremes of 10°C and 40°C (Schou et al. 2013). The optimum temperature for house flies is around 30°C (Schou et al. 2013).

Kjærsgaard et al. (2015) tested the effects of increased temperatures on house fly survival, activity levels, and flight performance using three distinct populations of house flies taken from areas with differing temperature profiles. Flies that were taken from the warmest area (Barcelona, Spain) which experienced the highest averaged daily temperatures, were also the most resistant (higher survival rate) to deleterious effects of high temperatures (42°C-45°C). Additionally, the flies collected from Spain were the most active at high temperatures (43°C), however, flies from all regions still had a significant decrease in activity levels at extreme temperatures. Interestingly, female flies tended to be more resistant (were more active and survived longer) to extreme temperatures than males.

House flies can thermoregulate by positioning themselves to either avoid or capture solar radiation but are still subject to wide variations in body temperatures. When temperatures

are cool, house flies are commonly seen warming themselves on vertical surfaces in direct sunlight (Keiding 1986), while under hot conditions flies seek shaded places (West 1951). In fact, studies on Egyptian house flies have demonstrated a distinct thermo-regulatory behavior, by moving into and out of homes as temperatures vary throughout the day. When given the choice flies will move to warmer areas when temperatures are below 20° C and into cooler areas when temperatures are above 30° C (Holway et al. 1951). In southern California, this corresponds to a general movement of flies diurnally into farm buildings during hot days in the summer, and out of farm buildings on days in the winter.

Intrinsically linked to house fly activity and sunlight is temperature. House flies are ectotherms and regulate their body temperature by moving to hotter or colder locations (West 1951) which are typically sunny or shady spots. It has been postulated that insect flight activity is likely controlled by an interaction of both light and temperature (Taylor 1963, Lewis and Taylor 1965). If temperatures never exceed the minimum flight threshold temperature, then insects are physiologically restricted and cannot take flight. But if temperatures are above this minimum, then the occurrence of flight becomes more independent of temperature (Taylor 1963) and is more likely modulated by other factors including light. For instance, the black aphid (*Doralis fabae*) was unable to take flight in the morning until temperatures reach 17.5°C, but flight was inhibited as dark fell even though temperatures remained above this threshold (Taylor 1963). Semakula et al. (1989) showed a similar response in house flies to decreasing levels of light, an indication that house fly flight activity is inhibited by darkness making light an important factor at dusk but not dawn.

The impact humidity has on house fly activity and behavior is not well understood, as humidity and temperature are not independent of each other (Lawrence 2005). In a laboratory

setting, adult house flies were given a choice of two humidities at a stable temperature of 25 °C; they selected the lower humidity and showed the most activity at higher temperatures and low humidity (Dakshinamurty 1948). House fly preferences for lower humidity might be a behavioral response to infection by entomopathogenic fungi. The risk for infection by *Entomophthora muscae* was reduced at humidity under 65% (Kramer 1980). By selecting areas of low humidity adult house flies might improve longevity by avoiding infection with pathogenic fungal spores.

The effect of population density on house fly activity has been widely studied. Schou et al. (2013), found that in the laboratory an increase in density (both sexes) resulted in a decrease in the locomotor activity of house flies at all temperatures tested, except for 15°C, suggesting that increased stresses at high temperatures (due to increased energetic costs) could be responsible for the decrease in activity. Increases in density also appear to impact each sex differently, as house flies placed into local activity monitors showed that females were most active (walking) at the lowest density while males were most active at the medium density (Bahrndorff et al. 2012). Additionally, when sexes were mixed, activity was highest in the vial with five males and one female. Perhaps males are harassing other males in an attempt to mate (Murvosh et al. 1964). When activity was measured for mating pairs of house flies, using more pairs (1-4 pairs) lowered the overall amount of activity (Schou et al. 2013). In contrast to increasing density. Social isolation significantly decreased male activity (walking) after 8 days of isolation as compared to isolated females, and socially maintained (2 flies/per cage) males (McCarthy et al. 2015). Male house flies were always more active than females over all densities tested (Bahrndorff et al. 2012, Schou et al. 2013, McCarthy et al. 2015). Within these limited laboratory trials increases in density appear to decrease the overall activity of house flies, the exception being males at moderate densities. It is unknown how this relates to field and the

overall activity of wild-type house flies, as wild flies can disperse and are not kept in close contact over time but can also be found in at extreme densities (personal observation). Additionally, most lab cage experiments use lab-adapted house fly strains, the densities are representative of a field population of house flies. However, it is unknown how density affects house flies in the field.

### **Diel Flight Activity of House Flies**

The diurnal activity of house flies is not well understood, though light intensity is putatively the most important variable affecting the initiation of diurnal flight activity for many insects (Lewis and Taylor 1965), and therefore likely a strong factor for house fly flight activity as well (Parker 1962). Adult house flies are most active during daylight hours and will seek resting locations at night (West 1951, Tsutsumi 1966, Semakula et al. 1989), but the presence of artificial lighting can shift and elongate the time periods when flies are the most active (Tsutsumi 1973).

House flies have been reported to prefer areas of bright sunlight over shade, but will move to shaded areas when it becomes too hot (Holway et al. 1951) and are able to fly at temperature ranging from 5-42°C (Tsutsumi 1968, Semakula et al. 1989, Schou et al. 2013) with higher flight activity when temperatures exceed 18.3°C (Pickens et al. 1967), and an optimum temperature range for flight activity of 30-35°C. At temperatures above these, activity levels decrease due to heat shock (Schou et al. 2013).

An early study on the diurnal activity of adult muscoid flies (this includes but is not limited to house flies) reports that in Japan during mid-May adult flies were most often collected around 1100 hours, and from late May into September flies were more often collected at 0900



hours and 1500 hours (Shinoda and Ando 1935 taken from West 1951), suggesting a shift to a bimodal (two peaks in activity) activity period during the hotter months in late summer. In a controlled laboratory study, teneral house flies held at 25 °C and 60% humidity with an artificial 16:8 (L:D) cycle, had low overall activity and were acyclic until about 48 hours after adult emergence at which point they began to show a bimodal activity pattern (Chabora and Shukis 1979). Activity would peak at 0700 hours, approximately 1 hour prior to lights on (0800 hours), decline, and increase to a second peak at 1700 hours. This suggests that a bimodal diel activity pattern might be an innate behavior for fully mature adult house flies.

These laboratory findings are corroborated by Semakula et al. (1989) who collected house flies from an enclosed loafing shed on a dairy in Manhattan, Kansas using Johnson-Taylor segregating suction traps. Traps were run continuously from July to October, with collections made every 30 min for 24 hours a day. The authors suggest that house fly activity was also bimodal with peaks occurring at 1330 hours and 1630 hours. However, a careful examination of their the data suggests that the distribution is likely unimodal with house fly numbers peaking at 1330 hours, which fell within their reported time range for maximal house fly collections (1300-1400 hours). Since the upper temperature threshold for flight (of house flies) was never reached, the peak(s) in activity coincided with peaks in temperature. Additionally, two smaller peaks at 0800 and 2000 hours were also evident, suggesting that house flies may have small crepuscular peaks in activity, but these two peaks were not addressed by the authors. Even though house flies were collected over a period of months, the data were pooled before analysis to obtain 48 estimates of activity over the 24-hour period and likely smoothed over any smaller peaks. This pooling of the data could have also hidden a shift in the time of peak activity as the

year progressed as was suggested by Shinoda and Ando (1935). Perhaps this is why Semakula et al. (1989) identified two peaks.

The effect of wind direction on house fly flight activity and direction of flight has been largely neglected, perhaps due to the large variability in wind speed and direction and to the large geographic scales and length of the time scales for most house fly release-recapture studies. The influence of wind speed on house fly activity is also poorly understood, as wind speed estimation is also problematic due to extreme spatial and temporal variability. It has been proposed that most flying insects reduce their flight activity when wind speeds exceed the insects' maximum flight speed (Lewis and Dibley 1970, Taylor 1974). The maximum flight speed of house flies has not been published, though the observed average flight speed for an adult house fly is approximately 2 m/s (West 1951, Shepard et al. 1972, Wagner 1986, Dahlem 2009). Therefore, it would be expected that house fly flight activity would not begin to decrease until wind speeds increased to some level above 2 m/s.

### **Height of Dispersal Flight**

The maximum height of flight for insects appears to be largely governed by what has been called the insect boundary layer (Taylor 1960). This is the maximum height at a given wind speed at which an insect can still maneuver under its own power. Above this height the forces of the wind are too great, and the insect's flight becomes uncontrolled. The taller and denser the substrate becomes (*e.g.* a dirt field versus a full-grown corn field) the higher the insect boundary becomes.

Wind speed, and to a lesser degree wind direction, also play a major role in modulating the flying height of insects. More precisely, it is how these two variables interact with physical

structures (buildings, fences, tree lines) on the ground which has the largest impact on the flying height of insects. The insects' flying height is generally governed by two principle variables, the wind speed and the maximum flight speed of the insect itself (this generally ignores planktonic insects like white flies or aphids). When the wind speed exceeds the insects' maximum flight speed, flight can no longer be self-controlled. Under such conditions, flight may be possible only near ground level where wind speeds remain slow enough to allow self-directed flight (Taylor 1960). The height of this boundary layer is primarily dependent on the wind speed and the relative flying strength (and flight speed) of the insect. The literature on the maximum flight speed for house flies is lacking but it has been reported that the average flight speed for house flies is 2 m/s (West 1951, Shepard et al. 1972, Wagner 1986, Dahlem 2009). It can then be assumed that the maximum flight speed lies somewhere above this velocity. Although people have stated that house flies are relatively slow fliers given their size so their maximum speed might not be as fast as expected.

To date, only Black and Krafsur (1985) have attempted to examine the height of flight for house flies in the field. House flies were collected using white sticky note cards positioned at 36 cm, 76 cm, and 116 cm above ground. Cards were placed in both dairy feed lots and cattle pastures. Flies were most concentrated in the airspace nearest to the ground, with the number of flies captured decreasing linearly with increasing trap height. An interaction between trap height and habitat was noted, indicating that flies may vary in their height of flight depending on the substrate or other habitat differences. This is in alignment with the consensus that flying height selection by insects can be altered by variation in substrate (Schulze et al. 1975) or by environmental characteristics, particularly wind speed (Taylor 1960, Lewis 1967, Lewis 1969, Snow 1977). A possible explanation for this interaction of site and height is that house flies are

taking short hopping flights as they search for food above an attractive substrate (cattle pen versus a pasture) so more flies are captured nearest the ground.

However, the low flying height reported by Black and Krafur (1985) seems to contradict observations that house flies will readily land on surfaces 1.8 m above ground (Gerry et al. 2011) and that flies often accumulate in overnight resting locations sometimes >6 m in height, (*e.g.* underneath barn roofs). It is unclear from these cursory observations whether house flies commonly fly at greater heights than those reported by Black and Krafur (1985) or if they fly low to the ground and then are redirected to fly upward upon encountering a vertical structure. That house flies may increase their dispersal height at some locations relative to others is immensely important if alternative management strategies that rely on the capture of dispersing flies are to be implemented.

### **Effect of Barriers on House Fly Dispersal**

Natural and manmade barriers such as fences and tree rows may influence the dispersal patterns of some insects by creating habitat corridors or by blocking direct access to a protected site. Both manmade (fences and walls) and natural barriers (hedge and tree rows) have been shown to impact the movements of some insect species including tabanids, stable flies, hover flies, and butterflies (Morgan and Lee 1977, Foil and Hogsette 1994, Wratten et al. 2003, Ingimarsdóttir et al. 2013).

Saltmarsh greenheads will look for gaps in a vegetative barrier (hedge row or similar) when encountered during flight (Schulze et al. 1975, Morgan and Lee 1977). When these gaps were blocked with a black colored burlap barrier there was no significant reduction in the number of greenheads captured beyond the barrier (Schulze et al. 1975). It was hypothesized

that the greenheads flew around the barrier. No height was given for the burlap barrier, however traps placed above the tree line indicated that greenheads were not flying over the barriers but were instead following corridors. This resulting difference in the effectiveness of barrier type may be attributed to the fact that the burlap barriers may not closely resemble a natural barrier in either shape or function. It may also be a case of the greenheads visualizing the barrier before it was encountered and simply moving around it. The flight of hover flies (Syrphidae) was obstructed when flies encountered both artificial and vegetative barriers one and two meters in height (Wratten et al. 2003). These studies suggest that artificial and vegetative barriers may be effective in managing the dispersal of the house fly.

This is not a behavior that is exclusively relegated to greenheads or hover flies. House flies encountering a dense forest edge turned and followed the linear edge of a forest rather than enter the forest (Fried et al. 2005). This suggests house fly dispersal follows the “drift fence hypothesis” where insects encountering a linear barrier (such as a habitat corridor) will turn to follow the barrier edge rather than moving through or over the barrier (Haddad and Baum 1999).

It has been proposed that presence of a vertical physical barrier might limit the dispersal of house flies beyond the boundary of the facility on which they are produced (Fried et al. 2005, Dubie 2014). There is some evidence that artificial barriers can impact the movement of house flies, which have been shown to become more aggregated in taller (3m) rather than shorter (1.5m) manmade corridors (Dubie 2014). Additionally, when traps were placed both inside and outside of the corridor the outside traps collected fewer marked flies when the barriers were in place, but became equal when the barriers were removed (Dubie 2014). The higher corridors also had fewer marked flies collected on traps placed at the top (Dubie 2014). This indicates that

house flies are interacting with the barrier and perhaps their movements can be altered through the placement of an artificial barrier, which serves to simply attract flies, or attract and hold them.

### **Relationship of Wind and Barriers on Insect Flight**

Vertical barriers create areas of turbulent air on the leeward side of the structure, commonly resulting in reduced wind velocities when compared with nearby open areas (Lewis 1966). These protected areas can provide a harborage for flying insects (Lewis 1965). In a series of experiments using both artificial and natural barriers, slower wind velocities increased turbulence. However, the permeability (openness) of the barrier and the size of insect (in order of importance) had the largest impact on the number of insects accumulated leeward of a barrier (Lewis 1965, 1966). But to what extent these factors influence accumulation is still unknown. Later work has shown that the number of insects accumulating leeward of the wind break is related to both permeability of the wind break and average wind speed. More permeable wind breaks collected fewer insects near the wind break (Lewis and Stephenson 1966). But as the permeability of the wind break decreased (becomes more closed) more insects were accumulated near the wind break (Lewis 1965). Depending on permeability and wind speed, the area of maximum accumulation (distance leeward of a barrier) can vary. In slow winds, insects accumulate closer to the barrier, and as winds increase so does the zone of maximum accumulation (Lewis and Stephenson 1966).

The angle of incidence (wind direction in relation to the fence) is also very important for smaller insects where the more perpendicular the wind is to the fence the more insects will accumulate leeward (Lewis 1966). These accumulations of insects are correlated with slower

wind speeds and an associated increase in turbulence (Lewis 1965), effectively extending the boundary layer for insects nearest the fence.

As would be expected, the height of the windbreak will also impact the flying height of insects collected leeward, where taller windbreaks result in an increase in the number of insects collected at heights equal to or less than the height of the windbreak relative to no windbreak at all (Lewis 1967). Thus, the presence of a taller windbreak results in more insects being accumulated leeward with a subsequent increase in flying height with increasing barrier height. Insects also have a greater accumulation at heights below the top of the windbreak, whereas insects collected above the windbreak are equally collected with and without the presence of the barrier. Lewis (1967) also provides evidence that the flying height of larger bodied insects is less impacted by the by the presence of a barrier as these insects are better able to control their flight paths under higher wind speeds. This is attributed to a deepening of the boundary layer for these insects, and if the boundary layer is already above the height of a windbreak then it stands to reason that the windbreak would not serve to deepen the boundary layer for larger insects. The height of the barrier itself does not appear to have a significant impact on the accumulation profile of insects captured leeward of the barrier. That is to say, the size of the affected area is larger but the relative proportions collected leeward of the barrier are still similar to that of a shorter barrier (Lewis 1967).

The relationship between the wind speed and the height of flight for house flies has never been directly addressed, but there are references to other dipterans with which parallels may be drawn. An artificial wind break was created to assess how flying insects accumulate both windward and leeward of the fence. There was a tendency for larger day-flying insects to accumulate in greater numbers nearest the fence on the leeward side, particularly for flies in

the family Syrphidae (Lewis 1965). The distribution resembled a logistic decline with most insects being collected closest to the fence. Smaller insects were much more evenly distributed by distance on the leeward side of the fence. Therefore, it is expected that house flies would likely accumulate like syrphids.

The purpose of this dissertation was to examine the relationship of house fly flight activity with changes in the environment (weather) and to examine the short-range dispersal behavior of house flies to support the development of novel management techniques which could be used to supplement existing control measures. In chapter 1, an observational study was conducted to investigate the diel flight activity of house flies on two different active dairies in southern California. In chapter 2, the flying height of house flies was examined using vertical 2.13m-tall sticky traps. In chapters 3 and 4, the relationship of house fly flight direction with environmental variables, and the impact a 1.83 m tall green visual target had on the direction of flight for house flies was investigated using a release-recapture study.



## REFERENCES CITED:

- Axtell, R. C. 1970.** Integrated Fly-Control Program for Caged-Poultry Houses. *Journal of Economic Entomology*. 63: 400–405.
- Axtell, R. C. 1986.** Fly Management in Poultry Production: Cultural, Biological, and Chemical. *Poultry Science*. 65: 657–667.
- Bahrndorff, S., A. Kjærsgaard, C. Pertoldi, V. Loeschcke, T. M. Schou, H. Skovgård, and B. Hald. 2012.** The Effects of Sex-Ratio and Density on Locomotor Activity in the House Fly, *Musca domestica*. *Journal of Insect Science*. 12: 71.
- Benecke, M. 2010.** Cases of Neglect Involving Entomological Evidence, p. 681. *In* *Forensic Entomology - The Utility of Arthropods in Legal Investigations*. CRC Press Taylor and Francis Group, Boca Raton, FL.
- Bishop, F. C., and E. W. Laake. 1921.** Dispersion of Flies by Flight. *Journal of Agricultural Research*. 21: 729–766.
- Black, W. C., and E. S. Krafur. 1985.** Use of Sticky Traps to Investigate Seasonal Trends in the Spatial Distribution of House Flies and Stable Flies (Diptera: Muscidae). *Journal of Medical Entomology*. 22: 550–557.
- Broce, A. B., J. R. Schwenke, and K. E. Hampton. 1991.** Landing Pattern of Stable Flies (Diptera: Muscidae) on the Alsynite Cylinder Trap: Effect of Wind Speed and Direction. *Journal of Medical Entomology*. 28: 730–733.
- Butler, J. F., A. Garcia-Maruniak, F. Meek, and J. E. Maruniak. 2010.** Wild Florida House Flies (*Musca domestica*) as Carriers of Pathogenic Bacteria. *Florida Entomologist*. 93: 218–223.
- Chabora, P. C., and A. A. Shukis. 1979.** The Automated Recording of Insect Activity: The House Fly. *Annals of the Entomological Society of America*. 72: 287–290.
- Chakrabarti, S., D. J. King, C. Afonso, D. Swayne, C. J. Cardona, D. R. Kuney, and A. C. Gerry. 2007.** Detection and Isolation of Exotic Newcastle Disease Virus from Field-Collected Flies. *Journal of Medical Entomology*. 44: 840–844.
- Chavasse, D., R. Shier, O. Murphy, S. Huttly, S. Cousens, and T. Akhtar. 1999.** Impact of Fly Control on Childhood Diarrhoea in Pakistan: Community-Randomised Trial. *The Lancet*. 353: 22–25.

- Cook, D. F., I. R. Dadour, and S. C. Voss. 2011.** Management of Stable Fly and Other Nuisance Flies Breeding in Rotting Vegetable Matter Associated with Horticultural Crop Production. *International Journal of Pest Management*. 57: 315–320.
- Cosse, A. A., and T. C. Baker. 1996.** House Flies and Pig Manure Volatiles: Wind Tunnel Behavioral Studies and Electrophysiological Evaluations. *Journal of Agricultural Entomology*. 13: 301–317.
- Dakshinamurty, S. 1948.** The common House-fly, *Musca domestica*, L., and its Behaviour to Temperature and Humidity. *Bulletin of Entomological Research*. 39: 339–357.
- Dubie, T. R. 2014.** Influence of Vegetation Height on the Dispersal of House Flies (*Musca domestica*) from Livestock Facilities and Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers (Dissertation). Oklahoma State University.
- Foil, L. D., and J. A. Hogsette. 1994.** Biology and Control of Tabanids, Stable Flies and Horn Flies. *Revue Scientifique et Technique de l'OIE*. 13: 1125–1158.
- Geden, C. J., and J. Hogsette. 1994.** Research and Extension Needs for Integrated Pest Management for Arthropods of Veterinary Importance. *Proceedings of a Workshop in Lincoln, Nebraska*. 328.
- Geden, C. J., D. A. Rutz, R. W. Miller, and D. C. Steinkraus. 1992.** Suppression of House Flies (Diptera: Muscidae) on New York and Maryland Dairies Using Releases of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) in an Integrated Management Program. *Environmental Entomology*. 21: 1419–1426.
- Gerry, A. C., G. E. Higginbotham, L. N. Periera, A. Lam, and C. R. Shelton. 2011.** Evaluation of Surveillance Methods for Monitoring House Fly Abundance and Activity on Large Commercial Dairy Operations. *Journal of Economic Entomology*. 104: 1093–1102.
- Godwin, R. M., D. G. Mayer, G. W. Brown, D. M. Leemon, and P. J. James. 2018.** Predicting Nuisance Fly Outbreaks on Cattle Feedlots in Subtropical Australia. *Animal Production Science*. 58: 343–349.
- Graczyk, T. K., R. Knight, R. H. Gilman, and M. R. Cranfield. 2001.** The Role of Non-Biting Flies in the Epidemiology of Human Infectious Diseases. *Microbes and Infection*. 3: 231–235.
- Hanec, W. 1956.** A Study of the Environmental Factors Affecting the Dispersion of House Flies (*Musca domestica* L.) in a Dairy Community Near Fort Whyte, Manitoba. *The Canadian Entomologist*. 88: 270–272.
- Hansson, L. 1991.** Dispersal and Connectivity in Metapopulations. *Biological journal of the Linnean Society*. 42: 89–103.

- Holway, R. T., W. A. Mitchell, and A. A. Salah. 1951.** Studies on the Seasonal Prevalence and Dispersal of the Egyptian Housefly. *Annals of the Entomological Society of America*. 44: 489–510.
- Hung, K., D. Theuret, and A. C. Gerry. 2013.** House Fly (*Musca domestica* L.). *Insect Pests of Animals*. [http://veterinaryentomology.ucr.edu/pdfs/house\\_fly.pdf](http://veterinaryentomology.ucr.edu/pdfs/house_fly.pdf)
- Ingimarsdóttir, M., J. Ripa, and K. Hedlund. 2013.** Corridor or drift fence? The Role of Medial Moraines for Fly Dispersal over Glacier. *Polar Biology*. 36: 925–932.
- Keiding, J. 1986.** The House Fly: Biology and Control. WHO VBC. 86.937: 1–62.
- Khamesipour, F., K. B. Lankarani, B. Honarvar, and T. E. Kwent. 2018.** A Systematic Review of Human Pathogens Carried by the Housefly (*Musca domestica* L.). *BMC Public Health*. 18: 1049.
- Kjærsgaard, A., W. U. Blanckenhorn, C. Pertoldi, V. Loeschcke, C. Kaufmann, B. Hald, N. Pagès, and S. Bahrndorff. 2015.** Plasticity in Behavioural Responses and Resistance to Temperature Stress in *Musca domestica*. *Animal Behaviour*. 99: 123–130.
- Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999.** Houseflies: not Simple Mechanical Vectors of Enterohemorrhagic *Escherichia coli* O157: H7. *American Journal of Tropical Medicine and Hygiene*. 61: 625–629.
- Krafsur, E. S., W. C. Black, C. J. Church, and D. A. Barnes. 1985.** Age Structure and Reproductive Biology of a Natural House fly (Diptera: Muscidae) Population. *Environmental Entomology*. 14: 159–164.
- Kramer, J. P. 1980.** The House-Fly Mycosis Caused by *Entomophthora muscae*: Influence of Relative Humidity on Infectivity and Conidial Germination. *Journal of the New York Entomological Society*. 88: 236–240.
- Lawrence, M. G. 2005.** The Relationship between Relative Humidity and the Dewpoint Temperature in Moist Air: A Simple Conversion and Applications. *Bulletin of the American Meteorological Society*. 86: 225–234.
- Lazarus, W. F., D. A. Rutz, R. W. Miller, and D. A. Brow. 1989.** Costs of Existing and Recommended Manure Management Practices for House Fly and Stable Fly (Diptera: Muscidae) Control on Dairy Farms. *Journal of economic entomology*. 82: 1145–1151.
- Legner, E. F., and C. W. McCoy. 1966.** The Housefly, *Musca domestica* Linnaeus, as an Exotic Species in the Western Hemisphere Incites Biological Control Studies. *The Canadian Entomologist*. 98: 243–248.
- Lewis, T. 1965.** The Effects of an Artificial wWnbreak on the Aerial Distribution of Flying Insects. *Annals of Applied Biology*. 55: 503–512.

- Lewis, T. 1966.** An Analysis of Components of Wind Affecting the Accumulation of Flying Insects Near Artificial Windbreaks. *Annals of Applied Biology*. 58: 365–370.
- Lewis, T. 1967.** The Horizontal and Vertical Distribution of Flying Insects Near Artificial Windbreaks. *Annals of Applied Biology*. 60: 23–31.
- Lewis, T., and J. W. Stephenson. 1966.** The Permeability of Artificial Windbreaks and the Distribution of Flying Insects in the Leeward Sheltered Zone. *Annals of Applied Biology*. 58: 355–363.
- Lewis, T., and L. R. Taylor. 1965.** Diurnal Periodicity of Flight by Insects. *Transactions of the Royal Entomological Society of London*. 116: 393–435.
- Lysyk, T. J., and R. C. Axtell. 1986.** Movement and Distribution of House Flies (Diptera: Muscidae) Between Habitats in Two Livestock Farms. *Journal of Economic Entomology*. 79: 993–998.
- Malik, A., N. Singh, and S. Satya. 2007.** House fly (*Musca domestica*): A Review of Control Strategies for a Challenging Pest. *Journal of Environmental Science and Health, Part B*. 42: 453–469.
- Marquez, J. G., and E. S. Krafur. 2002.** Gene Flow Among Geographically Diverse Housefly Populations (*Musca domestica* L.): A Worldwide Survey of Mitochondrial Diversity. *Journal of Heredity*. 93: 254–259.
- McCarthy, K., A. Kjærsgaard, S. Bahrndorff, T. M. Schou, T. Manenti, V. Loeschcke, and C. Pertoldi. 2015.** The Effect of Social Isolation on Locomotor Activity in the Houseflies (*Musca Domestica*). *Journal of Insect Behavior*. 28: 288–296.
- McKie, R. 2017.** The latest threat to Antarctica: An Insect and Plant Invasion. *The Observer*.
- Meyer, J. A., and J. J. Petersen. 1983.** Characterization and Seasonal Distribution of Breeding Sites of Stable Flies and House Flies (Diptera: Muscidae) on Eastern Nebraska Feedlots and Dairies. *Journal of Economic Entomology*. 76: 103–108.
- Morgan, N. O., and R. Lee. 1977.** Vegetative Barriers Influence Flight of Salt Marsh Greenheads. *Mosquito News*. 37: 263–267.
- Mullen, G. R., and L. A. Durden. 2009.** *Medical and Veterinary Entomology*, 2nd ed. Academic Press, China.
- Mullens, B. A., N. C. Hinkle, and S. E. Coralie. 1996.** Impact of Altering Manure Removal Schedules on Pest flies (Diptera: Muscidae) and Associated Predators (Coleoptera):

Histeridae, Staphylinidae; Acarina: Macrochelidae) in Caged-Layer Poultry Manure in Southern California. *Journal of Economic Entomology*. 89: 1406–1417.

**Mullens, B., N. Hinkle, C. Szijj, and D. Kuney. 2001.** Managing Manure and Conserving Predators Helps Control Flies in Caged-Layer Poultry Systems. *California Agriculture*. 55: 26–30.

**Murvosh, C. M., R. L. Fye, and G. C. Labrecque. 1964.** Studies on the Mating Behavior of the House Fly, *Musca domestica* L. *Ohio J. Sci.* 64: 264–71.

**Murvosh, C. M., and C. W. Thaggard. 1966.** Ecological Studies of the House Fly. *Annals of the Entomological Society of America*. 59: 533–547.

**Ngoen-klan, R., K. Moophayak, T. Klong-klaew, K. N. Irvine, K. L. Sukontason, C. Prangkio, P. Somboon, and K. Sukontason. 2011.** Do Climatic and Physical Factors Affect Populations of the Blow fly *Chrysomya megacephala* and House Fly *Musca domestica*? *Parasitology Research*. 109: 1279–1292.

**Nuttall, G., E. Hindle, and G. Merriman. 1914.** The Range of Flight of *Musca domestica*: Report upon Experiments. *The Journal of hygiene*. 14: 23.

**Olsen, A. R. 1998.** Regulatory Action Criteria for Filth and Other Extraneous Materials III. Review of Flies and Foodborne Enteric Disease. *Regulatory Toxicology and Pharmacology*. 28: 199–211.

**Parker, A. H. 1962.** Studies on the Diurnal Rhythms of the Housefly, “*Musca domestica*” L., in a Dry Tropical Environment.

**Parker, R. R. 1916.** Dispersion of *Musca domestica* Linnaeus Under City Conditions in Montana. *Journal of Economic Entomology*. 9: 325–354.

**Pickens, L. G., N. O. Morgan, J. G. Hartsock, and J. Smith. 1967.** Dispersal Patterns and Populations of the House Fly Affected by Sanitation and Weather in Rural Maryland. *Journal of Economic Entomology*. 60: 1250–1255.

**Quarterman, K. D., J. W. Kilpatrick, and Willis Mathis. 1954.** Fly Dispersal in a Rural Area Near Savannah, Georgia. *Journal of Economic Entomology*. 47: 413–419.

**Reddy, M. V. 1981.** Diurnal Variations in Abundance of the Housefly *Musca domestica* in a Tropical Village. *Oikos*. 36: 374–375.

**Schoof, H. F. 1959.** How Far do Flies Fly: and What Affect Does Flight Pattern Have on Their Control. *Pest Control*. 27: 16–24.

**Schoof, H. F., and R. E. Siverly. 1954.** Multiple Release Studies on the Dispersion of *Musca domestica* at Phoenix, Arizona. *Journal of Economic Entomology*. 47: 830–838.

- Schou, T. M., S. Faurby, A. Kjærsgaard, C. Pertoldi, V. Loeschcke, B. Hald, and S. Bahrndorff. 2013.** Temperature and Population Density Effects on Locomotor Activity of *Musca domestica* (Diptera: Muscidae). *Environmental Entomology*. 42: 1322–1328.
- Schulze, T. L., E. J. Hansens, and J. R. Trout. 1975.** Some Environmental Factors Affecting the Daily and Seasonal Movements of the Salt Marsh Greenhead, *Tabanus nigrovittatus*. *Environmental Entomology*. 4: 965–971.
- Schurrer, J. A., S. A. Dee, R. D. Moon, K. D. Rossow, C. Mahlum, E. Mondaca, S. Otake, E. Fano, J. E. Collins, and C. Pijoan. 2004.** Spatial Dispersal of Porcine Reproductive and Respiratory Syndrome Virus-Contaminated Flies after Contact with Experimentally Infected Pigs. *American Journal of Veterinary Research*. 65: 1284–1292.
- Semakula, L. M., R. A. J. Taylor, and C. W. Pitts. 1989.** Flight Behavior of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in a Kansas dairy barn. *Journal of medical entomology*. 26: 501–509.
- Shinoda, O., and T. Ando. 1935.** Diurnal Rhythm of Flies. *Botany and Zoology*. 3.
- Skovgard, H., and G. Nachman. 2004.** Biological Control of House Flies *Musca domestica* and Stable Flies *Stomoxys calcitrans* (Diptera Muscidae) by Means of Inundative Releases of *Spalangia cameroni* (Hymenoptera Pteromalidae). *Bulletin of Entomological Research*. 94: 555–567.
- Stamps, J. A., M. Buechner, and V. V. Krishnan. 1987.** The Effects of Edge Permeability and Habitat Geometry on Emigration from Patches of Habitat. *The American Naturalist*. 129: 533–554.
- Tabashnik, B. E. 1989.** Managing Resistance with Multiple Pesticide Tactics: Theory, Evidence, and Recommendations. *Journal of economic entomology*. 82: 1263–1269.
- Taylor, L. R. 1960.** The Distribution of Insects at Low Levels in the Air. *Journal of Animal Ecology*. 29: 45–63.
- Taylor, L. R. 1963.** Analysis of the Effect of Temperature on Insects in Flight. *The Journal of Animal Ecology*. 99–117.
- Tsutsumi, C. 1966.** Studies on the Behavior of the Housefly, *Musca domestica* L. I. The Behavior and Activity Patterns Under Experimental Conditions with Special Reference to the Nighttime Resting Habit. *Japanese Journal of Medical Science and Biology*. 19: 155–164.
- Tsutsumi, C. 1968.** Studies on the Behavior of the House Fly, *Musca Domestica* L. *Japanese Journal of Medical Science and Biology*. 21: 195–204.

**Tsutsumi, C. 1973.** Characteristics of the Daily Behavior and Activity Patterns of the Adult Housefly with Special Reference to Time-Keeping Device. *Japan J. Med. Sci. Biol.* 26: 119–141.

**Watt, J., and D. R. Lindsay. 1948.** Diarrheal Disease Control Studies. *Pub. Health Rep.* 63: 1319–1334.

**West, L. S. 1951.** *The Housefly, Its Natural History, Medical Importance and Control.* Comstock Publishing Co., Ithaca, New York.

**Wratten, S. D., M. H. Bowie, J. M. Hickman, A. M. Evans, J. R. Sedcole, and J. M. Tylianakis. 2003.** Field Boundaries as Barriers to Movement of Hover Flies (Diptera: Syrphidae) in Cultivated Land. *Oecologia.* 134: 605–611.

## CHAPTER 1

### Diel Activity of Adult House Flies on Two Southern California Dairies

#### ABSTRACT

House flies (*Musca domestica* L.) are common synanthropic pests associated with many confined animal operations, such as dairy farms, and are known carriers of many disease-causing pathogens of both human and animal. Given how common this insect pest is, surprisingly little is known about its daily flight activity on an active dairy. In July 2014, and July 2017 through November 2017, sticky traps placed on two southern California dairies were examined for house fly flight activity at hourly intervals from 30 minutes before civil dawn through civil dusk. The flight activity of house flies varied drastically over the course of a day. House flies were captured in greatest numbers during the mornings, but male house fly flight activity peaked before female flight activity. Peak flight activity periods were more similar among the sexes on days when temperature was lower. Temperature was correlated with changes in house fly flight activity throughout the day, while light intensity was correlated with flight activity from morning through midday and wind speed was correlated with flight activity during the late afternoon. Collection period (time of day) was a useful predictor of house fly activity, as time is essentially a proxy for diel changes in temperature, humidity, light intensity, and even wind speed in southern California. The diel variation in house fly flight activity noted in this study should be considered when developing a house fly monitoring program and may also be useful in determining appropriate timing of pesticide applications.



## INTRODUCTION

House flies (*Musca domestica* L.) are one of the most common and pestiferous insects found on confined animal production and waste disposal facilities. House flies are capable of mechanically transmitting pathogens that impact both human and animal health (Kobayashi et al. 1999, Graczyk et al. 2001, Pitkin et al. 2009, Pugh et al. 2014). Additionally, house flies can be a significant source of nuisance, in some cases prompting legal action, as flies disperse from concentrated development sites into surrounding neighborhoods (Thomas and Skoda 1993, Adams 2003, Lole 2005).

Many animal producers implement some form of pest management to control adult house flies, but there still is a need to develop and refine pest management methodologies for house flies while simultaneously easing the economic burden of pest control on producers. While residual insecticide sprays and granular fly baits will persist in the environment and thus can be applied for fly control independent of time of day, it is recommended that fast-acting, short-lived insecticides (thermal fogs and ultra-low volume sprays) be applied in the mornings when most flies have moved outdoors after leaving their overnight resting sites (Barnard 2003). More precise timing of application of fast acting insecticides (mists and thermal-fogs and ULV-sprays) to times of day when house flies are most vulnerable will result in better levels of control per application (Stevenson and Cocke 2000).

Changes in house fly flight activity throughout the day will likely have an impact on some house fly monitoring techniques, especially if they are implemented for short periods of time and are inconsistent in the time of day when the monitoring systems are deployed. For instance, Scudder grids have long been used to monitor house fly activity (Scudder 1947), but the grid is generally in place for only a short period of time (minutes) before making an

instantaneous count of fly activity. If monitoring counts are performed under dissimilar conditions over multiple days, it is anticipated that diel changes in house fly activity level could be misconstrued as changes in actual house fly abundance. Other house fly monitoring systems such as sticky fly ribbons, or spot cards are also used to measure of house fly flight activity (Gerry et al. 2011) and are just as susceptible to daily fluctuations in house fly flight activity if they are implemented only for short periods of time or are inconsistent in the time of day they are deployed or removed. Therefore, having a better understanding of daily house fly flight activity over a range of environmental conditions would directly impact commonly used surveillance technologies by optimizing times when traps are deployed to encompass time periods when house flies are most active.

The diurnal activity of house flies is not well understood. In Japan adult house fly activity was reported to shift from a unimodal pattern with a single activity peak around 1100 hr in mid-May to a bimodal activity pattern with peak flight activity at 0900 hr and 1500 hr from late May through September (Shinoda and Ando 1935, West 1951). It is suggested that this shift in peak activity pattern was a response by house flies to increasing daytime temperature during the summer months. A putative bimodal house fly activity pattern was also shown in a second study (Semakula et al. 1989), with the first peak in activity occurring in early afternoon followed by a second smaller activity peak in the late afternoon to early evening. Small crepuscular peaks in flight activity were also noted in the Semakula study in addition to the two dominant activity peaks. House flies collected indoors in a concrete building had a single activity peak in early afternoon coinciding when food was brought into the building. However, in a separate domicile where food was present for most of the day, house fly activity plateaued from 1300-1600 (Reddy 1981). Little information was given about trapping methods or the relationship of house

fly collections with changes in environmental variables, but the authors attributed changes in activity to the presence or absence of food as an attractant. Female house fly flight activity under laboratory conditions is reported to be unimodal with the peak in flight activity occurring approximately 5 hr after the lights turned on when flies were held under a 12:12 L:D cycle for the previous 3 days (Tsutsumi 1966).

House fly diurnal flight activity is positively correlated with temperature (Semakula et al. 1989), and negatively correlated with humidity (Dakshinamurty 1948), while the onset of flight activity of diurnal insects is more often correlated with light intensity (Taylor 1963), though in truth these variables are confounded and difficult to separate (Murvosh and Thaggard 1966). Taylor (1963) postulated that insect flight activity is controlled by an interaction of both light and temperature. If temperatures never exceeds the minimum flight threshold temperature, then insects are physiologically restricted and cannot take flight, but if temperatures are above this minimum then the occurrence of flight becomes independent of temperature (Taylor 1963) and is more likely modulated by other factors including light.

Temperature is often a good predictor of house fly capture rate (an indication of house fly activity) when house fly capture on sampling devices is aggregated over a week-long period (Goulson et al. 2005, Godwin et al. 2018). This weekly capture data are most useful as a record of relative change in adult house fly abundance across weeks or months, as longer-term changes in temperature will largely affect larval development rate as well as other life history traits.

The effect of temperature on daily flight activity rhythms under field conditions was examined for flies in a Kansas dairy barn by Semakula et al. (1989). In the Semakula study, unbaited suction traps showed that peak flight activity of mixed sex house flies occurred at the same time as temperature peaked in early-midafternoon, and flight activity was not inhibited at

the highest temperatures recorded (up to 42°C). Whether house fly flight activity in an outdoors setting would be similarly related to ambient temperature is unknown. In a laboratory setting, adult house fly locomotor activity has been positively correlated with temperature up to 30°C for males and 35°C for females, above which activity becomes negatively correlated with temperature (Schou et al. 2013). However, this was a lab study that looked at general walking activity and not flight activity.

The influence of wind speed on house fly activity is also poorly understood, as wind speed estimation is problematic due to extreme spatial and temporal variability. It has been proposed that most flying insects reduce their flight activity when wind speeds exceed the insects' maximum flight speed (Lewis and Dibley 1970, Taylor 1974). The maximum flight speed of house flies has not been published, however the observed average flight speed for an adult house fly is approximately 2 m/s (West 1951, Shepard et al. 1972, Wagner 1986, Dahlem 2009), therefore it would be expected that house fly flight activity would begin to decrease when wind speeds are above 2 m/s. The effect of wind direction on house fly flight activity and direction of flight has been largely neglected, perhaps due to the large variability in wind speed and direction and to the large geographic scales and to the length of the time scales for most house fly release-recapture studies.

The purpose of the current study is to determine the time of peak house fly flight activity on commercial dairies in southern California, and to determine how environmental factors impact flight activity. Identifying peak flight activity periods will inform recommendations for applications of non-persistent or fast-acting pesticides targeting these active flies. Furthermore, a better understanding of the relationship of house fly activity to environmental conditions will improve adult house fly surveillance methodologies. This is

particularly important when house fly surveillance is not conducted over longer time periods (several days to weeks) or at least at a consistent time of day when environmental factors might be expected to be most similar across days. When house fly surveillance is conducted over short periods of time (hours to days), a failure to appreciate environmental impacts on flight activity may result in misinterpretation of surveillance counts to suggest a real change in overall fly abundance rather than just short-term fluctuation in house fly activity driven by changes in environmental conditions.

## **METHODS**

Diurnal flight activity of adult house flies was examined on two large active dairy facilities (BS Dairy and OS Dairy; Figure 1.1) near the southern California town of San Jacinto. At the BS dairy, trapping was conducted over four days in July of 2014, with one additional day in July 2017 for confirmation of the earlier results. At the OS Dairy, trapping was conducted over seven days from June-November of 2017.

Flight activity was recorded hourly from 30 minutes before civil dawn (ranging from 5:50am to 7:07am over the study) through civil dusk (7pm to 9:00pm) using 12 sticky traps consisting of a wooden frame (30.5cm tall x 70cm wide) with a clear plastic sticky sheet (Olson Products, Medina, OH Part# B005BCST8M) attached to both the front and back of the frame (Figure 1.2A). The top of each trap was positioned 1m above ground and there was a minimum of 10m separation among traps. At the BS dairy (Figure 1.1A), traps were grouped into four trapping areas which were selected to avoid impacting normal dairy operations and to sample a variety of habitats starting from the center of the dairy moving out to the periphery (center of the dairy between two pens, east edge of the dairy with a cattle pen on the west, in the bottom

of a drainage ditch, and on the east side of the drainage ditch). All traps were positioned with the sticky faces oriented east and west, and trap locations remained the same over all trapping days at the BS dairy. At the OS dairy (Figure 1.1B), trap placement and trap face orientation by cardinal direction was randomly selected each day of the study. The layout of this dairy allowed for more flexibility in trap placement than at the BS dairy.

At the end of each one-hour collection period, captured flies were identified to species, sexed, counted, and removed from the trap. Thus, each hourly count provided a measure of house fly flight activity by sex during the preceding 1hr collection period. Depending upon day length (time from dawn-dusk) for each study date, there were 13-16 collection periods completed through civil dusk when trapping ceased.

## **DATA ANALYSIS**

For each dairy, the total number of male and female flies captured across all traps and dates was examined using a chi-square goodness-of-fit test for equal distribution by fly sex. The number of flies captured during each collection period across all traps and all collection dates was similarly examined using a chi-square goodness-of-fit test for equal distribution by collection period (time of day). Dairies were analyzed separately since trap placement and year of collection were different. These and all subsequent analyses were performed using the R Statistical Software version 3.5.1 (R Core Team 2017).

### **Flight Activity by Collection Period**

Differences in house fly flight activity by collection period for each dairy were evaluated using a LMM with the number of house flies pooled over house fly sex within each

collection period for each sampling date. The total number of house flies was square-root transformed before analysis to meet test assumptions. The collection date was included as a random effect since each dairy was sampled over multiple days. Due to autocorrelation of the residuals, the correlation structure of the random effect was modified to include lag 1 autoregressive (AR1) term. The main effect of collection period was evaluated using ANOVA (type II) tests using Satterthwaite's approximation of the denominator degrees of freedom on restricted maximum likelihood models (Luke 2017).

Since house flies were sampled at hourly intervals, temporal autocorrelation became an issue as it violates the underlying assumption of independence of errors (Crawley 2013). The presence of autocorrelation among the residuals within a LMM model was visually assessed using the *acf()* function from the 'stats' package (R Core Team 2017). If autocorrelation was found, then the correlation structure of the model was modified to include a lag 1 autoregressive term, using the 'nlme' package in R (Pinheiro et al. 2018). This method is preferable as it is not recommended to add a lagged term as a fixed effect to linear mixed models (Wilkins 2018). If no autocorrelation of the residuals was observed then the LMM's were fit using standard (Bolker et al. 2009, Crawley 2013) techniques.

For all analyses, initial full models were evaluated against the reduced model using likelihood ratio tests (LRT), to identify the simplest adequate description of the data (Crawley 2013) and assessed using Akaike Information Criterion (AIC) after each round of model reduction. A lower AIC equates to a more favorable combination of model fit or parsimony (Crawley 2013). The linear, and linear-mixed-effect, model assumptions of linearity and homoscedasticity of the residuals were visually checked using quantile-quantile plots (Q-Q plots) and residual versus fitted plots, respectively (Crawley 2013). Model overdispersion was checked

using both the residual versus fitted plot and using the method outlined in (<http://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion>) where the sum of the squared Pearson residuals should be  $\chi^2$  distributed (Bolker et al. 2009). Over dispersion is the occurrence of more variance in the data than predicted by the statistical model (Bolker et al. 2009). For LMM's the conditional R-squared ( $R^2_c$ ) was used as metric of model quality and was calculated for each mixed effect model using the R package 'piecewiseSEM' (Lefcheck 2016). The conditional R-squared is an estimate of the total variation explained by both the fixed and random effects in the model.

### **Sex Ratio by Collection Period**

Variation in house fly sex ratio among collection periods for each dairy was examined using a GLMM using the R package 'lme4' since the response was binomial (Bates et al. 2015) and the 'nlme' package does not allow for the fitting of GLMM's. The proportion of house flies collected within each collection period that were female was included as the response variable, with collection period included as a fixed effect, and date of collection as a random effect. A binomial error distribution with a logit link function was specified. Wald  $\chi^2$  tests were used to assess the significance of the model coefficients. All pairwise comparisons of house fly sex ratios at each collection period was performed with the R package 'emmeans' (Lenth 2018), using Tukey's correction of the p-value ( $\alpha=0.05$ ). Model assumptions were checked using the methods described above.



### **Effect of Environmental Variables on Period of Peak Flight Activity**

On each sampling date, the collection period with the greatest total number of fly captures for each sex was recorded as the sex-specific peak flight activity period for that sampling date. When two or more collection periods on the same day had equivalent numbers of house fly captures (by sex), the collection period with the greatest number of flies captured during the adjacent collection periods was recorded as the peak flight activity period (occurred on a single date for female flies). To assess if observed environmental variables were associated with a shift in the time of peak activity for house flies, the times of peak house fly activity were regressed using a linear model with peak collection period as the response variable and the day time mean values (not full 24hr period but during daylight hours) for temperature, light intensity, percent relative humidity, wind speed, date of collection, and house fly sex as main effects. The two-way interactions of house fly sex with each of the environmental variables (including date) were also included in the full model. The data from both dairies were combined for this analysis. The full model, including all fixed effects and interaction terms, was reduced using LRT tests, until a final minimally adequate model was found. Model assumptions of linearity and homoscedasticity of the residuals was visually checked using quantile-quantile plots (Q-Q plots) and residual versus fitted plots, respectively (Crawley 2013).

### **Effect of Environmental Variables on House Fly Activity**

To evaluate the effect of environmental variables on flight activity for each collection period, collection periods were categorized into one of three time of day categories: dawn (first two collection periods), dusk (last two collection periods), or midday (all remaining collection periods). Groupings were based upon similar overall flight activity patterns during these periods

where fly numbers increased over time during dawn, remained somewhat stable or had only a slight decrease over time during midday, and decreased over time during dusk. For each time of day category, the number of house flies by fly sex was pooled over all traps within each collection period for each sampling date to give a single value for each collection period from each date. Dairies were not separated for this analysis. Linear mixed effect models (LMM), using the R package 'nlme,' were created to assess the impact each observed environmental variable had on house fly flight activity. House fly sex, temperature, light intensity, relative humidity, and wind speed and their two-way interactions with house fly sex were included as fixed effects, while date of collection was included as a random effect. The number of house flies, by sex, was cube-root transformed to improve normality before analysis. Models were reduced following methods outlined above. The significance of individual model coefficients were assessed using Wald  $\chi^2$  tests (Luke 2017).

## RESULTS

A total of 9,233 house flies were captured during this study, with 3,572 house flies captured at the BS dairy and 5,661 house flies captured at the OS dairy. The sex ratio of captured flies was skewed toward greater capture of male flies at both dairies, with 55.9% male and 44.1% female ( $\chi^2=49.86$ ;  $df=1$ ;  $p<0.001$ ) at the BS Dairy and with 59.5% male and 40.5 % female ( $\chi^2=204.9$ ;  $df=1$ ;  $p<0.001$ ) at the OS Dairy. The number of flies captured was not distributed evenly across collection periods for either the BS dairy ( $\chi^2=748.83$ ;  $df=15$ ;  $p<0.001$ ) or the OS dairy ( $\chi^2=1116.5$ ,  $df=14$ ;  $p<0.001$ ), suggesting that house fly flight activity changes with time throughout the day.

### **Flight Activity by Collection Period**

House fly flight activity began very near or soon after civil dawn, with a single peak in overall flight activity during mid to late morning evident on most sampling dates (Figure S1.1). Collection period (time of day), was significantly related to overall house fly flight activity (Figure 1.3 A&B) at both the BS dairy ( $F_{15,140}=6.70$ ;  $p<0.001$ ;  $R^2_c=0.41$ ) and the OS dairy ( $F_{14,173}=7.28$ ;  $p<0.001$ ;  $R^2_c=0.49$ ). At the BS dairy a greater number of male flies were captured during the morning collection periods while female flies dominated in afternoon periods (Figure 1.3A). At the OS dairy, male flies were captured in greater number during nearly every collection period. Significant differences by house fly sex were seen in both models and were removed from the final model (above) and analyzed separately by looking at the proportional change in house fly sex by collection period.

### **Sex Ratio by Collection Period**

The sex ratio of captured house flies varied significantly by collection period at both the BS dairy (Wald:  $\chi^2_{15}=245.21$ ;  $p<0.001$ ) and OS dairy (Wald:  $\chi^2_{14}=119.91$ ;  $p<0.001$ , Figure 1.4). The proportion of house flies that were female was lowest during the first 3 collection periods at the BS dairy (<31.5% female,  $p<0.02$ ) and the first 2 collection periods at the OS dairy (<30.0% female,  $p<0.05$ ), increasing to  $57.0\pm 1.1\%$  (mean $\pm$ SE) during collection periods 7-13 at the BS dairy and  $44.6\pm 1.8\%$  female during collection periods 6-10 at the OS dairy.

### **Effect of Environmental Variables on Period of Peak Flight Activity**

With both dairies and all dates of collection combined, male flies had a significantly earlier peak in activity ( $t=2.80$ ,  $df=11$ ,  $p=0.017$ ), with mean peak activity during the 4 hour

( $3.58 \pm 0.50$ ) collection period corresponding to a clock time ranging from 0900 to 1000 hours when compared to females which on average peaked later in the day during the 7 hour collection period ( $7.00 \pm 0.95$ ) corresponding to a time ranging from 1200-1400 (Table 1.1). Because males peaked earlier, they also tended to be more active early in the day until 5-6 hr after dawn.

The main effects of house fly sex ( $F_{1,20}=13.35$ ;  $p=0.002$ ) and the interaction term of house fly sex with mean daily temperature ( $F_{1,20}=8.94$ ;  $p=0.007$ ) were both significant predictors of peak house fly flight activity ( $R^2=0.46$ ) while the main effect of mean daily temperature was not ( $F_{1,20}=0.20$ ;  $p=0.662$ ). The main effect of temperature was not significant since male and female house flies had opposite slopes. The collection period in which peak activity for female house flies occurred was positively related to temperature where for every  $1\text{C}^\circ$  increase in temperature increased the peak time (hours since dawn) by 0.27 hours, while the opposite effect was seen for male house flies, where every  $1\text{C}^\circ$  increase in temperature resulted in a 0.20 hour decrease in the peak time (Figure 1.5).

### **Effect of Environmental Variables on House Fly Activity**

House fly flight activity was significantly related to most of the observed environmental variables (Table 1.2). However, all environmental variables were significantly correlated with each other especially relative humidity which was had a strong negative correlation with temperature (Table S1.1) and was removed from all final models. Only wind speed and solar radiation were independent of one another.

Significant models were recovered for time periods categorized as dawn ( $R^2_c=0.88$ ), midday ( $R^2_c=0.55$ ) and dusk ( $R^2_c=0.67$ ) (Table 1.2 and Figures S1.2, 1.6, 1.7, 1.8). House fly sex

was an important interaction term during the morning and midday collection periods. Temperature was the most consistent predictor of house fly activity during dawn, midday and dusk (Table 1.2; Figures 1.6A, 1.7A, 1.8A respectively), though there was a significant interaction of temperature and house fly sex during midday. Light intensity was a significant predictor of house fly activity during morning and midday, but not during dusk (Figures 1.6B, 1.7B respectively). Light intensity had a significant interaction with house fly sex only during dawn. Wind speed was a significant predictor only during dusk (Figure 1.8B). During the morning collection period males and females both had positive responses to increases in temperature and light intensity, but the rate of increase significantly differed between the sexes in relation to light intensity where females had a slower response. During midday both males and females had a positive response to increasing light intensity, similar to the dawn collection periods but with a shallower slope. However, males and females appear to respond differently to temperature during this collection period where male activity decreased with increasing temperature and females had a slight increase in activity. In the dusk period temperature was again positively related to fly activity, as was wind speed.

## **DISCUSSION**

A study was undertaken to assess the diel flight activity of house flies, including differences between sexes, on active southern California dairies and to better understand the factors that may influence patterns in activity. House fly flight activity generally started near or shortly after civil dawn, increasing into the late morning when activity peaked. However, the time of peak activity was generally much earlier and more pronounced for male than for female house flies, whose activity commonly peaked later in the day relative to males. Conversely,

female activity plateaued in early afternoon before decreasing toward evening. Female flies did have a slight tendency to show a smaller peak of activity in the last 1 or 2 hours of the day, but it was not strong or consistent enough to be easily detected using the current methodology.

It was expected that house flies would exhibit a bimodal activity pattern, with a peak in fly activity in morning and again in the late afternoon (Shinoda and Ando 1935, Chabora and Shukis 1979, Semakula et al. 1989) because midday temperatures in the summer can commonly exceed 30 °C. Overall, house fly flight activity in the current study was consistently unimodal for both male and female house flies. However, if each day is observed independently some days do have a much smaller peak 1-3 hours before the end of the day, but it is difficult to distinguish these from noise in the data. It is also important to point out that previous studies specifically looked at diurnal house fly activity patterns in field-sampled house flies from within a fixed structure like a barn or other habitation (Reddy 1981, Semakula et al. 1989) and therefore might not be directly relatable to house flies collected in the current study's open-field setting. Additionally, the Reddy (1981) study had human food present during the day.

Overall, more male house flies were collected than female flies. It is expected that fly sex ratio at emergence is nearly equal (Krafsur 1985) so the skewed sex ratio on the traps may be the result of one or more of the following: female loss through dispersal from the dairy following emergence (Pickens et al. 1967), greater activity of male flies by location or height above ground (Ragland and Sohal 1973, Black and Krafsur 1985), greater flight activity of males relative to females generally across the dairy (clumping of the fly sexes), or attraction of males to the traps perhaps as resting or mating sites (Murvosh et al. 1964, Ragland and Sohal 1973). In all studies looking at house fly walking activity, males (under all test conditions) had high levels of activity when compared to females (Bahrndorff et al. 2012, Schou et al. 2013, McCarthy et al.

2015), possibly relating to the mating behavior of male house flies, where male house flies are reported to perch on vertical structures from which they will “strike” females that are flying by (Murvosh et al. 1964, Tobin and Stoffolano 1973). This suggests that some males may have used the traps in the current study as perching sites to wait for females to fly by.

Changes in house fly sex ratios were similar between dairies, with morning to early afternoon collection periods being male dominated, which evened out by the middle of the day, approximately coinciding with the collection periods when female house fly counts peaked. At the OS dairy females rarely accounted for more than 50% of the total captures per collection period; only around periods 9 and 10 were the sex ratios even. This contrasts with the BS dairy where females became predominant from collection periods 7-13, and then randomly fluctuated until the last time point. It must be pointed out that the sex ratios from the first and last collection periods may be skewed due to a smaller total number of house flies being captured.

It was expected that the time of peak fly activity would vary by date due to coarse changes in abiotic factors across months, particularly by shifts in the intensity of sunlight and changes in day length. However, date was not related to any shift in peak collection (data not shown). Only the average daytime temperature was correlated with the time of peak flight activity on any individual day, with male flight activity generally occurring earlier and female flight activity shifting later times on days with higher daytime temperature. This trend was magnified when the mean daytime temperature was above 35 °C, with male flight activity reaching a peak during the first two collection periods and female flight activity reaching a peak during the eighth collection period. House flies are clearly responding to changes in temperature

with males and females displaying differential behavioral responses during days with extreme heat.

House fly activity has been documented to change in response to environmental conditions including temperature, humidity, light intensity, and wind speed (Dakshinamurty 1948, West 1951, Murvosh and Thaggard 1966, Taylor 1974, Semakula et al. 1989, Schou et al. 2013), but these studies have largely been conducted under laboratory conditions or within protected enclosures (e.g. dairy barn) rather than in an exposed field environment. Relating these laboratory studies to the field environment is difficult due to the covarying nature of environmental variables in the field (*i.e.* an increase in temperature is often associated with a decrease in the relative humidity). As expected, in the current study most of the environmental variables observed were correlated with one another. Care must be taken when assessing the impact of a single variable on house fly flight activity because a single environmental variable cannot be controlled.

Semakula (et al. 1989) found that house flies collected from inside a dairy barn were in flight 50% of the time (*i.e.* 50% of the observed time points captured flies) when temperatures are ~21°C, but when temperatures rose to ~37°C house flies were in flight 95% of the time, demonstrating the effect temperature has on the likelihood a house fly will fly. Additionally, Kjærsgaard et al. (2015), found that house flies collected from warmer locations (Spain vs Denmark and Switzerland) flew the furthest when held at 41.5°C noting a trend for females to fly further than males. Schou et al. (2013) have shown that the walking activity of male house flies is significantly greater than females at all temperatures except at the two extremes of 10 °C and 40 °C, and that males had peak activity at 30 °C while females peaked at 35 °C. This suggests that male house flies are less likely to fly at high temperatures, perhaps because they are less



tolerant of heat stress than females. During midday in the current study, male flight activity declined over the range of observed temperatures while females had a slight increase in flight activity over the same range, suggesting that female house flies might be more tolerant of heat stress than males. Kjærsgaard et al. (2015) showed evidence of regional adaptation to heat stress in different populations of house flies in Europe. Since their most heat-stressed population came from Barcelona (average temp of 15°C and average high of 20°C), house flies in southern California may be even more adapted to high temperatures (average temp of 17.9°C and average high of 26.1°C: <https://en.climate-data.org>). If this adaptation to heat stress is true, it is possible that the upper threshold of heat tolerance was never reached for the current study. Additional studies looking at southern California house fly flight activity are needed to further test this hypothesis.

In controlled laboratory settings house fly walking activity has been shown to increase with increasing temperature from 10°C-35°C, but above 35°C activity began to decrease suggesting an upper threshold for house fly activity (Schou et al. 2013). Kjærsgaard et al. (2015) have also shown that heat tolerance differs among populations taken from regions experiencing different average temperatures, showing that house flies taken from warmer areas remain active at higher temperatures than flies taken from more temperate climates. In the current study house fly flight activity occurred at all temperatures recorded but was very reduced as temperatures neared 10°C. House fly flight activity was generally the highest when temperatures were over 15°C and peaked when temperatures were between 15°C and 30°C, but those temperatures also had the most variation in the number of house flies collected. Above 30°C the variation in the number of house flies collected decreased but the average number collected remained very stable, as seen in figure 1.6. This is an indication that house fly flight

activity for this population is not greatly affected by temperatures between 30°C and 45°C.

Minimum temperatures were never below 10.4 C° which is very near the estimated temperature where house flies are incapable of flight (West 1951, Schou et al. 2013), making a lower temperature flight threshold difficult to estimate from the present data.

The impact humidity has on house fly activity and longevity is not well understood. Overall, relative humidity is not a good predictor of house fly flight activity in southern California, although it has been suggested to have stronger impact on house fly behavior in tropical climates (Reddy 1981). The literature suggests that when relative humidity exceeds 80%, house fly mortality is significantly increased, and when temperatures are over 20°C, house flies would live longest at humidity levels around 50% (West 1951). Throughout this study relative humidity had a strong inverse relationship with temperature, and humidity was never as strong of a predictor as temperature.

Above the minimum flight threshold temperature for house flies, sunlight appears to be more strongly associated with the first onset of activity (Taylor 1963, Lewis and Taylor 1965, Semakula et al. 1989). This relationship was not observed for the current study since temperatures were never below the minimum temperature necessary for flight. Light intensity was more important at dawn and midday than during dusk. Therefore, it is likely that sunlight was the dominant cue for the onset of house fly activity as this environmental factor was a good predictor during the dawn and midday time periods.

At dusk, wind speed was a better predictor than solar radiation, perhaps because at our study location in southern California wind speeds are generally higher and more variable in the evenings than in the mornings. During the midday period there is a noticeable and significant increase in fly captures as light intensity increases, an indication that more flies are captured

when it is sunny versus cloudy. House fly flight activity has been shown to decrease during rain (Reddy 1981), but it only rained on one sampling date (August 2<sup>nd</sup>, 2017) so decreased activity during cloudy conditions was likely not a result of direct rainfall.

For many insect species, wind speeds above the insect's maximum flight speed decrease flight activity (Taylor 1974). The average flight speed of a house fly is approximately 2 m/s (West 1951, Shepard et al. 1972, Wagner 1986, Dahlem 2009) so it is somewhere above 2m/s where one would expect to see a decline in activity. At wind speeds under this, a house fly should be able to navigate freely. In the case of this study wind speed was not a very good predictor of house fly flight activity. The exception was during the dusk collection period, where there was a strong positive correlation between wind speed and house fly captures, a counterintuitive result. A possible explanation is that a single weather station was used, and wind speed is highly variable, therefore the speeds that were recorded may not accurately reflect the wind speed each individual trap experienced. Additionally, dairies are comprised of buildings and structures that would increase the boundary layer (area of slower moving air) allowing insects to keep flying even when winds speeds (as recorded by a weather station) are higher than controlled flight would allow. Therefore, wind speed is most likely to impact flying insects as they move across open country but become less impactful when these insects are near areas where there is a lot of structure like buildings or tree lines.

Using the information from this study it is recommended that point estimates of house fly populations in southern California should be made in the early afternoon, from 1200-1400 hours. As house fly activity is high and the most female house flies were active, as females are often the focus of management programs. However most important is to be consistent in the time when house fly monitoring is conducted. To alleviate most issues associated with collection

period house fly monitoring methods should be left in the field for at least 24 hours. This would smooth the daily variations of fly captures and give a better estimate of changes in the house fly population. Further work is necessary to see if these house fly behaviors are consistent among various locations (*i.e.* temperate and humid subtropical) throughout the United States and abroad.

However, if the concern is house fly dispersal or the spread of enteric pathogens, a focus on collections before noon is recommended, as house flies were most active during these times. It is there for assumed that the majority of house fly dispersal is also occurring during this time. Additional work looking at the age of house flies collected throughout the day may shed more light on the mechanisms behind the differences in peak activity by house fly sex, as it has been suggested that younger female house flies are more likely to disperse than older parous females (Pickens et al. 1967).

Based on these data any fast-acting pesticide should be applied in the morning hours when most house flies are active, as it is assumed that as activity decrease house flies are likely resting which may result in a less efficacious spray event. However, to manage house flies over a long period (summer) the most effective method is cultural control, and the removal or destruction of larval development sites, and using fast acting sprays as a last resort to manage sporadic outbreaks of pest flies like house flies.

## REFERENCES CITED

- Adams, J. 2003.** Vector: Filth Fly. *In* Poultry Training Manual. College of Agriculture, Forestry and Life Sciences: Clemson University, South Carolina.
- Bahrndorff, S., A. Kjærsgaard, C. Pertoldi, V. Loeschcke, T. M. Schou, H. Skovgård, and B. Hald. 2012.** The Effects of Sex-Ratio and Density on Locomotor Activity in the House Fly, *Musca domestica*. *Journal of Insect Science*. 12: 71.
- Barnard, D. R. 2003.** Control of Fly-Borne Diseases. *Pesticide Outlook*. 14: 222.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015.** Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software*. 67: 1–48.
- Black, W. C., and E. S. Krafur. 1985.** Use of Sticky Traps to Investigate Seasonal Trends in the Spatial Distribution of House Flies and Stable Flies (Diptera: Muscidae). *Journal of Medical Entomology*. 22: 550–557.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009.** Generalized Linear Mixed Models: A Practical Guide for Ecology and Evolution. *Trends in Ecology & Evolution*. 24: 127–135.
- Chabora, P. C., and A. A. Shukis. 1979.** The Automated Recording of Insect Activity: The House fly. *Annals of the Entomological Society of America*. 72: 287–290.
- Crawley, M. J. 2013.** *The R book*, Second edition. ed. Wiley, Chichester, West Sussex, United Kingdom.
- Dahlem, G. A. 2009.** Chapter 125 - House Fly: (*Musca domestica*), pp. 469–470. *In* Resh, V.H., Cardé, R.T. (eds.), *Encyclopedia of Insects (Second Edition)*. Academic Press, San Diego.
- Dakshinamurty, S. 1948.** The Common House-Fly, *Musca domestica*, L., and Its Behaviour to Temperature and Humidity. *Bulletin of Entomological Research*. 39: 339–357.
- Gerry, A. C., G. E. Higginbotham, L. N. Periera, A. Lam, and C. R. Shelton. 2011.** Evaluation of Surveillance Methods for Monitoring House Fly Abundance and Activity on Large Commercial Dairy Operations. *Journal of Economic Entomology*. 104: 1093–1102.
- Godwin, R. M., D. G. Mayer, G. W. Brown, D. M. Leemon, and P. J. James. 2018.** Predicting Nuisance Fly Outbreaks on Cattle Feedlots in Subtropical Australia. *Animal Production Science*. 58: 343–349.
- Goulson, D., L. C. Derwent, M. E. Hanley, D. W. Dunn, and S. R. Abolins. 2005.** Predicting Calyptate Fly Populations from the Weather, and Probable Consequences of Climate Change. *Journal of Applied Ecology*. 42: 795–804.

- Graczyk, T. K., R. Knight, R. H. Gilman, and M. R. Cranfield. 2001.** The Role of Non-Biting Flies in the Epidemiology of Human Infectious Diseases. *Microbes and Infection*. 3: 231–235.
- Kjærsgaard, A., W. U. Blanckenhorn, C. Pertoldi, V. Loeschcke, C. Kaufmann, B. Hald, N. Pagès, and S. Bahrndorff. 2015.** Plasticity in Behavioural Responses and Resistance to Temperature Stress in *Musca domestica*. *Animal Behaviour*. 99: 123–130.
- Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999.** Houseflies: Not Simple Mechanical Vectors of Enterohemorrhagic *Escherichia coli* O157:H7. *American Journal of Tropical Medicine and Hygiene*. 61: 625–629.
- Krafsur, E. S. 1985.** Age Composition and Seasonal Phenology of House-Fly (Diptera: Muscidae) Populations. *Journal of medical entomology*. 22: 515–523.
- Lefcheck, J. S. 2016.** piecewiseSEM: Piecewise Structural Equation Modelling in R for Ecology, Evolution, and Systematics. *Methods in Ecology and Evolution*. 7: 573–579.
- Russell Lenth. 2016.** Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*. 69: 1–33.
- Lewis, T., and G. C. Dibley. 1970.** Air Movement Near Windbreaks and a Hypothesis of the Mechanism of the Accumulation of Airborne Insects. *Annals of Applied Biology*. 66: 477–484.
- Lewis, T., and L. R. Taylor. 1965.** Diurnal Periodicity of Flight by Insects. *Transactions of the Royal Entomological Society of London*. 116: 393–435.
- Lole, M. J. 2005.** Nuisance Flies and Landfill Activities: An Investigation at a West Midlands Landfill Site. *Waste Management Research*. 23: 420–428.
- Luke, S. G. 2017.** Evaluating Significance in Linear Mixed-Effects Models in R. *Behavioral Research*. 49: 1494–1502.
- McCarthy, K., A. Kjærsgaard, S. Bahrndorff, T. M. Schou, T. Manenti, V. Loeschcke, and C. Pertoldi. 2015.** The Effect of Social Isolation on Locomotor Activity in the Houseflies (*Musca Domestica*). *J Insect Behavior*. 28: 288–296.
- Murvosh, C. M., R. L. Fye, and G. C. Labrecque. 1964.** Studies on the Mating Behavior of the House Fly, *Musca domestica* L. *Ohio Journal of Science*. 64: 264–71.
- Murvosh, C. M., and C. W. Thaggard. 1966.** Ecological Studies of the House fly. *Annals of the Entomological Society of America*. 59: 533–547.

- Pickens, L. G., N. O. Morgan, J. G. Hartsock, and J. Smith. 1967.** Dispersal Patterns and Populations of the House Fly Affected by Sanitation and Weather in Rural Maryland. *Journal of Economic Entomology*. 60: 1250–1255.
- Pinheiro, B. D., S. DebRoy, and D. Sarkar. 2018.** nlme: Linear and Nonlinear Mixed Effects Models.
- Pitkin, A., J. Deen, S. Otake, R. Moon, and S. Dee. 2009.** Further Assessment of Houseflies (*Musca domestica*) as Vectors for the Mechanical Transport and Transmission of Porcine Reproductive and Respiratory Syndrome Virus Under Field Conditions. *Canadian Journal of Veterinary Research*. 73: 91–96.
- Pugh, D. G., X. P. Hu, and B. Blagburn. 2014.** Habronemiasis: Biology, Signs, and Diagnosis, and Treatment and Prevention of the Nematodes and Vector Flies. *Journal of Equine Veterinary Science*. 34: 241–248.
- R Core Team. 2017.** R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ragland, S. S., and R. S. Sohal. 1973.** Mating Behavior, Physical Activity and Aging in the Housefly, *Musca domestica*. *Experimental Gerontology*. 8: 135–145.
- Reddy, M. V. 1981.** Diurnal Variations in Abundance of the Housefly *Musca domestica* in a Tropical Village. *Oikos*. 36: 374–375.
- Schou, T. M., S. Faurby, A. Kjærsgaard, C. Pertoldi, V. Loeschcke, B. Hald, and S. Bahrndorff. 2013.** Temperature and Population Density Effects on Locomotor Activity of *Musca domestica* (Diptera: Muscidae). *Environmental Entomology*. 42: 1322–1328.
- Scudder, H. I. 1947.** A New Technique for Sampling the Density of Housefly Populations. *Public Health Reports (1896-1970)*. 62: 681–686.
- Semakula, L. M., R. A. J. Taylor, and C. W. Pitts. 1989.** Flight Behavior of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in a Kansas Dairy Barn. *Journal of Medical Entomology*. 26: 501–509.
- Shepard, M., R. Noblet, and P. E. Hunter. 1972.** Biology and Flight Ability of the House Fly Following Irradiation of the Pupae. *Journal of Economic Entomology*. 65: 1721–1725.
- Shinoda, O., and T. Ando. 1935.** Diurnal Rhythm of Flies. *Botany and Zoology*. 3.
- Stevenson, D., and J. Cocke. 2000.** Integrated Pest Management of Flies in Texas Dairies. Available electronically from <http://hdl.handle.net/1969.1/87804>.

- Taylor, L. R. 1963.** Analysis of the effect of temperature on insects in flight. *The Journal of Animal Ecology*. 99–117.
- Taylor, L. R. 1974.** Insect Migration, Flight Periodicity and the Boundary Layer. *Journal of Animal Ecology*. 43: 225–238.
- Thomas, G. D., and S. R. Skoda. 1993.** Rural Flies in the Urban Environment? Proceedings of a Symposium Presented at the Annual Meeting of the Entomological Society of America, December 1989, San Antonio, TX. Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, Lincoln NE.
- Tobin, E. N., and J. G. Stoffolano. 1973.** The Courtship of *Musca* Species Found in North America. 1. The House Fly, *Musca domestica*. *Annals of the Entomological Society of America*. 66: 1249–1257.
- Tsutsumi, C. 1966.** Studies on the Behavior of the Housefly, *Musca domestica* L. I. The Behavior and Activity Patterns Under Experimental Conditions with Special Reference to the Nighttime Resting Habit. *Japanese Journal of Medical Science and Biology*. 19: 155–164.
- Wagner, H. 1986.** Flight Performance and Visual Control of Flight of the Free-Flying Housefly (*Musca Domestica* L.) III. Interactions Between Angular Movement Induced by Wide-and Smallfield Stimuli. *Philosophical Transactions of the Royal Society. London. B*. 312: 581–595.
- West, L. S. 1951.** *The Housefly, Its Natural History, Medical Importance and Control*. Comstock Publishing Co., Ithaca, New York.
- Wilkins, A. S. 2018.** To Lag or Not to Lag?: Re-Evaluating the Use of Lagged Dependent Variables in Regression Analysis. *Political Science Research and Methods*. 6: 393–411.



**Table 1.1:** Summary for each day of fly sampling showing the start time, hours since the start time when fly numbers peaked, the decimal clock time of the peak for each day, and summary statistics for each environmental variable collected on that day. Environmental statistics include min, max, mean, and standard error.

Date	<u>Female</u>			<u>Male</u>			<u>Temperature (°C)</u>			<u>Humidity (%)</u>			<u>Light Intensity (w/m<sup>2</sup>)</u>			<u>Wind Speed (m/s)</u>		
	Start Time	Coll. Period	Peak Time	Coll. Period	Peak Time	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	
17-Jul-14	5:50	7	12:50	7	12:50	16.72	32.28	24.66±1.42	33.40	77.90	52.3±3.63	0.00	978.00	427.13±97.81	1.10	7.60	3.2±0.42	
22-Jul-14	5:55	5	10:55	5	10:55	14.50	37.00	29.44±1.81	20.40	77.10	39.4±4.17	0.00	979.00	508.69±91.89	0.00	4.60	2.04±0.34	
24-Jul-14	5:56	8	13:56	2	7:56	17.61	44.00	35.3±2.21	12.60	44.90	23.81±2.24	0.00	980.00	501.44±92.02	0.00	5.50	2.26±0.44	
29-Jul-14	6:00	5	11:00	2	8:00	18.50	40.00	31.65±1.63	19.20	44.10	31.08±1.87	0.00	864.00	409.69±78.07	0.00	4.10	1.84±0.34	
6-Jul-17	5:38	13	18:38	3	8:38	17.22	41.67	32.36±1.94	26.10	63.00	44.18±2.21	1.00	934.50	471.41±87.66	0.00	3.40	1.53±0.33	
2-Aug-17	6:00	11	17:00	2	8:00	25.56	35.00	31.15±0.78	52.80	81.70	64.39±2.61	0.00	496.00	215.67±46.01	0.00	3.70	1.52±0.42	
29-Aug-17	6:24	11	17:24	1	7:24	20.70	43.80	36.83±1.99	16.00	68.30	26.54±4.67	2.00	847.50	387.75±75.93	0.00	6.60	2.47±0.56	
6-Sep-17	6:30	3	9:30	3	9:30	21.30	43.60	31.22±1.51	18.30	80.20	40.69±5.15	0.50	834.00	441.46±82.58	0.00	5.50	2.66±0.54	
14-Sep-17	6:35	6	12:35	5	11:35	12.10	25.30	20.54±0.99	44.50	86.50	57.47±3.67	0.00	728.50	315.64±61.56	0.40	5.70	2.7±0.43	
20-Sep-17	6:40	8	14:40	5	11:40	17.40	26.70	22.35±0.78	41.30	74.80	55.98±2.79	0.00	666.00	311.79±66.22	0.80	4.30	2.16±0.29	
19-Oct-17	7:00	3	10:00	4	11:00	10.40	29.40	21.96±1.52	35.70	87.20	51.01±4.5	0.00	636.00	249±59.94	0.00	3.10	1.17±0.29	
2-Nov-17	7:07	4	11:07	4	11:07	14.40	21.10	18.3±0.57	43.30	78.20	54.02±3.41	0.00	574.00	253.31±63.5	0.00	2.90	1.4±0.28	

**Table 1.2:** Linear mixed effects models of the effect of environmental variables on house fly flying activity. The response variable (number of flies) was cube-root transformed to normalize the residuals errors, values have not been back transformed into the normal scale. Data was separated into three distinct time periods based on the collection period. Dawn= first 2 collection periods, Dusk= last 2 collection periods, and Midday= all collection periods in between. Individual analyses were then run on each collection-period. Estimate is the size of the effect of each variable on the house fly number, standard error gives the relative accuracy of this estimate, the t value is the estimate divided by its standard error, and the Pr(>|t|) gives probability of observing this result in a random set of data.

Dawn	Fixed Effects	Estimate	Standard Error	t value	Pr(> t )	Wald $\chi^2$	p-value
	<b>Initial Model (AIC=155.78)</b>						
	Intercept	-2.271	1.386	-1.639	0.113		
	Sex	0.816	1.134	0.719	0.479	42.210	0.000
	Temperature	0.176	0.051	3.428	0.002	18.220	0.000
	Light Intensity	0.005	0.002	3.018	0.006	24.590	0.000
	Relative Humidity	0.009	0.014	0.633	0.533	0.000	0.950
	Wind Speed	0.016	0.204	0.081	0.937	0.070	0.780
	Sex <sup>x</sup> Temperature	0.057	0.037	1.513	0.142	2.290	0.130
	Sex <sup>x</sup> Light Intensity	0.003	0.002	1.525	0.139	2.330	0.130
	Sex <sup>x</sup> Relative Humidity	-0.019	0.012	-1.580	0.126	2.500	0.110
	Sex <sup>x</sup> Wind Speed	0.066	0.189	0.349	0.730	0.120	0.730
	<b>Best Fit Model (AIC=129.08)</b>						
	Intercept	-1.982	0.801	-2.476	0.014		
	Sex	0.331	0.232	1.427	0.154	40.390	0.000
	Temperature	0.199	0.045	4.394	<0.001	19.310	0.000
	Light Intensity	0.004	0.002	2.919	0.004	35.720	0.000
	Sex <sup>x</sup> Light Intensity	0.006	0.002	3.010	0.003	9.060	0.000

**Table 1.2 Contd.**

<b>Midday</b>	<b>Fixed Effects</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>t value</b>	<b>Pr(&gt; t )</b>	<b>Wald <math>\chi^2</math></b>	<b>p-value</b>
	<b>Initial Model (AIC=498.47)</b>						
	Intercept	2.861	0.557	5.134	<0.001		
	Sex	0.466	0.585	0.797	0.427	5.310	0.020
	Temperature	-0.026	0.011	-2.277	0.024	9.670	0.000
	Light Intensity	0.001	0.000	5.359	<0.001	49.220	0.000
	Relative Humidity	0.003	0.006	0.554	0.580	1.090	0.300
	Wind Speed	0.063	0.032	1.956	0.052	1.590	0.210
	Sex <sup>x</sup> Temperature	-0.009	0.011	-0.818	0.415	0.670	0.410
	Sex <sup>x</sup> Light Intensity	0.000	0.000	-0.295	0.769	0.090	0.770
	Sex <sup>x</sup> Relative Humidity	0.004	0.006	0.625	0.533	0.390	0.530
	Sex <sup>x</sup> Wind Speed	-0.065	0.042	-1.535	0.127	2.360	0.120
	<b>Best Fit Model (AIC=451.88)</b>						
	Intercept	3.126	0.276	11.325	<0.001		
	Sex	0.718	0.253	2.841	0.005	5.280	0.020
	Temperature	-0.024	0.008	-2.980	0.003	23.370	0.000
	Light Intensity	0.001	0.000	6.907	<0.001	47.710	0.000
	Sex <sup>x</sup> Temperature	-0.019	0.008	-2.351	0.019	5.530	0.020

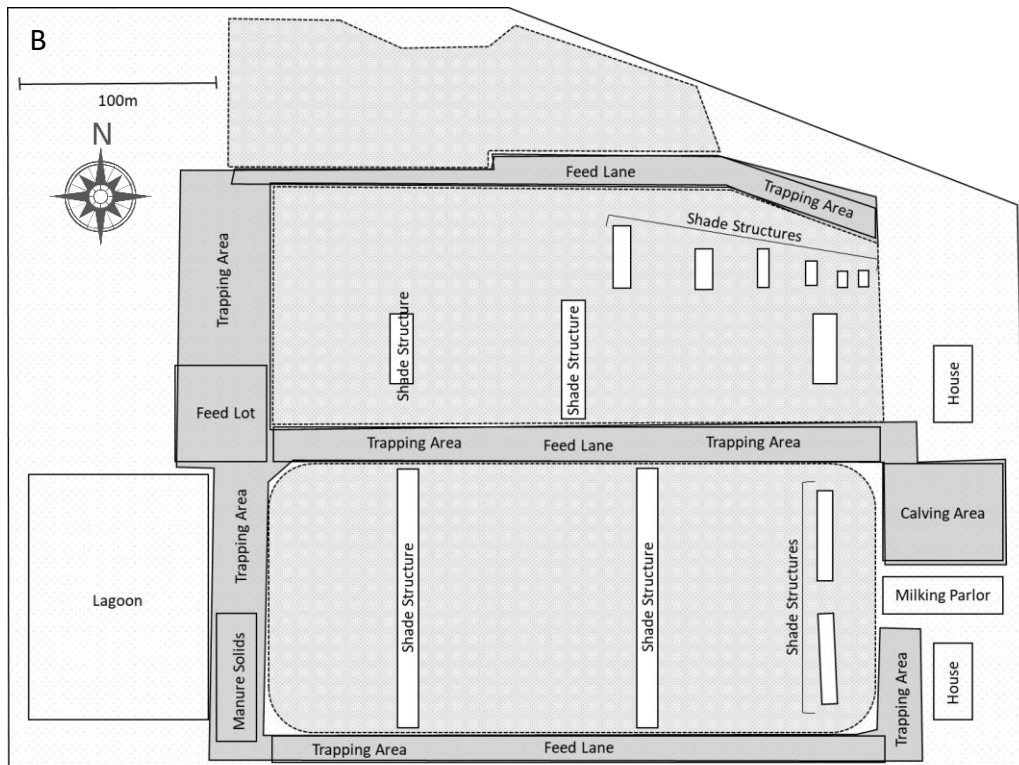
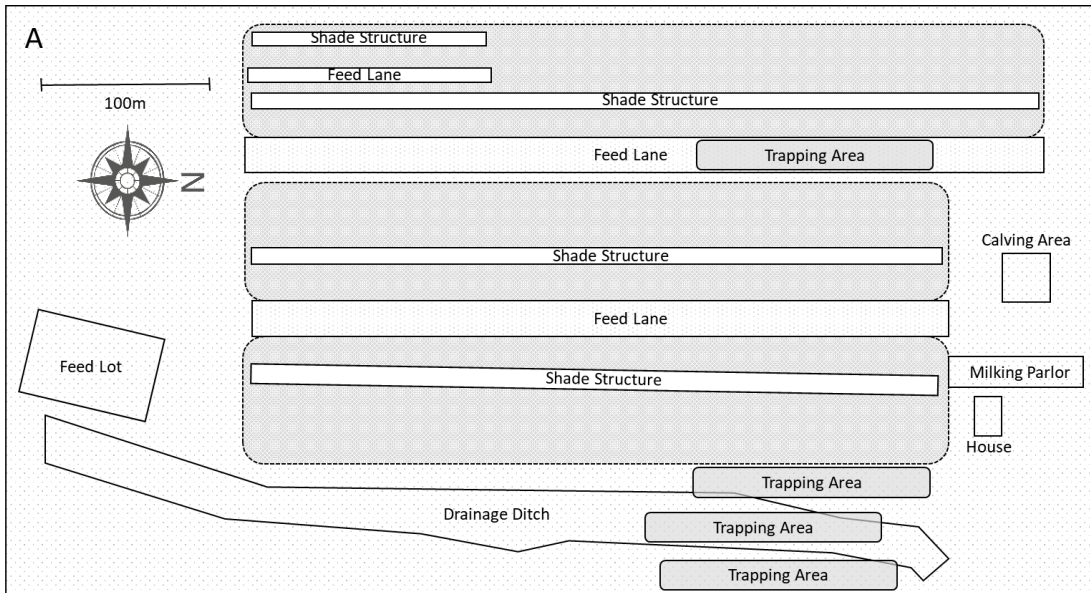
**Table 1.2 Contd.**

<b>Dusk</b>	<b>Fixed Effects</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>t value</b>	<b>Pr(&gt; t )</b>	<b>Wald <math>\chi^2</math></b>	<b>p-value</b>
	<b>Initial Model (AIC=153.29)</b>						
	Intercept	-0.827	1.892	-0.437	0.666		
	Sex	1.495	2.091	0.715	0.481	0.130	0.720
	Temperature	0.093	0.045	2.050	0.051	3.220	0.070
	Light Intensity	0.001	0.011	0.072	0.943	0.140	0.710
	Relative Humidity	-0.005	0.020	-0.245	0.809	0.440	0.510
	Wind Speed	0.153	0.107	1.426	0.166	10.160	0.000
	Sex <sup>x</sup> Temperature	-0.050	0.050	-1.001	0.326	1.000	0.320
	Sex <sup>x</sup> Light Intensity	0.004	0.014	0.314	0.757	0.100	0.750
	Sex <sup>x</sup> Relative Humidity	-0.012	0.022	-0.532	0.600	0.280	0.590
	Sex <sup>x</sup> Wind Speed	0.200	0.144	1.384	0.178	1.920	0.170
	<b>Initial Model (AIC=107.53)</b>						
	Intercept	-1.143	0.622	-1.836	0.067		
	Temperature	0.091	0.023	3.978	<0.001	15.830	0.000
	Wind Speed	0.246	0.060	4.080	<0.001	16.650	0.000

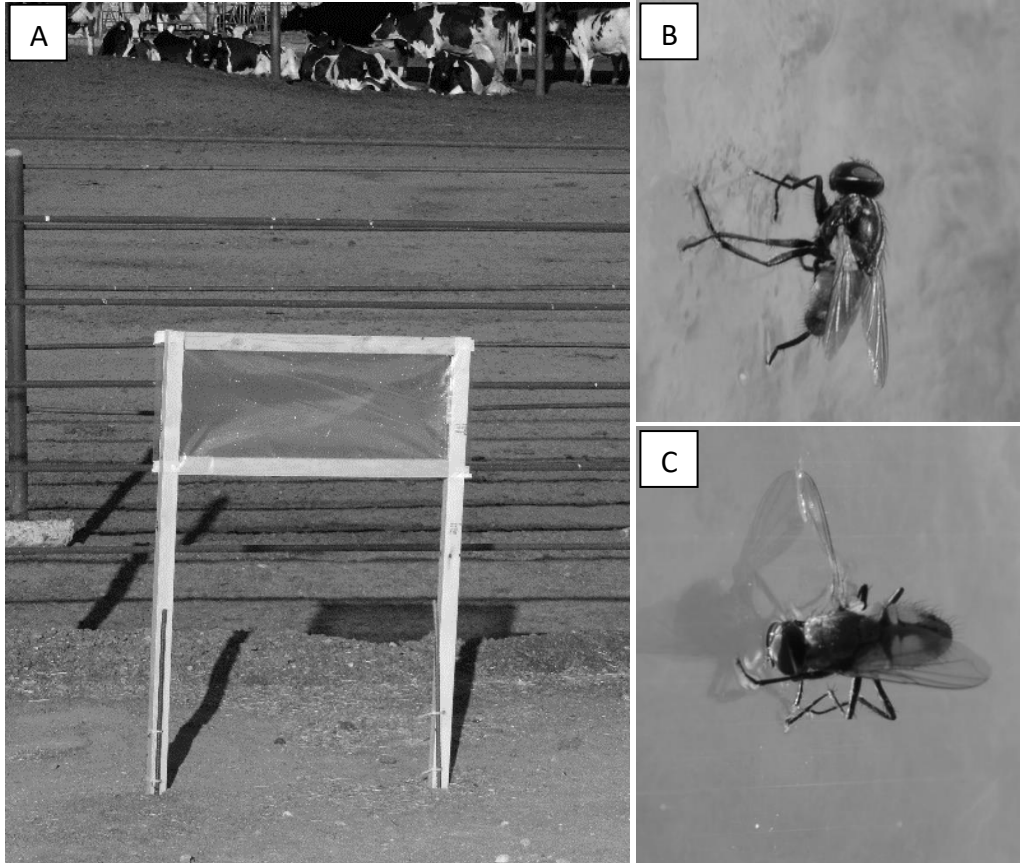
**Table S1.1:** Correlation matrix of environmental variables showing Pearson’s coefficient of correlation (r) and the significance of the correlation after adjustment for multiple comparisons.

	Temp		RH		Sol.Rad		Wnd.Spnd	
	r	p-val	r	p-val	r	p-val	r	p-val
<b>Temp</b>	1.00	0.00	-0.76	0.00	0.42	0.00	0.37	0.00
<b>RH</b>	-0.76	0.00	1.00	0.00	-0.44	0.00	-0.35	0.00
<b>Sol.Rad</b>	0.42	0.00	-0.44	0.00	1.00	0.00	0.12	0.48
<b>Wnd.Spnd</b>	0.37	0.00	-0.35	0.00	0.12	0.12	1.00	0.00

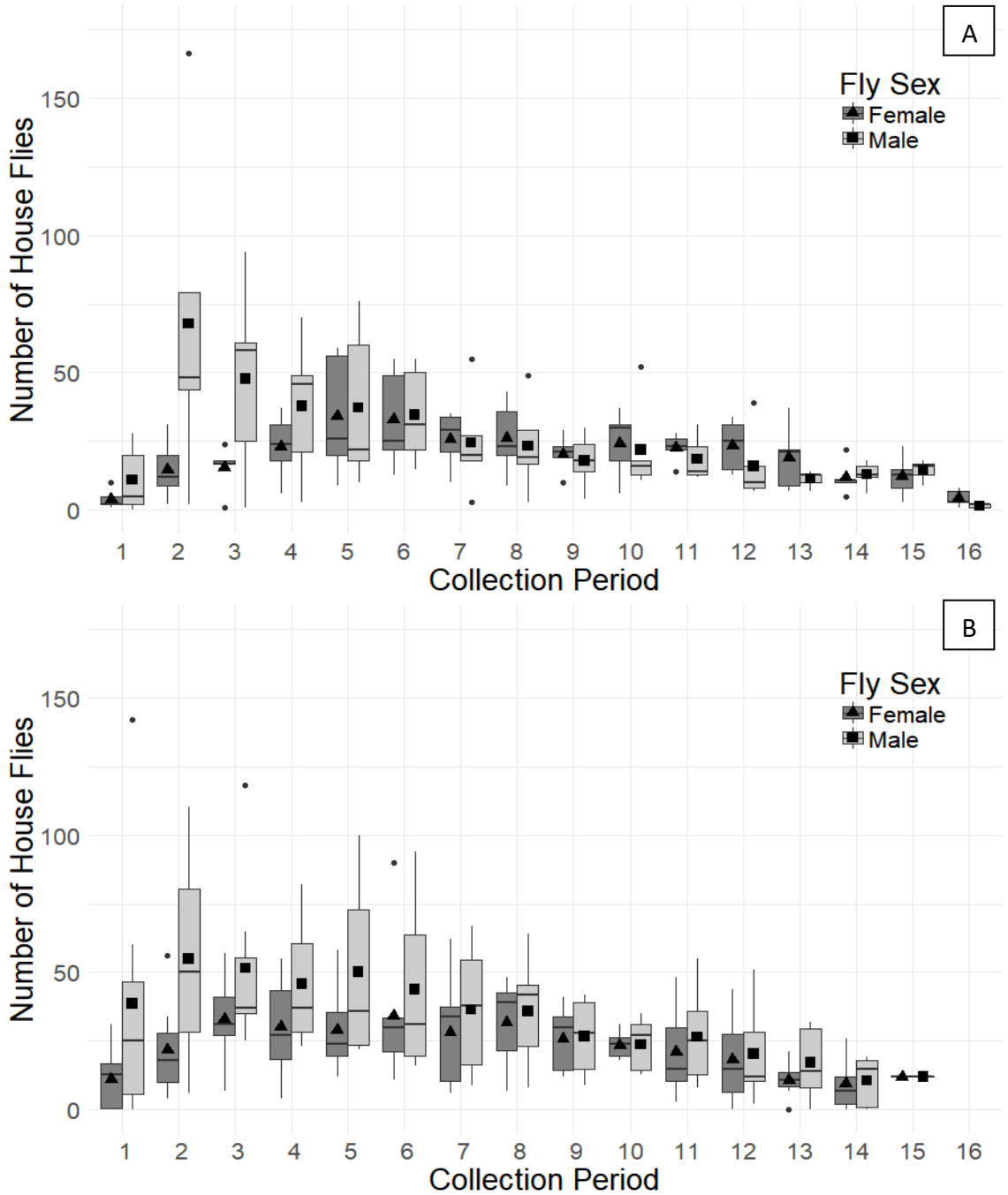
**Figure 1.1:** Diagrammatic sketches of the dairies and sites sampled. A) BS Dairy: Three traps were placed into each of 4 trapping areas (solid dark grey shaded areas) arranged from the dairy center to the periphery of the dairy (12 traps per day). B) OS dairy: Twelve traps were placed randomly each day within the trapping area (solid dark grey shaded areas) at locations where facility workers and cattle could not interfere with them.



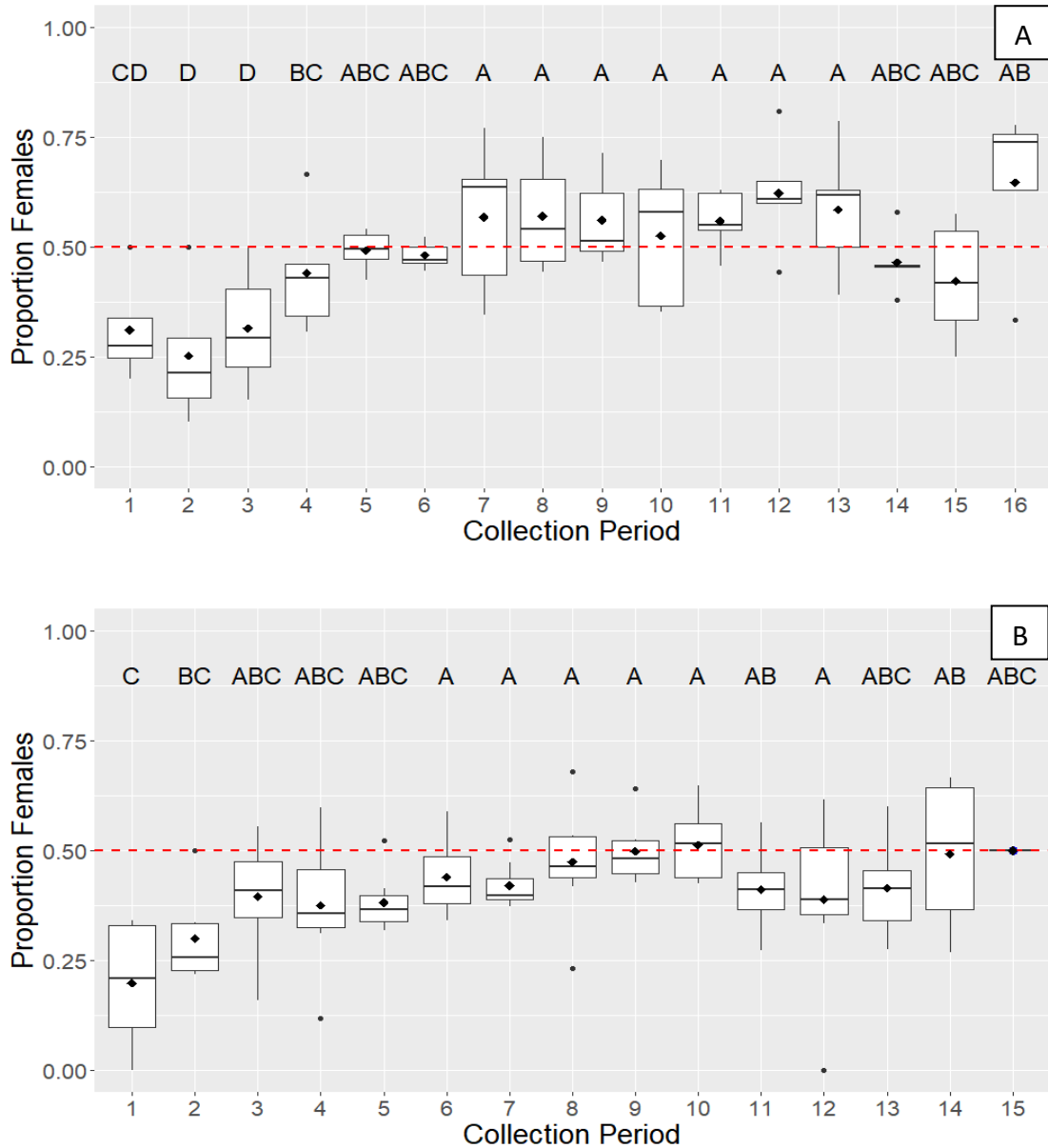
**Figure 1.2:** Figure showing A) trap used to capture house flies, B) house fly stuck to adhesive surface of sticky trap, and C) house fly that appears to have flown directly into the adhesive surface of the sticky trap.



**Figure 1.3:** Box plots showing house fly activity by collection period at the **A) BS dairy** and the **B) OS dairy**. Collection periods were hourly intervals starting from 30 min before civil dawn through civil dusk. House flies were not collected during period 16 at the OS dairy.

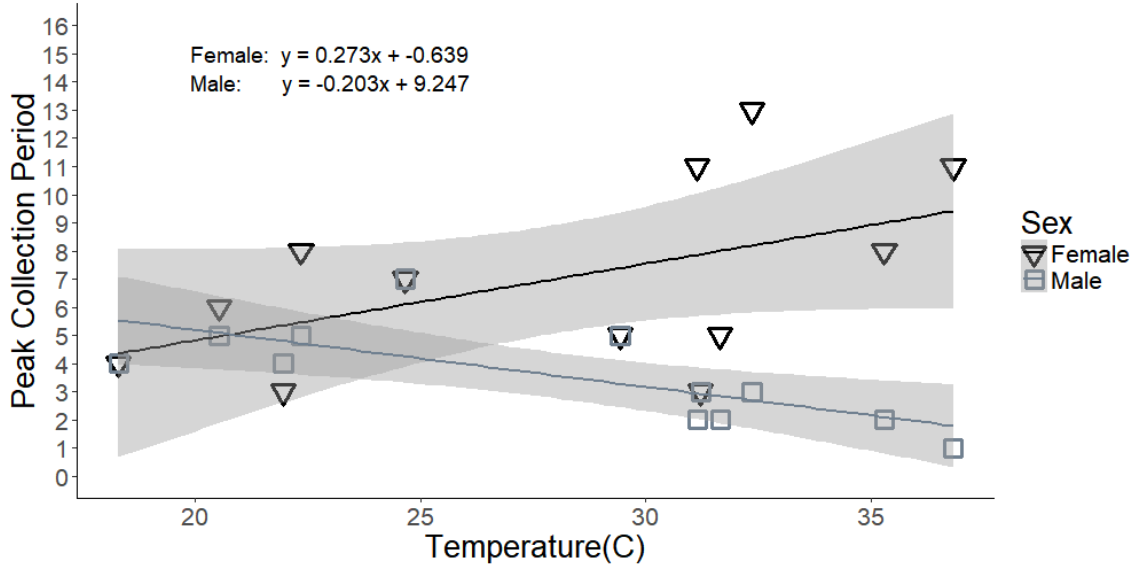


**Figure 1.4:** Proportion of house flies captured on sticky traps that were female during each hour at the A) BS Dairy and B) OS Dairy. Black diamonds indicate raw mean proportions. Collection periods with the same letter are not significantly different after Tukey's correction of the p-value ( $\alpha=0.05$ ).

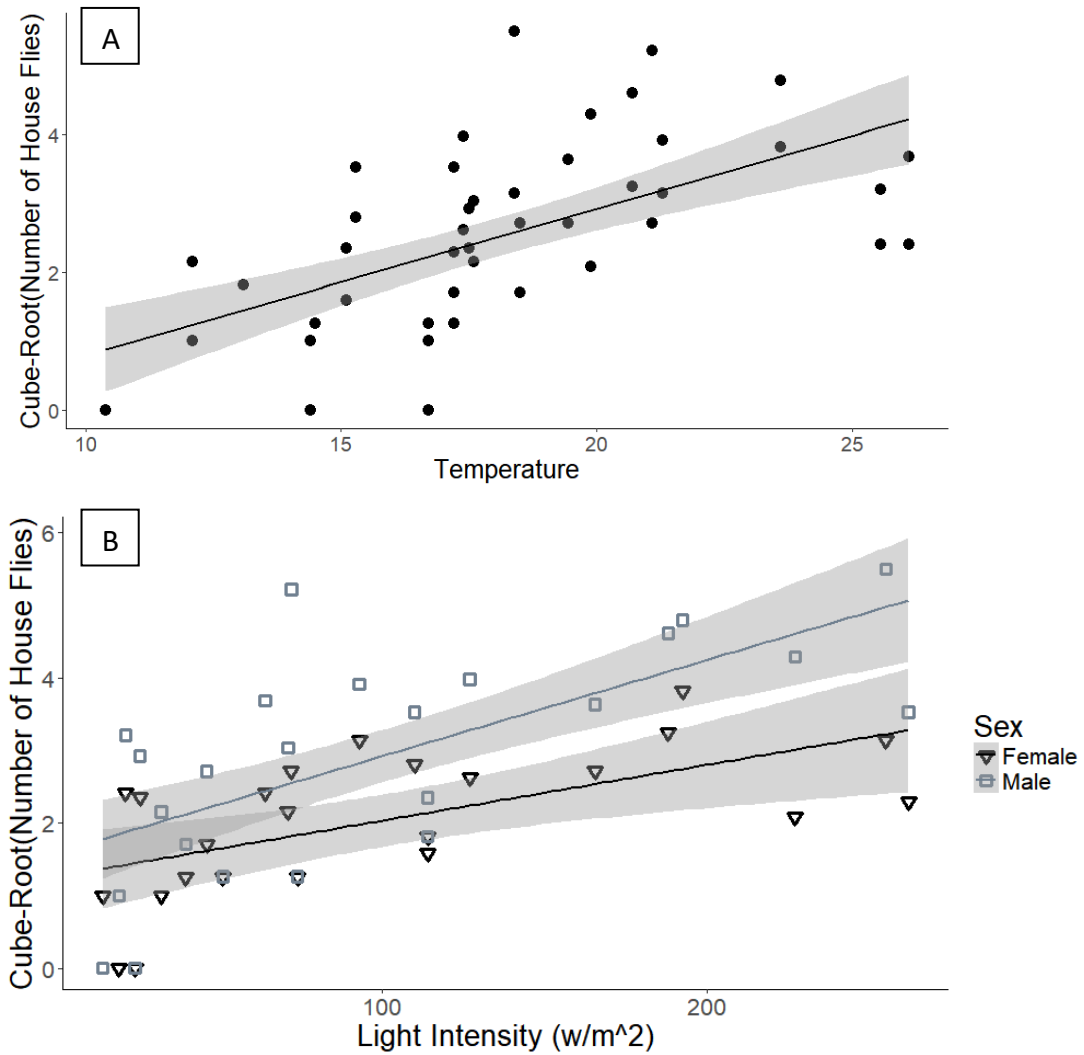




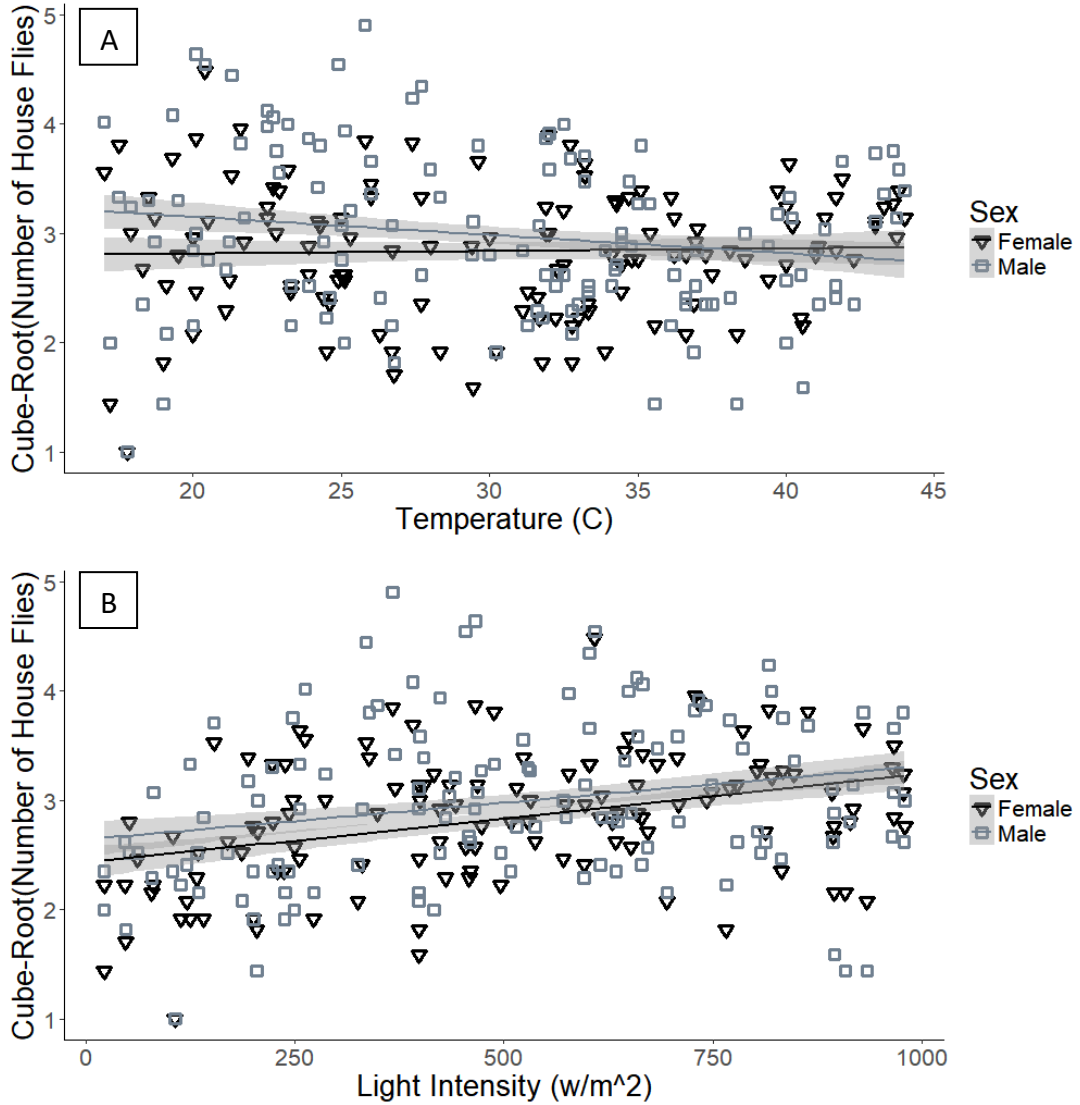
**Figure 1.5:** Collection period with peak house fly activity by mean daytime temperature for both male (grey triangles) and female (black circles) house flies. Light grey bands around each line indicate the 95% C.I. by fly sex.



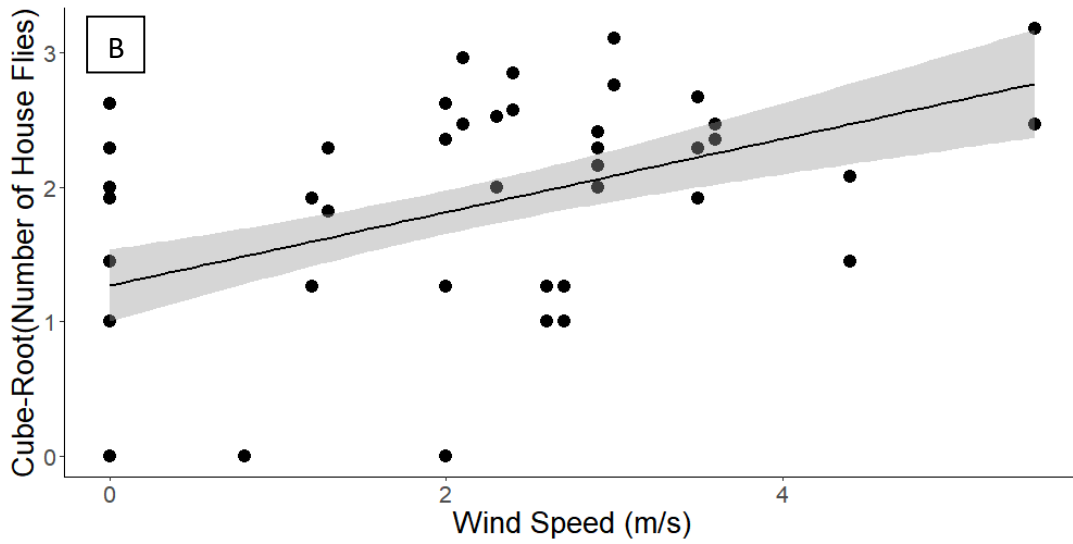
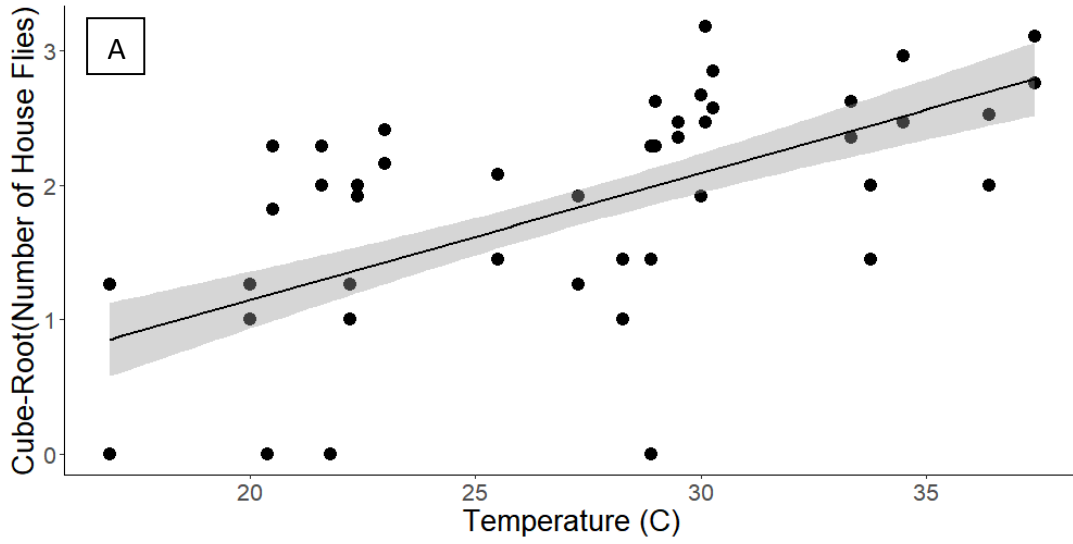
**Figure 1.6:** Significant predictors for house fly activity near dawn for male (grey; triangle) and female (black; circles). Light grey bands around each line indicate the 95% C.I. for each fly sex.



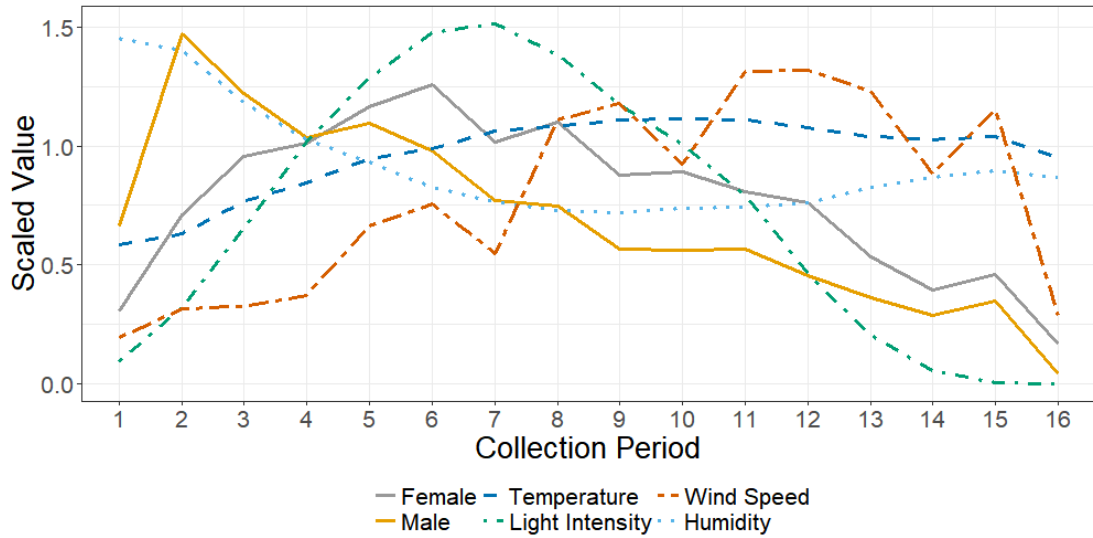
**Figure 1.7:** Significant predictors for house fly activity during midday for male (grey; triangle) and female (black; circles). Light grey bands around each line indicate the 95% C.I. for each fly sex.



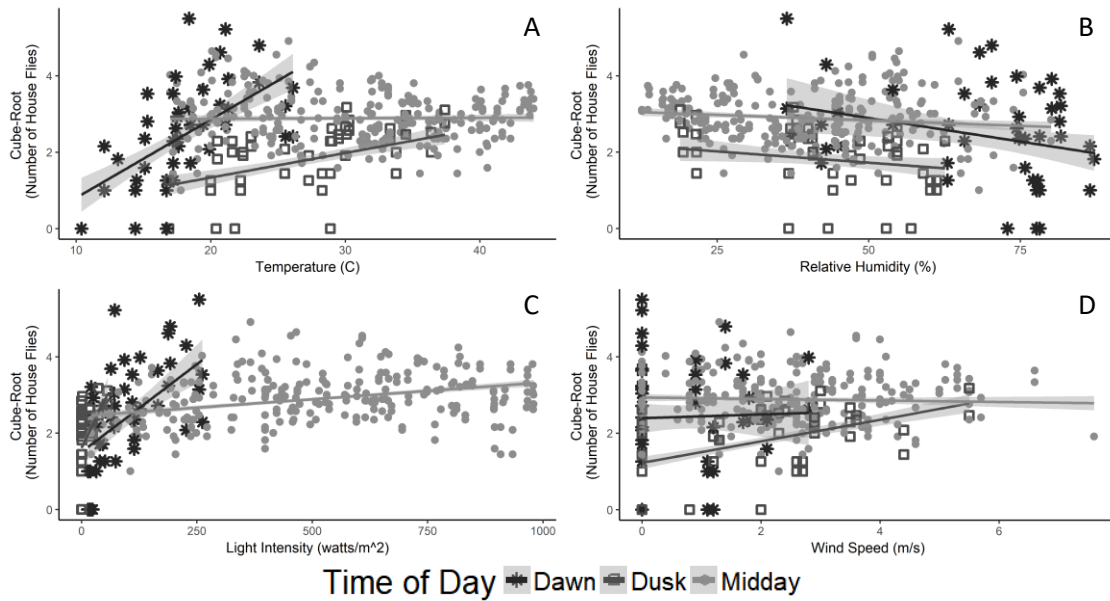
**Figure 1.8:** Significant environmental predictors for house fly activity near dusk. Light grey bands around the regression line indicate the 95% C.I.



**Figure S1.1:** Mean Z-scores (pooled over all collection dates) for the number of female (solid; grey) and male (solid; gold) house flies along with, temperature (long-dash; blue), light intensity (dot-dash; teal), wind speed (dash; orange), and relative humidity (dotted; blue). Panel shows how each variable changed on average over the course of day. Z-scores are calculated by dividing observed value for each observation by the SD. Plot is for visual comparisons only to show how each variable would change in relation to another.



**Figure S1.2:** Combined data from both the BS and OS dairies and for both fly sexes, showing the relationship of house fly activity to the recorded environmental conditions at dawn (Orange), midday (Blue), and dusk (Green). Dawn is the first two collection periods each day, dusk is the last two collection periods each day, and midday is all remaining collection periods. Environmental conditions recorded were A) temperature, B) relative humidity, C) light intensity, and D) wind speed. Light grey bands around each line indicate the 95% C.I. for each time of day category.



## CHAPTER 2

### Vertical Distribution of House Flies (*Musca domestica*) During Flight on a Southern California Dairy

#### ABSTRACT

House flies are a common pest insect and can become a public health hazard if flies disperse in large numbers. House flies are best controlled using integrated pest management (IPM) techniques, including methods to reduce fly dispersal from fly development sites to nearby sensitive locations such as residential homes or schools. Vertical barriers like fence lines or tree lines have been proposed to limit house fly dispersal, with suggestions including surrounding animal production facilities with 1.8m tall fencing with windscreen. In this study, the flying height of house flies on an active southern California dairy was determined using 2.13m-tall sticky traps designed to capture flies during flight. House flies were evenly distributed over this trap height, except for flies captured on traps positioned in or near the edge of an alfalfa field. In the alfalfa field, more house flies were collected towards the top of the trap while more flies were collected at the bottom of the traps at the location adjacent to the alfalfa field. The relatively even distribution of house flies over the full height of the traps indicates house flies are almost certainly also flying above the trap height. The direction of flight only had a small impact on flying height, but temperature, relative humidity, and wind speed were linearly associated with changes in flying height. It is apparent from this study that a barrier to dispersal of house flies would likely need to be taller than 2.13 m and further study at greater height is needed to determine if there is a height above which house fly flight activity is reduced.

## **INTRODUCTION:**

House flies (*Musca domestica* Linn.) are a common pest and are associated with confined animal production and waste storage facilities where suitable immature development habitat can be available (Keiding 1986). When adult house flies are present in larger numbers, their dispersal from these facilities to human residences, schools, or other sensitive sites can often result in considerable nuisance complaints (Lole 2005, Winpisinger et al. 2005). Lawsuits, zoning limitations, and animosity between farmers and home owners have resulted (Campbell 1993). Furthermore, house flies can carry more than 65 disease organisms affecting humans or animals (Greenberg 1971), such as the virulent *Escherichia coli* strain O157:H7 (Kobayashi et al. 1999, Sasaki et al. 2000, Junqueira et al. 2017).

Control efforts to reduce house fly nuisance focuses on eliminating house fly developmental habitat (e.g., wet manure) and reducing the dispersal of house flies away from development sites and into surrounding sensitive areas. It has been proposed that presence of a physical barrier may limit dispersal of house flies beyond the boundary of the facility on which they are produced (Fried et al. 2005, Dubie 2014), and both manmade (fences and walls) and natural barriers (hedge and tree rows) have been shown to impact the movements of some insect species including tabanids, stable flies, hover flies, and butterflies (Morgan and Lee 1977, Foil and Hogsette 1994, Wratten et al. 2003, Ingimarsdóttir et al. 2013). An effective barrier to dispersal would be expected to intercept and/or alter the flight direction of house flies as they encounter the barrier during flight. During dispersal flight, house flies encountering a dense forest edge were reported to turn and follow the linear edge of the forest rather than enter the forest (Fried et al. 2005), suggesting house fly dispersal follows the “drift fence hypothesis” where insects encountering a linear barrier (such as a habitat corridor) will turn to follow the



barrier edge rather than moving through or over the barrier (Haddad and Baum 1999). However, there is little research evidence to date supporting the use of barriers to limit house fly dispersal from house fly development sites, and even basic aspects of house fly flight behavior, such as flying height, are not known.

House flies are reported to concentrate in the airspace nearest to the ground during flight, with the number of flies decreasing linearly with increasing height above ground (Black and Krafur 1985). However, in this study house flies were captured on white index cards placed at fixed heights of 36, 76, and 116 cm above ground; considerably lower than the height of a typical screened construction fence (182-243 cm) that might be erected as a barrier to fly dispersal. The flying height reported by Black and Krafur for the house fly is similar to the 61-91 cm above ground flying height recorded for the horse fly *Tabanus nigrovittatus* (Joyce and Hansens 1968, Schulze et al. 1975). In the Black and Krafur (1985) study, an interaction between trap height and trap location suggested that house flies vary flying height in response to substrate variation or other collection site or environmental characteristics, in agreement with the general consensus that flying height selection by insects can be altered by variation in substrate (Schulze et al. 1975) or by environmental characteristics, particularly wind speed (Taylor 1960, Lewis 1967, Lewis 1969, Snow 1977). The low flying height for house fly reported in the Black and Krafur (1985) study seems to contradict observations that house flies readily land on surfaces 1.8 m above ground (Gerry et al. 2011) and that flies often accumulate in overnight resting locations often  $\geq 6$  m in height, such as beneath barn roofs (personal observation). It is unclear whether house flies commonly fly at greater heights than those reported by Black and Krafur (1985) or if they adjust their height of flight based upon location.

The flying height of insects is also influenced by wind speed, particularly when wind speed is high. When wind speed exceeds the insects' maximum flight speed, flight can no longer be self-controlled. Under such conditions, flight may be restricted to near ground level (the insect boundary layer) where wind speeds remain slow enough to allow self-directed flight (Taylor 1960). The height of this insect boundary layer is primarily dependent on the wind speed and the relative flying strength (and flight speed) of the insect. The literature on the maximum flight speed for house flies is lacking but has been reported to be 2 m/s on average (West 1951, Shepard et al. 1972, Wagner 1986, Dahlem 2009). Thus, the height where wind speed exceed 2 m/s would be the lower extent of the boundary layer for house flies.

The objective of this study was to characterize the flying height of house flies in relation to a range of factors (spatial, temporal, and environmental) which might impact total trap catches and height of flight. Understanding the flight behavior of house flies is immensely important to developing management strategies that rely on the capture or redirection of house flies during flight.

## **METHODS:**

House flies were captured on a large commercial dairy (BS Dairy) near the town of San Jacinto in southern California using tall sticky traps placed at six different locations across the dairy (Figure 2.1A). The tall sticky traps were constructed of four pine furring strip boards (5.08 cm x 5.08 cm x 244 cm) held in a vertical position by wooden trim board to form a rectangular "tower trap" that was 31 cm wide on each side and 2.4 m high (Figure 2.1B). Three clear sticky panels (30.5 cm x 70 cm) (Biting Fly Trap Replacement Sheets, Olson Products, Medina OH, Part# MBX10-1227SSR) were placed end-to-end on each face of the trap to give a vertical sticky

surface extending from ~2 cm to 2.1 m above ground level on each of the four sides of the tower trap. Because the edges of each sticky panel lacked adhesive, there was a small (~2-4cm) non-sticky gap between the three panels on each trap side where flies were not captured. This non-sticky gap can be seen in most figures of fly captures (e.g., Figure 2.3) and provides an easy visual separation of the three sticky panels that comprised each trap face.

Trapping was conducted on six days during June-August 2014, with four tower traps placed on each sampling day at one of the six trapping locations sampled. The six trapping locations were distributed around the entire dairy and placed to avoid disturbance by cattle or dairy facility employees. Trapping locations were located on dirt roads at the periphery of the dairy to the east and south, in the center of the dairy in an empty dry lot cattle pen (Corral), next to a hay storage pad (Haystack), and to the west of the dairy, and just inside an adjacent alfalfa field to the west (Alfalfa; Figure 2.1A). At each trapping location, the four tower traps were placed in a line, oriented north-south, except for the south location where the line was oriented east-west due to site constraints. The four traps in each trap line were separated by 20 m, with individual traps positioned so that each trap side faced a cardinal direction (N, S, E, & W) so that house fly flight direction could be inferred by house fly capture on each side of each trap.

Flies were captured on the tower traps during three 2-hr collection periods: 8-10am, 12-2pm, and 4-6pm (morning, afternoon, and evening collection periods respectively). At the end of each two-hour collection period, each trap face was examined, and captured flies were identified to species and sex, with the height above ground for each individual house fly recorded prior to removing the fly from the trap. Sticky sheets were replaced prior to the start of the next collection period. At the start and end of each collection period, environmental data including temperature, humidity, wind speed and wind direction was recorded using a hand-

held sensor (Mini Thermo-Anemometer with Humidity - model 45158, Extech Instruments, Nashua, NH) at a height of ca. 1.5 m above ground and within 20m of the trapping sites.

## **DATA ANALYSIS**

All statistics were analyzed using R version 3.4.3 (R Core Team 2017). Due to trap design and sampling methodology the data are theoretically spatially and temporally autocorrelated, where flies collected on each side of a single trap are more like each other than to flies collected on the same side of another trap (spatial autocorrelation), and house flies were repeatedly sampled over three time periods each day using static traps (temporal autocorrelation). To address these issues a unique identifier called trap ID was given to each side (4) of each trap (4) within each location (6), giving a total 96 trap IDs, and trap ID was included as a random effect in mixed effect models

A generalized linear mixed model (lme4; Bates et al. 2015) was used to compare the total number of flies captured with the fixed effects of trap face (direction of flight), collection period (time of day), fly sex, and the interaction of collection period with fly sex, and trap ID and collection period as correlated random effects with a Poisson error term. Including collection period within the random effects portion of the model allows the slopes to vary for each of the three collection periods to give a better estimate of the true difference in means for each of the model factors. Multiple comparisons were performed using the R package 'emmeans' (Lenth 2018) with the Tukey method for p-value adjustment.

The mean flying height was determined for flies of each sex, captured on each side of each trap (direction), during each collection period, within a trap location. Calculating mean flying height in this way addresses issues with pseudoreplication when using the raw data of

individual fly capture height (Crawley 2013). Variation in mean flying height was assessed using a linear model with house fly sex, trap location, direction (cardinal direction side of the trap faced), collection period, and the three environmental variables (temperature, relative humidity, and wind speed) included as main effects, and with all two-way interactions included in the initial full model. Models were reduced using a likelihood ratio test (LRT) by comparing the fit of a full model to a series of simplified models, first removing two-way interactions and then removing any nonsignificant main effects (Crawley 2013). After model reduction the main effects of location, direction, temperature, relative humidity, and wind speed were retained in the model. No interaction terms were included in the final model. Model residuals were assessed visually using normal Q-Q plots and residual versus fitted plots (Crawley 2013). Multiple comparisons were performed using the R package 'emmeans' (Lenth 2018) with the Tukey method for p-value adjustment.

## RESULTS

Sticky traps were adequate in collecting house flies (Figure 2.2A) and flies were observed impacting the traps head-first on many occasions (Figure 2.2B). A total of 4,241 house flies (52.1% female) were captured from all traps combined during this study. The number of house flies collected varied significantly by trap face (Wald:  $\chi^2=75.258$ ;  $df=3$ ;  $P<0.001$ , Figure 2.3), collection period (Wald:  $\chi^2=64.714$ ;  $df=2$ ;  $P<0.001$ ), and fly sex (Wald:  $\chi^2=10.069$ ;  $df=1$ ;  $P=0.002$ ) with a significant interaction between collection period and fly sex (Wald:  $\chi^2=13.407$ ;  $df=2$ ;  $P=0.001$ , Table 2.1). The east facing side of the trap captured significantly more house flies than north, south, or west ( $P\leq 0.001$ , all comparisons), and the west facing side captured significantly fewer flies than north or south ( $P<0.001$ ,  $P=0.011$ , respectively). The morning

collection period captured significantly more flies than the afternoon or evening ( $P \leq 0.001$ , all comparisons), but fly numbers were similar between the afternoon and the evening periods ( $P=0.482$ ). Within both the morning and evening collection periods the number of males and females house flies collected were equal ( $P=0.458$ ,  $P=0.547$ , respectively), but significantly fewer males than females were collected during the afternoon collection period ( $P < 0.001$ ). Since location and date are confounded, no statistical analyses were performed to evaluate differences in overall number of flies captured among trap locations/dates.

House flies were captured over the entire height of the tower trap (Figures 2.4 & 2.5) with the mean height of fly capture for both females ( $110.36 \pm 0.93$  cm) and males ( $109.8 \pm 1.4$  cm) being very close to the center of the trap (106.5 cm). Ninety-five percent of the captured flies were captured at a flying height of 12-204 cm (Quartiles: 0-55cm; 56-109cm; 110-167cm; 168-213cm), an indication that flies are well distributed across the entire 213 cm trap height.

Flying height did not vary between the fly sexes (Figures 2.5) or among the collection periods and were dropped from the final model, but mean height did vary by trap location ( $F_{5,423}=17.19$ ; 423;  $P < 0.001$ , Figure 2.6) and direction ( $F_{3,423}=3.54$ ;  $P=0.015$ , Figure 2.7). Mean flying height was significantly lowest at the West location ( $84.2 \pm 4.4$  cm;  $p < 0.001$ ) and significantly highest at the Alfalfa location ( $144.0 \pm 8.8$  cm;  $p < 0.018$ ). Mean flying height at other locations was near the middle of the trap, with Corral ( $106.8 \pm 3.3$  cm), East ( $110.4 \pm 2.6$  cm), Haystack ( $108.4 \pm 3.3$  cm), and South ( $118.5 \pm 3.8$  cm) locations being similar ( $p > 0.35$ ) to each other. Flying height also varied by trap face orientation (Figure 2.6) with house flies captured at significantly greater height on the east facing side of the trap ( $118.0 \pm 2.4$  cm) relative to the west ( $102.7 \pm 4.7$  cm;  $p=0.018$ ) and north sides ( $104.2 \pm 3.0$  cm;  $p=0.048$ ). No other differences in flying height among trap face orientation were noted. Temperature ( $F_{1,423}=0.353$ ;  $P=0.553$ ), and

relative humidity ( $F_{1,423}=0.462$ ;  $P=0.497$ ) did not significantly impact the flying height of house flies. Wind speed ( $F_{1,423}=3.476$ ;  $P=0.063$ ) was marginally significant with a negative impact on house fly flying height ( $\beta=-3.99$ ;  $SE=2.14$ , Figure 2.8). However, the coefficient of correlation ( $R^2=0.17$ ) was relatively low suggesting that this model does not adequately describe the observed data. Observed environmental variables by location and collection period are presented in supplemental tables (Tables S2.1 & S2.2). The wind direction was not evaluated since measurements were only taken at the beginning and end of each collection period and wind direction likely varied over the two-hour trapping periods.

#### **DISCUSSION:**

The trapping technique used in this study was adequate in sampling house flies while in flight as many of the captured house flies had apparently impacted the traps head first showing that at least some individuals were unable to detect the presence of the clear sticky sheets. Additionally, observations by the authors did not suggest house flies were using the structures as perching sites, however this cannot be ruled out as the traps themselves act as a sink, where flies that land cannot fly again. But the use of sticky impaction traps (often employing glue applied to a clear surface) to assess the vertical distribution of insects has been used on multiple occasions (Williams and Rogers 1976). Though the heights previously examined were either at high altitudes to examine aerial dispersal or were very close to the ground to study boundary layer effects on insect flight (Johnson 1957, Taylor 1960).

Both stable flies and house flies are reported to fly generally close to the ground, with stable flies mostly at or below 1.2 m (Williams and Rogers 1976) and house flies increasing in number as trap height is reduced from 116 cm to 36 cm (Black and Krafur 1985). Based on

these studies, it was anticipated that the current study would show an inverse relationship between trap height and number of house flies captured and that house flies would be captured in greatest numbers below 1 m in trap height. However, this was not observed in the current study, and house flies were generally evenly distributed over the full 2.13 m height of the tower traps suggesting that flies must also commonly fly at heights greater than the maximum height sampled by these traps.

Using the average height of fly capture was suitable for examining changes in house fly flying height but tended to mask some of finer differences in the vertical distributions of house flies, especially at locations which collected large numbers of house flies (locations in the interior of the dairy and the East location). Both the East and South locations had slight upward shifts in their average heights of fly capture but were not drastically different from each other or the Corral and the Haystack locations.

The two locations which had an even distribution of house flies were within the interior of the dairy and situated either inside a dry lot pen or placed near concrete slabs (Corral and Haystack, respectively) and were surrounded by various structures (buildings, shade covers, and open sided barns). Locations on the periphery of the dairy (Alfalfa, East, and South) had fly distributions skewed either toward the top of the trap (3 locations) or bottom of the trap (1 location) perhaps due to more variable ground cover and lack of nearby structures. However, it is not yet possible to draw many other strong conclusions from these results as the East location was also on the edge of the dairy but did not experience significant change in the height of flies as did the South location.

The two locations where height distributions were not uniform were located on the western edge of the dairy adjacent to, or just within, a crop field consisting mostly of grasses



mixed with ~20 cm tall flowering alfalfa (Alfalfa and West, respectively). The visual contrast between vegetation at the field edge and the adjacent open ground may be attractive to house flies which are reported to be attracted to areas of light and dark contrast (Scudder 1947). Within the crop field, flies may adjust flying height upward in accordance with the vegetation height. However, only a small number of house flies were captured within the alfalfa field which was approximately 100 m away from the nearest pen with cattle. These are contrasted by the other four locations which were all near cattle pens and graveled or paved roads which were devoid of vegetation. Differences in mean flying height among these locations may be an indication that flying height depends on the substrate over which house flies are moving.

Perhaps unsurprisingly, temperature and relative humidity were not strongly associated with changes in the mean flying height of house flies. To our knowledge this is the first report on the relationship of flying height of house flies to temperature or humidity. Wind speed had a negative trend on the mean flying of house flies, where the mean height decreased as wind speeds increased which aligns with results reported for other insects (Taylor 1960). However, this trend was not strong, perhaps due the high variability in wind speeds between the three collection periods used, where winds were much higher in the afternoons when compared to the morning period. Additionally, a location effect cannot be ruled out.

It is likely that the boundary layer for house flies was elevated above the tops of many of the traps due to the abundance of tall structures on the dairy and the generally low observed windspeeds, but there was a clear linear decrease in flying height as wind speeds increased. This indicates that wind speed is likely modulating the height of flight for house flies as has been proposed by (Lewis 1965). Additionally, winds in the morning collection period (when most house flies were collected) were below the average 2 m/s flight speed of house flies (West 1951,

Shepard et al. 1972, Wagner 1986, Dahlem 2009) likely pushing the upper extent of the boundary layer above the tops of the traps, allowing flies to move freely over the entire 2.13-meter height sampled by the traps.

Perhaps the combination of wind breaks and low wind speeds allowed house flies to fly more freely over a greater height as compared to Black and Krafur (1985), where many of the traps were placed away from structures and perhaps were subject to higher wind speeds pushing the height of flight for house flies closer to ground level, similar to the flying heights seen at the West location in the current study.

House fly numbers declined as the days progressed, with the morning collection period accounting for the most house fly captures and the evening collection period the least. This supports the diel activity pattern described in chapter 1 of this dissertation where house fly flight activity peaked during the mid to late morning and steadily declined over the rest of the day. It is a little surprising that there were no significant differences in the number of male versus female house flies collected during the morning collection period which is when the diel flight activity between males and female was most dissimilar (chapter 1). Likely the peak in house fly activity was shifted to later in the day when between collection periods, which is why significant differences by house fly sex were seen in the afternoon or evening collection periods.

House fly captures varied according to side of the trap they were captured on. Apart from Alfalfa, which captured comparatively few house flies, the east facing side of each trap always captured the most house flies, an indication that house flies were moving towards the west (perhaps upwind). Alternatively, the greater accumulation of flies on the east side of the trap could be caused by the trap itself acting as a wind break where the turbulent airflow acts to hold the flies near the trap. These results are similar to those of Lewis (1965, 1966) where flying

insects were shown to accumulate in greatest numbers nearest the windbreak on the leeward side. Like Lewis (1967) the average height of flight for house flies was also elevated on the east side of the traps when compared with the other three sides, another indication that the traps may be acting as a wind break. It should be noted that the insects Lewis collected were not house flies and were much smaller. Therefore, direction of travel likely does not greatly influence the height of flight for house flies.

House fly sex ratio was similar over the entire height of the trap, however, around 100 cm the collections became slightly female biased but not significantly so. This contrasts what Black and Krafur observed, where house flies were males were proportionally greater at 116 cm (their highest collection) compared with lower heights at 36 cm or 76 cm. The authors state that this change is likely the result of females avoiding male perching sites as male house flies have been shown to harass males and females flying near vertical resting sites (Murvosh and Thaggard 1966, Ragland and Sohal 1973). Observed differences between studies may be due to differences in trapping methods, as Black and Krafur (1985) used sticky white note cards, as compared to the clear sticky sheets used in the current study. Perhaps house flies did not sense the clear sticky sheets and were impacting them in midflight rather than simply landing on them to rest or wait for female house flies.

The sex ratios of house flies did vary by the location in which they were collected, with only alfalfa and west having a larger proportion of male flies when compared with the rest of the dairy, which is similar to Black and Krafur (1985) where they collected a higher proportion of male flies in the grassy pastures when compared to areas located more centrally on the dairy. Although, all their catches were male biased, possibly a result of using white sticky note cards to collect house flies. Locations close to, and within, cattle pens were more female biased as there

is a preponderance of larval development sites, which presumably attracts female flies looking for an ovipositional site. Similarly, after sweep netting house flies near feeding troughs, calf hutches, and along a silo, Krafur et al. (1985) found that male house flies were more commonly collected (though not statistically different) near shaded resting areas like the side of the silo when compared to flies collected from feeding troughs or near calf hutches. The authors suspected there was a site-by-date interaction which was masking a true difference in sex ratio by location.

Based on the findings from this study it is apparent that any barrier intending to mitigate house fly dispersal would need to be over 2.1 meters in height and the height of the barrier needed to mitigate dispersal would likely be dependent on the side of the dairy it is to be placed. Given this limitation it is unlikely that most commercial fences would be tall enough to prevent flies from dispersing, or maybe to affect dispersal at all. However, it is still possible that vegetative barriers may be effective barriers as many types of trees and shrubs used in tree rows will grow taller than 2 meters. Additional sampling of the flying height of house flies just off the dairy is necessary to better understand the vertical distribution of house flies in flight, however the number of house flies collected appear to quickly decline as traps are placed further away from dairy (Lysyk and Axtell 1986) making assessments of flying height away from active larval development sites difficult.

## REFERENCES CITED

- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015.** Fitting Linear Mixed-Effects Models using lme4. *J. Stat. Softw.* 67: 1–48.
- Black, W. C., and E. S. Krafur. 1985.** Use of Sticky Traps to Investigate Seasonal Trends in the Spatial Distribution of House Flies and Stable Flies (diptera: Muscidae). *Journal of Medical Entomology* 22: 550–557.
- Campbell, J. B. 1993.** The Economics of the Fly Problem, p. 97. *In Rural Flies Urban Environ., Research Bulletin.* University of Nebraska Lincoln.
- Crawley, M. J. 2013.** The R book, Second edition. ed. Wiley, Chichester, West Sussex, United Kingdom.
- Dahlem, G. A. 2009.** Chapter 125 - House Fly: (*Musca domestica*), pp. 469–470. *In* Resh, V.H., Cardé, R.T. (eds.), *Encyclopedia of Insects* Second Ed. Academic Press, San Diego.
- Dubie, T. R. 2014.** Influence of Vegetation Height on the Dispersal of House Flies (*Musca domestica*) from Livestock Facilities and Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers (Dissertation). University of Oklahoma.
- Foil, L. D., and J. A. Hogsette. 1994.** Biology and Control of Tabanids, Stable Flies and Horn Flies. *Revue Scientifique et Technique de l'OIE.* 13: 1125–1158.
- Fried, J. H., D. J. Levey, and J. A. Hogsette. 2005.** Habitat Corridors Function as Both Drift Fences and Movement Conduits for Dispersing Flies. *Oecologia.* 143: 645–651.
- Gerry, A. C., G. E. Higginbotham, L. N. Periera, A. Lam, and C. R. Shelton. 2011.** Evaluation of Surveillance Methods for Monitoring House Fly Abundance and Activity on Large Commercial Dairy Operations. *Journal of Economic Entomology.* 104: 1093–1102.
- Greenberg, B. 1971.** Flies and Disease. Vol. I. Ecology, Classification and Biotic Associations. Princeton University Press, Princeton, New Jersey, U.S.A.
- Haddad, N. M., and K. A. Baum. 1999.** An Experimental Test of Corridor Effects on Butterfly Densities. *Ecol. Appl.* 9: 623–633.
- Ingimarsdóttir, M., J. Ripa, and K. Hedlund. 2013.** Corridor or Drift Fence? The Role of Medial Moraines for Fly Dispersal Over Glacier. *Polar Biol.* 36: 925–932.
- Johnson, C. G. 1957.** The Distribution of Insects in the Air and the Empirical Relation of Density to Height. *J. Anim. Ecol.* 26: 479–494.

- Joyce, J. M., and E. J. Hansens. 1968.** The Influence of Weather on the Activity and Behavior of Greenhead Flies, *Tabanus nigrovittatus* Macquart and *Tabanus lineola* Fabricius. *Journal of the New York Entomological Society*. 76: 72–80.
- Junqueira, A. C. M., A. Ratan, E. Acerbi, D. I. Drautz-Moses, B. N. V. Premkrishnan, P. I. Costea, B. Linz, R. W. Purbojati, D. F. Paulo, N. E. Gaultier, P. Subramanian, N. A. Hasan, R. R. Colwell, P. Bork, A. M. L. Azeredo-Espin, D. A. Bryant, and S. C. Schuster. 2017.** The Microbiomes of Blowflies and Houseflies as Bacterial Transmission Reservoirs. *Sci. Rep.* 7: 16324.
- Keiding, J. 1986.** The House Fly: Biology and Control. WHO VBC. 86.937: 1–62.
- Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999.** Houseflies: Not Simple Mechanical Vectors of Enterohemorrhagic *Escherichia Coli* O157: H7. *Am. Journal of Tropical Medicine and Hygiene*. 61: 625–629.
- Krafsur, E. S., W. C. Black, C. J. Church, and D. A. Barnes. 1985.** Age Structure and Reproductive biology of a Natural House Fly (Diptera: Muscidae) Population. *Environmental Entomology*. 14: 159–164.
- Russell Lenth. 2016.** Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*. 69: 1–33.
- Lewis, T. 1965.** The Effects of an Artificial Windbreak on the Aerial Distribution of Flying Insects. *Ann. Appl. Biol.* 55: 503–512.
- Lewis, T. 1966.** An Analysis of Components of Wind Affecting the Accumulation of Flying Insects Near Artificial Windbreaks. *Ann. Appl. Biol.* 58: 365–370.
- Lewis, T. 1967.** The Horizontal and Vertical Distribution of Flying Insects Near Artificial Windbreaks. *Ann. Appl. Biol.* 60: 23–31.
- Lewis, T. 1969.** The Distribution of Flying Insects Near a Low Hedgerow. *J. Appl. Ecol.* 6: 443–452.
- Lole, M. J. 2005.** Nuisance Flies and Landfill Activities: An Investigation at a West Midlands Landfill Site. *Waste Manag. Res.* 23: 420–428.
- Lysyk, T. J., and R. C. Axtell. 1986.** Movement and Distribution of House Flies (Diptera: Muscidae) Between Habitats in Two Livestock Farms. *Journal of Economic Entomology*. 79: 993–998.

- Morgan, N. O., and R. Lee. 1977.** Vegetative Barriers Influence Flight of Salt Marsh Greenheads. *Mosquito News*. 37: 263–267.
- Murvosh, C. M., and C. W. Thaggard. 1966.** Ecological Studies of the House Fly. *Annals of the Entomological Society of America*. 59: 533–547.
- R Core Team. 2017.** R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ragland, S. S., and R. S. Sohal. 1973.** Mating Behavior, Physical Activity and Aging in the Housefly, *Musca domestica*. *Experimental Gerontology*. 8: 135–145.
- Sasaki, T., M. Kobayashi, and N. Agui. 2000.** Epidemiological Potential of Excretion and Regurgitation by *Musca Domestica* (Diptera: Muscidae) in the Dissemination of *Escherichia Coli* O157: H7 to Food. *J. Med. Entomol.* 37: 945–949.
- Schulze, T. L., E. J. Hansens, and J. R. Trout. 1975.** Some Environmental Factors Affecting the Daily and Seasonal Movements of the Salt Marsh Greenhead, *Tabanus nigrovittatus*. *Environmental Entomology*. 4: 965–971.
- Scudder, H. I. 1947.** A New Technique for Sampling the Density of Housefly Populations. *Public Health Rep.* 1896-1970. 62: 681–686.
- Shepard, M., R. Noblet, and P. E. Hunter. 1972.** Biology and Flight Ability of the House Fly Following Irradiation of the Pupae. *Journal of Economic Entomology*. 65: 1721–1725.
- Snow, W. F. 1977.** The Height and Direction of Flight of Mosquitoes in West African Savanna, in Relation to Wind Speed and Direction. *Bulletin of Entomological Research*. 67: 271–279.
- Taylor, L. R. 1960.** The Distribution of Insects at Low Levels in the Air. *J. Anim. Ecol.* 29: 45–63.
- Wagner, H. 1986.** Flight Performance and Visual Control of Flight of the Free-Flying Housefly (*Musca Domestica* L.) III. Interactions Between Angular Movement Induced by Wide- and Smallfield Stimuli. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*. 312: 581–595.
- West, L. S. 1951.** *The Housefly, Its Natural History, Medical Importance and Control*. Comstock Publishing Co., Ithaca, New York.
- Williams, D. F., and A. J. Rogers. 1976.** The Vertical and Lateral Distribution of Stable Flies Northwestern Florida. *J. Med. Entomol.* 13: 95–98.

**Winpisinger, K. A., A. K. Ferketich, R. L. Berry, and M. L. Moeschberger. 2005.** Spread of *Musca domestica* (Diptera: Muscidae), from Two Caged Layer Facilities to Neighboring Residences in Rural Ohio. *Journal of Medical Entomology*. 42: 732–738.

**Wratten, S. D., M. H. Bowie, J. M. Hickman, A. M. Evans, J. R. Sedcole, and J. M. Tylanakis. 2003.** Field Boundaries as Barriers to Movement of Hover Flies (Diptera: Syrphidae) in Cultivated Land. *Oecologia*. 134: 605–611.



**Table 2.1:** Total number of male and female house flies captured by collection period and trap location on a southern California dairy. Collection periods with ‡ symbol indicate significant difference in mean values ( $\mu$ ) by fly sex within that collection period at  $\alpha=0.05$ . Since location and date are confounded, no statistical analyses were performed to evaluate differences in overall number of flies captured among trap locations/dates.

Location	Morning (8am-10am)			Afternoon (12pm-2pm) ‡			Evening (4pm-6pm)		
	Male	Female	% Female (Mean±SE)	Male	Female	% Female (Mean±SE)	Male	Female	% Female (Mean±SE)
<b>Alfalfa</b>	31	6	16.8±7.0	7	6	45.8±14.4	0	0	
<b>Corral</b>	155	131	47.9±3.8	57	87	60.2±4.2	71	96	62.3±4.7
<b>East</b>	542	648	53.9±2.4	86	113	55.8±4.1	88	95	49.9±5
<b>Haystack</b>	202	217	55.6±3.3	117	175	56.7±4.8	40	70	67.6±6
<b>South</b>	162	220	55.9±3.3	133	203	64.2±3.9	0	0	
<b>West</b>	237	89	30.6±4.3	52	42	47.8±7.2	52	11	22.5±7.1
<b>Average</b>	5.8±0.4	6.1±0.5		2.6±0.2	3.4±0.2		2.3±0.2	2.5±0.2	

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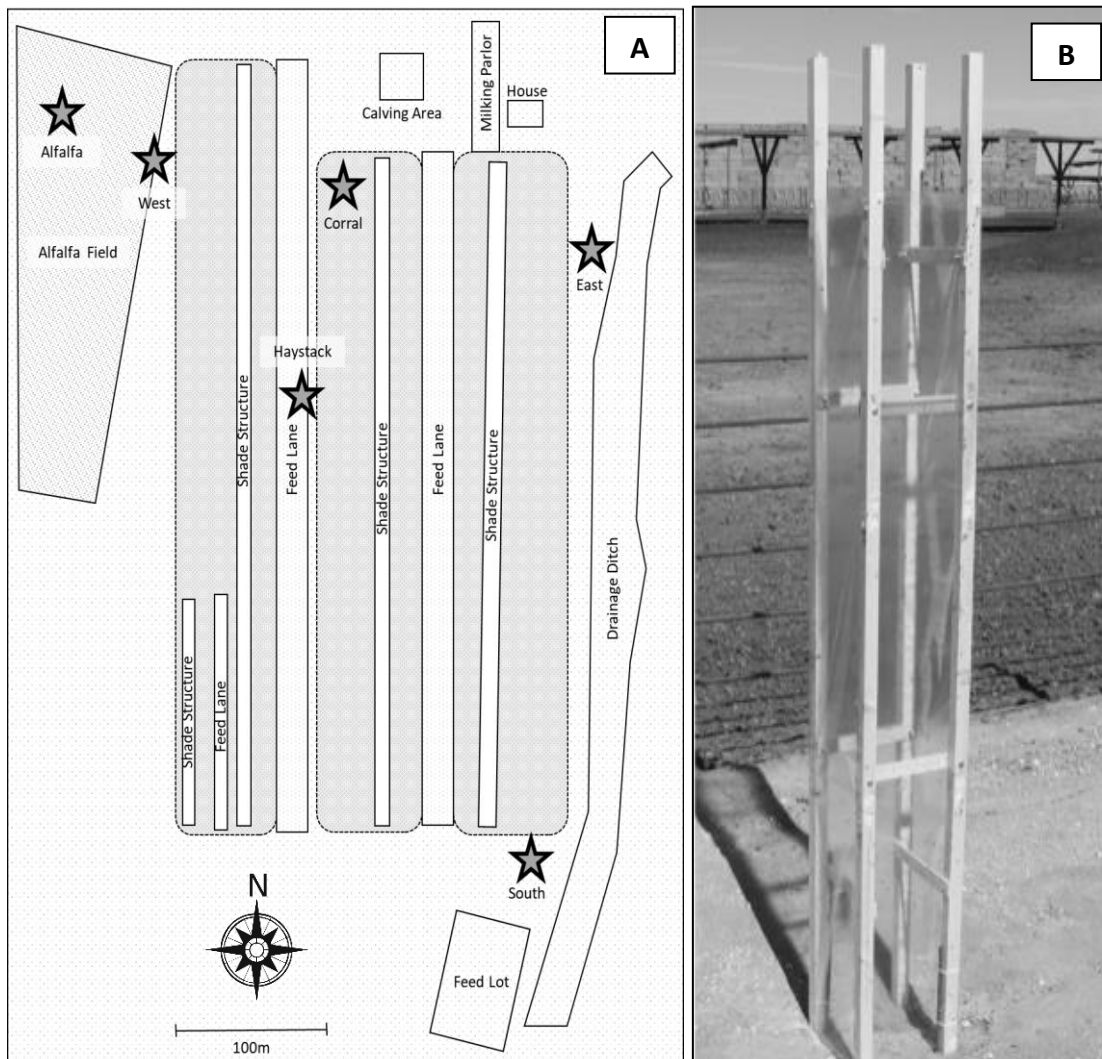
**Table S2.1:** Raw means, and their standard error, for temperature, relative humidity, and wind speed for each location/date across all collection periods.

Location	Date	Temperature °C (Mean±SE)	Humidity % (Mean±SE)	Wind Speed m/s (Mean±SE)	Wind Direction ° (Mean±SE)
<b>Alfalfa</b>	2-Jul-14	28.68±0.71	50.88±1.01	1.49±0.08	136.8±11.28
<b>Corral</b>	1-Jul-14	32.04±0.2	41.98±0.3	2.91±0.04	243.02±0.78
<b>East</b>	4-Jun-14	26.62±0.11	31.52±0.2	2.17±0.02	252.97±0.24
<b>Haystack</b>	7-Aug-14	24.05±0.22	47.97±0.48	2.36±0.05	209.1±2.65
<b>South</b>	12-Aug-14	29.18±0.21	46.81±0.59	3.57±0.04	246.06±0.84
<b>West</b>	13-Jun-14	26.8±0.18	37.31±0.33	2.4±0.05	225±0

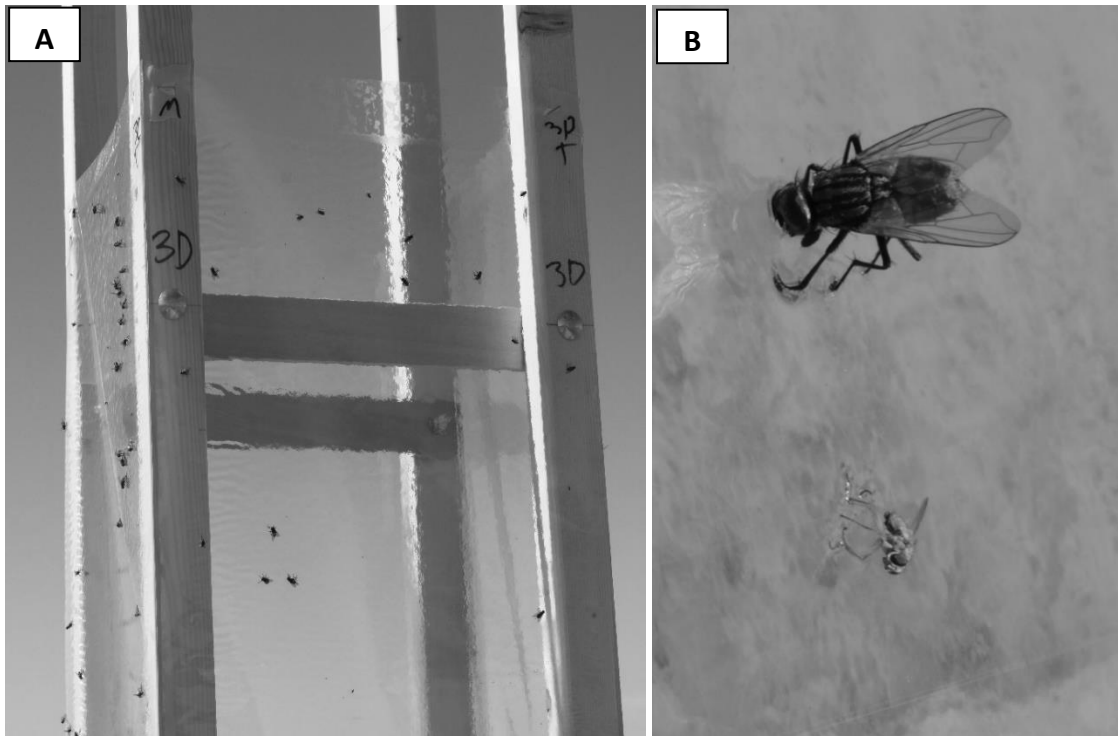
**Table S2.2:** Observed environmental variables for each collection period within each location sampled. Raw means and standard error for each variable within a collection period are shown.

Collection Period	Location	Temperature °C	Humidity %	Wind Speed m/s	Wind Direction °
<b>Morning</b>	Alfalfa	25.72	55.05	1.15	90
	Corral	27	47.55	2.05	225
	East	24.17	35.95	1.7	247.5
	Haystack	17.94	61.35	1.15	135
	South	23.83	61.55	2.45	225
	West	24.11	42.3	2.2	225
			$\mu=23.54\pm0.14$	$\mu=49.86.2\pm0.48$	$\mu=1.85\pm0.02$
<b>Afternoon</b>	Alfalfa	37.08	39	2.45	270
	Corral	35.94	29.2	3.05	247.5
	East	35.36	15	3.6	270
	Haystack	30.19	33.95	3.3	292.5
	South	35.25	30.05	4.85	270
	West	33.42	28.05	1.35	225
			$\mu=34.15\pm0.12$	$\mu=34.15\pm0.37$	$\mu=3.37\pm0.05$
<b>Evening</b>	Corral	37.31	43.45	4.25	270
	East	33.08	20.7	3.65	270
	Haystack	31.03	34.2	4.5	270
	West	30.83	25.3	5	225
			$\mu=33.58\pm0.18$	$\mu=33.58\pm0.63$	$\mu=4.22\pm0.03$

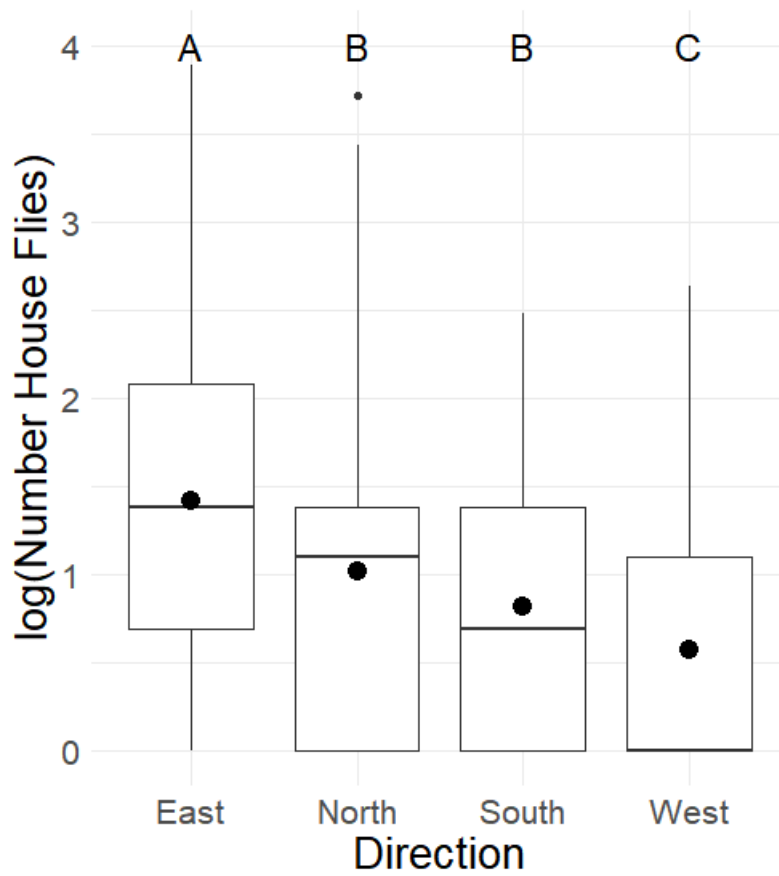
**Figure 2.1:** A) Diagram of the southern California dairy used in this study, with black stars indicating the locations of six house fly trapping locations; shaded areas are drylot pens (corrals) holding dairy cattle. B) Example of 2.13 m high tall sticky trap (“tower trap”) suspending clear sticky sheets applied to a wooden frame to capture flies during flight.



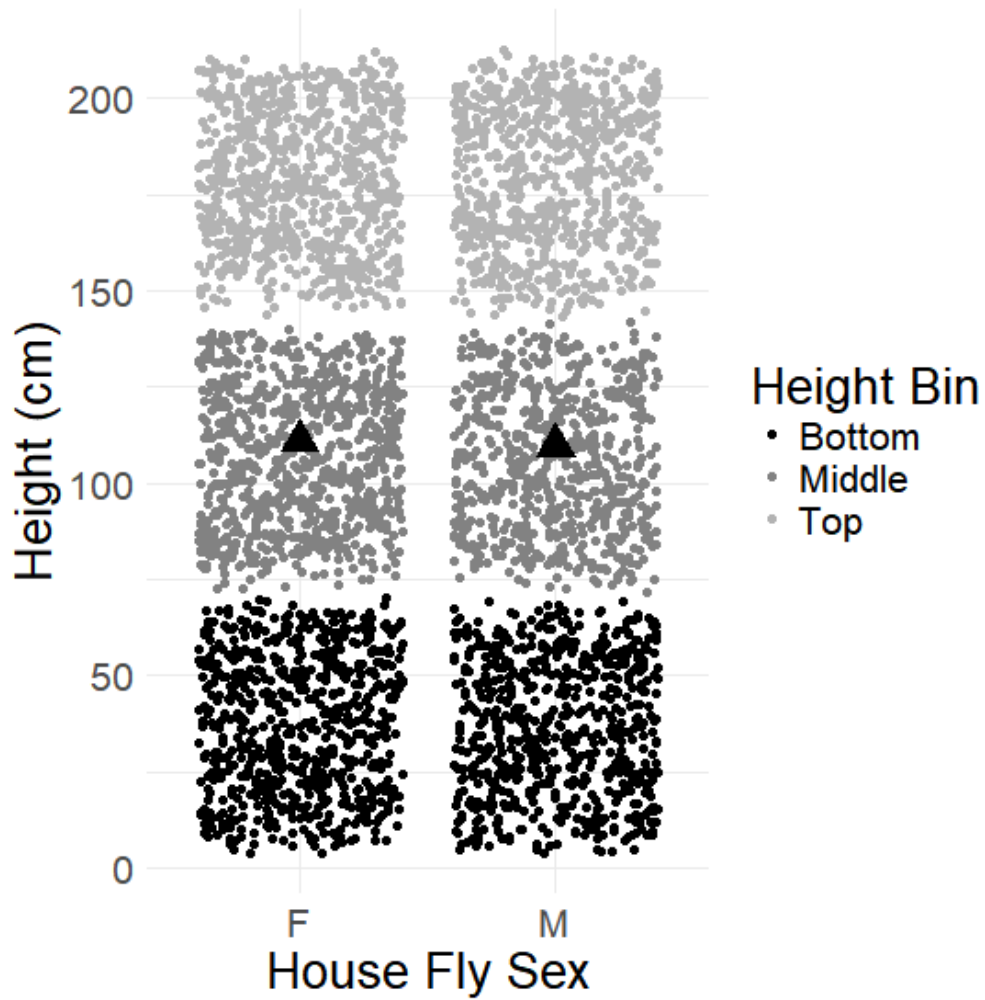
**Figure 2.2:** A) Top of a single trap, looking south. House flies can be seen strewn across the sticky sheets. B) Example of a house fly with its head stuck to the stick sheet. An indication that house flies may not have perceived the sheet as a solid object and were attempting to fly through.



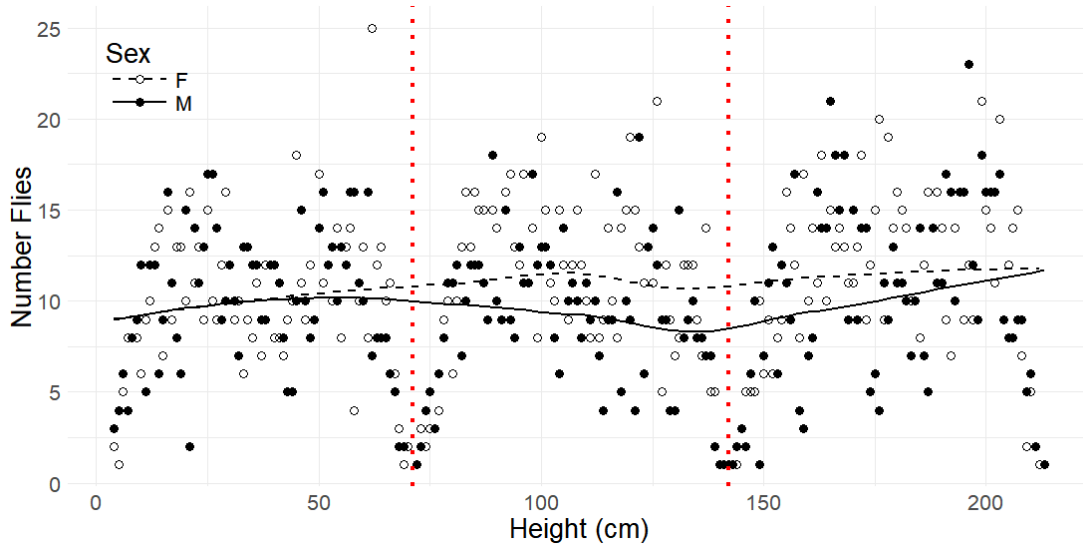
**Figure 2.3:** Log number of house flies captured by trap face (direction). Black circles represent the mean value for each direction. Letters above columns indicate significant differences at  $\alpha=0.05$  after Tukey's adjustment of the p-values.



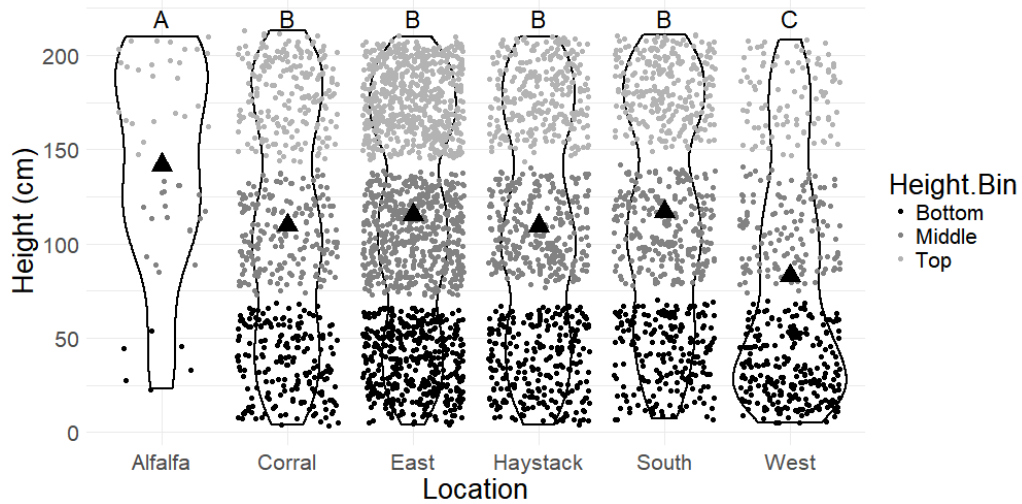
**Figure 2.4:** Distribution by flying height of house flies captured on a southern California dairy. Each dot represents an individual fly captured on 2.1 m tall “tower traps”. Data is combined for all traps, across all locations and collection periods. Gaps between height bins are an artifact due to lack of adhesive at the edge of each sticky sheet comprising each height bin of the tower trap. Black triangles indicate the mean capture height for females and males.



**Figure 2.5:** Scatter plot showing the A) total number of male (closed circle) and female (open circle) house flies collected at each 1 cm height increment. Red vertical lines indicate areas between each height bin (sticky sheet) where no sticky material was applied. The number of male and female house flies collected over each height were similar over the entire height of the trap. Horizontal lines are a local regression fitted to the data for each sex (male: solid, female: dotted) using a LOESS smoother. Data are combined for all traps from all locations.

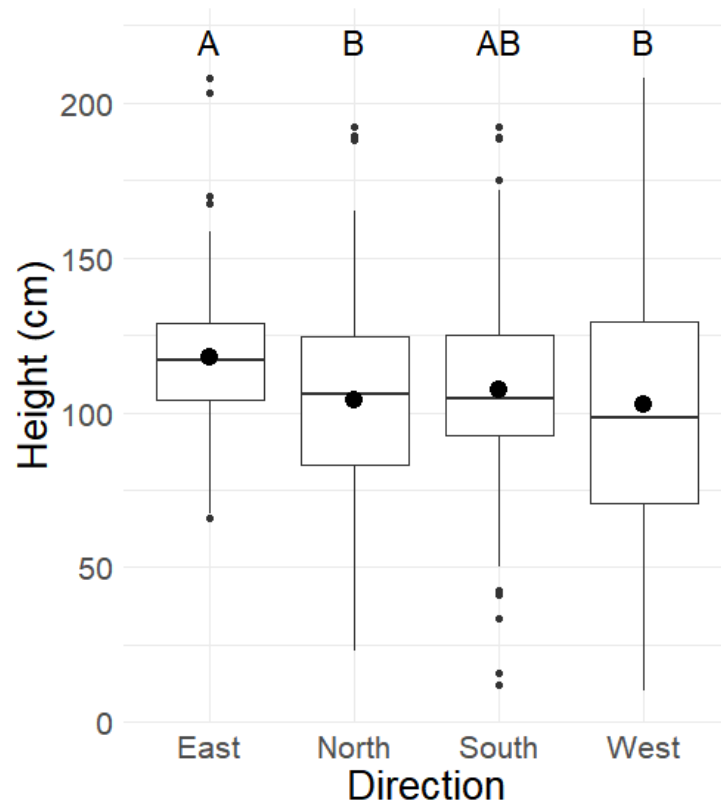


**Figure 2.6:** Violin plots showing the number of house flies (both sexes pooled) captured over various heights for traps at six different locations. Data points were jittered to allow for a better appreciation of the vertical spread of the data. Black triangles represent the mean capture height for flies at each trapping location. Letters above columns indicate significant differences in mean heights. The order of trapping locations does not indicate a cline or trend across the dairy.

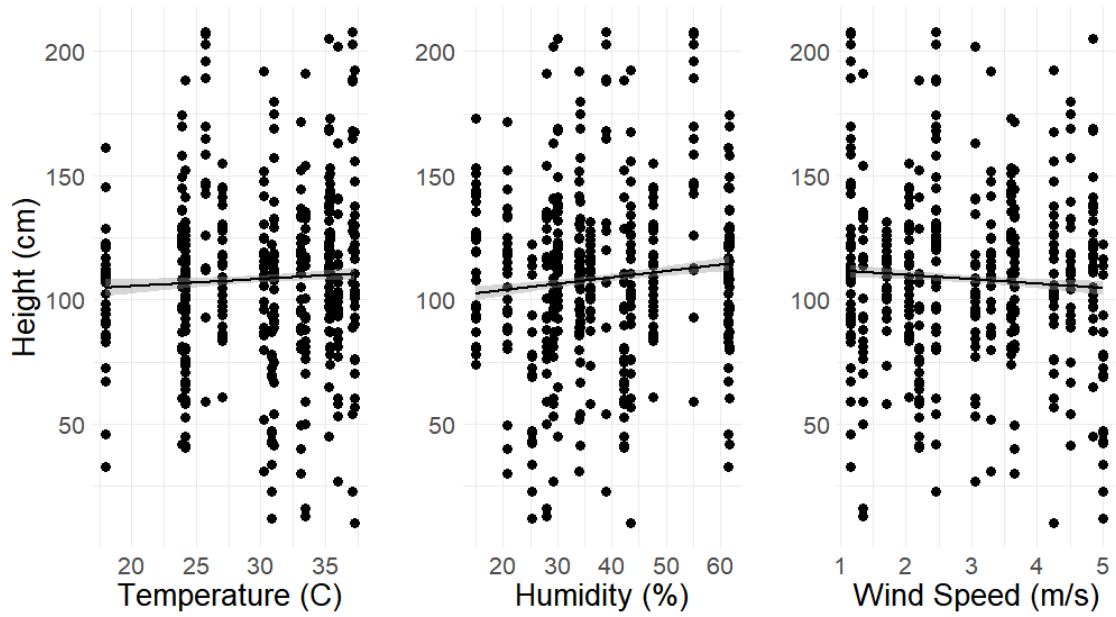




**Figure 2.7:** Boxplots showing the vertical distribution of captured house flies by trap face orientation, with means indicated by the black circles and medians by the black horizontal lines. Letters above columns indicate significant differences at  $\alpha=0.05$  after Tukey's adjustment of the p-values.



**Figure 2.8:** Regression of temperature, relative humidity, and wind speed on the flying height of house flies. The black line denotes the model fit and the gray area around it represents the standard error.



## CHAPTER 3

### House Fly Directional Flight Following Disturbance under Field Conditions

#### ABSTRACT

Flight behavior of adult house flies impacts pathogen transmission and nuisance to humans and animals near fly development sites. Yet, short-range directional flight of house flies has been overlooked as a research focus. A release-recapture study was conducted to characterize house fly flight direction and to assess the impact of environmental conditions on the short-range flight direction. House flies were liberated from a specially designed release chamber and allowed to disperse for 15 min. Flies were recaptured using white PVC pipe covered with Tanglefoot and placed in two concentric circles with radii of 10 m and 20 m. House flies were not dispersing randomly, and the short-range flight of both male and female house flies was generally toward the southeast, with somewhat greater variability in flight direction for males relative to females. Mean flight direction differed by time of day with a decrease in the variation around the mean flight direction at later times in the day. Flight direction was significantly correlated with time of release, solar position, wind speed, humidity, and temperature. The covariates of time and solar position had the strongest correlation with flight direction, followed by wind speed. Humidity and temperature were more weakly correlated with flight direction. The correlation of flight direction with solar position may be spurious as a small group of trees was also southeast of the release point. Dispersing flies may have been using this visual cue as a stripe fixation point or flying towards this area to seek shade. Flight direction was not correlated with wind direction; the mean flight direction was approx. 136° from a direct upwind flight, anecdotally supporting a crosswind flight behavior for

dispersing house flies. Further research at a variety of locations under a more diverse array of environmental conditions is necessary to create a more complete picture of short-range dispersal for house flies, including the role of wind direction.

## **INTRODUCTION**

House flies are mechanical vectors of enteric pathogens that affect both humans and animals, including enterohemorrhagic *E. coli* O157:H7 and *Shigella* spp. (Watt and Lindsay 1948, Lindsay et al. 1953, Kobayashi et al. 1999, Sasaki et al. 2000), both of which are associated with diarrheal disease. Consequently, application of insecticides to reduce fly numbers in small rural towns has successfully reduced the incidence of diarrheal disease (Watt and Lindsay 1948, Lindsay et al. 1953). However, the overuse of insecticides has led to selection for insecticide resistance in house fly populations (Chapman 1985, Bull and Pryor 1990, Scott et al. 2013), and alternative management strategies that reduce fly dispersal from development sites into nearby sensitive residential communities are needed. Physical barriers (“flight barriers”) that impede the normal flight pattern of house flies have been suggested as a means to reduce fly dispersal from development sites or to direct dispersing flies away from more sensitive areas (Fried et al. 2005, Dubie 2014). However, little is known about the flight characteristics of house flies under field conditions, making design and placement of flight barriers for the management of house flies challenging.

While house flies have been recorded to fly over 20 km in a single flight (Bishopp and Laake 1921), typical flight distance is expected to be less than 3.2 km (Parker 1916, Bishopp and Laake 1921, Schoof and Siverly 1954a, Schurrer et al. 2004, Winpisinger et al. 2005) and fly dispersal may simply be the culmination of a series of short (<1km) disjointed circuitous flights

resulting in the aggregation of house flies at some more distant point which flies find attractive (Schoof and Siverly 1954a, 1954b). If dispersal is principally through a series of short flights, then flight barriers may be an effective means to direct dispersing house flies away from sensitive sites (e.g. homes and schools) and toward areas where house flies can concentrate without negatively impacting nearby humans or animals.

While it might be reasonable to expect that house fly dispersal flight would be impacted by changing environmental conditions, only wind direction (and wind speed included as an observation) has been given any significant attention as to its impact on house fly flight direction (Nuttall et al. 1914, Parker 1916, Bishopp and Laake 1921, Hanec 1956, Pickens et al. 1967, Cosse and Baker 1996). There is much uncertainty about how wind speed and wind direction affect the directional flight of house flies. Many of the conclusions from the literature are not empirical but generally anecdotal and often contradictory. House flies have been found to disperse against the wind (Nuttall et al. 1914, Hanec 1956, Pickens et al. 1967), at an angle in relation to wind direction (Nuttall et al. 1914), or with no relationship to wind direction at all (Parker 1916, Quarterman et al. 1954a, Quarterman, et al. 1954b). With one study observing house flies moving with, against, and perpendicular to the wind (Bishopp and Laake 1921). Perhaps this is because the study was conducted over multiple years adding a high amount of noise to the data. House flies in a wind tunnel will fly upwind to locate an odor source Cosse and Baker (1996), but this may be typical only of an appetitive search behavior in a constrained system rather than of more general dispersal flight behavior as a result of other factors present under natural field conditions. No study to date has ever examined the relationship of temperature or humidity to direction of flight for house flies.

Parker (1916) theorized that each individual environmental factor (wind speed, temperature, humidity, and wind direction) will vary frequently over time (weeks, months, seasons) making direction appear random, and leading to house fly directional flight that also appears to be random over the scale of a fly season. However, if house fly flight direction is observed using a small-time scale (minutes) then it is likely that environmental conditions will be more consistent and may become a better descriptor of flight direction. For instance, if wind direction is a strong predictor of flight direction, manmade or vegetative barriers could be constructed on the side of the farm from which winds prevail. The objective of the current study was to determine house fly flight direction during a short flight following fly disturbance at a release point, and to evaluate the impact of environmental conditions (temperature, humidity, wind speed, wind direction) and solar position on this flight.

## **METHODS**

Adult house flies were collected by sweep net from a large commercial dairy near San Jacinto, California during summer of 2015 and subsequently reared in the laboratory using standard rearing practices (Zahn and Gerry 2018). House fly pupae from several separate immature rearing pans were placed into a temperature-controlled room at 16 °C for up to 7 days until >5,000 pupae were accumulated. All pupae were then mixed together and 5,000 pupae (measured volumetrically) were randomly selected and placed into an adult fly cage provisioned with food (1:1 powdered milk and granular sugar) and water. Cages were held in an insectary at 26.7 °C and 45% RH for emergence of adult flies.

House fly flight direction was determined for 18 separate fly release trials performed over 10 days primarily from April-July 2016 (one release occurred during October 2015).

Typically, groups of flies were released at two separate times on each date, though three fly releases occurred on one day and a single fly release occurred on three days. Where multiple fly releases occurred on the same date, releases were separated by  $\geq 1$  hr. Release times were deliberately varied within and among days to provide a range of environmental and solar position conditions across all trial dates. Flight direction trials were conducted at the University of California at Riverside (UCR) Agricultural Operations in a large dirt lot (70m x 200m) that lacked vegetation or other structure that might impede or alter house fly flight within the study area (Figure 3.1). Flies were released from a single location (release point) and subsequently recaptured using sticky pole traps placed to form two trap circles with a radius of 10m and 20m from the release point. Two trapping circles were used since it was unknown how consistent flight direction would be after house flies were released.

Sticky pole traps were 2.4 m long white PVC pipe (19 mm outside dia.) poles liberally coated with a sticky material (Tanglefoot, Grand Rapids MI, #300000685) and placed on 0.5 m long rebar supports to stand vertically. Each trap circle contained 8 sticky poles evenly spaced around the trap circle at 45-degree increments starting at magnetic north (Figures 3.2 & 3.3). After site set up, and before each house fly release, every pole was visually examined, and any insects captured during site set up were removed. Tanglefoot was reapplied to poles as needed. Natural populations of house fly in this area are very low and there was negligible (approx. <2 fly per pole on average) capture of house flies on poles during site set up period as well as between fly releases that occurred on the same date. Thus, the house flies captured during these studies were those that were released during these trials at the study site.

On each trial date, an adult fly cage containing 5,000 3-5-day-old mixed sex flies (based on volume of pupae measured) was placed in a shaded location near the release site for at least

1 hr to acclimate flies to the field environment. Flies were then anesthetized with CO<sub>2</sub>, transferred into a specially designed fly release chamber (Figure 3.4), and held in the shaded location for an additional 30 min to allow flies to recover. The release chamber was a triangular pyramid constructed of 0.5 cm thick shatter resistant plexiglass cut into triangles of equal dimension (45.5 x 45.5 x 45.5 cm). The three side walls attached to a base triangle using hinges such that when closed the release chamber had a total volume of 0.011 m<sup>3</sup>. Air vents were cut into each side wall of the chamber and were covered with mesh to provide air flow. An elastic band was fixed from the base of the pyramid to each side wall, placing all three sides of the pyramid under tension when the side walls were raised to close the release chamber. The release chamber was held in a closed position using a small bungee cord wrapped around the top of the release chamber to hold the side walls together.

A few minutes before each fly release, the release chamber was placed on the open ground in the center of the study site. A 40 m long chamber release string was tied to the bungee cord holding the release chamber closed. The researcher then moved in a randomly selected direction to a position 40m from the release chamber (outside the study area) while carrying the end of this chamber release string. Upon pulling the string, the bungee cord securing the release chamber was released, allowing the sides of the release chamber to fall quickly to the ground. The sudden and complete opening of the release chamber startled the contained flies into immediate flight (see video in supporting documents). This startle flight is typical under natural field conditions where flies are numerous and movement of nearby animals or objects (or even a gust of wind) will startle a concentrated group of flies to take flight simultaneously. Once released, flies were given 15 min to be captured on a sticky pole or to disperse from the study area, after which each sticky pole was visually examined and the



number of house flies on each pole was recorded by sex. At the end of the 15 min flight period, very few flies remained within the study area and those that did were usually on the ground very near the release chamber, suggesting that they never took flight. Wind speed (m/s), wind direction (degrees from north), temperature (°C), and relative humidity (percent) were recorded at the start and end of each 15 min dispersal period, with the average of the two recordings used for analysis.

## **DATA ANALYSIS**

Because time of day is known to impact house fly flight activity and perhaps flight direction (see Chapter 1 of this dissertation), trials were grouped by time of fly release into three periods: morning (0815-1045, N=4), afternoon (1145-1255, N=8), and evening (1400-1820, N=6). The total number of house flies of each sex that were recaptured during each fly release trial was square-root transformed to meet the test assumption of normality and then subjected to ANOVA to identify differences in recapture rate among release periods, by trap distance (diameter of trapping circle), by house fly sex, and for interaction of house fly sex with release period or trap distance. Post-hoc comparisons were made using a Tukey's adjustment of the p-value (Lenth 2018). Model assumptions of linearity and homoscedasticity of the residuals was visually checked using quantile-quantile plots (Q-Q plots) and residual versus fitted plots, respectively (Crawley 2013).

For each release trial, the mean direction of flight and the circular concentration around the mean direction of flight (mean resultant length) was determined for each fly sex and trap distance using the R package *circular* version 0.4-93 (Agostinelli and Lund 2017) following the methods given by Pewsey et al. (2013). Mean direction of flight is the average of the individual

angles recorded for each individual fly captured on a sticky pole following a release. The average direction of fly travel is calculated by converting the angle of each observation to a polar coordinate (radians) before calculating the mean angle using the equation,

$$\bar{\theta} = \text{atan2} \left( \frac{1}{n} \sum_{i=1}^n \cos \theta_i, \frac{1}{n} \sum_{i=1}^n \sin \theta_i \right)$$

where  $\theta_i$  = the angle (converted to radians) of each observation, and  $n$  = number of observations. The circular concentration around the mean direction of flight is an estimate of homoscedasticity and calculated using the equation,

$$\bar{R} = \sqrt{\left( \frac{1}{n} \sum_{i=1}^n \cos \theta_i \right)^2 + \left( \frac{1}{n} \sum_{i=1}^n \sin \theta_i \right)^2}$$

where  $\bar{R}$  takes a value ranging from [0,1]. A circular concentration value close to 1 indicates that most flies were flying in the same direction (similar angle of flight) while a value close to 0 indicates that flies were not uniform in their flight direction (varied flight angles). Following the recommendations of Pewsey et al. (2013), no corrections to the estimated means or circular concentration estimates were made since the data consisted of at least eight observations (number of poles in each circle).

Watson's large-sample nonparametric test ( $Y_g$ ) was used (Pewsey et al. 2013) to test the null hypothesis of similar flight direction for males and females among the three release periods and the two trap distances. Similarity in flight direction was also assessed between males and females within each of the three release periods and for both trap distances (Figure 3.5). If comparisons of groups with three or more returned a significant p-value (large  $Y_g$ ), then individual pairwise comparisons were made using the same Watson's large-sample nonparametric test using a Bonferroni correction of the p-values to account for the multiple comparisons.

To determine if house fly flight direction was correlated with any of the observed linear predictors (time of release, temperature, humidity, and wind speed) or circular predictors (wind direction, solar azimuth), the mean direction of flight for each house fly sex at each trap distance (10m and 20m) was determined for all 18 fly releases. All correlations were pooled over fly sex. Circular-linear correlations were analyzed using the *R2xtCorrCoeff()* function provided by Pewsey et al. (2013) which produces a correlation coefficient ( $R^2_{\chi\theta}$ ) between one and zero, and is analogous to Pearson's coefficient of correlation. Circular-circular correlations were analyzed using the *cor.circular()* function found in the 'circular' package. This function calculates the Jammalamadaka-Sarma correlation coefficient (JSCoef) which gives a test-statistic and a p-value used to assess the likelihood that the correlation coefficient is zero.

## RESULTS

A total of 4,013 flies (min=33, max=813, average=222.9±40.7) were recaptured out of approx. 100,000 house flies released over all trials. While similar numbers of male and female house flies were released, more female (65.53±6.48; mean± SE) than male (45.94±12.59) house flies were recaptured ( $F_{1,64}=16.51$ ;  $P<0.001$ ) over all trials, with more house flies captured in the morning (98.06±23.07), compared to evening (58.75±8.91) or afternoon (32.31±6.47,  $F_{2,64}=14.40$ ;  $P<0.001$ ). However, there was a significant interaction of fly sex and release period ( $F_{2,64}=7.45$ ;  $P=0.001$ , Figure 3.6), with males recaptured in greater numbers during the morning relative to afternoon or evening release periods ( $P<0.032$ ) while females were captured in similar numbers throughout the day ( $P<0.348$ ). The mean number of flies captured at each trap distance also significantly varied ( $F_{1,64}=19.87$ ;  $P<0.001$ ), with more house flies recaptured at 10m

(76.75±11.69) relative to 20m (34.72±6.65), and with no significant interaction between sex and distance ( $F_{1,64}=1.11$ ;  $P=0.197$ ).

### **Flight Direction by Trap Distance**

Mean flight direction significantly varied by trap distance ( $Y_g=7.198$ ;  $p=0.007$ ) but was generally to the southeast for both the 10 m and 20 m trap distances (Table 3.1, Figure 3.7). At each trap distance, mean flight direction was similar between the sexes (10m:  $Y_g=0.172$ ;  $p=0.679$ , and 20m:  $Y_g=2.432$ ;  $p=0.119$ ). Males had a slight, but significant, eastward shift in mean flight direction from the 10 m to the 20 m trap distance ( $Y_g=6.375$ ;  $p=0.012$ ) while females had a similar flight direction at both distances ( $Y_g=2.062$ ;  $p=0.151$ ).

### **Flight Direction by Release Period**

Mean flight direction varied by release period ( $Y_g=57.295$ ;  $p < 0.001$ ), with significant differences among all three release periods ( $p \leq 0.009$ ) (Table 3.2, Figure 3.8). However, flight direction was quite variable in the morning (angular range [AR] = 289.06, which is the arc containing all mean flight direction values) while becoming more directed towards the southeast during the afternoon (AR = 273.76 degrees) and especially during the evening (AR = 73.65 degrees). Between the sexes, mean flight direction differed significantly during morning ( $Y_g=41.949$ ;  $p < 0.001$ ), afternoon ( $Y_g=4.211$ ;  $p=0.04$ ) and evening ( $Y_g=10.361$ ;  $p=0.001$ ) with females exhibiting a more southward flight relative to males during the afternoon and evening. Within the sexes, the flight direction for males was similar during afternoon and evening ( $p > 0.05$ ), while female flight direction had a significant shift eastward between afternoon and evening ( $p=0.028$ ). Due to the wide range of observed mean flight directions during the morning

period and the low values obtained for  $\bar{R}$ , differences in mean flight direction were not tested.

### **Flight Direction by Predictor Variables**

Mean flight direction was significantly correlated with time of fly release ( $R^2_{x\theta}=0.446$ ;  $P=0.008$ ; Figure 3.9A) with mean flight direction of the combined fly sexes shifting from the south towards the east as the time of day progressed. Mean flight direction was also correlated with most of the environmental factors examined including a significant correlation with wind speed ( $R^2_{x\theta}=0.472$ ;  $P=0.026$ ; Figure 3.10A), solar position (JSCoef=-0.361;  $P=0.031$ ; Figure 3.9B), and temperature ( $R^2_{x\theta}=0.328$ ;  $P=0.04$ ; Figure 3.10C), but not relative humidity ( $R^2_{x\theta}=0.192$ ;  $P=0.204$ ; Figure 3.10B). Flight direction was not correlated with wind direction (JSCoef=0.163;  $p=0.669$ , Figure 3.11A & B), though wind direction was relatively consistent in blowing from the west during 16 of the 18 fly release trials. Many of the linear and circular variables are also significantly correlated with each other: time and solar position ( $R^2_{x\theta}=0.854$ ;  $P<0.001$ ), time and wind speed ( $R^2=0.76$ ;  $P<0.001$ ), and solar position and wind speed ( $R^2_{x\theta}=0.438$ ;  $P=0.01$ ).

### **DISCUSSION**

Over the last century there has been little work on the directionality of house fly flight, and is often viewed as a teleological problem, focusing on locations where house flies aggregate and ignoring how house flies arrived at that location. The purpose of this study was to characterize house fly flight by examining the relationship of flight direction with commonly observed environmental factors. This is the first semi-field study to investigate house fly flight direction on a small spatial and temporal scale.

Even though distance of recapture for this study was comparatively short in relation to other works, the recapture rate of 4% is similar to many release-recapture studies using house flies (Nuttall et al. 1914, Parker 1916, Bishopp and Laake 1921, Quarterman, J. W. Kilpatrick, et al. 1954, Quarterman, Mathis, et al. 1954, Hanec 1956, Pickens et al. 1967). Over all releases more females were recaptured than males. In chapter one of this dissertation it was observed that male house flies were more commonly collected in the mornings with house fly sex ratios equalizing from late morning until early evening; this was attributed to an increase in flight activity. However, a similar trend in house fly recaptures was observed in the current study, casting doubt on this earlier conclusion since similar numbers of house flies (and equal sex ratios) were released during each replicate and the number of flies in flight should have been similar. If more flies of one sex are not flying, then more must be landing. Perhaps the differences in recapture of flies for each sex are the result of a differential behavioral response, where males are more likely to land on traps (perhaps any structure) early in the day as part of a mate location strategy (Murvosh et al. 1964, Ragland and Sohal 1973), while female house flies are landing at equal rates throughout the day.

It is apparent that house fly flight direction is not a series of random flights as suggested by Schoof and Siverly (1954a, 1954b). If true, then the pattern of dispersal should have been uniform around the circle, which was never observed. The average direction of flight for both male and female house flies was to the southeast, and was relatively consistent over the two trapping distances, of 10 m and 20 m from the release point. It was anticipated that house flies might display a random direction of flight upon initial release (startle flight) before exhibiting any directionality. However, the similarity in flight direction recorded at both 10 and 20 m shows no indication of a random direction of flight, and that flies are likely continuing in the

same direction between 10 m and 20 m. The mean direction of flight between the two distances did significantly differ for males, but the means were similar enough to suggest that after being released, males are consistently moving the same direction over both distances. Flight direction remained the same for females. Flies likely made their direction choice within the first 10 m as both trap circles generally provided similar mean directions of flight for house flies.

There was a lot of variation in the average direction of flight between each of the three release periods, but flights became more directed towards the east (starting from south) as the time of day increased. House fly flight was distributed nearly uniformly around the release site during the morning, making any assessment of mean flight direction during this release period conjectural (even though means were obtained). At the latter two collection periods (later in the day) the direction of flight became more consistent, with both male and female flies moving towards the southeast. Even though there were some statistically significant differences in flight directions between sexes, the difference appears to be small enough to conclude that both males and females are generally moving in the same direction.

Both solar position and time of day were correlated with the mean direction of flight over all fly releases. However, clock time and solar position are covariates and resulted in similar correlational values with the mean direction of flight (they are on different scales). It is likely better to use the solar position as a predictor of fly movement rather than time, since solar position is better predictor over a variety of locations and environments and is not an anthropological construct like time. Given the significance of the correlations with solar position (and time) it is possible that house flies are using the sun as a solar compass, as observed in *Drosophila* spp., (Beetz and el Jundi 2018) to maintain a straight flight path. Alternatively, the correlation solar position is simply circumstantial, as house flies could have moving to the

southeast in response a small group of trees lying in the same direction, using stripe fixation making the correlation with the sun circumstantial as the mean direction of fly travel happened to be the same as the observed azimuths of the sun. Stripe fixation or simply fixation, is the alignment of an insects' flight path towards a fixed object to maintain a straight flight path, similar to *Drosophila* spp. (Gotz 1987, Giraldo et al. 2018), a short-range dispersal behavior.

It was thought that the group of trees to the southeast of the release site was outside the range that would have a visual impact on house flies, but it is possible that upon release flies detected the shaded area and moved in that direction, at least later in the day. However, this leaves the morning period unresolved. Perhaps, in the morning period house flies were not orienting towards any target and were not flying in a straight line or simply flew in an arbitrary direction. Since temperature was poorly correlated with direction it is likely not a shade seeking response. Rather it may be the position of the sun in the evening relative to the morning influences how house flies perceive the tree line (*i.e.* lower contrast in the morning but higher in the evening) as house flies have been shown to orient to contrasting objects (Howard and Wall 1998).

Since all the linear environmental variables except relative humidity were significantly correlated with changes in the mean direction of fly movement, it becomes difficult to identify if any have a causal effect on the mean direction of fly movement. Temperature does impact house fly flight activity (chapter 1) as well as the number of flies in flight (Semakula et al. 1989). There was a strong correlation between wind speed and mean direction of fly travel even though no wind speeds were recorded between 0 and 2 m/s. As wind speed increased, the mean flight direction shifted from the south to the southeast and became more consistent. However, windspeed is also a covariate of release time and solar position, with average wind



speeds increasing with time (later in the day). This makes it difficult to draw a definitive conclusion about the impact of windspeed on house fly flight direction.

It has been suggested that house fly flight direction is a direct response to wind direction, but all previous field studies have been conducted over days or weeks (Nuttall et al. 1914, Parker 1916, Bishopp and Laake 1921, Quarterman, J. W. Kilpatrick, et al. 1954, Quarterman, Mathis, et al. 1954, Hanec 1956, Pickens et al. 1967), making the observed wind directions unreliable for determining how house flies are orienting over short flights and distances and time periods. In the current study, the short flight time (15 min) ensured that wind direction remained consistent over the entire flight period. However, there is not enough evidence to support any correlation between wind direction and mean flight direction even when removing the two releases where winds were not from the west (data not shown).

Anecdotally, house flies in the current study appeared to move down and slightly crosswind as evidenced by a mean difference in wind direction and fly movement of  $136^\circ$ , similar to what was observed by Nuttall et al. (1914) and Bishopp and Laake (1921). An average angle of  $\sim 0^\circ$  would indicate upwind travel,  $\sim 90^\circ$  indicates crosswind movement, and  $\sim 180^\circ$  a downwind movement. It has been suggested that if wind direction is highly variable (changes greater than  $60^\circ$  change) it becomes advantageous for a flying insect to move up or downwind rather than to move crosswind as it increase the chances of intercepting an airborne odor (Cardé and Willis 2008), but this hypothesis remains untested for house flies. Further work on the relationship between flight direction and wind direction is necessary. It would be advantageous to continue this research at multiple release sites using more accurate wind sensing equipment that is paired with video footage of dispersing house flies. Doing this would allow more precise correlations between direction of flight and instantaneous wind direction.

## REFERENCES CITED

- Agostinelli, C., and U. Lund. 2017.** R package “circular”: Circular Statistics.
- Beetz, M. J., and B. el Jundi. 2018.** Insect Orientation: Stay on Course with the Sun. *Current Biology*. 28: R933–R936.
- Bishopp, F. C., and E. W. Laake. 1921.** Dispersion of Flies by Flight. *Journal of Agricultural Research*. 21: 729–766.
- Bull, D. L., and N. W. Pryor. 1990.** Characteristics of Resistance in House Flies Subjected to Long-term Concurrent Selection with Malathion and Permethrin. *Pesticide Biochemistry and Physiology*. 37: 101–115.
- Cardé, R. T., and M. A. Willis. 2008.** Navigational Strategies Used by Insects to Find Distant, Wind-Borne Sources of Odor. *Journal of Chemical Ecology*. 34: 854–866.
- Chapman, P. A. 1985.** The Resistance to Eighteen Toxicants of a Strain of *Musca domestica* L. Collected from a Farm in England. *Pesticide Science*. 16: 271–276.
- Cosse, A. A., and T. C. Baker. 1996.** House Flies and Pig Manure Volatiles: Wind Tunnel Behavioral Studies and Electrophysiological Evaluations. *Journal of Agricultural Entomology*. 13: 301–317.
- Crawley, M. J. 2013.** *The R book*, Second edition. ed. Wiley, Chichester, West Sussex, United Kingdom.
- Dubie, T. R. 2014.** Influence of Vegetation Height on the Dispersal of House Flies (*Musca Domestica*) from Livestock Facilities and Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers (Dissertation). Oklahoma State University.
- Fried, J. H., D. J. Levey, and J. A. Hogsette. 2005.** Habitat Corridors Function as Both Drift Fences and Movement Conduits for Dispersing Flies. *Oecologia*. 143: 645–651.
- Giraldo, Y. M., K. J. Leitch, I. G. Ros, T. L. Warren, P. T. Weir, and M. H. Dickinson. 2018.** Sun Navigation Requires Compass Neurons in *Drosophila*. *Current Biology*. 28: 2845–2852.e4.
- Gotz, K. G. 1987.** Course-Control, Metabolism and Wing Interference During Ultralong Tethered Flight in *Drosophila melanogaster*. *J. Exp. Biol.* 128: 35–46.
- Hanec, W. 1956.** A Study of the Environmental Factors Affecting the Dispersion of House Flies (*Musca domestica* L.) in a Dairy Community Near Fort Whyte, Manitoba. *Canadian Entomologist*. 88: 270–272.

- Howard, J. J., and R. Wall. 1998.** Effects of Contrast on Attraction of the Housefly, *Musca domestica*, to Visual Targets. *Medical and Veterinary Entomology*. 12: 322–324.
- Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999.** Houseflies: Not Simple Mechanical Vectors of Enterohemorrhagic *Escherichia coli* O157: H7. *American Journal of Tropical Medicine and Hygiene*. 61: 625–629.
- R. Lenth. 2016.** Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*. 69: 1–33.
- Lindsay, D. R., W. H. Stewart, and J. Watt. 1953.** Effect of Fly Control on Diarrheal Disease in an Area of Moderate Morbidity. *Public Health Rep*. 68: 361.
- Murvosh, C. M., R. L. Fye, and G. C. Labrecque. 1964.** Studies on the Mating Behavior of the House Fly, *Musca Domestica* L. *Ohio Journal of Science*. 64: 264–71.
- Nuttall, G., E. Hindle, and G. Merriman. 1914.** The Range of Flight of *Musca domestica*: Report upon Experiments. *The Journal of Hygiene*. 14: 23.
- Parker, R. R. 1916.** Dispersion of *Musca domestica* Linnaeus Under City Conditions in Montana. *Journal of Economic Entomology*. 9: 325–354.
- Pewsey, A., M. Neuhauser, and G. Ruxton. 2013.** *Circular Statistics in R*. Oxford University Press.
- Pickens, L. G., N. O. Morgan, J. G. Hartsock, and J. Smith. 1967.** Dispersal Patterns and Populations of the House Fly Affected by Sanitation and Weather in Rural Maryland. *Journal of Economic Entomology*. 60: 1250–1255.
- Quarterman, K. D., J. W. Kilpatrick, and M. Willis. 1954a.** Fly Dispersal in a Rural Area Near Savannah, Georgia. *Journal of Economic Entomology*. 47: 413–419.
- Quarterman, K. D., W. Mathis, and J. W. Kilpatrick. 1954b.** Urban Fly Dispersal in the Area of Savannah, Georgia. *Journal of Economic Entomology*. 47: 404–412.
- Ragland, S. S., and R. S. Sohal. 1973.** Mating Behavior, Physical Activity and Aging in the Housefly, *Musca domestica*. *Experimental Gerontology*. 8: 135–145.
- Sasaki, T., M. Kobayashi, and N. Agui. 2000.** Epidemiological Potential of Excretion and Regurgitation by *Musca Domestica* (Diptera: Muscidae) in the Dissemination of *Escherichia coli* O157: H7 to Food. *Journal of Medical Entomology*. 37: 945–949.
- Schoof, H. F., and R. E. Siverly. 1954a.** Multiple Release Studies on the Dispersion of *Musca domestica* at Phoenix, Arizona. *Journal of Economic Entomology*. 47: 830–838.

- Schoof, H. F., and R. E. Siverly. 1954b.** Urban Fly Dispersion Studies with Special Reference to Movement Pattern of *Musca Domestica*. *American Journal of Tropical Medicine and Hygiene*. 3: 539–547.
- Schurrer, J. A., S. A. Dee, R. D. Moon, K. D. Rossow, C. Mahlum, E. Mondaca, S. Otake, E. Fano, J. E. Collins, and C. Pijoan. 2004.** Spatial Dispersal of Porcine Reproductive and Respiratory Syndrome Virus-Contaminated Flies after Contact with Experimentally Infected Pigs. *American Journal of Veterinary Research*. 65: 1284–1292.
- Scott, J. G., C. A. Leichter, F. D. Rinkevihc, S. A. Harris, C. Su, L. C. Aberegg, R. Moon, C. J. Geden, A. C. Gerry, D. B. Taylor, R. L. Byford, W. Watson, G. Johnson, D. Boxler, and L. Zurek. 2013.** Insecticide Resistance in House Flies from the United States: Resistance Levels and Frequency of Pyrethroid Resistance Alleles. *Pesticide Biochemistry and Physiology*. 107: 377–384.
- Semakula, L. M., R. A. J. Taylor, and C. W. Pitts. 1989.** Flight behavior of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in a Kansas dairy barn. *Journal of Medical Entomology*. 26: 501–509.
- Watt, J., and D. R. Lindsay. 1948.** Diarrheal Disease Control Studies. *Pub Health Rep*. 63: 1319–1334.
- Winpisinger, K. A., A. K. Ferketich, R. L. Berry, and M. L. Moeschberger. 2005.** Spread of *Musca domestica* (Diptera: Muscidae), from Two Caged Layer Facilities to Neighboring Residences in Rural Ohio. *Journal of Medical Entomology*. 42: 732–738.
- Zahn, L., and A. Gerry. 2018.** House Fly (*Musca domestica*) Laboratory Rearing Protocol - Gerry Lab (UC Riverside). protocols.io. (<https://www.protocols.io/view/house-fly-musca-domestica-laboratory-rearing-proto-vbse2ne/abstract>).

**Table 3.1:** Mean flight direction of house flies by trap distance. Circular concentration (mean resultant length) indicates the variation around the observed mean direction of flight.

Distance	Sex	Mean Direction (Deg.)	95% Confidence Interval		Circular Concentration ( $\bar{R}$ )	95% Confidence Interval	
			Lower	Upper		Lower	Upper
10m		136.54	131.07	142.02	0.26	0.23	0.28
20m		122.82	113.87	131.77	0.25	0.21	0.28
10m	Female	‡135.80 Aa	129.76	141.83	0.30	0.27	0.33
	Male	‡138.34 Aa	126.63	150.04	0.19	0.15	0.24
20m	Female	‡127.85 Aa	119.17	136.52	0.33	0.29	0.38
	Male	‡108.62 Ab	85.34	131.90	0.14	0.08	0.20

‡ Capital letters indicate significant differences between fly sex within distance. Lower case letters denote differences between trap distances within fly sex.

**Table 3.2:** Mean flight direction of house flies by release period. Circular concentration (mean resultant length) indicates the variation around the observed mean direction of flight.

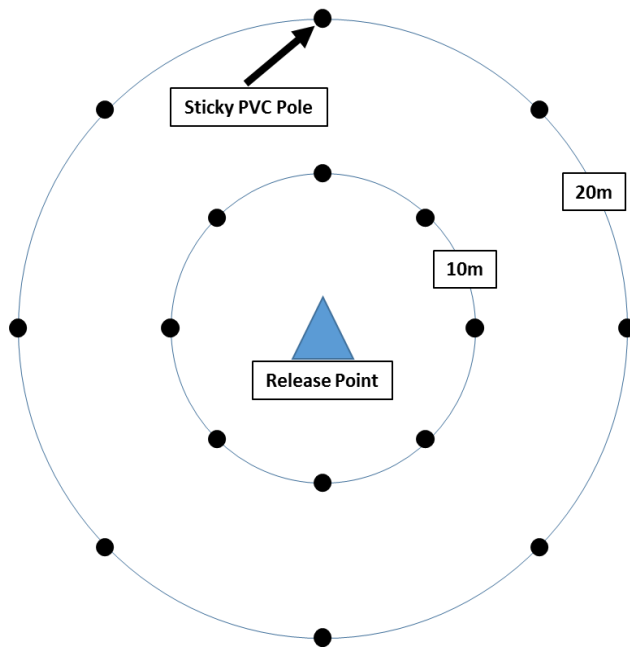
Period	Sex	Mean Direction (Deg.)	95% Confidence Interval		Circular Concentration ( $\bar{R}$ )	95% Confidence Interval	
			Lower	Upper		Lower	Upper
Morning		209.29	169.55	249.02	0.05	0.01	0.08
Afternoon		147.39	139.89	154.88	0.30	0.26	0.34
Evening		119.65	115.85	123.44	0.50	0.47	0.53
Morning	Female	113.24	83.44	143.04	0.11	0.05	0.16
	Male	245.57	220.97	270.16	0.10	0.06	0.15
Afternoon	Female	‡150.19 Aa	142.30	158.08	0.31	0.26	0.35
	Male	‡127.96 Bb	105.82	150.11	0.27	0.15	0.38
Evening	Female	‡125.22 Ab	119.92	130.52	0.46	0.42	0.50
	Male	‡112.07 Bb	106.95	117.20	0.60	0.55	0.64

‡ Capital letters indicate significant differences between fly sex within release period. Lower case letters denote differences between release periods within fly sex.

**Figure 3.1:** Google Earth photo of the study area used to release house flies. Release area is shown in the within the white outlined box and is represented by the triangle within the two concentric circles. Size of the circles are to scale. Area surrounding release site was devoid of vegetation for at least 25m from the outermost ring of traps. Buildings to the north are greenhouses, and houses lie to the east and south, with the remainder of the agricultural operations to the west. A small copse of trees and shrubs lies to the south before entering the residential neighborhood. The dark area in the bottom left of the white box are rows of old smudge pots used for citrus growing.



**Figure 3.2:** Diagrammatic sketch of the experimental design used to recapture released unmarked house flies. The inner circle has a radius of 10 meters and the outer a radius of 20 m. Eight pvc poles covered in tangle foot were evenly spaced around each circle with poles placed according to true north. The release chamber (blue triangle) was placed in the center of the concentric rings.

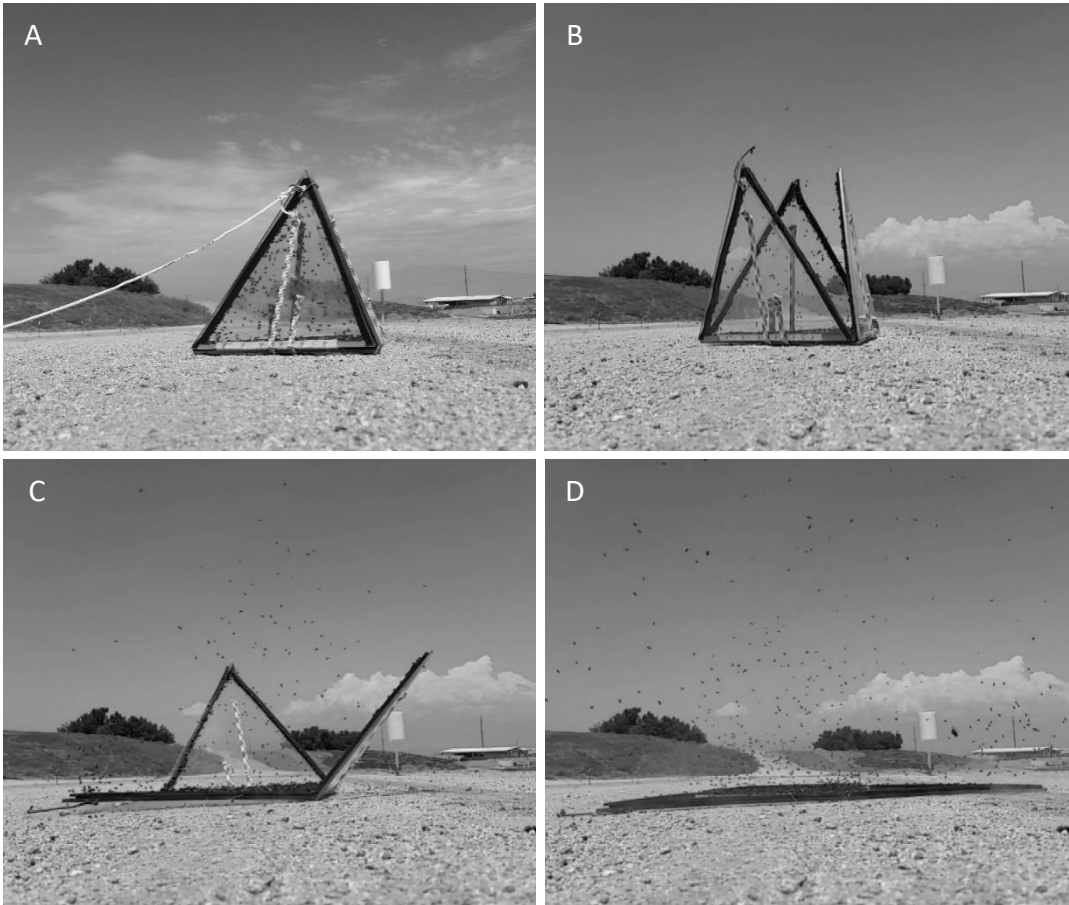


**Figure 3.3:** Arrangement of PVC poles covered in tangle trap. This method of recapture was a cheap but effective method for collecting house flies in flight. A) Ground level looking southeast, and B) photo taken from adjacent hill looking east, subject (5'10" tall) is standing at the fly release point in the center of the trap circles.

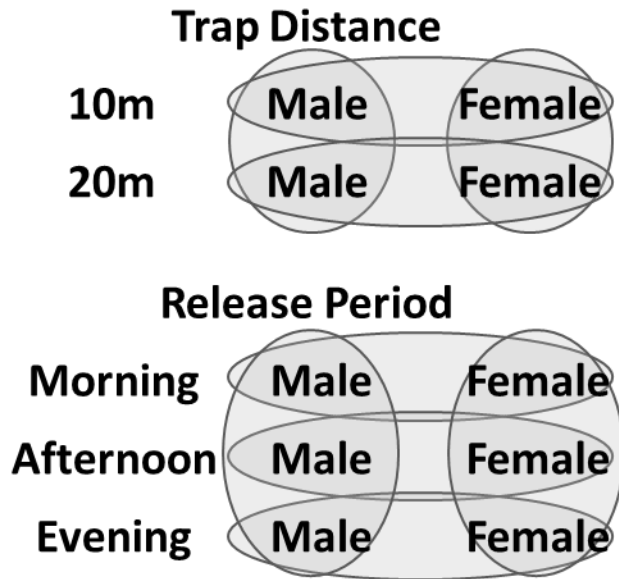




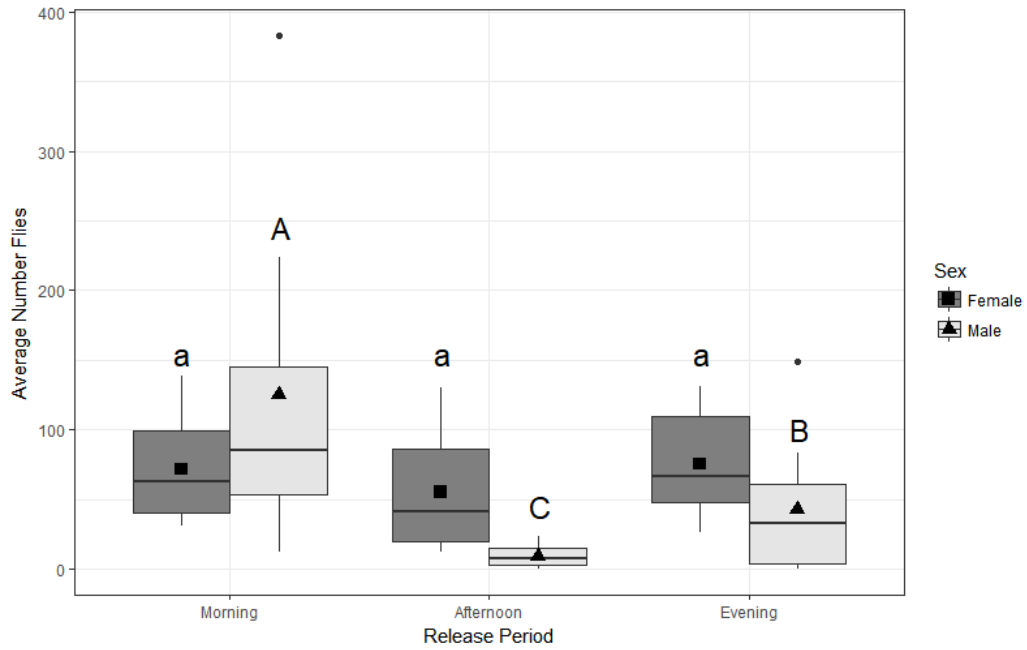
**Figure 3.4:** Pyramidal fly release chamber. After opening, the release chamber would lie flat on the ground to encourage house flies to leave the release site. Panels A through D show still shots taken from a video recording of the release chamber. Release chamber was “spring loaded” so that all three sides would open at similar rates.



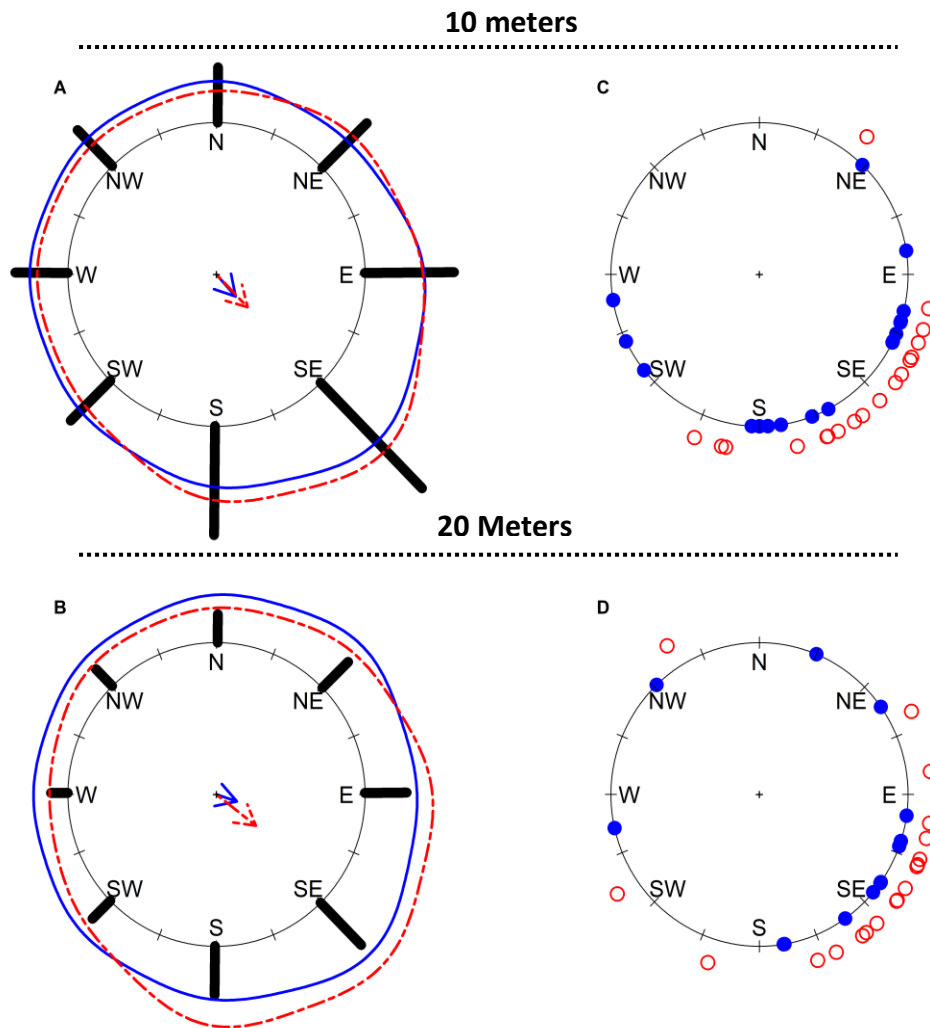
**Figure 3.5:** Diagrammatic sketch of groupings used to examine the mean direction of flight for male and female house flies under field conditions. Grey circles indicate a single independent comparison between/among the levels encompassed.



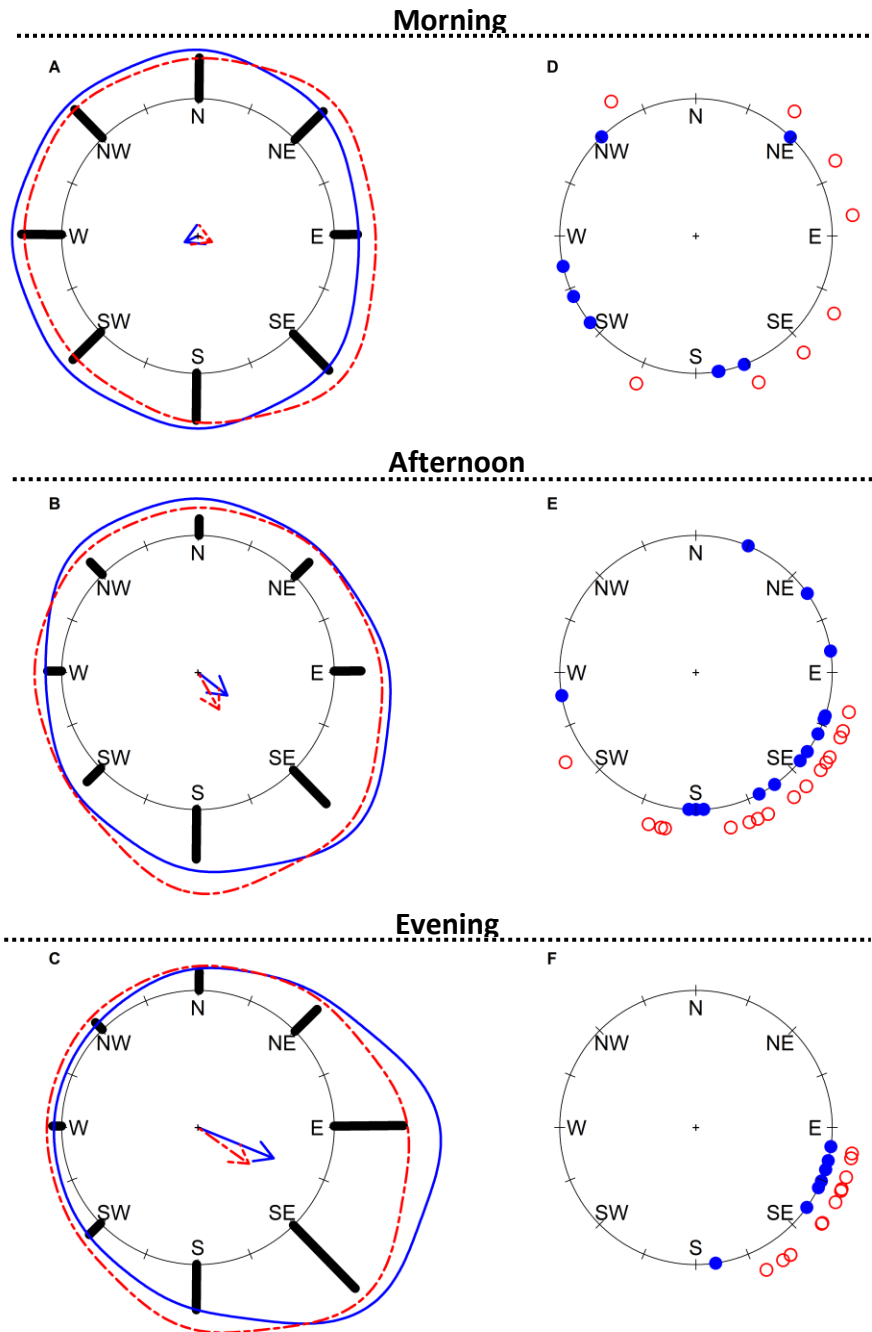
**Figure 3.6:** Boxplots showing the mean number and quartile range for female and male house flies recaptured following fly releases performed during morning, afternoon, or evening release periods. Plots with the same letter are not significantly different ( $\alpha=0.05$ ). Lower case letters are differences among females, and uppercase are differences among males.



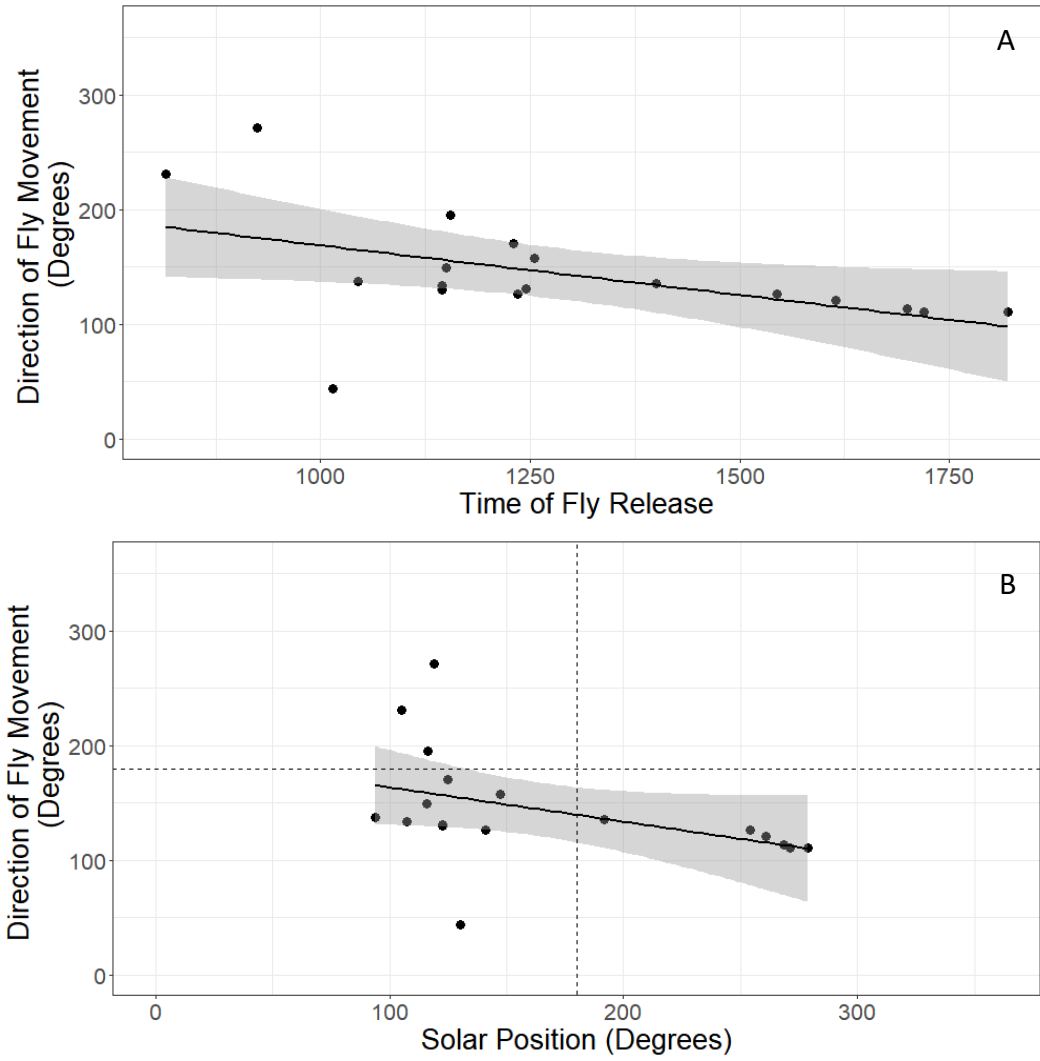
**Figure 3.7:** Distribution of house fly flight at A) 10m and B) 20m from a central release point with lines showing the smoothed kernel density estimate (solid blue lines and closed dots: males, dotted red lines and open circles: females), and circular scatter plots showing the observed mean flight direction by fly sex for each release at C) 10m D) 20m from the release point. Black lines radiating from plots on the left indicate the total number of house flies (sexes combined) collected per pole. Arrows are vector showing the mean direction of travel and mean resultant length (homoscedasticity). The longer the arrow the more tightly grouped the points are.



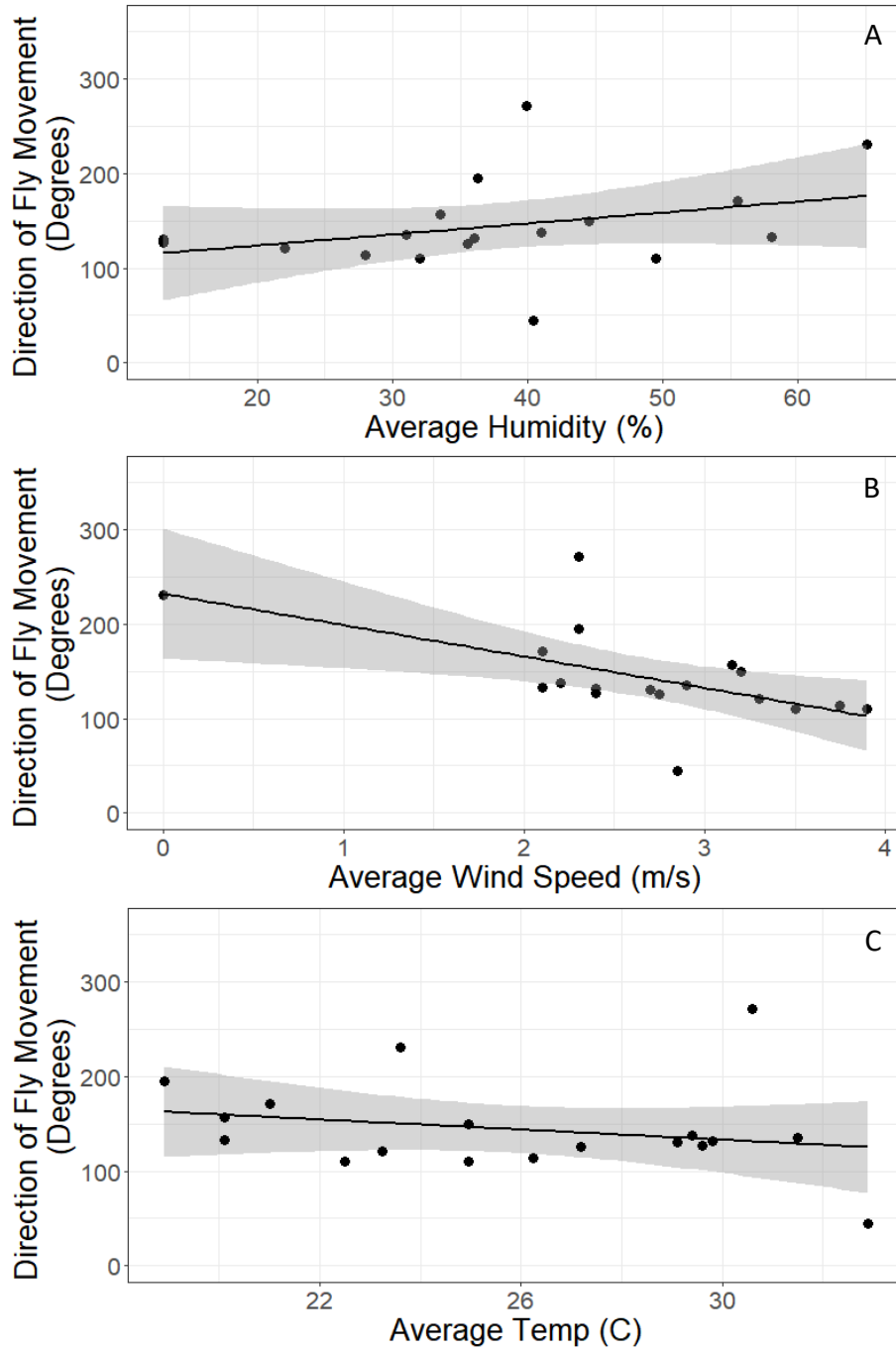
**Figure 3.8:** Distribution of fly movement during A) morning, B) afternoon, and C) evening release periods. Circular scatter plots showing the distribution of mean direction of fly movement from each release at D) morning, E) afternoon, and F) evening release periods (solid blue lines and closed dots: males, dotted red lines and open circles: females).



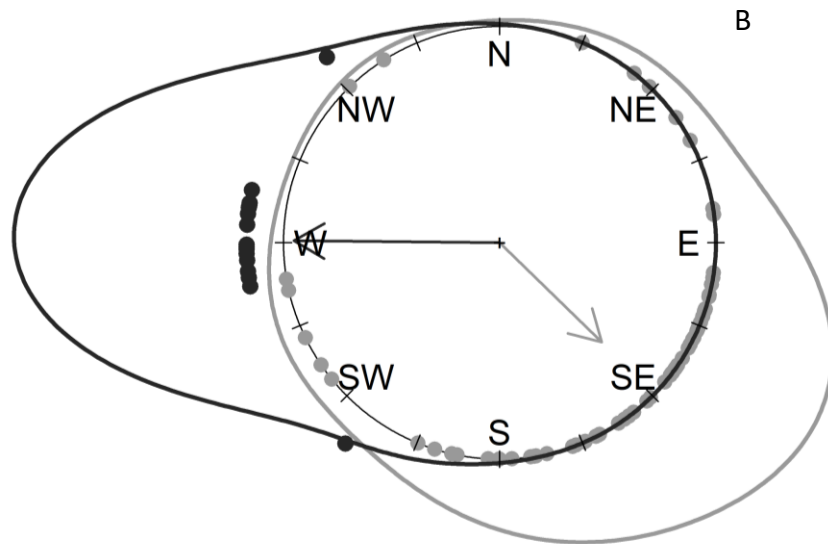
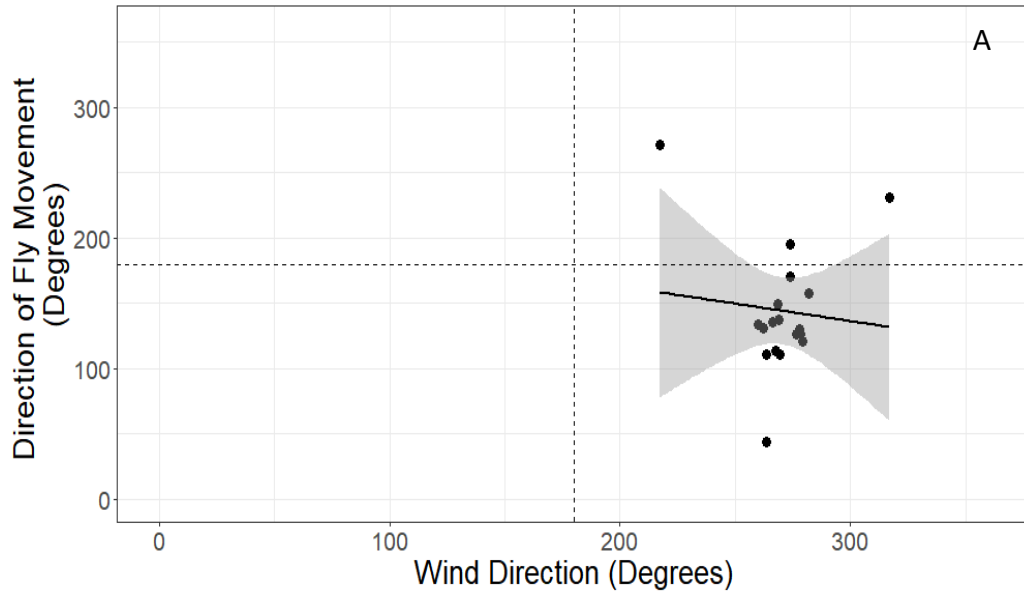
**Figure 3.9:** Plots showing the relationship of average direction of flight for all house flies in relation to the A) time of house fly release, and B) the position of the sun with the average. Regression line is included to give an overall impression of the trends in the data.



**Figure 3.10:** Scatter plots showing the relationship of mean fly direction with A) humidity, B) wind speed, and C) temperature over the 15-minute dispersal period. Regression lines are included to give an overall impression of the trends in the data.



**Figure 3.11:** A) Plot showing the circular-circular correlation of mean wind direction and mean flight direction. Wind direction was consistently from the west for all releases except for two. Regression line is included to give an overall impression of the trends in the data. B) Circular plot of mean wind direction (direction from which the wind blew) (dark grey) and mean direction of house fly flight (light grey), showing that winds were predominantly from the west and that house flies generally flew towards the southeast.





## CHAPTER 4

### Response of Released House Flies (*Musca domestica*) to the Presence of a Visual Target

#### ABSTRACT

House flies (*Musca domestica* L.) are one of the most common pestiferous insects found on and around confined animal production facilities. Their propensity to bother humans and their ability to carry enteric pathogens can make house flies a public health risk and make it necessary for producers to implement some form of house fly management. It has been proposed that house flies may fly toward nearby visual targets following disturbance, and that this behavior might be exploited to develop methods for trapping flies or reducing their dispersal from a point source. A study was undertaken to assess changes in house fly flight direction in response to raising a 1.83 m tall green construction fence as a visual target placed along a 90-degree arc at 20 m from a single fly release point. The position of the target on the circle was randomly selected for each paired release. House fly releases were paired with the visual target raised or lowered. Flies were captured on sticky poles placed in a 10 m radius circle around the fly release point. The mean flight direction and concentration of captured flies relative to the position of the visual target was determined and compared for paired releases when the fence was raised or lowered. Over all paired releases, the mean direction of house fly flight was altered by the raising or lowering of the target but did not result in a significantly smaller absolute angular difference (difference between the target center and mean direction of flight) when the barrier was raised versus lowered. Number of flies captured on sticky poles adjacent to the fence location was also compared when the fence was raised or lowered. House flies were captured

on sticky poles in greater numbers when the fence was raised and the aspect of the fence facing the release point was sunward (reflecting sunlight) but not when shaded.

## **INTRODUCTION**

The movement of house flies (*Musca domestica* L.) away from development sites and into more sensitive areas, such as residential neighborhoods, schools, restaurants can result in animosity between home owners and animal producers (Campbell 1993). House flies are also a public health nuisance when they disperse away from development sites in large numbers (Lindsay et al. 1953). The presence of house flies landing on stored food products has been associated with an increase in food contamination by *E. coli* and *Shigella* spp. (Lindeberg et al. 2018) in addition to vectoring enteric pathogens like enterohemorrhagic *E. coli* O157:H7 (Kobayashi et al. 1999, Burrus et al. 2016).

House fly management will often focus on the use of insecticides which may culminate in house fly populations becoming resistant to these insecticides (Keiding 1975, Gerry and Zhang 2009, Abbas et al. 2015) and under intense selective pressure resistance can develop in only a few generations (Kaufman et al. 2010). Therefore, it is often recommended that multiple management techniques be used simultaneously by following the tenets of an integrated pest management program (Stevenson and Cocke 2000). The most effective non-insecticidal method is the removal of suitable ovipositional and developmental sites (Mullens et al. 1996, Mullens et al. 2001) which often consists of animal manures or food wastes (Keiding 1975). Under optimal conditions, house flies can have a generation time in as little as 7 days (Mullen and Durden 2009), requiring at least weekly removal of development substrates to interrupt house fly development. This requirement can often be impractical due to the frequency, equipment, and

labor costs. Alternative management strategies are necessary to overcome some of these limitations.

The leveraging innate insect behaviors has been a valuable tool in creating new management techniques. Among hematophagous insects, like mosquitoes, the use of CO<sub>2</sub> as an attractant has proven to be highly effective for both surveillance and insecticidal purposes (Xue et al. 2008, Mullen and Durden 2009). Among house flies, the putative female sex pheromone (Z)-9-tricosesne (muscalure) has been shown to increase the number of male and female catches on traps impregnated with the pheromone (Butler et al. 2009), and has also been added to existing fly baits (Butler et al. 2007). Visual targets with dark/light contrasts have been shown to increase the landing rate of house flies along the contrasting edges (Howard and Wall 1998). Combinations of an putative attractants with pesticides have been successful in reducing house fly numbers and decreasing annoyance (Diclaro, et al. 2012).

The use of physical barriers (natural or manmade) has been suggested as a low maintenance supplement to support a house fly management program (Dubie 2014) by reducing the number of house flies that disperse from locations supporting fly development. But there is little research on the use, placement, or type of physical barrier needed to significantly alter house fly moments. It is anticipated that physical barriers may work through redirection of house fly flight (Fried et al. 2005) or perhaps may serve as attractive targets to encourage house fly resting thereby reducing dispersal distance. However, evidence from Chapter 1 and Dubie (2014) suggests that the height of a barrier would need to be at least 3 meters in height. Work by Fried et al. (2005) suggests that vegetative barriers (tree lines) may also be effective in redirecting house fly flight.

The previous chapter (Chapter 3) showed that house flies predominantly flew towards the southeast and that flight direction though correlated with some environmental variables, was most correlated with time and therefore the position of the sun. However, the correlation of flight direction and sun position may have been incidental, as there was a small group of trees and foliage about 75 meters to the southeast of the release sites in this study and house flies may have been flying towards this visual target. House flies have been shown to be attracted to contrasting edges (*e.g.* white against black) using three-dimensional structures (Scudder 1947) and black and white two-dimensional images (Hecht 1963, Howard and Wall 1998).

A study was conducted to assess the impact a contrasting green construction fence would have on the direction of dispersal flight for house flies released in a field setting. It is anticipated that house flies will alter their direction of flight based on the presence of a target and show a tendency to move around the target. House flies will be more abundant on traps placed in front of the target.

## **METHODS**

Adult house flies were collected by sweep net from a large commercial dairy near San Jacinto, California during summer of 2015 and subsequently reared in the laboratory using standard rearing practices (Zahn and Gerry 2018). House fly pupae from several separate immature rearing pans were placed into a temperature-controlled room at 16 °C for up to 7 days until >5,000 pupae were accumulated. All pupae were then mixed together and 5,000 pupae (measured volumetrically) were randomly selected and placed into an adult fly cage provisioned with food (1:1 powdered milk and granular sugar) and water. Cages were held in an insectary at 26.7 °C and 45% RH for emergence of adult flies.

Adult flies were subsequently released for flight direction studies at the University of California at Riverside (UCR) Agricultural Operations (AgOps) in a large dirt lot that lacked vegetation or other structures that might impede or influence house fly flight within the study area (Figure 4.1). Flight direction was examined for 12 paired trials (visual target raised or lowered; N=24 total releases). Flies were released between 0800 and 1300 over six individual days from July through September 2017. When two paired release trials occurred on the same day, approx. 1 hr of separation was given for each trial.

Flies were captured using 16 sticky poles evenly spaced at 22.5-degree intervals starting at magnetic north and forming a 10 m radius circle (Figures 4.2). Sticky poles were made from 2.4 m long white PVC pipe (19 mm outside dia.) liberally coated with a sticky material (Tanglefoot, Grand Rapids MI, #300000685) and placed on 0.5 m long rebar supports to stand vertically. The visual target was created using an unmodified 35 m long by 1.83 m tall dark green privacy fence (88-90% light blockage, Aleko plk06150A) to span a 90° arc on an outer concentric arc with a radius of ~20m from the fly release point and with the clockwise edge of the fence assigned to a position behind a randomly selected sticky pole (Figure 4.2). Construction fence was used due to its commonality and green was chosen since, of the color choices available, most closely simulated a line of vegetation. The fence was positioned behind 5 adjacent sticky poles. The starting state of the target (raised vs. lowered) for the first release each day was randomized and then alternated for each successive release on the same day.

After site set up, and before each house fly release, every pole was visually examined, and any insects captured were removed. Tanglefoot was reapplied to poles as needed. House flies were not marked to ensure the fly behavior was not unnecessarily impaired, and the release area was nearly free of wild house flies. Natural populations of house fly in this area

were very low according to preliminary fly collections at the study site and there was a negligible capture of house flies on poles during site set up and between fly releases on the same day. Thus, the house flies captured during these studies were assumed to be those that were released during these trials at the study site.

On each trial date, adult fly cages containing ~5,000 mixed sex flies (3-5 d-old) were placed in a shaded location near the release site for at least 1 hr to acclimate flies to the field environment. Flies were then transferred into a specially designed fly release chamber (Figure 4.3). The release chamber was a triangular pyramid constructed of 0.5 cm thick shatter resistant plexiglass cut into triangles of equal dimension (45.5 x 45.5 x 45.5 cm) with three side walls attached to a base triangle using hinges. When closed the release chamber had a total volume of 0.011 m<sup>3</sup>. Air vents were cut into each side wall of the chamber and covered with window screen to provide air flow. An elastic band was fixed from the base of the pyramid to each side wall, placing all three sides of the pyramid under tension when the side walls were raised. The release chamber was held in a closed position using a small bungee cord wrapped around the top of the release chamber to hold the side walls together. Flies were directly aspirated into the release chamber by sealing all unnecessary holes and applying suction directly to the chamber, using a modified shop vac to reduce the amount of suction to a non-lethal level. The aspiration method was used instead of anesthetization with CO<sub>2</sub> so flies could recover quicker and eliminate any behavioral effects due to CO<sub>2</sub>. The release chamber was kept shaded for an additional 30 min to ensure flies fully recovered.

A few minutes before each fly release, the release chamber was placed in the center of the study site. A 40 m long chamber release string was tied to a bungee cord closing the release chamber. The researcher then moved in a randomly selected direction to a position  $\geq 40$ m from

the release chamber (outside the study area). Upon pulling the string, the bungee cord holding the release chamber closed was released, allowing the sides of the chamber to fall quickly to the ground. The sudden and complete opening of the chamber startled the contained flies into immediate flight. This startle flight is typical under natural field conditions where flies are numerous and the movement of nearby animals or objects (or even a gust of wind) will startle a concentrated group of flies to simultaneously take flight (personal observation). Once released, flies were given 15 min to be captured or to disperse from the study area, after which each sticky pole was visually examined. The number and sex of house flies on each pole was recorded. At the end of the 15 min flight period, very few flies remained within the study area and those that did were usually on the ground very near the release chamber, suggesting that they never took flight. Mortality was low but was never quantified. Wind speed (m/s), wind direction (degrees from north), temperature (°C), and relative humidity (percent) were recorded using a Kestrel® 5500 portable weather station (sku# 0855). Readings were taken at 1 min intervals and averaged over the 15 min release period. This short release period was chosen to mitigate the chances of large changes in any of the environmental variables. Wind speed and direction are both potentially variable in time and space, and the readings are rough approximations of what the house flies likely experienced.

## **DATA ANALYSIS**

For each fly release, the mean flight direction and the circular concentration around the mean was calculated using the R package 'circular' (Agostinelli and Lund 2017). The methods and code given in the book 'Circular Statistics in R' (Pewsey et al. 2013) were followed, with

differences among treatment groups (fence raised or lowered) determined using the same R statistical package.

Mean flight direction was calculated by converting the polar coordinate (angle in degrees clockwise from north) of each observation (individual fly captured on a sticky pole) to radians before converting to cartesian coordinates, as follows:

$$\bar{\theta} = \text{atan2} \left( \frac{1}{n} \sum_{i=1}^n \cos \theta_i, \frac{1}{n} \sum_{i=1}^n \sin \theta_i \right)$$

where  $\theta_i$  = the angle (converted to radians) of each observation, and  $n$  = total number of observations. The circular concentration around the mean direction of flight is an estimate of homoscedasticity and calculated using the equation,

$$\bar{R} = \frac{1}{n} \sqrt{\left( \sum_{i=1}^n \cos \theta_i \right)^2 + \left( \sum_{i=1}^n \sin \theta_i \right)^2}$$

where  $\bar{R}$  takes a value ranging from [0,1]. A circular concentration value close to 1 indicates that most flies were flying in the same direction (similar angle of flight) while a value close to 0 indicates that flies were not uniform in their flight direction (varied flight angles). Circular variance can be obtained by subtracting the calculated value for circular concentration from one ( $V = 1 - \bar{R}$ ). Following the recommendations of Pewsey et al. (2013), no corrections to the estimated means or circular concentration estimates were made since the data consisted of more than eight observations.

Watson's large-sample nonparametric test ( $Y_g$ ) was used (Pewsey et al. 2013) to test the null hypothesis of similar mean flight direction for all releases pooled over fly sex, and for each paired release (target up vs. down). This test does not assume the underlying distributions have a common dispersion or shape (Pewsey et al. 2013). Similarly, Wallraff's nonparametric test,



which internally implements a Kruskal-Wallis test, was used to test the null hypothesis that flies would have similar circular concentrations around a circle for all releases pooled over fly sex, and for each paired release.

To test if having the target raised resulted in a shift of the mean direction of flight towards the target, the absolute angular difference (since the shift could be clockwise or counter clockwise) between the center of the target and the mean direction of flight was calculated for each house fly release. The absolute angular difference among treatment groups (target raised or lowered) was then tested using a paired one-sided Wilcoxon signed rank test. If house flies are shifting their flight path towards the target, the absolute angular distance should be reduced when the target is raised, as the visual target would “pull” flies towards the target. A decrease in the circular concentration ( $\bar{R}$ ) should also be associated with a smaller absolute angular difference. Differences in the value of  $\bar{R}$  between the two groups (raised vs lowered) was assessed using a paired one-sided Wilcoxon signed rank test, to test the hypothesis that the data would be more dispersed (smaller  $\bar{R}$ ) when the target was raised.

Differences in the number of house flies collected on the 5 traps positioned in front of the target were analyzed using an ANOVA to assess differences in fly catch with the target raised and lowered. This was done to remove any random noise associated with collections from poles not directly manipulated by altering the target. The side of the target facing the release point was categorized as having been shaded (N=5 paired releases), sunlit (N=6 paired releases), or neither (N=7 paired releases). Only the shaded and sunlight traps were retained for analysis. Separate analyses were performed for the shaded and sunlit categories since there was significant interaction with barrier state (raised/lowered). The response was  $\log_e(n+1)$  transformed for normality before analysis. House fly sex and barrier state were included as main

effects. Data were checked visually for linearity and equal variances using Q-Q plots and residual versus fitted plots (Crawley 2013).

## RESULTS

A total of 6,388 house flies were recaptured out of approx. 120,000 flies liberated over all paired releases. Overall, male and female house flies had similar mean flight directions towards the south-southeast ( $Y_g=0.800$ ;  $P=0.371$ , Figure 4.4). Over all paired releases the mean directions of flight significantly differed when the target was raised versus lowered ( $Y_g=16.980$ ;  $P<0.001$ ) with house flies flying more southerly when the target was raised and easterly when lowered (Table 4.1). Circular concentrations were also significantly lower when the target was raised ( $H=13.932$ ,  $df=1$ ,  $P<0.001$ ). When the mean direction of flight was compared between paired fly releases, the mean direction of flight differed for only three of 12 paired releases ( $Y_g\geq 15.770$ ;  $P\leq 0.001$ , Figure 4.5: 3A&B, 6A&B, 11A&B). When circular concentration was assessed (Table 4.1), 7 of 12 paired releases significantly differed ( $H<4.392$ ;  $df=1$ ;  $P<0.036$ , Figure 4.5: 3A&B, 4A&B, 6A&B, 7A&B, 10A&B, 11A&B, 12A&B).

Over all paired releases, having the target raised ( $88.37^\circ\pm 12.56^\circ$ , mean $\pm$ se) did not result in a significant shift in the mean direction of flight towards the target ( $W=52$ ;  $P=0.170$ , Figure 4.6A) versus lowered ( $94.90^\circ\pm 15.59^\circ$ ). Similar results were obtained for changes in the average value of the circular concentration ( $W=60$ ,  $P=0.055$ , Figure 4.6B) for when the target was raised ( $0.233\pm 0.024$ ) and lowered ( $0.298\pm 0.021$ ).

For traps that were sunlit, significantly more house flies were collected when the target was raised ( $F_{1,57}=17.392$ ;  $P<0.001$ ), and these collected more female flies ( $F_{1,57}=4.938$ ;  $P=0.03$ ;  $R^2=0.26$ , Figure 4.7). However, when the target was shaded, there were no significant

differences between the barrier raised versus lowered ( $F_{1,57}=1.393$ ;  $P=0.241$ ) or by house fly sex ( $F_{1,57}=0.839$ ;  $P=0.362$ ,  $R^2=0.002$ ).

## **DISCUSSION**

A study was undertaken to assess the impact a contrasting visual target would have on the direction of flight for lab reared house flies released under field conditions. Previous work suggested that house flies released under similar conditions may have been orienting towards a group of trees to the southeast of the study site (see chapter 3). The placement of a visual target nearer to the release point was tested to see if the direction of flight might be significantly altered towards the target.

House flies have been shown to have differential landing responses based on the contrast of a visual target with more flies landing on targets with more contrast (Howard and Wall 1998). Flies also show an increase in landings on white and blue targets (Diclaro, et al. 2012). The effect of wind direction on house fly flight direction is inconclusive (Nuttall et al. 1914, Parker 1916, Bishopp and Laake 1921, Quarterman et al. 1954, Hanec 1956, Pickens et al. 1967), with evidence for multiple response to wind direction. The effects of temperature, humidity, and wind speed have not been assessed in relation to house fly dispersal direction in the field.

Over all releases house flies preferentially moved towards the south-southeast, even though the mean direction of flight did have a significant shift towards the south when the target was raised. The placement of a 1.83-meter-tall manmade visual target did significantly alter the mean direction of flight and circular concentrations over all paired releases. However, it was not clear what the change in mean direction indicated as the target over time was located

on both sides of the mean direction of flight, and the target was placed an equal number of times on both the east and west halves of the circle. Similar numbers of flies were also collected on each half of the circle, making it unlikely that a numerical difference was responsible for the southerly change in mean direction. Additionally, there was no significant change in the absolute angular difference between the target position and the mean direction of flight for when the target was raised versus lowered. This is likely a better metric than using mean direction of flight, as it would indicate a detectable flight preference in relation to the target. Three of the paired releases had significantly different mean directions of flight when those were examined, two releases (3 and 6) had decreases in their absolute angular distances, while the third increased. But two out of three is not enough to draw any conclusions.

The significant difference in overall circular concentration for raised and lowered targets only indicates that, when the target was raised, direction of flight became more random. But it is not known if a more random flight pattern means more flies were moving towards the target itself. There was a slightly stronger decrease in the circular concentration around the mean direction of travel when the target was raised, which suggested that the presence of the target caused a greater number of flies to fly in the direction of the raised fence. But when comparing  $\bar{R}$  between the two groups using the more conservative paired Wilcoxon test, this difference again became nonsignificant. If the target was having any effect on the mean direction of flight for house flies, it was not readily detected using these methods.

The presence of the target did have a significant effect on the number of flies collected on the five poles directly in front, but only when the target was under sunlit conditions. House fly releases occurred between the hours of 0800 and 1300. This placed the target in partial or direct sunlight when positioned on the western side of the circle, and the sunlit portion was

facing away from the release point when the target was on the eastern side of the circle.

Perhaps house flies are moving towards the fence to sun themselves before dispersing further, as house flies are known to seek bright sunlit locations to warm themselves (West 1951, Keiding 1986).

Alternatively, direct sunlight could be an important factor for the detection of the target/poles by house flies. Contrast of the white poles against the darker background also might be attractive to house flies. This may be due to a navigational strategy used by flies (*Drosophila* sp.), where flies orient towards a nearby feature and fly towards that object to maintain a straight flight path, commonly called stripe fixation (Warren et al. 2019). It is likely that house flies use a similar behavior and that the poles became more visible when in direct sunlight against a darkened background. Also, it has been shown that edge contrast is an important property of a visual target for capturing house flies and that vertical white lines against a black background are also related to increased landing responses (Howard and Wall 1998).

The green color of the construction fence may also have impacted the targets attractiveness. The color was chosen since it contrasted with much of the substrate in the study area and hopefully simulated a line of green vegetation. While house flies have never been demonstrated to be particularly attracted to various shades of green (Freeborn and Berry 1935, Burg and Axtell 1984, Diclaro, et al. 2012), green was never the least attractive color. It is probable that if the target color were changed to blue or white a stronger response to the presence of the barrier may have been seen (Diclaro, et al. 2012). However, these colors of construction fence are not easily obtainable, and it was desirable to have the fence color be as homogenous as possible in the present tests.

Lastly, variation in recaptures might have been slightly influenced by minor variation in the number of house flies released. However, care was taken to ensure that a similar numbers of house flies were used for each release. It was also assumed that paired releases were close enough together in time that the environmental effects were the same. However, this assumption may not be true and could account for much of the variation seen in both the mean direction of flight and the number of flies recaptured. The possibility of the target acting as a wind break was also explored, as the prevailing winds were mostly from the west, however there was no significant correlation between number captures and wind direction.

If all three metrics used to examine changes in house fly flight direction are viewed together, it appears that a visual target does impact flight direction. House flies (overall) did change their direction of flight when the target was raised, the change in direction skewed towards the target itself (but not significantly), and more house flies were collected when the target was raised and in direct sunlight. However, it is not clear how house flies are interacting with the poles and target (when raised). Changes in mean flight direction may be caused by house flies avoiding the target and flying around the target and not towards it. When the target was placed in the path of the overall mean flight direction (south to southeast) either the circular concentration decreased, or the mean direction of flight changed. However only three paired releases met these criteria.

It remains unclear how the addition of a visual target is impacting the direction of flight for house flies. It was anticipated that a closer contrasting target would have a greater impact on the flight direction of dispersing house flies. Based on these results and those of chapter 3, it is apparent that the field site is having an impact on the mean direction of flight for house flies, but because the target position was changing it was desirable to use the same study location for

all releases. It is likely that other environmental characteristics are also impacting dispersal in some yet unknown manner, as does the surrounding environment. The use of sticky poles for examining dispersal direction appears to be adequate as sample mean directions were readily obtained and are consistent over multiple releases, however future work should still use a variety of study locations ideally with even less substrate structure than in this study. Lastly, it is possible that house flies do simply fly haphazardly before encountering an attractive site which causes them to become aggregated as suggested by Schoof and Siverly (1954).

## REFERENCES CITED

- Abbas, N., H. Khan, and S. A. Shad. 2015.** Cross-Resistance, Stability, and Fitness Cost of Resistance to Imidacloprid in *Musca domestica* L., (Diptera: Muscidae). *Parasitology Research*. 114: 247–255.
- Agostinelli, C., and U. Lund. 2017.** R package “circular”: Circular Statistics.
- Bishopp, F. C., and E. W. Laake. 1921.** Dispersion of Flies by Flight. *Journal of Agricultural Research*. 21: 729–766.
- Burg, J. G., and R. C. Axtell. 1984.** Monitoring house fly, *Musca domestica* (Diptera: Muscidae), populations in caged-layer poultry houses using a baited jug-trap. *Environmental Entomology*. 13: 1083–1090.
- Burrus, R. G., J. A. Hogsette, P. E. Kaufman, J. E. Maruniak, A. H. Simonne, and V. Mai. 2016.** Prevalence of *Escherichia coli* O157:H7 From House Flies (Diptera: Muscidae) and Dairy Samples in North Central Florida. *Journal of Medical Entomology*. 0: 1–9.
- Butler, S. M., A. C. Gerry, and B. A. Mullens. 2007.** House Fly (Diptera: Muscidae) Activity Near Baits Containing (Z)-9-tricosene and Efficacy of Commercial Toxic Fly Baits on a Southern California Dairy. *Journal of Economic Entomology*. 100: 1489–1495.
- Butler, S. M., R. D. Moon, N. C. Hinkle, J. G. Millar, J. S. McElfresh, and B. A. Mullens. 2009.** Characterization of Age and Cuticular Hydrocarbon Variation in Mating Pairs of House Fly, *Musca domestica*, Collected in the Field. *Medical and Veterinary Entomology*. 23: 426–442.
- Campbell, J. B. 1993.** The Economics of the Fly Problem, p. 97. *In* *Rural Flies in the Urban Environment?*, Research Bulletin. University of Nebraska Lincoln.
- Crawley, M. J. 2013.** *The R book*, Second edition. ed. Wiley, Chichester, West Sussex, United Kingdom.
- Diclaro, J. W., L. W. Cohnstaedt, R. M. Pereira, S. A. Allan, and P. G. Koehler. 2012.** Behavioral and Physiological Response of *Musca domestica* to Colored Visual Targets. *Journal of Medical Entomology*. 49: 94–100.
- Diclaro, J. W., J. C. Hertz, R. M. Welch, P. G. Koehler, and R. M. Pereira. 2012.** Integration of Fly Baits, Traps, and Cords to Kill House Flies (Diptera: Muscidae) and Reduce Annoyance. *Journal of Entomological Science*. 47: 56–64.
- Dubie, T. R. 2014.** Influence of Vegetation Height on the Dispersal of House Flies (*Musca domestica*) from Livestock Facilities and Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers (Dissertation). Oklahoma State University.



- Freeborn, S. B., and L. J. Berry. 1935.** Color Preferences of the House Fly, *Musca Domestica* L. Journal of Economic Entomology. 28: 913–916.
- Fried, J. H., D. J. Levey, and J. A. Hogsette. 2005.** Habitat Corridors Function as Both Drift Fences and Movement Conduits for Dispersing Flies. Oecologia. 143: 645–651.
- Gerry, A. C., and D. Zhang. 2009.** Behavioral Resistance of House Flies, *Musca domestica* (Diptera: Muscidae) to Imidacloprid. US Army Medical Department Journal. 54–59.
- Hanec, W. 1956.** A Study of the Environmental Factors Affecting the Dispersion of House Flies (*Musca domestica* L.) in a Dairy Community Near Fort Whyte, Manitoba. The Canadian Entomologist. 88: 270–272.
- Hecht, O. 1963.** On the Visual Orientation of House-Flies in Their Search of Resting Sites. Entomologia Experimentalis et Applicata. 6: 107–113.
- Howard, J. J., and R. Wall. 1998.** Effects of Contrast on Attraction of the Housefly, *Musca domestica*, to Visual Targets. Medical and Veterinary Entomology. 12: 322–324.
- Kaufman, P. E., S. C. Nunez, C. J. Geden, and M. E. Scharf. 2010.** Selection for Resistance to Imidacloprid in the House Fly (Diptera: Muscidae). Journal of Economic Entomology. 103: 1937–1942.
- Keiding, J. 1975.** Problems of Housefly (*Musca domestica*) Control Due to Multiresistance to Insecticides. Journal of Hygiene, Epidemiology, Microbiology, and Immunology. 19: 340–355.
- Keiding, J. 1986.** The House Fly: Biology and Control. WHO VBC. 86.937: 1–62.
- Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999.** Houseflies: Not Simple Mechanical Vectors of Enterohemorrhagic *Escherichia coli* O157:H7. American Journal of Tropical Medicine and Hygiene. 61: 625–629.
- Lindeberg, Y. L., K. Egedal, Z. Z. Hossain, M. Phelps, S. Tulsiani, I. Farhana, A. Begum, and P. K. M. Jensen. 2018.** Can *Escherichia coli* fly? The Role of Flies as Transmitters of *E. coli* to Food in an Urban Slum in Bangladesh. Tropical Medicine & International Health. 23: 2–9.
- Lindsay, D. R., W. H. Stewart, and J. Watt. 1953.** Effect of Fly Control on Diarrheal Disease in an Area of Moderate Morbidity. Public health reports. 68: 361.
- Mullen, G. R., and L. A. Durden. 2009.** Medical and Veterinary Entomology, 2nd ed. Academic Press, China.

- Mullens, B. A., N. C. Hinkle, and C. E. Szijj E. 1996.** Impact of Altering Manure Removal Schedules on Pest Flies (Diptera: Muscidae) and Associated Predators (Coleoptera: Histeridae, Staphylinidae; Acarina: Macrochelidae) in Caged-Layer Poultry Manure in Southern California. *Journal of Economic Entomology*. 89: 1406–1417.
- Mullens, B., N. Hinkle, C. Szijj, and D. Kuney. 2001.** Managing Manure and Conserving Predators Helps Control Flies in Caged-Layer Poultry Systems. *California Agriculture*. 55: 26–30.
- Nuttall, G., E. Hindle, and G. Merriman. 1914.** The Range of Flight of *Musca domestica*: Report Upon Experiments. *The Journal of Hygiene*. 14: 23.
- Parker, R. R. 1916.** Dispersion of *Musca domestica* Linnaeus Under City Conditions in Montana. *Journal of Economic Entomology*. 9: 325–354.
- Pewsey, A., M. Neuhauser, and G. Ruxton. 2013.** *Circular Statistics In R*. Oxford University Press.
- Pickens, L. G., N. O. Morgan, J. G. Hartsock, and J. Smith. 1967.** Dispersal Patterns and Populations of the House Fly Affected by Sanitation and Weather in Rural Maryland. *Journal of Economic Entomology*. 60: 1250–1255.
- Quarterman, K. D., W. Mathis, and J. W. Kilpatrick. 1954.** Urban Fly Dispersal in the Area of Savannah, Georgia. *Journal of Economic Entomology*. 47: 405–412.
- Schoof, H. F., and R. E. Siverly. 1954.** Multiple Release Studies on the Dispersion of *Musca domestica* at Phoenix, Arizona. *Journal of Economic Entomology*. 47: 830–838.
- Scudder, H. I. 1947.** A New Technique for Sampling the Density of Housefly Populations. *Public Health Reports (1896-1970)*. 62: 681–686.
- Stevenson, D., and J. Cocke. 2000.** Integrated Pest Management of Flies in Texas Dairies.
- Warren, T. L., Y. M. Giraldo, and M. H. Dickinson. 2019.** Celestial Navigation in *Drosophila*. *The Journal of Experimental Biology*. 222: 1–10.
- West, L. S. 1951.** *The Housefly, Its Natural History, Medical Importance and Control*. Comstock Publishing Co., Ithaca, New York.
- Xue, R.-D., M. A. Doyle, and D. L. Kline. 2008.** Field Evaluation of CDC and Mosquito Magnet® X Traps Baited with Dry Ice, Co2 Sachet, and Octenol Against Mosquitoes. *Journal of the American Mosquito Control Association*. 24: 249–252.
- Zahn, L., and A. Gerry. 2018.** House Fly (*Musca domestica*) Laboratory Rearing Protocol - Gerry Lab (UC Riverside). protocols.io. (<https://www.protocols.io/view/house-fly-musca-domestica-laboratory-rearing-PROTO-VBSE2NE/abstract>).

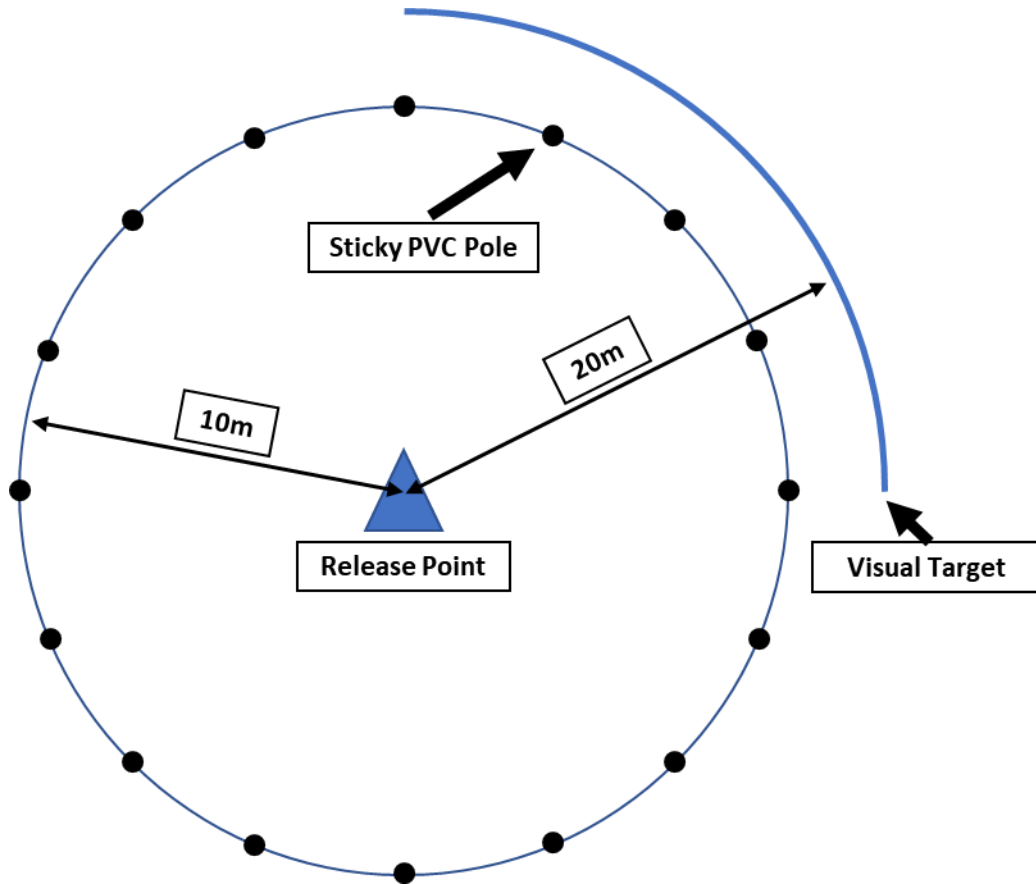
**Table 4.1:** Table showing the mean direction of travel and the circular concentration (mean resultant length) for each individual release, with pairwise comparisons of the means and circular concentrations within each replicate. Values in bold indicate significant differences at  $\alpha=0.05$ .

Replicate	Release	Date	Time	Barrier Up	Comparison of Circular Means			Comparison of Circular Concentration			
					Mean Direction (°)	$Y_g$	p-value	$\bar{R}$	$H$	df	p-value
1	A	7/31/2017	930	Raised	159.4	0.153	0.696	0.30	0.587	1	0.443
	B		1020	Lowered	166.6			0.32			
2	A	7/31/2017	1115	Lowered	165.1	0.305	0.581	0.22	0.074	1	0.786
	B		1245	Raised	175.0			0.25			
3	A	8/4/2017	1000	Lowered	<b>187.3</b>	<b>34.766</b>	<b>0.000</b>	<b>0.27</b>	<b>68.991</b>	<b>1</b>	<b>0.000</b>
	B		1100	Raised	<b>132.5</b>			<b>0.37</b>			
4	A	8/4/2017	1200	Raised	140.8	1.283	0.257	<b>0.27</b>	<b>5.891</b>	<b>1</b>	<b>0.015</b>
	B		1255	Lowered	126.8			<b>0.46</b>			
5	A	8/7/2017	845	Raised	196.8	0.176	0.675	0.20	0.312	1	0.576
	B		940	Lowered	202.3			0.24			
6	A	8/7/2017	1035	Raised	<b>191.9</b>	<b>28.476</b>	<b>0.000</b>	<b>0.28</b>	<b>70.94</b>	<b>1</b>	<b>0.000</b>
	B		1130	Lowered	<b>129.7</b>			<b>0.22</b>			
7	A	8/12/2017	907	Lowered	<b>228.4</b>	<b>3.149</b>	<b>0.076</b>	<b>0.30</b>	<b>20.793</b>	<b>1</b>	<b>0.000</b>
	B		950	Raised	<b>177.9</b>			<b>0.12</b>			
8	A	8/12/2017	1025	Raised	155.6	0	0.995	0.30	0.891	1	0.345
	B		1108	Lowered	155.7			0.36			
9	A	8/14/2017	920	Raised	168.9	1.386	0.239	0.17	1.696	1	0.193
	B		1000	Lowered	143.2			0.30			
10	A	8/14/2017	1049	Raised	157.3	1.014	0.314	<b>0.06</b>	<b>4.392</b>	<b>1</b>	<b>0.036</b>
	B		1130	Lowered	118.3			<b>0.31</b>			
11	A	9/7/2017	835	Raised	<b>222.4</b>	<b>15.77</b>	<b>0.000</b>	<b>0.19</b>	<b>101.304</b>	<b>1</b>	<b>0.000</b>
	B		955	Lowered	<b>174.5</b>			<b>0.14</b>			
12	A	9/7/2017	1055	Raised	144.8	1.386	0.239	<b>0.20</b>	<b>27.443</b>	<b>1</b>	<b>0.000</b>
	B		1155	Lowered	135.0			<b>0.42</b>			
<b>Pooled Over all Releases</b>				<b>Raised</b>	<b>175.9</b>	<b>16.980</b>	<b>0.000</b>	<b>0.18</b>	<b>13.932</b>	<b>1</b>	<b>0.000</b>
				<b>Lowered</b>	<b>157.5</b>			<b>0.25</b>			

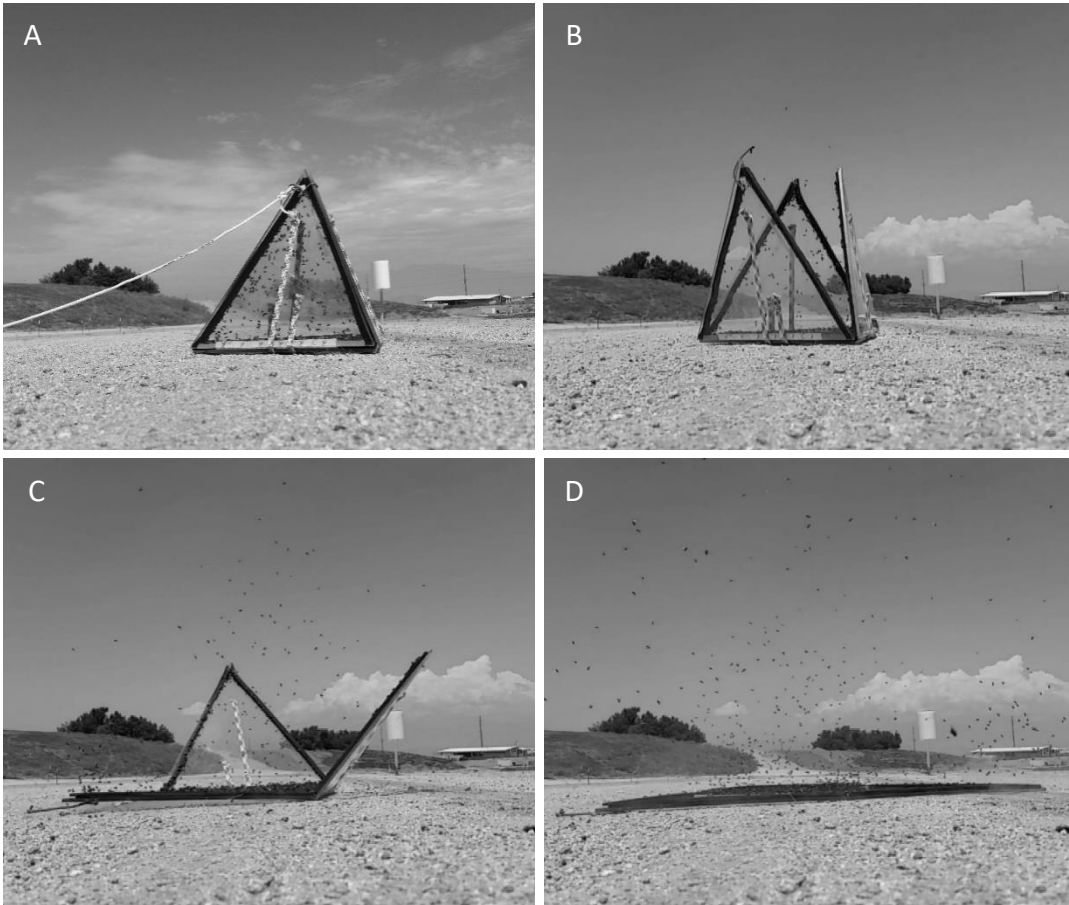
**Figure 4.1:** Google Earth photo of the study area used to release house flies. Release area is shown within the white outlined box and is represented by the triangle within the circle. Size of the circle is to scale. Area surrounding release site was devoid of vegetation for at least 25m from the ring of traps. Buildings to the north are greenhouses, and houses lie to the east and south, with the remainder of the agricultural operations to the west. A small copse of trees and shrubs lies to the south before entering the residential neighborhood. The dark area in the bottom left of the white box are rows of old smudge pots used for citrus.



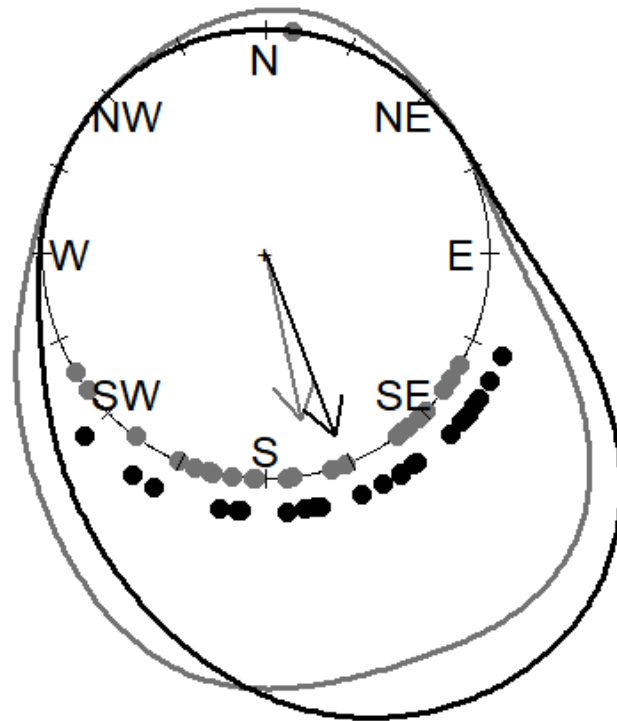
**Figure 4.2:** Diagrammatic sketch of the experimental design used to recapture released unmarked house flies. The circle has a radius of 10 m. Sixteen pvc poles covered in tanglefoot were evenly spaced around the circle with poles placed according to true north. The release chamber (blue triangle) was placed in the center.



**Figure 4.3:** Pyramidal fly release chamber. After opening, the release chamber would lie flat on the ground to encourage house flies to leave the release site. Panels A through D show still shots taken from a video recording of the release chamber opening. The release chamber was “spring loaded” so that all three sides would open at similar rates. Traps in the background were not the ones used in this study.



**Figure 4.4:** Mean direction of house fly travel for males (grey) and females (black) over all paired releases. Arrows indicate mean direction; the length of the arrow indicates the concentration of points around the mean, and the lines are circular kernel density estimates showing the distribution of points around the circle.



**Figure 4.5:** Circular diagrams showing the mean direction of house fly movements as indicated by the direction of the blue arrow in the center of each circle. The length of the arrow (from center of circle to point) is an indication of the concentration of the points around the mean ( $\bar{R}$ ). The longer the arrow the more concentrated the points are around the mean. The blue line surrounding each plot is a circular kernel density estimate showing the underlying distribution of points. Black dots around the circle indicate individual observations and are stacked. Red dots placed in an arc indicate poles (and release) behind which the target was placed. Numbers indicate replicate and the letters indicate individual release. The symbol ( $\oplus$ ) indicates the mean wind direction and the asterisk symbol (\*) indicates the position of the sun for each release.

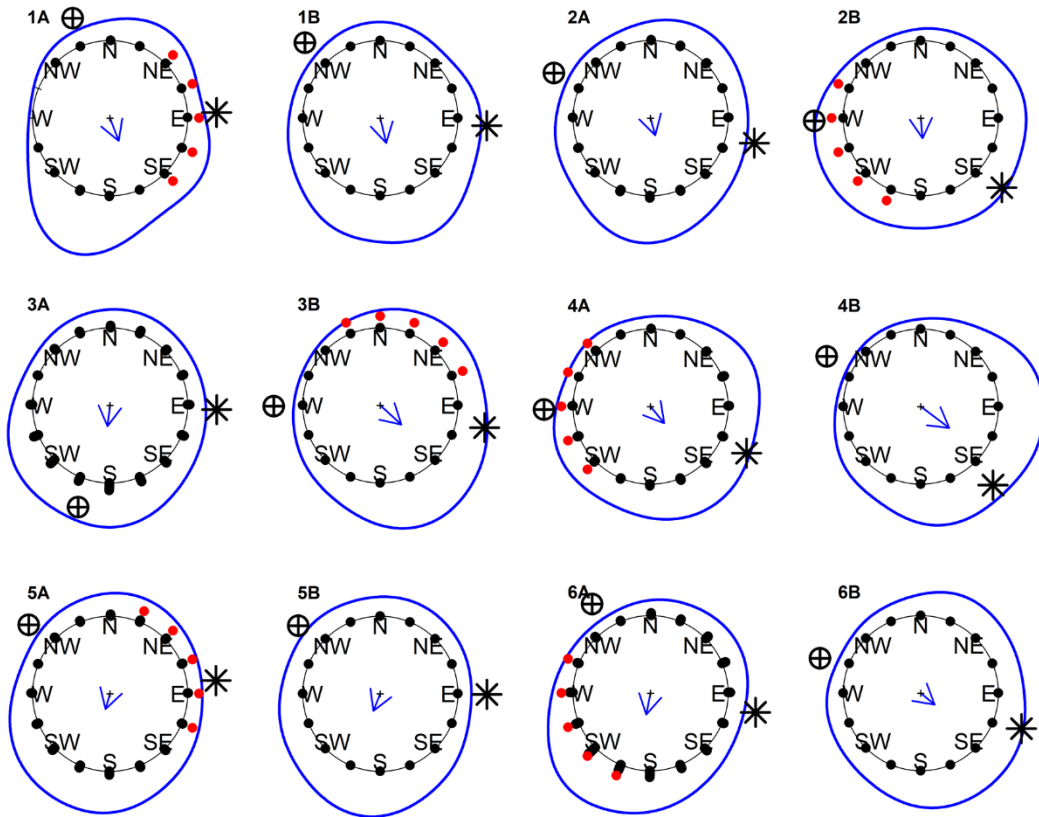
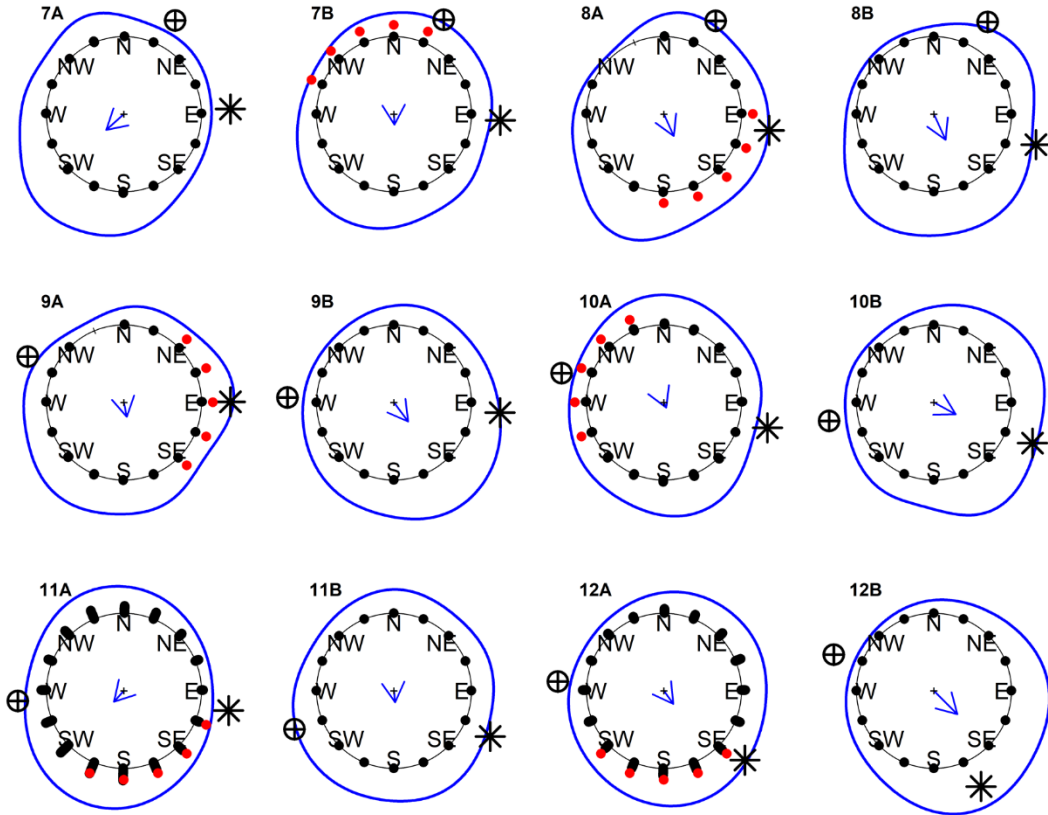
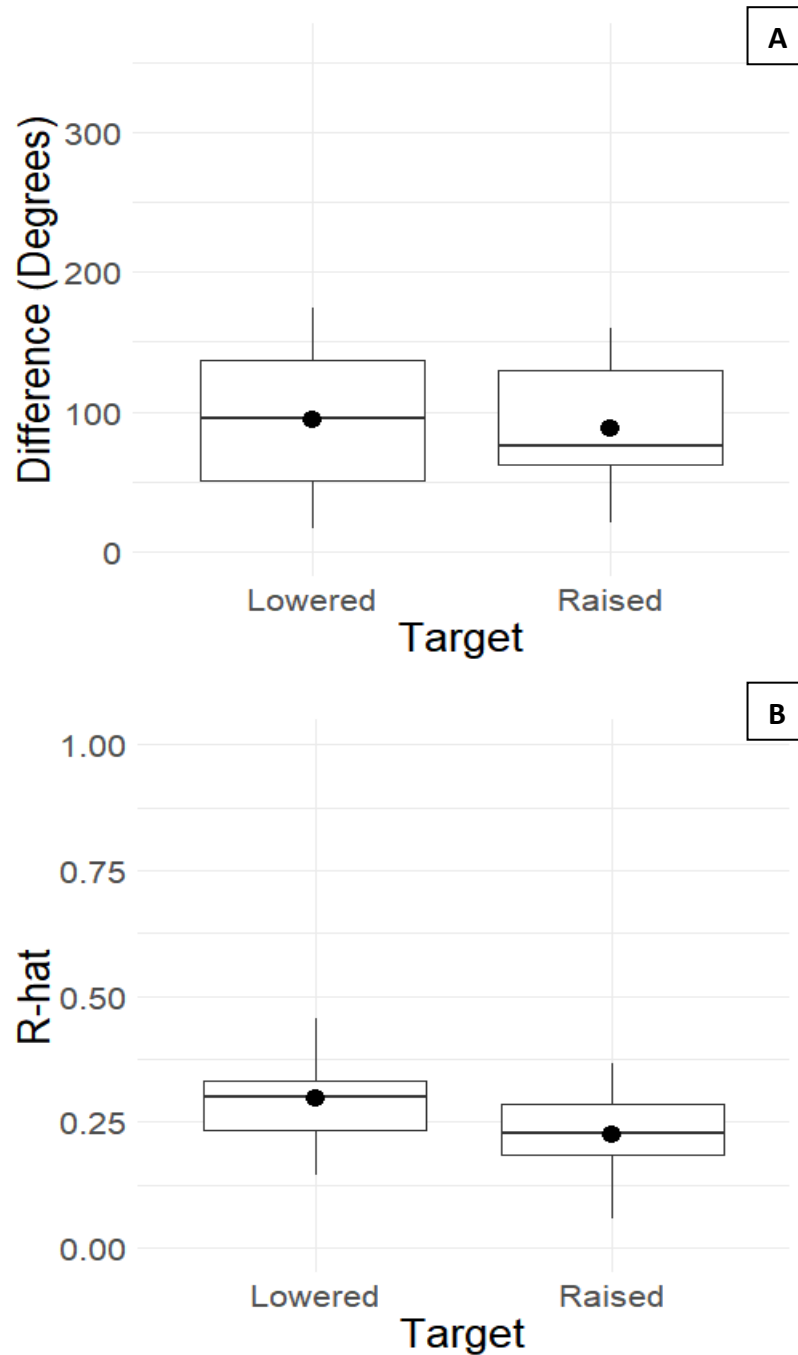




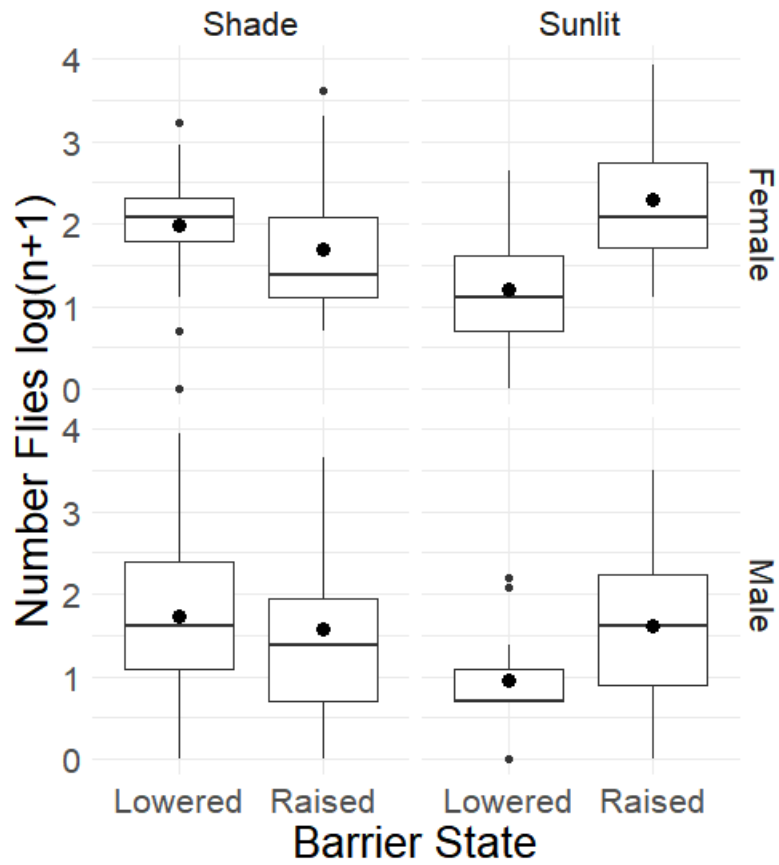
Figure 4.5: Contd.



**Figure 4.6:** Box plots showing A) the absolute angular difference (in degrees) between the center position of the barrier and the mean direction of house fly travel, calculated for when the target was raised and lowered, and B) the estimated values of Rho (circular concentration around the circular mean) for when the target was raised and lowered. The box plot indicates the quartile ranges, with the median represented by the black horizontal line, while black dots show the means.



**Figure 4.7:** Box plots showing the transformed ( $\log n+1$ ) number of male and female house flies collected on sticky traps for when the side of the target facing the release point was shaded or sunlit. The box plot indicates the quartile ranges, with the median represented by the black horizontal line, while black dots show the means.



## CONCLUSION

House fly (*Musca domestica* L.) production on dairies and other confined animal production facilities remains a significant problem for animal producers (Thomas and Skoda 1993, Adams 2003, Lole 2005). Cultural control methods may fail to effectively reduce the number of nuisance flies (Lazarus et al. 1989), and when coupled with the possibility of pesticide resistance (Keiding 1986), current management practices can fall short in maintaining house fly populations low enough to prevent lawsuits or litigation against producers (Campbell 1993, Lole 2005, Winpisinger et al. 2005). As such, novel control techniques need to be developed to supplement existing control measures. One possible novel control method is the use of barriers or visual targets to prevent house flies from emigrating from development sites and into surrounding urban areas (Fried et al. 2005, Dubie 2014). House flies may exhibit behaviors that can be manipulated to capture or divert emigrating flies away from sensitive areas using barriers and/or visual targets.

In order to implement new control strategies, it is important to understand the biology and behavior of *Musca domestica* in a field environment. To that end, a series of observational studies were undertaken to assess the diel flight activity of house flies, identify the height above ground at which flies generally fly, assess which environmental factors are important in determining flight direction, and assess the response house flies have when encountering a visual target. Using these data, we hope to supplement current house fly management methods on confined animal production facilities.

House flies are active daytime fliers and will begin flight activity within 30 minutes (before or after) of civil dawn, with increasing activity for the first two to five hours after. Only a single peak in activity was observed for house flies in the field, however activity levels fluctuated

over the course of a day and may have obscured other smaller peaks at other times during the day. The period of peak activity occurred on average four hours after dawn for male house flies, and seven hours for females. Higher daytime average temperatures resulted in earlier peaks in activity for male house flies but resulted in later peaks in activity for females. Likely these are behavioral responses to avoid activity during the hottest part of the day. For males this equates to increasing flight activity to earlier in the day before temperatures would peak. Females shifted their peaks in activity to on or just after the highest temperatures. At and below average daytime temperatures of 25°C, the peak times of activity became similar for both sexes. It would be interesting to see if peak times of activity are similar when average daytime temperatures fall below 18°C. Since the time of peak activity was different for each sex, it is not surprising that sex ratios also varied throughout the day with males being predominantly collected before noon. Sex ratios equalized from noon until dusk.

Regarding overall fly activity, temperature had the largest impact, and was a significant predictor of house fly activity over a daytime period. Temperature had a strong positive correlation with activity during the dawn and dusk periods and was strongest during dawn. This is also when the largest changes in temperature occurred. During the middle of the day there was slight negative effect of temperature on activity, but this likely caused by temperatures >35°C where activity levels begin to decline due to overheating (Schou et al. 2013). Light intensity was also a very good predictor of activity for house flies but only from dawn through midday. Male house flies had a significantly stronger positive response to increasing light intensity than females. Perhaps this is related to a sunbathing behavior by male house flies which are smaller and can warm more quickly. During dusk, light intensity did not affect house fly activity. But wind speed had a (unexpected) positive associated with house fly activity. Most

insects show less activity with increasing wind speeds. Given this assumption, wind speed might be a spurious result since wind speeds sharply declined at the same time house fly activity declined, hence the strong correlation.

Due to the shifting levels of activity throughout an average southern California summer day, any house fly monitoring programs should make a considerable effort in being consistent in the timing of trap placement and collection if point estimates (over a short time span) are being used. If traps are placed inconsistently in time, then it would be very easy to draw incorrect conclusions about changes in house population levels. Traps which are placed over longer periods of time (weekly) would overcome daily fluctuations and provide a better estimate of population changes. Additionally, any management program using fast acting pesticides delivered as a spray or ultra-low volume spray (ULV) should try to time spray event to when the most house flies are flying, approximately 4-7 hours after dawn. It is anticipated that through a better understanding of house fly diel activity current management programs will be refined to better target adult house flies, which have the potential to disperse into surrounding neighborhoods.

Future work on diel activity should focus on the interaction of environmental variables with house fly flight activity. New surveillance technologies which can sample and identify insects while in flight are currently in development (Chen et al. 2014) and could potentially provide large amounts of data that can be used to model and perhaps predict daily house fly activity levels.

House flies are thought to be strong dispersers capable of flying many kilometers in a single flight (Thomas and Skoda 1993). However, little information is available on the maximum flight distance for house flies and would be a fertile area for future research. It has been

proposed that the use of barriers could slow or prevent house flies from dispersing away from development sites and into unwanted areas (Fried et al. 2005). A key component in assessing the success of a flight barrier is to first evaluate the height above ground house flies commonly fly. Except for two locations, it was found that house flies were evenly distributed over the entire height of a 2.13-meter-tall trap. Both locations were on the western side of the dairy with one site in an alfalfa field approx. 100 meters from the nearest cattle pen, while the second was along the edge of the field approx. 20 meters from the nearest pen (closer to the dairy).

Within the field site more house flies were collected towards the top of the trap, while the opposite was observed for the site along the field, with more collected towards the bottom. It is unclear why flying height was different for these two locations. Perhaps house flies are adjusting their flying height in response to the type of ground cover through/over which they were flying, or in response to wind speed and/or wind direction. Low wind speeds, which were observed at the location within the alfalfa field, may have elevated the insect boundary layer (vertical airspace nearest the ground which an insect can control its own flight path) above the tops of the traps, while the trap along the edge of the field experience higher average wind speeds may have decreased the height of the boundary layer and resulted in more house flies being collected towards the bottom of the trap. The other four locations, which were more centrally located on the dairy, were all near barns, haystacks, and other structures which may have increased the boundary layer height for house flies at these locations, perhaps explaining why the height distributions at these four locations are all very similar.

The sex ratio of house flies was the same over the entire height of the traps. The number of house flies collected varied by the time of day they were collected, with more flies captured in the mornings, and decreasing towards evening in agreement with findings from

chapter one. However, more females than males were collected and might reflect a collection bias due to trapping methods. The most house flies were collected on the east facing sides of the traps, which subsequently had the highest average height of collected house flies. Since winds were predominantly out of this west this suggests an interaction with wind, where either the flies are flying into the wind before encountering the trap, or the trap acts as a wind barrier resulting in an increase in flying height and accumulating more flies than the other sides of the traps.

Given the height distribution observed at most of the locations used, a barrier would likely need to be taller than 2.13 meters in height, which is taller than most commercially available products. It was anticipated that trap height used would be high enough to detect a decrease in the number of house flies collected (*i.e.* the upper extent of the boundary layer for house flies), but this was only observed at the site along the alfalfa field. Additionally, wind speed does vary by height and a similar number of flies collected over the entire height of the trap would indicate a decrease in house fly density (per unit of airspace) with increasing height. This is because higher wind speeds sample more air per unit time. However, wind speed was only recorded at 1.5 meters in height and changes can only be estimated.

Future work should focus on sampling a variety of dairy locations on the same day to eliminate the confounding effect of location and time that was present in the current study. Trapping should also extend above 2.13 meters to identify the inflection point where house fly numbers begin to decline. It may even be worthwhile to perform a release recapture study looking at flying height over different types and heights of substrate.

The direction of flight for dispersing house flies had always been difficult topic to study in a field setting. It has been proposed that house fly dispersal is essentially random, and that



flies simply aggregate at attractive sites, given the impression of directed flight. From the study performed in this dissertation it is apparent that house flies are not dispersing randomly but are orienting their flight in relation to some cue. Over all releases house flies flew towards the southeast of the release site.

Flight direction was not correlated with wind direction in any meaningful way. However, on average house flies generally flew at a 45° (with wind direction) angle down wind and does not conform to any previously known insect search behavior. Direction of flight was significantly correlated with the position of the sun and clock time, but it is expected that solar position would be a better predictor of flight direction than time. But this correlation could also be spurious as house flies could have been flying towards a group of trees located to the south east of the release site. In the mornings house flies appeared to disperse more randomly than during the afternoon (as shown by the correlation with time). It is assumed that this change in the concentration of flights is related to some external environmental factor. Flight direction was also correlated with temperature, humidity and wind speed. Increasing humidity is correlated with a clockwise shift in the mean direction of flight from the southeast towards the south, while increases in temperature and wind speed both are correlated with counterclockwise shifts in mean direction of flight from the south towards the south east. Wind speed like clock time is also a covariate of solar position as windspeeds became higher later in the day. As such the linear-circular associations are very similar. As wind speeds increased it appeared that the amount of variation around the mean direction of flight decreased. However, this is only speculation based on the figures alone. It is not clear which if any of these observed variables are impacting the mean flight direction of house flies. Over all releases more male house flies were collected than females. Since the sex ratios and number of flies were the same for each

release, differences are likely due to differential landing behaviors between the sexes. Males might simply land more often perhaps as a mate location strategy, while females continue to disperse away from the study area.

Since house flies were thought to be orienting towards the group of trees to the southeast, another study was conducted to see if the presence of a closer target would change the mean direction of flight away from the southeast towards the new manmade target. Over all the releases the mean direction of flight and the circular concentration around the means did significantly differ with house flies shifting their mean direction of flight clockwise from the southeast to the south when a visual target was raised. However, it is not clear what a change in mean direction indicates since the target was randomly placed at positions all around the circle. The change in the circular concentration is slightly more interpretable as the observed decrease in concentration likely indicates that the flight directions of house flies are becoming more random when the target is raised. Wind direction was not associated with differences in mean direction of flight or the number of house flies collected, suggesting that the target used was not acting as windbreak for house flies. The placement of a visual target did impact the number of house flies collected, but only when the target and traps were in direct sunlight (in relation to the release point).

It appears that the methods used to assess the direction of flight for dispersing house flies are adequate, but it still assumes that the trap(s) which collected the most flies are indicative of the preferred direction of flight. It is possible that flight is not being measured, but landing preferences are. Future work using video tracking or various types remote sensing (radar or lidar) could be used to gain a much clearer picture by simply observing house flies in a natural environment. Secondly, both the directionality study and the target study should be

replicated using a larger variety of locations preferably in differing habitats as well. The time of the fly releases should be kept similar as well, as changes in environmental variables should be more similar (*i.e.* temperatures are always rising when releases are performed). This would help eliminate some random noise in the recapture of house flies.

Overall, house fly flight behaviors in the field are complex, and are likely impacted by changes in all the environmental variables observed in this dissertation, including their interactions with each other. Changes in temperature and sunlight (time of day) appear to have the broadest impact on house fly behavior, affecting flight activity and directionality. It is anticipated that as sampling techniques move into the digital age, the higher resolution of the resulting data will allow research's to better understand the effect weather has on the movements of house flies over each day. As with most observational studies more questions are created than answers, and further research is always warranted.

## REFERENCES CITED

- Adams, J. 2003.** Vector: Filth Fly. *In* Poultry Training Manual. College of Agriculture, Forestry and Life Sciences: Clemson University, South Carolina.
- Campbell, J. B. 1993.** The Economics of the Fly Problem, p. 97. *In* Rural Flies Urban Environ., Research Bulletin. University of Nebraska Lincoln.
- Chen, Y., A. Why, G. Batista, A. Mafrá-Neto, and E. Keogh. 2014.** Flying Insect Classification with Inexpensive Sensors. *Journal of Insect Behavior*. 27: 657–677.
- Dubie, T. R. 2014.** Influence of Vegetation Height on the Dispersal of House Flies (*Musca domestica*) from Livestock Facilities and Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers (Dissertation). Oklahoma State University.
- Fried, J. H., D. J. Levey, and J. A. Hogsette. 2005.** Habitat Corridors Function as Both Drift Fences and Movement Conduits for Dispersing Flies. *Oecologia*. 143: 645–651.
- Keiding, J. 1986.** The House Fly: Biology and Control. WHO VBC. 86.937: 1–62.
- Lazarus, W. F., D. A. Rutz, R. W. Miller, and D. A. Brow. 1989.** Costs of Existing and Recommended Manure Management Practices for House Fly and Stable fly (Diptera: Muscidae) Control on Dairy Farms. *Journal of Economic Entomology*. 82: 1145–1151.
- Lole, M. J. 2005.** Nuisance flies and landfill activities: an investigation at a West Midlands Landfill site. *Waste Management & Research*. 23: 420–428.
- Schou, T. M., S. Faurby, A. Kjærsgaard, C. Pertoldi, V. Loeschcke, B. Hald, and S. Bahrndorff. 2013.** Temperature and Population Density Effects on Locomotor Activity of *Musca domestica* (Diptera: Muscidae). *Environmental Entomology*. 42: 1322–1328.
- Thomas, G. D., and S. R. Skoda. 1993.** Rural Flies in the Urban Environment? Proceedings of a Symposium Presented at the Annual Meeting of the Entomological Society of America. Institute of Agriculture and Natural Resources. University of Nebraska-Lincoln, Lincoln NE.
- Winpisinger, K. A., A. K. Ferketich, R. L. Berry, and M. L. Moeschberger. 2005.** Spread of *Musca domestica* (Diptera: Muscidae), from Two Caged Layer Facilities to Neighboring Residences in Rural Ohio. *Journal of Medical Entomology*. 42: 732–738.