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Competition among three forensically important blow fly species (Diptera: Calliphoridae): *Phormia regina, Lucilia sericata,* and *Chrysomya rufifacies*

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COMPETITION AMONG THREE SPECIES OF FORENSICALLY IMPORTANT BLOW FLY SPECIES (DIPTERA: CALLIPHORIDAE): *PHORMIA REGINA, LUCILIA SERICATA*, AND *CHRYSOMYA RUFIFACIES*

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COMPETITION AMONG THREE SPECIES OF FORENSICALLY IMPORTANT BLOW FLY SPECIES (DIPTERA: CALLIPHORIDAE): *PHORMIA REGINA, LUCILIA SERICATA*, AND *CHRYSOMYA RUFIFACIES*

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The aim of this study was to use interspecific competition between three species of blow flies (Diptera: Calliphoridae) to determine if interspecific competition might explain the successional patterns. A replacement series model was used for three species of blowflies: *Phormia regina*, *Lucilia sericata*, and *Chrysomya rufifacies*. A total of 20 maggots were used for each treatment and the proportion of each species was varied. The graphic evidence and the relative crowding coefficient of *P. regina* versus *L. sericata* indicated a significant competitive advantage of *P. regina*. One of the life history traits of *L. sericata* is that it oviposits on carrion without any delay, while *P. regina* delays oviposition on carrion by up to 24 hours. Differences in oviposition times might represent a mechanism for *L. sericata* to avoid potential competition. *C. rufifacies* are known predators on other maggot species in the presence of limited food. With *P. regina* versus *C. rufifacies*, the later killed all *P. regina* in mixed treatments, showing a huge competitive advantage. These two species do not overlap often because of seasonal distributions. However, with the warming climate, *C. rufifacies* is likely to occur later in fall, earlier in the spring, and to extend its range north, so these two species could find themselves overlapping in the future. Consequently, *C. rufifacies* is likely to present a

strong selective force on *P. regina*, and competitive displacement of *P. regina* seems likely. The relative crowding coefficient and modified relative crowding coefficient did not show distinguishable differences in competition between *L. sericata* and *C. rufifacies. L. sericata* has been shown to form clusters away from predaceous maggots allowing a better chance for survival, which may account for the absence of predation by *C. rufifacies*. Finally, this study shows that replacement series models are a useful tool in measuring competition of blow flies, and interspecific competition between species might explain the life history traits used by forensic entomologists and could be useful in predicting future situations.

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Forensic Entomology

Forensic entomology uses arthropods, usually insects, to aid in civil or criminal investigations. There are three primary areas of forensic entomology: (1) urban entomology which focuses on insects that infest human environments, (2) food storage entomology which focuses on insects or parts in products of consumption, and (3) medico-legal entomology which focuses on using arthropods in criminal cases (Amendt et al. 2011; Gennard 2012).

Urban entomology seeks to determine the cause or source of a particular infestation and methods to mitigate or eliminate infestations. Of greatest economic importance in urban entomology is the protection of structures from insect attack (primarily from termites) and the elimination of insects in homes, particularly cockroaches. Some workers also include other entomological aspects of the urban environment such as turf and ornamental plantings in urban entomology. Forensically, lawsuits associated with termite damage or failed management are of greatest importance.

The forensic importance of food storage entomology largely pertains to insect infestation of raw and processed food. Stored-product entomology usually involves identifying and confirming the presence of insects (such as cockroaches, flies, beetles, and ants) in food. Since preventing all insects in food is nearly impossible, many countries provide legal standards of allowable insect parts in food, and those standards must be followed. Therefore, stored product entomologists work closely with those agencies. (Gennard 2012).

Medicolegal entomology is likely the most well-known area of forensic entomology due to fictional crime dramas, such as the television shows CSI, Bones, and the movie *The Silence of the Lambs*; the maggots help to determine the time of death and the suspect who seemingly had an alibi, no longer has an alibi for the new time of death. This field of forensic entomology is the focus of this study.

Forensic entomology is an old science. Many people like to think of it as a newer science only because it hasn't been talked about as long or as much as other types of evidence like fingerprints and firearms. In fact, much of the public still has no idea about forensic entomology, and those that have heard of it only know what they have seen on TV or read in books. However, the first reported case of insects used in "forensics" dates back to 13th century China. The chief of village wrote about this account in the *Washing Away of Wrongs* (Tz'u 1981). Sun Tz'u used fly evidence to find a man that slashed another villager to death. He had the men lay out their sickles in the center of the village. On this warm day, flies attracted to microscopic traces of blood and tissue left behind on the sickle began to gather (Benecke 2001; Gennard 2012; Greenberg 1991). This later lead to the man's confession. In 1668, Francesco Redi developed an experiment to show that eggs hatched into maggots and the eggs came from flies. In his experiment, he had two jars of meat. One exposed jar was accessible to flies, and the sealed jar was inaccessible to insects. In the exposed jar, the flies laid eggs which hatched into maggots while the sealed jar had no maggots. This allowed him to disprove the idea that maggots arise through spontaneous generation (Gennard 2012).

In 1850 understandings of insect development on carrion were applied to a murder case, where the workers on a remodel discovered the body of a murdered

newborn baby behind the mantle of a house during renovation. Moth larvae and blow fly puparia on the body placed the time of death in 1848. This information was used to exonerate the current occupants and implicating the previous occupants (Gennard 2012, Greenberg 1991). This information was based on two flawed assumptions: first that insects took a full year for development, secondly that insects only laid eggs in the summer. This helped to lay the foundation for modern forensic entomology. However, forensic entomology was only used sporadically after that until the 20th century.

Today, insects found on a corpse are most commonly used to estimate the postmortem interval (PMI), or time since death (Hall and Huntington 2008). In particular, the larval stage of the insect provides the most clues in an investigation.

Insects develop with a predictable pattern from egg to adult, making them useful in PMI calculations. Insects are poikilotherms, meaning they cannot internally regulate their own body temperature, so they are dependent on the ambient temperature for development. Probably the most important developmental pattern in forensic entomology is that of blowflies, the Calliphoridae, which are typically the first insects to occur on a dead animal.

Adult blow flies are attracted to a corpse and lay eggs. As an example, once oviposited, eggs of the blow fly *Calliphora vicina* maintained at 15 °C or above will usually hatch within 24 hours (Donovan et al. 2006). The hatched larvae feed on body tissues and grow passing through three larval stages, called larval instars (by definition, an instar is an insect between two molts, so blowflies have three larval instars and one pupal instar). Different groups of insects will have a varying number of instars, but blow flies have only three instars before the pupal stage. To transition to the next larval stage,

the insect molts through the process of apolysis (pulling away from old cuticle and creating new) and ecdysis (shedding the old cuticle), to reveal the next stage (Amendt et al. 2011). Once the third stage larvae finish feeding, they usually migrate away from the body and into the soil where they pupate forming a casing from the third larval exoskeleton (called a puparium). After a given amount of time and temperature, adults emerge from the puparia to begin the cycle over again.

As with all insects, development is temperature dependent. Within limits, at higher temperatures larvae will develop more quickly than at lower temperatures. Different species of flies also vary in development time. Also, a body in direct sunlight will warm up, decreasing the development time of the insects. Typically insect development is measured as a combination of time and temperature called a heat unit or degree day (DD). Degree days take into account the average air temperature as well as the amount of time the insect experienced that temperature since in nature a constant temperature is unlikely. To reach the next developmental stage, the insect must experience a certain number of these degree days. The degree days for one day are added to the previous day's total which is called accumulated degree days (ADD). The formula for DD and ADD is:

$$DD = (\text{average daily temperature} - \text{developmental threshold}) * \text{days}$$

$$ADD = DD_1 + DD_2 + DD_3 \ldots$$

Each species of insect has a specific developmental threshold or minimum temperature for development. Below that temperature, development ceases or slows and becomes negligible (Higley and Haskell 2001).

The Blow Flies: *Phormia regina*, *Lucilia sericata*, and *Chrysomya rufifacies*

Phormia regina. *Phormia regina* (Meigen), the black blow fly, is common and widespread throughout the United States and has a Holarctic distribution. This species is most prevalent on carrion throughout the northern United States in the fall and spring months and during the summer months in the southern United States (Byrd and Allen 2001, Greenberg 1971, Hall 1948). The adult fly is easily distinguished from other blow flies by its olive-green color, orange anterior thoracic spiracle, and lack of setae on the stem vein (Whitworth 2006). On livestock, *P. regina* has been reported to cause myiasis and has been shown to invade healthy tissues when used in maggot therapy (Knipling and Rainwater 1937, Greenberg 1971). In larval stages, *P. regina* may wander away from the food (observations from Dr. Amanda Roe and Dr. Christian Elowsky), but do not tend to migrate away from the food source during pupation (personal observations). Also, *P. regina* appears to arrive later or delay oviposition upon a corpse instead of immediate oviposition as occurs with *Lucilia sericata* (Norris 1959).

Lucilia sericata. *Lucilia sericata* (Meigen), is one of the most common and widespread species of blow fly in the United States. They are bright green or copper colored metallic blowflies that are commonly confused with other members of the same genus, such as

Lucilia cuprina (Whitworth 2006). The adults can be distinguished by the presence of setae on the stem vein, 3 postsutural setae, and 2–5 central occipital setae (Whitworth 2006). *Lucilia sericata* is a primary fly involved in myiasis, but depending on the location it may be more heavily involved in myiasis in some countries than in other which is thought to be due to the lack of receptors in some parts (Norris 1959). The adults of this species are found in open fields, in sunny weather (Greenberg 1971) and are usually one of the first species to arrive at a carcass and begin oviposition (Byrd and Castner 2010).

Chrysomya rufifacies. *Chyrsomya rufifacies* (Macquart), the hairy maggot blow fly, is native to Australia, but was introduced to the continental United States in the 1980s (Baumgartner 1986) and favors warm weather (Norris 1959). Wells and Greenberg (1994) demonstrated preference of *C. rufifacies* for larger carcasses of rabbit and goat, as compared to rat carcasses. Unlike many other blow flies which rapidly find and use carrion, *C. rufifacies* may delay host finding or oviposition (Norris 1959). The larvae of *C. rufifacies* can be predators on other species of Diptera, which reduces the numbers of other species present on carrion (Wells and Greenberg 1992). Alternatively, with scare food, larvae of *C. rufifacies* have been shown to consume the larvae of other species, however, in the presence of sufficient food, the larvae have not been shown to harm other species (Subramanian and Mohan 1980). In addition to their feeding on carrion, *C. rufifacies* has been recorded causing secondary myiasis on sheep (Norris 1959). (Myiasis is the infestation of living tissue by fly larvae; secondary myiasis is an infestation after the previous myiasis from another species).

Larvae of *C. rufifacies* have many fleshy tubercles or spines which they can use as a defense. They prey upon smooth bodied maggots wrapping their bodies around their prey and using their mouth hooks to pierce and kill their prey (Norris 1959). As adults, *C. rufifacies* are easily recognized by their metallic green and blue bodies with the black posterior margins on the first couple abdominal segments and pale genal dilations (Whitworth 2006).

Among unique characteristics of C. *rufifacies* is that females exhibit monogeny, or laying eggs of only one sex, which appears to be controlled by the mother's chromosomes (Ullerich and Schottke 2006, Roy and Siddons 1939). Females lay the same sex of offspring in successive egg batches (Roy and Siddons 1939).

Intraspecific and Interspecific Competition

There have been a few studies detailing the effects of interspecific competition on blow fly species and the effects of larval crowding. The impact of this competition has been shown to cause smaller adults, longer development times, and smaller puparium (citation). Adults of *L. sericata*, when placed in high densities for development, typically smaller in size than those at a lower density (Martinez- Sanchez et al. 2007).

However, there have been very few studies looking at the effects of interspecific competition among Calliphorid species, and some those that have looked at it contain flaws in their experimental design which make establishing any weight to their study nearly impossible. In fly populations consisting of species of *Lucilia*, *Lucilia illustris* became the dominant species over the other in the same genus. The other species tend to emerge later, rather than competing with the dominant species (Hanski 1987). Smith and

Wall (1997) examined the asymmetric competition between *Calliphora vicina* and *Lucilia sericata*. They found in populations of individuals less than 150 there was no evidence of competition because the proportion of survivors in the pure and mixed cultures were not significantly different. However, when the number of larvae was between 150 and 300 individuals, the number of adults that emerged was greater for *L. sericata* than *C. vicina*. In this range, the number of *L. sericata* was higher in the pure cultures than in the mixed cultures meaning that *L. sericata* experienced more interspecific competition in the mixed cultures while *C. vicina* experienced high intraspecific competition in the pure cultures. When blowflies of the species *Hemipyrellia ligurriens* (Diptera: Calliphoridae) and *Boettcherisca formosensis* (Diptera: Sarcophagidae) were placed in mixed cultures, the larvae of *B. formosensis* were higher in numbers than *H. ligurriens*, which were also smaller in relative size. This suggests the *B. formosensis* is a better competitor, or better at exploiting the limited resource first (So and Dudgeon 1990). However, most of these competition studies have only conducted their mixed grouping at one ratio, an even mixture of the two species. They have no way of determining what would happen if they skewed the mixture with one species dominating the ratio which is a flaw in the studies. Also, most of these studies are not looking at competition between forensically important blow fly species, but rather those of medical and veterinary importance. Prinkkila and Hanski (1995) examined interspecific competition between four species of blow fly in the genus *Lucilia*. They found that the density affected the outcome of the superior competitor. At the intermediate densities some were found to be better competitors than the other, but it was reversed at a high density. However, this study had very few replicates, 5 at low

densities, 2-3 in the intermediate density, and only 1 at higher densities. Additionally, this study used 5 grams of liver as a food source. Because of this, it is hard to assign any weight to this study. *Chrysomya putoria* (Weidmann) was found to be a superior competitor over *Cochliomyia macellaria* (Fabricius) at low densities, and still able to outcompete *C. macellaria* at higher densities just not as efficiently (dos Reis et al. 1999). Again, due to the lack of replication (N=2), and the lack of variable ratios, it is hard to put weight behind the conclusions of this study. *Chrysomya albiceps* (Weidmann) exhibits similar behavior as *Chrysomya rufifacies* in terms of predation of temperature tolerance. When *C. albiceps* was placed into containers at higher temperatures with *L. sericata,* nearly all the *L. sericata* were wiped out except in containers where the ratio was 25 *L. sericata* per 1 *C. albiceps*. The *C. albiceps* were exhibited predatory behavior on the *L. sericata* (Kheirallah et al. 2007).

Goals

To explain the succession of blowflies on carrion, this study examined intraspecific competition among three species of forensically important blow fly species: *Lucilia sericata*, *Chrysomya rufifacies*, and *Phormia regina.*

References

Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J. (2011). Forensic entomology: Applications and limitations. *Forensic Science, Medicine, and Pathology, 7*(4), 379-392.

Baumgartner, D. L. (1986). The hairy maggot blow fly *Chrysomya rufifacies* (Macquart) confirmed in Arizona. *Journal of Entomological Science, 21*(2), 130-132.

Benecke, M. (2001). A brief history of forensic entomology. *Forensic Science International, 120*(1-2), 2-14.

Byrd, J. H., & Allen, J. C. (2001). The development of the black blow fly, *Phormia regina* (Meigen). *Forensic Science International, 120*(1-2), 79-88.

Bryd, J. H., & Castner, J. L. (2010). Insects of forensic importance. In: Byrd JH, Castner JL, (Eds.). Forensic Entomology: The Utility of Arthropods in Legal Investigations, 2nd ed. 39-126, Florida: CRC Press.

Donovan, S. E., Hall, M. J., Turner, B. D., & Moncrieff, C. B. (2006). Larval growth rates of the blow fly, *Calliphora vicina*, over a range of temperatures. *Medical and Veterinary Entomology,20*(1), 106-114

Gennard, D. E. (2012). *Forensic Entomology: An Introduction* (2nd ed.). Chichester, West Sussex: John Wiley & Sons.

Greenberg, B. (1971). *Flies and Disease* (Vol. 2). Princeton, NJ: University Press.

Greenberg, B. (1991). Flies as forensic indicators. *Journal of Medical Entomology, 28*(5), 565-577.

Hall, D. G. (1948). *The Blowflies of North America*. Springfield, Il: Thomas.

Hall, R. D., & Huntington, T. E. (2008). Medicocriminal entomology. In *Entomology & Death: A Procedural Guide* (2nd ed.). Clemson, South Carolina: Joyce's Print Shop.

Hanski, I. (1987). Carrion fly community dynamics: patchiness, seasonality, and coexistence. *Ecological Entomology*, 12:257-266.

Higley, L. G., & Haskell, N. H. (2001). Insect development and forensic entomology. In: Byrd JH, Castner JL, (Eds.). *Forensic Entomology: The Utility of Arthropods in Legal Investigations*(pp. 287-302). Boca Raton, FL: CRC Press.

Kheirallah, A., Tantawi, T., Aly, A., & El-Moaty, Z. (2007). Competitive interaction between larvae of *Lucilia sericata* (Meigen) and *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae). *Pakistan Journal of Biological Sciences,10*(7), 1001-1010.

Knipling, E. F., & Rainwater, H. T. (1937). Species and incidence of dipterous larvae concerned in wound myiasis. *The Journal of Parasitology,23*(5), 451.

Martínez-Sánchez, A., Smith, K. E., Rojo, S., Marcos-García, M. A., & Wall, R. (2006). Geographic origin affects larval competitive ability in European populations of the blow fly, *Lucilia sericata*. *Entomologia Experimentalis et Applicata,* 0(0), 93-98.

Norris, K. R. (1959). The ecology of sheep blowflies in Australia. *Monographiae Biologicae Biogeography and Ecology in Australia,* 514-544.

Prinkkilá, M., & Hanski, I. (1995). Complex competitive interactions in four species of *Lucilia* blowflies. *Ecological Entomology, 20*(3), 261-272.

Reis, S. F., Zuben, C. J., & Godoy, W. A. (1999). Larval aggregation and competition for food in experimental populations of *Chrysomya putoria* (Wied.) and *Cochliomyia macellaria* (F.) (Dipt., Calliphoridae). *Journal of Applied Entomology, 123*(8), 485-489.

Roy, D. N., & Siddons, L. B. (1939). On the life history and bionomics of *Chrysomyia rufifacies* Macq. (Order Diptera, Family Calliphoridae). Parasitology, 31(04), 442-447.

Smith, K. E., & Wall, R. (1997). Asymmetric competition between larvae of the blowflies *Calliphora vicina* and *Lucilia sericata* in carrion. *Ecological Entomology,22*(4), 468-474.

So, P., & Dudgeon, D. (1990). Interspecific competition among larvae of *Hemipyrellia ligurriens* (Calliphoridae) and *Boettcherisca formosensis* (Sarcophagidae) (Diptera). *Researches on Population Ecology,32*(2), 337-348.

Subramanian, H., & Raja Mohan, K. (1980). Biology of the blowflies *Chrysomya megacephala, Chrysomya rufifacies,* and *Lucilia cuprina*. *Kerala Journal of Veterinary Science,11*(2), 252-261.

Tzu, S. (1981). *The Washing Away of Wrongs: Forensic Medicine in 13th-century China* (B. E. McKnight, Trans.). Ann Arbor: University of Mich., Center for Chinese studies.

Ullerich, F., & Schöttke, M. (2006). Karyotypes, constitutive heterochromatin, and genomic DNA values in the blow fly genera *Chrysomya, Lucilia*, and *Protophormia* (Diptera: Calliphoridae). *Genome,49*(6), 584-597.

Wells, J. D., & Greenberg, B. (1992). Laboratory interaction between introduced *Chrysomya rufifacies* and native *Cochliomyia macellaria* (Diptera: Calliphoridae). *Environmental Entomology,21*(3), 640-645.

Wells, J. D., & Greenberg, B. (1994). Resource use by an introduced and native carrion flies. *Oecologia,99*(1-2), 181-187.

Whitworth, T. (2006). Keys to the genera and species of blowflies (Diptera: Calliphoridae) of America North of Mexico. *Proceedings of the Entomological Society of Washington,108*(3), 689-725.

CHAPTER 2: MATERIALS AND METHODS

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Fly Colonies and Egg Collection

Two species of blowflies, *Phormia regina* and *Lucilia sericata*, were received as eggs from the same colony of Dr. Amanda Roe at College of Saint Mary in Omaha, NE (Roe and Higley 2015). Pupae of *Chrysomya rufifacies* were received from Dr. Jeff Wells at Florida International University in Miami, FL. The colony of C. *rufifacies* was initially gathered from Homestead, FL in 2017 and reared in a laboratory. The number of generations was not counted. Colonies were kept in mesh cages approximately 46 cm^3 (Bioquip products, California) and maintained at 22 °C on a 12:12 photoperiod using a lamp. The flies were given water through the use of a quail waterer and cotton, and sugar as a food source. At least five days prior to egg collection, adult flies were given beef liver as a protein meal to help develop the female ovaries.

During egg collection, the flies were provided beef liver inside a five-ounce paper cup, half covered with aluminum foil. The flies were allowed to oviposit for approximately 18 hours before removing the eggs. Eggs were used from multiple clusters to ensure variation. Also, this helps to ensure that both genders of *Chyrsomya rufifacies* would be represented as a female will only lay one sex of eggs. Eggs were placed inside a small glass petri dish and covered with a moist paper towel before putting the cover over the petri dish. The covered dish was placed inside a sandwich bag and placed in the growth chamber at 27 °C with a 12:12 photoperiod until hatch.

Growth Chambers

The growth chamber used were *DigiTherm*® 38-liter Heating/Cooling Incubators to allow for temperature regulation within 0.1°C of a constant temperature and a 12:12 photoperiod until egg eclosion. The incubators have internal lighting and a recirculating air system to ensure air flow.

Experimental Design

The experimental design used was a randomized block with ten replicates. Each replicate was set up as a replacement series with five treatments, or ratios, of larvae: 1:0, 3:1, 1:1, 1:3, 0:1. The treatment set up for one of the three pairings is shown in Figure 1 (Appendix). The column on the left indicates the replicate number and the other 5 column shows which species and how many belong in each treatment. Each treatment was placed in a plastic 7 × 7 × 10 cm box with approximately 2.5cm of vermiculite in the bottom. There were three different competition pairings. The first pairing was *P. regina*/C. *rufifacies*. The second was *P. regina/L. sericata* and the third was *L. sericata*/C. *rufifacies*.

For each box, one dead, immature mouse (popularly called a fuzzy), had the chest and abdomen sliced open and the maggots were placed inside then it was laid incision side up in the box. Each mouse weighed approximately 5–7 grams. A total of 20 maggots were place inside each mouse. Newly hatched maggots were transferred using a moistened paintbrush. The ratios were as follows: 20:0, 15:5, 10:10, 5:15, 0:20. The mice were chosen as a natural food source and to help minimize mold and desiccation.

The boxes were placed in a growth chamber at 25 °C until adult emergence. After the adults emerged, they were collected and placed in ethanol for storage. Each adult was

identified to the species level using a modified version of Keys to the Genera and Species of Blow Flies (Diptera: Calliphoridae) of America north of Mexico (Whitworth 2006) and a Leica Stereo microscope. Then the total numbers for each box were recorded. A fine mesh sieve was used to sort through the contents of each container to look for any maggots that had migrated away from the food source and died.

For each of the pairings, the relative crowding coefficient (RCC) and modified relative crowding coefficient (RCCM) were calculated. The formula used to calculate the RCC value based on Harper (1977) is:

$$RCC = \frac{\left(\frac{A_{1:1}}{B_{1:1}}\right)}{\left(\frac{A_{1:0}}{B_{0:1}}\right)}$$

The RCCM proposed in Novek et al. (1993) is:

$$RCCM = \frac{\frac{1}{3}\left[\left(\frac{1}{3} \times \frac{A_{3:1}}{B_{3:1}}\right) + \left(\frac{A_{1:1}}{B_{1:1}}\right) + \left(3 \times \frac{A_{1:3}}{B_{1:3}}\right)\right]}{3\left(\frac{A_{1:0}}{B_{0:1}}\right)}$$

Both the RCC and RCCM values provide evidence of competition. If the calculated value were 1, it would indicate competition between species (interspecific competition) was identical to competition within species (intraspecific competition). However, when the calculated RCC or RCCM varies from one, it indicates competitive differences between species. A t-test was calculated on each RCC and RCCM to determine if it was

significantly different from one. To assess the significance of competition, if the p-value was below 0.05, there was a significant difference from 1 indicating competition. The modified form of RCC was chosen because this takes into account variation throughout all the ratios. In the RCC calculations, only the two pure cultures and the even mixture ratios are used, which leaves some two ratios of data that are not used in the calculations.

References

Harper, J. L. (1977). *Population Biology of Plants*. London: Academic Press.

Novak, M. G., Higley, L. G., Christianssen, C. A., & Rowley, W. A. (1993). Evaluating larval competition between *Aedes albopictus* and *A. triseriatus* (Diptera: Culicidae) through replacement series experiments. *Environmental Entomology, 22*(2), 311-318.

Roe, A., & Higley, L. G. (2015). Development modeling of *Lucilia sericata* (Diptera: Calliphoridae). *PeerJ,* 3:e803 https://dx.doi.org/10.7717/peerj.803.

Whitworth, T. (2006). Keys the genera and species of blow flies (Diptera: Calliphoridae) of America North of Mexico. *Proceedings of the Entomological Society of Washington,108*(3), 689-725.

CHAPTER 3: RESULTS

CHAPTER 3: RESULTS

Phormia regina versus *Lucilia sericata*

In treatment 1 (1:0), there was an average of 14.3 ± 2.81 *P. regina* and 0.0 ± 0.0 *L. sericata* in each replicate. In treatment 2 (3:1), there were 13.3 ± 2.05 *P. regina* and 3.8 ± 1.07 *L. sericata* in each container. In treatment 3 (1:1), there were 7.7 ± 1.70 *P. regina* and 6.0 ± 2.38 *L. sericata* in each container. In treatment 4 (1:3), there were 5.0 ± 2.24 *P. regina* and 8.8 ± 3.13 *L. sericata* in each container. In treatment 5 (0:1), there were 0.0 ± 0.0 *P. regina* and 16.2 ± 2.36 *L. sericata* in each container.

Figure 2 shows a replacement diagram using adult eclosion for numbers of *P. regina* and *L. sericata*. This diagram represents a model II replacement diagram (Orberg et al. 1996) and indicates there is competition between the two species with *Phormia regina* being the superior larval competitor. The calculated relative crowding coefficient (RCC) and modified relative crowding coefficient (RCCM) values are shown in Table 4. The calculated RCC of 1.56 ± 0.231 (pr > |t |= 0.039, DF=9) and an RCCM value of 1.83 ± 0.517 (pr > |t |= 0.14, Df=9) support the interpretation from Figure 2. Specifically, in mixed treatments *P. regina* was a superior competitor as compared to *L. sericata*.

Chrysomya rufifacies versus *Phormia regina*

In treatment 1 (1:0), there was an average of 12.5 ± 4.69 *P. regina* and 0.0 ± 0.0 *C. rufifacies* in each replicate. In treatment 2 (3:1), there was an average of 0.1 ± 0.30 *P. regina* and 3.0 ± 1.26 *C. rufifacies* in each replicate. In treatment 3 (1:1), there was an average of 0.0 ± 0.0 *P. regina* and 7.0 ± 2.35 *C. rufifacies* in each replicate. In treatment 4 (1:3), there was an average of 0.0 ± 0.0 *P. regina* and 10.0 ± 2.32 *C. rufifacies* in each

replicate. In treatment 5 (0:1), there was an average of 0.0 ± 0.0 *P. regina* and 12.0 ± 3.87 *C. rufifacies* in each replicate.

Because in the mixed treatment groups, there were no *P. regina* survivors, the RCC and RCCM values were not calculated.

Lucilia sericata* versus *Chrysomya rufifacies

In treatment 1 (1:0), there was an average of 7.9 ± 6.41 *L. sericata* maggots and 0.0 ± 0.00 *C. rufifacies* in each replicate. In treatment 2 (3:1), there was an average of 6.2 ± 3.13 *L. sericata* and 3.2 ± 1.86 *C. rufifacies* in each container. In treatment 3 (1:1), there was an average of 4.0 ± 2.00 *L. sericata* and 5.5 ± 2.06 *C. rufifacies* in each container. In treatment 4 (1:3), there was an average of 1.7 ± 0.94 *L. sericata* and 9.3 ± 2.92 *C. rufifacies* in each container. In treatment 5 (0:1), there was an average of 0.0 ± 0.00 *L. sericata* and 10.6 ± 2.50 *C. rufifacies* in each container.

Figure 3 shows a replacement series diagram for the number of eclosed adults from *L. sericata* and *C. rufifacies*. This figure is a model I replacement diagram (Orberg et al. 1996). Figure 2 indicates that C. *rufifacies* is a slightly better competitor than *L. sericata*. The calculated RCC and RCCM values are shown in Table 4. The calculated RCC value of 2.91 ± 1.190 (pr > |t |= 0.17, DF=5) and RCCM value of 2.17± 0.723 (pr > |t |= 0.17, Df=5) show that both are not significantly different from 1, so there is no evidence of interspecific competition between the *L. sericata* and *C. rufifacies*, even though the intersection of the graph appears slightly shifted to the left. The levels of intraspecific competition and interspecific competition are approximately equal.

The raw data for each block and treatment are shown in Tables 5, 6, and 7 in the appendix. The RCC and RCCM calculated per block is shown in Tables 8 and 9 in the appendix.

References

Oberg, A. L., Young, L. J., & Higley, L. G. (1996). A comparison of two measures of competition. *Journal of Agricultural, Biological, and Environmental Statistics,1*(4), 393.

Figures

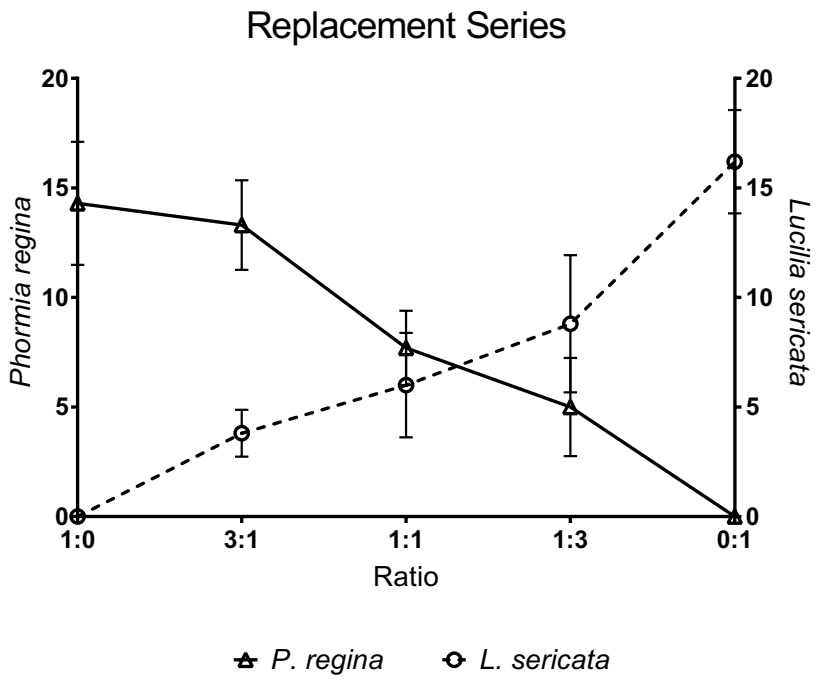

Figure 2. Replacement diagram for reared adult survivors of *Phormia regina* and *Lucilia sericata.*

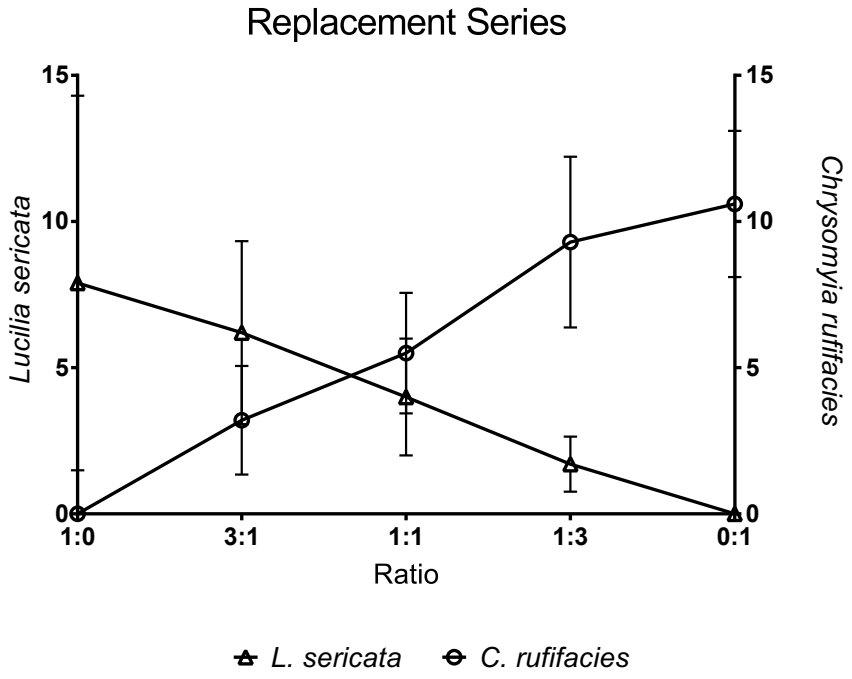

Figure 3. Replacement diagram for reared adult survivors of *Lucilia sericata* and *Chrysomya rufifacies.*

Tables

Table 1. The mean and standard error for the replacement series of *P. regina* and *L. sericata* shown by treatment for replications 1–10.

		Reps 1-10			
		Mean	SE	Mean	SE
Trt	Ratio	*P. regina*		*L. sericata*	
1	1:0	14.3	2.81	0.0	0.00
2	3:1	13.3	2.05	3.8	1.07
3	1:1	7.7	1.70	6.0	2.38
4	1:3	5.0	2.24	8.8	3.13
5	0:1	0.0	0.00	16.2	2.36

Table 2. The mean and standard error for the replacement series of *L. sericata* and *C. rufifacies* shown by treatment for reps 1–6. Reps 7–10 were excluded due to missing values.

		Reps 1–6			
		Mean	SE	Mean	SE
Trt	Ratio	*L. sericata*		*C. rufifacies*	
1	1:0	7.9	6.41	0.0	0.00
2	3:1	6.2	3.13	3.2	1.86
3	1:1	4.0	2.00	5.5	2.06
4	1:3	1.7	0.94	9.3	2.92
5	0:1	0.0	0.00	10.6	2.50

Table 3. The mean and standard error for the replacement series of *P. regina* and *C. rufifacies* shown by treatment for replications 1–7, 8, &10. Replications 7 and nine were excluded due to missing values.

		Reps 1–6, 8, 10			
		Means	SE	Mean	SE
Trt	Ratio	*P. regina*		*C. rufifacies*	
1	1:0	12.5	4.69	0.0	0.00
2	3:1	0.1	0.30	3.0	1.26
3	1:1	0.0	0.00	7.0	2.35
4	1:3	0.0	0.00	10.0	2.32
5	0:1	0.0	0.00	12.0	3.87

Table 4. The average RCC and RCCM along with the standard error for each pairing: *P. regina* vs. *L. sericata*, *L. sericata* vs. *C. rufifacies*, and *P. regina* vs. *C. rufifacies*.

P.regina vs. *L. sericata*				*L. sericata* vs. *C. rufifacies*				*P. regina* vs. *C. rufifacies*			
RCC		RCCM		RCC		RCCM		RCC		RCCM	
Mean ß	Std Error ß	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean†	Std Error†	Mean†	Std Error†
1.56	0.231	1.83	0.517	2.91	1.189	2.17	0.723	--	--	--	--

† The RCC and RCCM could not be determined since there were no *P. regina* survivors in the mixed ratios.
ß Significant value ($p < 0.05$)

CHAPTER 4: DISCUSSION

CHAPTER 4: DISCUSSION

There was a significant difference in the competitive abilities of *Phormia regina* and *Lucilia sericata*; however, there did not appear to be competitive differences between *Chrysomya rufifacies* and *Lucilia sericata*. Because no *Phormia* remained in mixed treatments between *Phormia regina* and *Chrysomya rufifacies*, we can conclude that *C. rufifacies* is a superior competitor, if we interpret killing of *P. regina* by *C. rufifacies* as an ultimate type of competitive interaction.

I would argue the most severe form of intraspecific competition is cannibalism, and predation of *C. rufifacies* on *P. regina* is analogous with interspecific competition between larvae of these species. There is evidence to support *C. rufifacies* predation on other maggot species. Subramanian and Mohan (1980) found that predation occurred usually when little food was available the predation. In my experiment, the mass of the mice used (5-7g) , is limiting for 20 blow fly larvae (Roe & Higley 2015). Frequently, carrion available to blow flies can be a limited food source. This vertebrate carrion, for example, a large portion of the flesh is usually removed by vertebrate scavengers leaving the insects to feed on the "scraps" left behind.

A further indicator of potential competitive advantage is that *C. rufifacies* develop more quickly than *P. regina,* requiring only 180.6 ADD at 25 °C (Byrd and Butler 1997) the complete their life cycle compared to *P. regina* which requires 215 ADD at 26.7 °C (Kamal 1958). Because *Chrysomya rufifacies* hatch before *P. regina,* if eggs of both species are laid at the same time, *C. rufifacies* will be able to establish themselves on the

carrion and begin to use the limited resource first. Also, since *P. regina* are smaller and further behind in development, they make easy prey for larger *C. rufifacies* maggots.

Phormia regina was able to outcompete *L. sericata*. From Figure 2, the intersection of the graph was shifted right, indicating *P. regina* was the superior competitor. Similarly, the RCC value indicates significant differences interspecific competition. Interestingly, Roe and Higley (2015), report that *L. sericata* requires 221.2 ADD when reared at 25 ° C, which is slightly slower than that of *P. regina* which requires 215 ADD. This slight difference in development rate might give *P. regina* a advantage in feeding, which could magnify through time. While this competition is not as drastic as that between *P. regina* and *C. rufifacies,* differences in development rates might be a factor in the evolution of the succession pattern of these two species. Specifically, *L. sericata* is known to arrive early to carrion and begins laying eggs almost immediately given ideal conditions. In contrast, *P. regina*, delays oviposition on carrion by up to 24 hours even under ideal conditions. Differences in competition between ancestors of these species might have selected *L. sericata* to earlier arrival times on carrion in to exploit the resource and avoid interspecific competition.

However, if development time was the only factor at play in determining competition, we should have seen the ability of C. *rufifacies* to outcompete or even wholly predate upon *L. sericata*, but that did not occur. Although Figure 3 clearly shows a shifted intersection of the two lines to the left of center (indicating *C. rufifacies* was the superior competitor), the lack of significance in the the t-test of RCC or RCCM values versus one, leads to the conclusion that any competitive difference between *L. sericata* and *C. rufifacies* are minor at most.

Fuller (1934) and Waterhouse (1947) described a repulsive effect of *C. rufifacies* on maggots from the genus *Lucilia*, in which *Lucilia* larvae move away from *C. rufifacies* and form a mass. If we consider the mass as the maggot equivalent to herding, as in animal species such as cattle and fish, then presumable such clusters are an aid in defense from predators. This conclusion seems likely with *Lucilia sericata* in my experiment. The small mouse might not have been enough distance for the *L. sericata* maggots to wander to escape predation entirely, but it might have been enough to allow a few survivors. Previous work shows that predation by *C. rufifacies* is more likely as *C. rufifacies* larvae are larger than other prey/predator maggots. Formation of a mass by *L. serciata* could reasonably deter aggression by *C. rufifacies* based on size.

I have heard it said that competition is the largest driving force behind speciation which is counter to what many ecology textbooks teach which is geographic isolation is the key driving factor (Personal Communication, Higley 2018). Since the competitive exclusion principle states that no two species can coexist and occupy the same niche, I would argue that it is what drives species to either select for genes to adapt to their own niche or die off. In nature, there are ways of niche partitioning for species to coexist. If the two species occupy the same niche, competition happens over the same resource. That level of competition cannot be sustained for long especially over a limited resource. In the case of blowflies, there are differences in arrival times and active times of the year. Since *L. sericata* prefers the warmer summer months, and *P. regina* the colder autumn and winter in the south, the species do not coexist often, but there are times of overlap. However, *P. regina* has been observed to delay oviposition so that might be one way to

combat the competitive nature of carrion. Perhaps, even *L. sericata* arrives early to occupy the niche first and get a head start in development.

The observed competitive differences between *P. regina* and *C. rufifacies* could become problematic in the future. *P. regina* is a spring and fall fly avoiding the hotter and colder times of the year, and *C. rufifacies* is a summer fly. Currently, there is little overlap between the two species of flies. However, given global warming, in the future, *C. rufifacies* will likely overlap more with *P. regina*. As seen in this experiment, *C. rufifacies* are predators on *P. regina* and killed virtually all *P.* regina larvae in my experiments (save for one individual in a 1:1 treatment). If interactions between *P. regina* and *C. rufifacies* are correspondingly severe in natural settings, this means either *P. regina* would have to evolve to deal with this competition or go extinct. For example, selection saving more cold-tolerant *P. regina* could reduce or eliminate seasonal overlap with *C. rufifacies*. Alternatively, selection for greater delays in oviposition by P. regina, might also provide a means to avoid C. rufifacies, although it would leave P. regina open to competition from other later occurring species. Irrespective of the possible evolution of *P. regina*, my results imply that as seasons are extended with global warming, *C. rufifacies* seems likely to become more common and possibly displace *P. regina* in much of its range.

This work shows that experimental examinations of competition among blow fly species offers a fruitful approach for considering life history differences among species and potential interactions among blow flies with changing ranges and environmental conditions. In these studies, both species of maggots were placed on the rearing medium at the same time. However, delaying the addition of maggots in mixed treatments, could

correspond to delayed oviposition as seen with *P. regina* and *C. rufifacies* as compared to *L. sericata*. Also, delayed infestation studies offer the potential to characterize the role maggot age/size plays in competitive relationship, especially regarding predatory behavior by *C. ruficacies*.. Another possible option would be to alter environmental conditions, especially rearing temperature. For example, because *P. regina* is adapted to cooler temperatures, would the competitive advantage of *C. rufifacies* over *P. regina* I observed disappear at colder temperatures climate.

While this study was designed with small maggot populations in a laboratory, examining competition with large maggot masses in a field setting would obviously be of value. Large maggot masses generate more metabolic heat than small masses, and the behaviors of larvae in such masses can be different than in smaller groups. Unfortunately, the technical challenges in conducting such experiments are formidable, especially initially quantifying maggots, timing oviposition, and collecting individuals after larval development.

References

1. Byrd, J. H., & Butler, J. F. (1997). Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development. *Journal of Medical Entomology,34*(3), 353-358.

2. Kamal, A. S. (1958). Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera)1 I. Bionomics. *Annals of the Entomological Society of America, 51*(3), 261-271.

3. Subramanian, H., & Raja Mohan, K. (1980). Biology of the blowflies *Chrysomya megacephala*, *Chrysomya rufifacies*, and *Lucilia cuprina*. Kerala Journal of Veterinary Science,11(2), 252-261.

4. Roe, A., & Higley, L. G. (2015). Development modeling of *Lucilia sericata* (Diptera: Calliphoridae). *PeerJ, 3*:e803 https://dx.doi.org/10.7717/peerj.803.

APPENDIX

APPENDIX

1	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
2	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
3	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
4	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
5	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
6	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
7	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
8	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
9	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*
10	1:0 20 *P. regina* 0 *L. sericata*	3:1 15 *P. regina* 5 *L. sericata*	1:1 10 *P. regina* 10 *L. sericata*	1:3 5 *P. regina* 15 *L. sericata*	0:1 0 *P. regina* 20 *L. sericata*

Figure 1. Graphic showing how one pairing of the experiment, *P. regina and C. rufifacies.* was set up. Each row represents one replicate and the boxes in each row are the different treatments. The numbers of each species placed in the treatment are shown below the ratio.

Table 5. The raw data for each container by block and treatment for *Phormia regina* and *Lucilia sericata*. Species A is *P. regina*, and species B is *L. sericata*.

Block	Treatment	A total	B total
1	1	12	0
1	2	15	4
1	3	5	2
1	4	10	3
1	5	0	16
2	1	20	0
2	2	15	5
2	3	8	6
2	4	4	10
2	5	0	14
3	1	16	0
3	2	11	4
3	3	6	5
3	4	4	13
3	5	0	13
4	1	17	0
4	2	14	3
4	3	9	6
4	4	4	7
4	5	0	18
5	1	20	0
5	2	15	2
5	3	10	10
5	4	4	10
5	5	0	20
6	1	15	0
6	2	10	5
6	3	8	7
6	4	4	10
6	5	0	17
7	1	11	0
7	2	11	5
7	3	9	5
7	4	4	13
7	5	0	11

8	1	9	0
8	2	10	5
8	3	6	3
8	4	4	11
8	5	0	8
9	1	15	0
9	2	12	1
9	3	10	6
9	4	5	7
9	5	0	17
10	1	16	0
10	2	14	3
10	3	9	4
10	4	4	9
10	5	0	7

Table 6. The raw data for each container by block and treatment for *Phormia regina* and *Chrysomya rufifacies*. Species A is *P. regina*, and species B is *C. rufifacies*.

Block	Treatment	A total	B total
1	1	14	0
1	2	0	5
1	3	0	4
1	4	0	13
1	5	0	15
2	1	7	0
2	2	0	3
2	3	0	5
2	4	0	12
2	5	0	12
3	1	17	0
3	2	0	3
3	3	0	7
3	4	0	10
3	5	0	17
4	1	13	0
4	2	0	1
4	3	0	5
4	4	0	10
4	5	0	15
5	1	10	0
5	2	0	2
5	3	0	8
5	4	0	12
5	5	0	10
6	1	5	0
6	2	0	5
6	3	0	11
6	4	0	10
6	5	0	10
7	1	20	0
7	2	0	2
7	3	.	.
7	4	0	8
7	5	0	11
8	1	17	0

8	2	0	4
8	3	0	6
8	4	0	8
8	5	0	9
9	1	18	0
9	2	1	2
9	3	.	.
9	4	0	12
9	5	0	17
10	1	16	0
10	2	0	3
10	3	0	10
10	4	0	5
10	5	0	4

Table 7. The raw data for each container by block and treatment for *Lucilia sericata* and *Chrysomya rufifacies*. Species A is *L. sericata*, and species B is *C. rufifacies*.

Block	Treatment	A total	B total
1	1	18	0
1	2	10	2
1	3	5	1
1	4	3	9
1	5	0	12
2	1	10	0
2	2	5	1
2	3	7	7
2	4	1	11
2	5	0	13
3	1	1	0
3	2	1	1
3	3	4	7
3	4	1	11
3	5	0	13
4	1	15	0
4	2	8	5
4	3	2	6
4	4	1	11
4	5	0	9
5	1	1	0
5	2	9	5
5	3	5	6
5	4	1	11
5	5	0	6
6	1	10	0
6	2	4	5
6	3	1	6
6	4	3	3
6	5	0	10
7	1	12	0
7	2	3	6
7	3	0	9
7	4	0	14
7	5	0	15
8	1	12	0

8	2	4	1
8	3	.	.
8	4	1	12
8	5	0	17
9	1	12	0
9	2	7	3
9	3	0	9
9	4	3	5
9	5	0	13
10	1	0	0
10	2	0	2
10	3	3	5
10	4	3	4
10	5	0	13

Table 8. The RCC and RCCM (and adjusted RCCM) by block (replicate) for *P. regina* vs. *L. sericata.* The RCCMM are adjusted for any missing values which in this case was zero.

	Phormia to *Lucilia* (adj)		
Block	RCC	RCCM	RCCMM
1	3.33	18.33	18.33
2	0.93	2.96	2.96
3	0.98	2.47	2.47
4	1.59	5.05	5.05
5	1.00	4.70	4.70
6	1.30	3.41	3.41
7	1.80	3.46	3.46
8	1.78	3.34	3.34
9	1.89	8.85	8.85
10	0.98	2.25	2.25

Table 9. The RCC and RCCM (and adjusted RCCM) by block (replicate) for *L. sericata* vs. *C. rufifacies.* The RCCMM are adjusted for any missing values which in this case was zero.

L. sericata to *C. rufifacies* (adj)			
Blk	RCC	RCCM	RCCMM
1	3.33	5.11	5.11
2	1.30	4.32	4.32
3	7.43	15.31	15.31
4	0.20	0.68	0.68
5	5.00	10.24	10.24
6	0.17	3.43	3.43
7	.	0.21	.
8	.	2.24	.
9	.	2.79	.
10	.	.	.