Impact of field application of liquid mink manure on *Fannia canicularis* L. (Fannidae, Diptera) population in Cavendish, NL

by

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"Do what we can, summer will have its flies"

– Ralph Waldo Emerson, *Prudence*

ABSTRACT

The Cavendish mink fur producer was blamed by area residents for causing a severe annoyance of lesser housefly (*Fannia canicularis* L). To address the problem, the farm had installed a mechanized liquid mink manure production system in 2014 which significantly lowered the number of flies. The community's concern then shifted to the application of the resulting liquid manure to fertilize fields to grow grass (mink bedding). The aim of this project includes assessments of the attraction of *F. canicularis* to a liquid manure applied field. I explored two hypotheses: i) does liquid manure attract *F. canicularis* to the field, and ii) does liquid manure enable these flies to breed in the field? A demonstration strip plot in which fly populations were assessed using initially yellow (in 2015), and, thereafter in 2016 with yellow, blue and transparent sticky cards. SLAM traps were deployed throughout both sampling seasons. Soil samples were surveyed for evidence of *F. canicularis* breeding. Only 22 *F. canicularis* were trapped in sticky cards during 2015 and zero in 2016. In SLAM traps only two in 2015 with none captured in 2016. There was no evidence of breeding in the manure-treated field. This near absence in 2015 and complete absence in 2016 negated a need for statistical analyses or further assessments of *F. canicularis* activity using a more powerful experimental design. However, a de novo technique was introduced in chapter 3: stratification of strips via Autocorrelation function which permitted the development of a rigid statistical model to analyze the treatment effects on frequently captured flies during seven sampling periods in 2015. Results showed significant interaction effects among treatments and sampling periods for overall flies, Anthomyiidae and Muscidae. For Fannidae, the

interaction effect was not significant whereas treatment effects were. Overall fly numbers especially Anthomyiidae increased after liquid manure application, with declining abundance thereafter. However, in conclusion, liquid mink manure will be safe for field application neither will be an issue in breeding or attracting *F. canicularis* nor any other group of flies.

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Chapter 1. General Introduction

In Cavendish, Newfoundland and Labrador, for over 15 years occupants are known to have complained that their homes were covered in approximately tens of thousands of flies. They assumed that this phenomenon arose from the local fur (mink) farm (Viking Fur Inc.). Their concerns and suspicions were shared by other communities in which mink fur production has been occurring. These complaints motivated a fly monitoring study conducted by the Newfoundland and Labrador provincial government (Madore and Madore 2010). The significant conclusion of this study was that the lesser housefly, *Fannia canicularis* Linnaeus (Fannidae, Diptera) was by far the most dominant fly species present in these incidents. This nuisance fly and a Viking Fur's Cavendish mink farm's efforts to reduce the fly population became my main research focus. In this general introduction, I will be introducing the *F. canicularis* in terms of its taxonomy, morphology, distribution, abundance, adult habitats, larval habitats, and its growth and development. I will describe my study site, Viking Fur Inc. in Cavendish, NL. I will finish by highlighting the main questions and the organization of my thesis.

1.1 Taxonomy, Morphology, Distribution and Abundance

The *Fannia canicularis* belongs to the family Fannidae Townsend, 1935, which has six genera, five of which are found in North America (Chillcott 1960). One of the five genera, *Fannia*, has the most species and, therefore, the most commonly encountered genus in the family. Typical of the genus' wing venation: the first anal vein is shorter than the second and the second anal vein is significantly curved towards the first such that an extended imaginary line would cross over the

first vein before the wing margin (Figure 1.1 B) (Chillcott 1960, McAlpine et al. 1981, Marshall 2006). The genus presently has 285 species (Wang et al. 2007) with 51 *Fannia* species recorded in North America; 16 of which were collected in Labrador (Huckett 1965). One species *Fannia postica* Stein (Fanniadae, Diptera) from Huckett's (1965) list recorded for the island of Newfoundland. A second species, *F. canicularis* was identified from collections from mink farms on the island of Newfoundland (Madore and Madore 2010). In the absence of any concerted effort to collect *Fannia* on Newfoundland, it seems likely that there are more *Fannia* species present on the island.

The focus of my studies, *Fannia canicularis,* is commonly known as the lesser, or the little, housefly due to its frequent occurrence in houses. The majority of *F. canicularis*' distribution is in Eurasia and North America (Chillcott 1960, Wang et al. 2007). In these regions, this species is synanthropous and cosmopolitan like the common housefly, *Musca domestica* Linnaeus (Muscidae, Diptera) (Chillcott 1960, Steve 1960, Zhang et al. 2013). Although superficially similar to the common housefly, *F. canicularis* has a number of distinguishing characteristics. These flies are smaller and more slender than the common housefly (Figure 1.1 A). For the most part, their bodies are blackish in color with three or four obvious yellow stripes on the basal-lateral parts of the male abdomen and only on the basal parts of the female abdomen (Figure 1.1). Additionally, there are three brownish-black stripes on the thorax that are more prominent on female (James 1947, Steve 1960). Although difficult to observe, *F. canicularis* also has a straight fourth longitudinal wing vein and an open first posterior cell (Steve 1960). However, the narrow 'V' like appearance of *F. canicularis*' wings at rest (Steve 1960) makes it easier to identify in living specimens without a microscope.

A **B**

Figure 1.1 A: Side view of an adult *Fannia canicularis* (Linnaeus) male, captured from the experimental field; B: close up view of the wing venation and yellow stripes (in circle) on the abdomen. These photos were taken in Chapman lab using a Leica DFC420 917 digital microscope camera mounted on a Leica MZ95 compound microscope.

Fannia canicularis is spring and early summer pest (Mullens et al. 2001); Lewallen (1954) and Hall et al. (1972) noted their occurrence during spring and late fall. Steve (1960) also confirms in Massachusetts poultry farms that *F. canicularis* is very active in early spring, but, in contrast, maintains high numbers throughout the summer until mid-October. The difference in seasonal activity between these studies may be due to the regional variation and/or differences, variation in seasonal abundance among years. Overall, however, these studies suggest that *F. canicularis* have poor tolerance of hot temperatures and they are active throughout the normal growing season. They prefer temperatures below 28^0 C (Steve 1960), for this reason, their numbers may decline or sometimes seem to disappear in midsummer (Lewallen 1954).

Fannia canicularis usually overwinter as pupa two to three inches below the surface of the soil and less commonly, they may overwinter as larvae (Mellor 1919). They can also overwinter as adults in warm rooms Hansens (1963); Chilcott (1960) also reports overwintering adults by citing Wilhelmi (1920).

1.2 Adult Habits

Due to the *Fannia canicularis*'s synanthropic behaviour, they are commonly encountered in dwellings and farms. According to Hewitt (2011), of the total flies captured in homes, *F. canicularis* makes up 2-25 % and, if captured in upper-storey rooms of homes, *F. canicularis* makes up 100% of the sample. Uebel (1977) similarly reported that *F. canicularis* constitutes 1-50% of the total fly population in the average house. Lesser houseflies are annoying in houses, but it is on farms in which they are most abundant, likely due to the abundance of larval habitat (see section 1.3 below). Majority of lesser houseflies have been recorded in dairy farms (Ogden and Kilpatrick 1958, Landolt et al. 2015) and poultry farms (Lewallen 1954, Steve 1960, Fay et al. 1963, Hansens 1963, Hall et al. 1972, Legner et al. 1973, Uebel 1977, Axtell and Arends 1990, DuPonte and Larish 2003). Some researchers may have focused on poultry farms because the *F. canicularis* has been implicated in the transmission of Newcastle disease, which impacts poultry (Rogoff et al. 1975), although, there is some evidence that the *F. canicularis* is more attracted to poultry manure over other manure (Steve 1960). Some studies (Funder and Mourier 1965, Madore and Madore 2010, Prieto et al. 2018) including a Government report (Madore and Madore 2010) documented *F. canicularis* as a dominant fly species in mink farms (the focus of this thesis). Moreover, observation of some twitted pictures, the statement of the agitated

complainants and disturbed homeowners confirmed the abundance of *F. canicularis* near the mink farm at Cavendish, NL.

Male *Fannia canicularis* have a stereotypical behaviour in which they congregate in the center of a room, typically underneath a pendulous object like a hanging light, and fly in an erratic circular pattern sometimes called a dancing flight (Lewallen 1954, Chillcott 1960, Steve 1960, Anderson and Poorbaugh 1964, Hunter 1979). The swarming behavior occurs from sunrise to sunset at temperatures above 15^0C (Hunter 1979). Females swarm less (Hunter 1979) and are usually seen resting with a preference for vertical surfaces such as walls, doors and ceilings of rooms or barns (Lewallen 1954, Steve 1960). Resting females contain partially developed eggs (Hunter 1979).

1.3 Larval Habitats

Lesser housefly larvae have been found in a variety of decaying matter (James 1947, Chillcott 1960). Larvae have been found in decaying vegetables, iris buds, cabbage stalks, plums, tomatoes, peas, grass, corn, grass clippings, canola stalks, leaf mould, forest floor litter and decomposing onion (Chillcott 1960, Dindonis and Miller 1981) as well as decomposing animal material such as snails, locusts, fish meal applied to soil and high-protein food wastes and different types of excrement such as human, pig, chicken, horse, rabbit and even white-mouse dung (Chillcott 1960). Larvae have also been found inside the human body; it has been frequently recorded in the intestine as well as vesicular and aural myiasis (Chillcott 1960). Many studies conclude that fresh animal dung is the preferred media for larval development (Lewallen

1954, Mullens et al. 2001, DuPonte and Larish 2003, Landolt et al. 2015). It would seem to be a safe conclusion that any organically rich substrate is potentially larval habitat for this fly.

1.4 Growth and Development

Usually, the fly life cycle has four stages: egg, larva, pupa and adult. The total developmental time for *Fannia canicularis* from egg to adult is 18 to 29 days and 27 days from egg to egg (Steve 1960, Fay et al. 1963). The total developmental time broken down by each developmental stage is: eggs 1 to 2 days, larvae 8 to 10 days and pupae 9 to 10 days (Steve 1960).

The eggs (0.88-2 mm in length) of the *F. canicularis* are white, and oval with a narrower anterior end. The lateral and ventral aspects have longitudinal ridges that extend along the length of the egg (Steve 1960). Lewallen (1954) describes two wing-like processes extending laterally that allows the egg to float. The average time from laying to hatching is 24 to 36 hours (Fay et al. 1963).

The larvae are very distinct from all other fly larvae. They are dorso-ventrally flattened and, most distinctly, they bear fleshy processes or spine-like structures dorsally and laterally (Greene 1956, Steve 1960, DuPonte and Larish 2003). Larval stage have three instars (Lewallen 1954, Chillcott 1960, Steve 1960) that can be distinguished by relative size and by the number of openings of the posterior spiracles (the number of openings is equal to the number of instars). The first and second instars are translucent white, while the third instar is leathery and pale brown (Lewallen 1954). Very detail description of larval morphology is given by (Greene 1956), Hewitt (1912) and Lewallen (1954). The larval body consists of eleven segments in addition to

the head, which is small and retractile into the under part of the first segment (Greene 1956). Overall, larvae are 5 to 7 mm long and approximately 5 mm wide.

The *Fannia canicularis* usually leaves their very moist larval habitat to seek somewhat dryer places to pupate (Chillcott 1960). The pupal stage is described as unusual because it has the appearance of a quiescent larva that has become more robust and shortened, retracted the cephalic region, ceased feeding, and with the integument hardened and darkened (Steve 1960).

Very little is known about adult feeding. According to Chillcott (1960) and Bennett (2009) adults feed on honeydew and plant sap. Different studies used different materials for their rearing purpose, such as cellulose cotton pads soaked with a mixture of powdered whole milk, and extracted honey (Fay et al. 1963) and lump sugar and water (Fay et al. 1963). Separate 1: 1 solutions of molasses-water and of evaporated-milk/water (Steve 1960) or sugar water (20%) and protein-hydrolysate water solution (4%) (Lewallen 1954) are used.

1.5 Viking Fur Inc. as a Source of Flies

Viking Fur Inc. established in 2004 one of the major mink fur producers in Newfoundland & Labrador. The farm produces 80,000 mink pelts annually with a value at peak season as much as 100 dollars/pelt, worth between $1.6 million and $8 million in international markets. The farm employs more than 100 people each year with 15-30 full time workers resulting in about one million dollars per year in labour. However, residents and people who own cabins in the area were adamantly against the farm; they had complaints of foul smells and the fly population. “You won’t be able to sit out on your deck because of the flies,” owner of a cabin at Cavendish

told during an interview with a Telegram. Personal communication of local entomologist (Peggy Dixon, personal communication, January 2015) and previous researcher who found *Fannia canicularis*'s larvae in the mink feces (Kate Carson, personal communication, January 2015), it was confirmed that *Fannia canicularis* was the main culprit who was upsetting the occupants of the homes near the farm.

The farm has invested more than $10 million as of December 2014 to mitigate environmental impacts from mink farming. In 2014 Viking Fur Inc. installed mechanized feces removal and liquefying system costing $2.2 million which was, in part, a response to concerns voiced by residents surrounding the farm. This liquid manure production system mechanically moves all the mink feces from under the mink cages to a subterranean system of pipes. The feces is then liquefied, filtered and delivered to above ground reservoirs where this liquid is aged and stored until the resulting liquid manure is needed to fertilize fields (for the production of bedding for the mink).

The reduction of mink feces (preferred breeding media of lesser houseflies) from the barns has reduced the fly population as no fly complaint received from the residents since the system was implemented (communication with Viking Fur's staff). However, concern from angry residents quickly refocused on the application of this liquid mink manure to the field. The citizens have argued that this practice of liquid manure application would exacerbate the problem by attracting flies and creating fly breeding habitat in the applied field.

1.6 Thesis Questions

I have focused my research efforts on the flies associated with this mink farm. The focus of chapter 2: (i) Whether the application of liquid mink manure attract *Fannia canicularis* to a manure applied forage field, more precisely, investigating their abundance through frequently used traps in the surrounding area of manure applied field. (ii) Whether the application of liquid mink manure enhances any breeding habitat of *F. canicularis* to the forage field, more precisely, investigating the presence of any immature stages of *F. canicularis* in the soil treated with liquid manure and compare with the soil that was untreated. As the installation of liquid manure production system in 2014 has already reduced the fly problem in the area, I started with a very basic experimental design (Demonstration Strip Design) for preliminary observation of the presence of *F. canicularis* in a manure treated field. Most effective fly trapping techniques were utilized such as yellow sticky cards, sweep netting, SLAM trapping along with visual investigation. I also investigated potential bias in the adult trapping method (yellow sticky cards) initially used to address question one above described in chapter 2.

In addition, the experimental design utilized in chapter 2 normally precludes rigorous quantification; however, in chapter 3, I introduced an analytical approach that could conceivably increase the knowledge that could be extracted from such a simple non-replicated experimental design.

Chapter 2. Impact of Liquid Mink Manure Application on *Fannia canicularis*'s Behaviour

2.1 Introduction

Fannia canicularis L is a dominant fly species in mink farms (Funder and Mourier 1965, Madore and Madore 2010, Prieto et al. 2018). Females lay eggs in fresh mink feces and diverse array of organically rich materials especially manures; thus it is plausible that liquid mink manure and the solid mink compost attracts adult flies and is suitable as larval habitat (see section 1.3 for the range of larval habitats).

The liquid mink manure is produced from mink feces and urine, and it is filtered such that any hard particles (accretions of bone fragments, feather pieces, and fish scales that are in the mink diet) are removed. The liquid is then stored in tanks and matured by bubbling air through it. The resulting liquid manure (Appendix 2.1) contains ammonia and given that ammonia is a well-known attractant for *Fannia canicularis* (Steve 1960) I predicted that *F. canicularis* could be caught in areas of the field in which liquid manure was applied. However, it is unclear as to whether this attraction will result in more larvae being found in areas of the field in which liquid manure has been applied. Furthermore, the other type of solid mink compost was used in my experiment to compare the differences in captured fly numbers with liquid manure. Mink compost is made of mink carcasses (natural deaths and the end product of skinning) and mink feces both are combined with wood chips (as an additional carbon source) and composted

until carcases are completely broken down. Using a manure spreader, the compost has been applied in surrounding fields. Given the long history of compost application in the fields surrounding this farm, it would seem plausible that *F. canicularis* are both attracted to the fields and breeding there.

To investigate the *Fannia canicularis*'s attraction in the manure applied field an initial basic field experiment was attempted which is called a demonstration strip design (Ministry of Agriculture and Forestry Alberta 2002), or sometimes it is called a farm strip trial ("On-farm strip trials — tips" 2016). This design precludes formal analysis (although see chapter 3), but it may provide some evidence to the value of conducting a replicated experimental design (e.g. random block design) with its associated higher effort and cost. Given that the mink farm that motivated my thesis work had already put in place automated feces removal equipment in 2014 which have lowered the fly population on the farm, it seemed prudent to take a cautious first step with my research aims and establish if the *F. canicularis* is present in large numbers and potentially breeding in a field near the mink farm.

My strip plot had three long strips (Figure 2.3 D): one untreated, one had mink compost applied (as has been done in this region for decades) and one with liquid mink manure applied (which is novel for this area). To assess breeding in the field, I surveyed soil to look for immature stages of *F. canicularis* such as eggs, larvae and pupae in the soil or associated roots of grasses and clover in this experimental field. To assess adult attraction to these strips I used an array of yellow sticky cards attached to one metre-high stakes.

The yellow sticky trap is a standard technique for sampling flies (Black and Krafsur 1985, Hogsette et al. 1993, Burgess 2012). Flies belong to the genus *Fannia* were captured using

yellow sticky traps (Goulson et al. 1999). In a study by Steve (1960), considerable numbers of *Fannia canicularis* were collected from homes near some of Massachusetts poultry farms using sticky ribbons. Similarly, Madore and Madore (2010) caught *F. canicularis* from the mink barn using sticky ribbons and established that sticky ribbons are the best fly monitoring technique for the fur farms across the province of Newfoundland and Labrador. These previous research has proven that *F. canicularis* can be sampled sufficiently by using yellow sticky cards.

Moreover, multiple trapping techniques were used considering trap type biases to maximize precision. Malaise traps are made for active flying insects and have known very effective for collecting Diptera (Martin 1977). A modified malaise trap called SLAM trap was used to observe the *F. canicularis* population. In addition, to survey the surrounding area of the experimental field the sweep net was used.

2.2 Materials and Methods

2.2.1 Location and experimental setup

The Viking Fur Inc. mink farm is located near Cavendish, NL (47° 43' 24.1104" N 53° 29' 33.9828" W) which is approximately 100 km northwest of St. John's (Figure 2.2). The 14.29 acre field used for this study was 1 km northeast of the main mink farm facility (Figure 2.3) and has had mink compost applied to it to produce forage (grass and clover) for decades. A demonstration strip plot design was applied along the major axis of this field (Figure 2.3). The field is 1 to 15 m higher in elevation than the surrounding forest, fen and bog.

A team of six (including myself) conducted all fieldwork. To ensure consistent observational behavior within the team, written procedures were given in to all field assistants and all workers viewed demonstrations of those procedures in the field, and were assessed by myself.

The strip plot used in this study was 180 meters long approximately west to east and 60 meters wide (approximately north to south; Fig. 2.3, D). The plot was demarcated using 1 m tall surveyor stakes spray painted orange or pink with orange flagging tape attached to the tops of each stake. The total plot was divided into three strips using two internal rows of stakes producing three 20m wide strips. This width of strip allowed the tractor and compost and liquid manure application equipment to easily maneuver within the plot. To simplify the application of treatments, the north side strip of the plot was to be untreated, the middle strip was to have liquid

mink manure applied, and the south side strip was to be treated with mink compost (Figure 2.3, D).

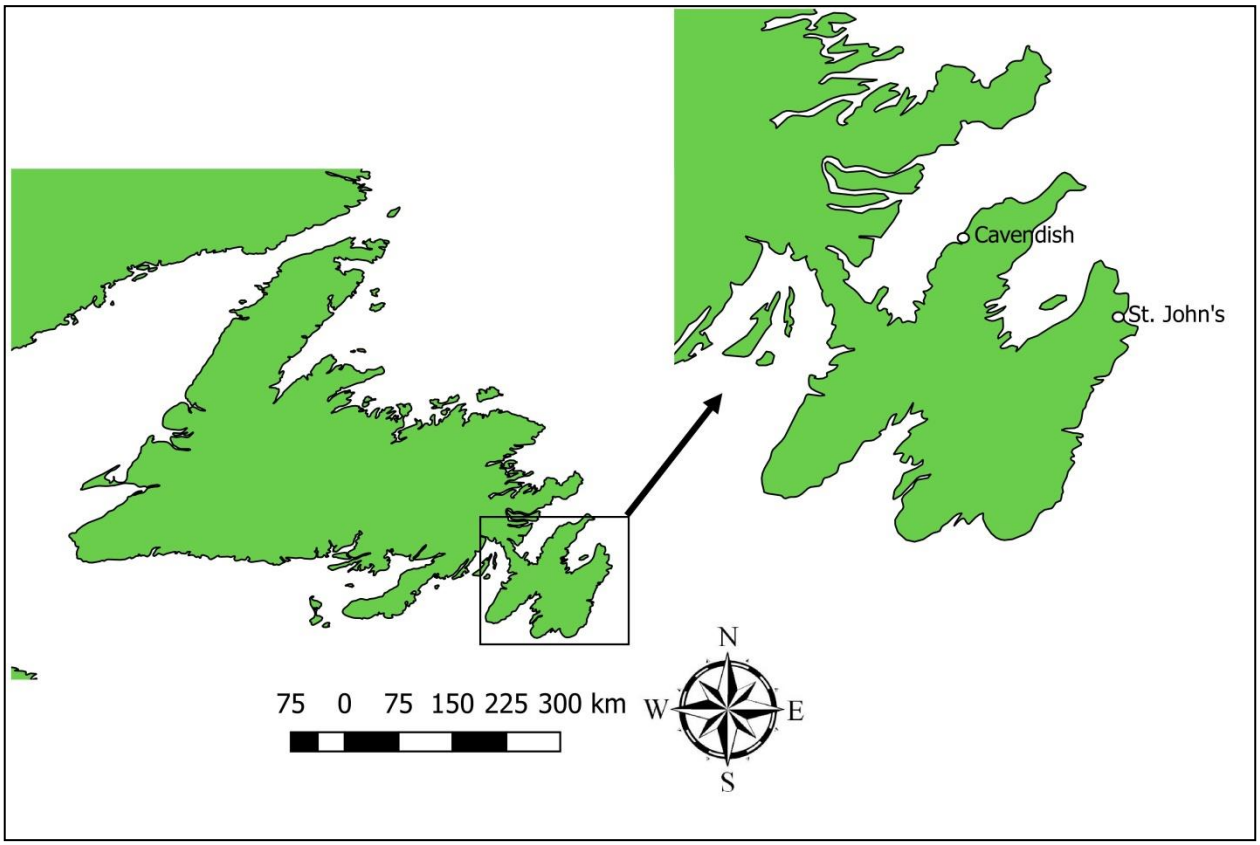

Figure 2.2: The Island of Newfoundland (left), and an expansion of the Avalon Peninsula to the right showing Cavendish in relation to the province of Newfoundland and Labrador's capital city, St. John's. Viking Fur Inc. is located immediately north of Cavendish and along Trinity Road South 80.

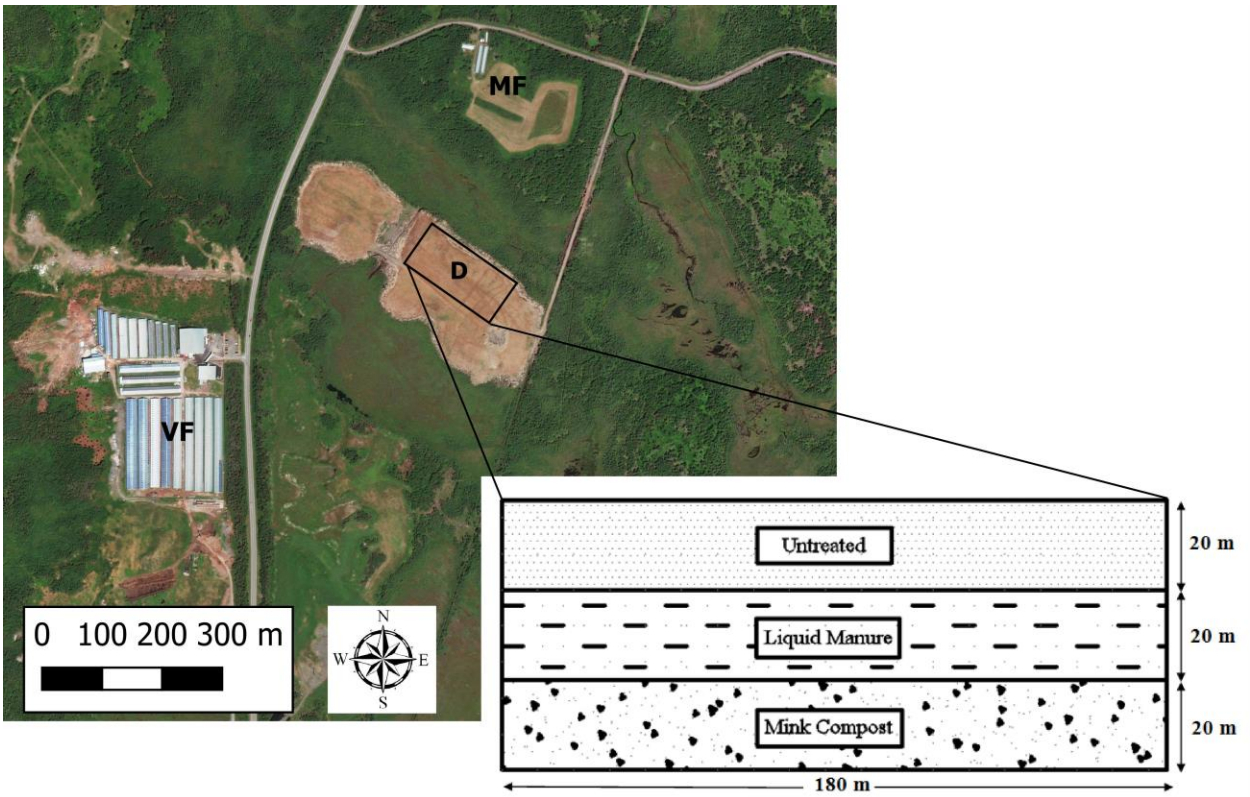

Figure 2.3: A satellite image (downloaded from Bing and edited in Arc GIS) of the Viking Fur Inc. facility and the study field. VF: indicates the main Viking Fur Inc. facility buildings (21 barns housing 15,000 female breeders). D: indicates the demonstration strip plot. The expansion shows the treatment strips: Untreated strip (UN), Liquid manure treated strip (LQ) and Mink compost treated strip (MC). Each of the strips is 180 meter in length, and 20 meter in width. MF: indicates another mink farm operating northern side of our experimental field.

2.2.2 Detection of adult *Fannia canicularis* to study plot

2.2.2.1 Treatment applications

Mink compost was applied to the research field on May 15, 2015 (Table 2.1) using a manure spreader towed behind a tractor. Viking Fur Inc. could not provide an exact application rate, but my observation suggests that compost is applied to an average depth of 1cm. The liquid manure was applied to the liquid manure treatment strip on May 21, 2015 (Table 2.1).

2.2.2.2 Trapping flies with yellow sticky cards (2015)

To trap flies, double-sided 25 cm in length by 10 cm width yellow sticky cards, were purchased from Natural Insect Control, Ontario, Canada. Yellow sticky cards have been shown to attract a variety of insects because of the attractive yellow colour; particularly, many studies have used yellow sticky cards to trap a wide variety of fly taxa (Black and Krafsur 1985, Parrella and Jones 1985, Sanderson et al. 1989, Hogsette et al. 1993, Lance and Gates 1994, Beresford and Sutcliffe 2006) especially *Fannia* spp (Goulson et al. 1999, Burgess 2012).

In the field, ten 1-m tall surveyor stakes were hammered 10 cm into the ground along the centerline for each strip in the plot (Figure 2.4). Yellow sticky cards were stapled near the top of each stake such that the card did not extend above the stake. Attaching the cards this way was necessary to protect them from the high winds that can periodically occur in this field, but consequently it meant that only one side of the card had its sticky surface exposed. All sticky cards had their sticky side facing to the west of the field.

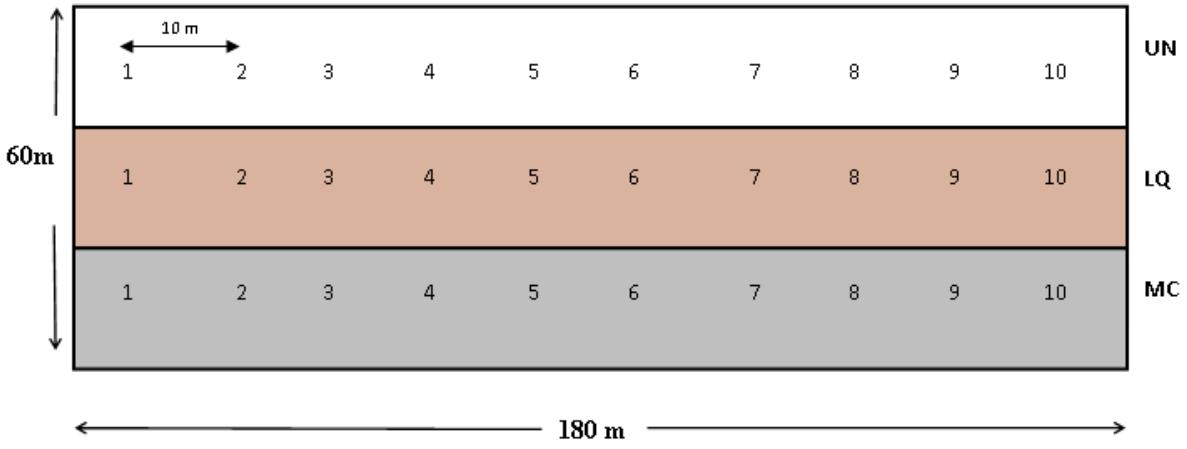

Figure 2. 4: The study plot with numbers indicating the positions of staked sticky cards. Stakes were placed equidistant from each other. The experimental treatments are shown: UN (Untreated), LQ (Liquid Manure), MC (Mink compost).

A full array of 30 cards was deployed seven times (P1 to P7) during the 2015 field season: May through to September (Table 2.1). Cards were left in the field for 7 to 15 days before being taken down from the stakes (Table 2.1). Wooden boards with two rows of nails enabled the cards to be placed on their long edge such that they would not come in contact with another card. The boards with cards were then placed in plastic bins with covers for transport back to Memorial University of Newfoundland.

Table 2.1: Date of total seven fly trapping/collection periods, using yellow sticky cards, during the 2015 sampling season (Cavendish, NL).

Collection periods	Cards setup (2015)	Cards collected (2015)	Duration (Days)
*P1	May, 14	May, 21	7
**P2	June, 03	June, 11	8
P3	June, 11	June, 25	14
P4	June, 25	July, 03	8
P5	July, 03	July, 17	11
P6	July, 17	July, 24	7
P7	Sep, 02	Sep, 17	15

* Mink compost was applied to the research field on May 15, 2015, the day after cards were deployed (P1)

** The liquid manure was applied on May 21, 2015, but after cards from P1 were removed from the field

2.2.2.3 Identification of collected specimens

2.2.2.3.1 Taxonomic Identification

Cards were examined under a Leica MZ95 dissecting microscope. Identification of flies (other insects were ignored) was aided by (Chillcott 1960, McAlpine et al. 1981, Marshall 2006). Three families were the focus: Fannidae, Muscidae and Anthomyiidae (family level numbers are presented and discussed in chapter 3). Specimens suspected to be *Fannia canicularis* were the only specimens that were identified to species; numbers were counted.

Most specimens of *Fannia canicularis* were easy to identify while they were still stuck to the card. However, some specimens required to be removed from the card to verify their identity. HISTO-CLLEAR II (National Diagnostics), a citrus oil derived solvent, was used to remove these specimens (Yeargan and Quate 1996, Liburd et al. 1998, Reng-Moss et al. 1998, Schaeffer et al. 2011). A drop of HISTO-CLLEAR II was applied to a fly, and with tweezers the fly was carefully lifted from the card. Most specimens that were removed from cards were placed in 70% alcohol in labeled vials that are stored at Memorial University of Newfoundland (room SN4113). And, some of these specimens were pinned as vouchers and are stored at Memorial University of Newfoundland (room SN4113). To confirm the identification, few samples from each species of Fannidae were sent to the Canadian National Collection of Insects (CNC); identification was confirmed by entomologist Dr. Bradley J. Sinclair.

2.2.2.3.2 Molecular Identification

Molecular techniques were used to confirm the taxonomic identification. I extracted DNA from a total of three adult lesser housefly specimens and photographed each specimen using a Leica DFC420 917 digital microscope camera mounted on a Leica MZ95 compound microscope. DNA was extracted using Qiagen DNA easy Tissue kit (DNA easy Tissue Kit, Qiagen, Valencia, CA). Body parts were broken with forceps and placed into a 1.5ml centrifuge tube containing 180 µL of ATL buffer and crushed well. 20 µL proteinase K was used for lysing tissue and 200µL of AE buffer was used for elution. All protocols followed those given in the Qiagen DNA easy kit manual. The quantity of the extracted DNA was checked using a Nanodrop 1000 spectrophotometer. Samples were stored at -20 °C until needed for the amplification.

PCR reactions were carried out with 15 µL total volume; 7.5 µL GoTaq Master Mix (promega), 0.6 µL of each primer (15 µL concentration of stocks), 1.0 µL of template DNA and 5.3 µL of DNAse free water. The mitochondrial Cytochrome Oxidase subunit 1 (COI) gene region were used as primers sequences following (Folmer et al. 1994); 710 base pair (bp) of LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3'). The PCR was carried out in an Eppendorf Authorized thermal cycler, Mastercycler EPGradients. PCR cycles were run at the following conditions: 47.50 C (45min), 720 C (1:02min), 920 C (30min). The quality of PCR product was checked by gel electrophoresis using Red Safe nucleic 953 acid staining solution (iNtRON Biotechnology Inc., Sungnam-Si, Gyunggi-Do).

PCR samples were cleaned up using the QIAquick PCR purification kit (Qiagen, Valencia,

CA) to remove excess primers. I modified the protocol as required by the DNA sequencing facility that substitutes DNAse free H_2O for the elution buffer. Sequences were obtained from the Center for Applied Genomics (TCAG) DNA sequencing Facility in Toronto, Ontario. FASTA files were made using BioEdit v. 7.2.5 by combining forward and reverse sequences. BLAST searches were made using the GenBank database to identify similar sequences. Sequences were deposited in GenBank; the accession number is pending.

2.2.2.4 Data analysis

The numbers of *Fannia canicularis* (the main focus of this chapter) including other frequently captured flies were counted and recorded (see 2.3.1 in results section). Numbers of *F. canicularis* was not enough to conduct a statistical analysis.

However, a new analytical approach (Post-hoc stratification of strips) was introduced to analyze the treatment effects on frequently captured flies which I presented in chapter 3. This approach describes how one could quantify the data using such a simple non-replicated experimental design.

2.2.3 SLAM trap and Sweep net sampling

Considering trapping biases alternative trapping techniques were used. Many studies have used SLAM traps to catch flies (Brown 2005, Skvarla et al. 2016). SLAM traps (The Sea, Land, and Air Malaise) were purchased from "Bug Dorm cages/stores" online store (MegaView Science Co., Ltd). The self-supporting polyester SLAM trap is 110 cm in height with four wings (each one 110 cm in length). A SLAM trap is a multi-directional malaise trap that does not attract insects like the yellow cards might. It is designed to use the natural tendencies of insects to fly over objects in their flight path. SLAM traps were deployed to examine local Dipteran diversity in the surrounding area of the experimental field.

During 2015 sampling, one trap was placed at the North-east edge of the experimental field closest to the untreated strip. This side of the field has an earth and stone berm (about 2 m high) that could act to funnel low flying insects along the field until they encountered a wing of the SLAM trap. During 2016 two traps were deployed; one on the same location as 2015 and the other one was placed at the South-west edge of the experimental field. With each visit to the field during 2015 and 2016 (Table 2.1) the SLAM trap(s) was/were inspected and captured insects were transferred to vials containing 70% alcohol and labeled. Samples were later inspected using a Leica MZ95 dissecting microscope for the presence of *Fannia canicularis*.

In addition, sweep net was used to investigate the presence of *F. canicularis* in surrounding area of the experimental field. Sweep net samples were brought back to the Memorial University of Newfoundland and looked for the *F. canicularis*. Except for *F. canicularis*, the other captured flies were not recorded.

2.2.4 Assessment of trap bias of yellow sticky cards (2016)

In 2016, an experiment was conducted to test whether there is different colour preference of *F. canicularis* which might be the reason of these flies were not efficiently attracting in yellow colour card. To test the effect of card colour, yellow (same as in 2015), blue (same size and vendor as the yellow cards) and transparent (made from clear polypropylene cut to the same size as yellow and blue cards and painted on both sides with Tangle-Trap sticky coating) (Contech, USA) cards were deployed in the same plot as the 2015 experiment (Figure 2.3). However, the field was treated homogenously with compost and liquid manure in 2016 meaning that the treatment strips were no longer meaningful. In each of the 30 collection locations (Figure 2.4) the three colour cards were suspended lengthwise between two wooden stakes. The position (top, middle, and bottom) of each colour card was chosen randomly. By being suspended between two stakes, both sides of the cards were used to trap flies. However, for yellow and blue cards, one side left unmodified, for the other side a clear polypropylene card with Tangle Trap was applied chosen at random. This was done to allow a comparison of the stickiness (how well flies are held) of factory-manufactured cards (unmodified side) to that of a similar coloured card with a Tangle-Trap coating. The direction of each card facing was random. Tangle-Trap coating was done in the field after the cards were mounted to stakes. This array of sticky cards was set up on August 10, 2016 and the cards were collected and brought back to Memorial University on August 17, 2016. Flies were identified, and recorded.

2.2.4.1 Data analysis

No *Fannia canicularis* was caught on any of the sticky cards during 2016, but other species of *Fannia* were caught. The ANOVA (Negative Binomial with Identity link) was conducted to examine i) card colour preference of *Fannia* spp., ii) the effect of direction of the card facing, and iii) the difference between the stickiness of commercially bought cards (blue and yellow) and the tangle trap coated clear cards.

No. of *Fannia* spp. = $\beta_o + \beta_C . X_C + \beta_S . X_S + \beta_D . X_D +$ error

In this model, C indicates the color of cards (Three categories: blue, yellow and transparent), S is stickiness (Five categories: blue-original, blue-Tanglefoot, yellow-original, yellow-Tanglefoot, and transparent-Tanglefoot), and D is direction. Homogeneity and normality were checked with deviance residual versus fit plot and normal probability plot (Q-Q plot). The residuals were homogenous and normal.

2.2.5 Examination of *Fannia canicularis*'s breeding

Field vegetation and the underlying soil were sampled from the experimental plot throughout the 2015 field season. Twelve sets of samples were taken over the 2015 field season (see Appendix 2.2 for sampling dates). For each soil-sampling day, a set of thirty samples was taken (ten per treatment strip). The location of each sample within a strip was determined randomly by producing three sets of ten pairs of random numbers. An example of random coordinates generated for one day of sampling is given in Table 2.2. Always starting from the west end of the plot, sampling locations were identified by using a measuring tape.

Soil samples were taken with a 20 cm^2 spade which was dug 15 cm into the soil. Each plug of plants and underlying soil was deposited into a large shallow white tray. The samples were then visually inspected for eggs, larvae and pupae.

Two different methods for searching soil samples were tested in the field and rejected: sieving and floating (Martin 1977). Sieving the soil was thought to be too rough and could possibly destroy delicate specimens, and the difficulty of getting water in the experimental field was too time consuming and laborious. Instead, the sample was broken by hand and spread out on to a plastic tray (1.2 x 1.5 m). Each sample was inspected for 20 minutes. Any eggs, larvae or pupae were removed with flexible forceps and deposited into labeled vials containing 70% alcohol. Putative specimens of immature fly stages were inspected, in the lab at Memorial University of Newfoundland using a dissecting microscope and taxonomic keys (James 1947, Marshall 2006)

Table 2.2: A single set of randomly produced soil sampling coordinates (length and width measurements in metres). Starting from the west end of the study plot, a field measuring tape was used to locate each sampling location or "Spot".

Untreated strip			**Liquid Manure treated strip**		**Mink Compost treated strip**	
Spot	**Length(m)**	**Width (m)**	**Length (m)**	**Width (m)**	**Length (m)**	**Width (m)**
1	10	18	21	1	12	10
2	25	14	50	7	18	4
3	37	14	57	8	22	3
4	44	9	68	11	28	6
5	54	5	91	12	32	16
6	64	10	100	14	67	18
7	87	10	140	16	105	12
8	108	13	141	17	132	8
9	144	19	144	17	145	9
10	152	11	160	18	152	5

2.3 Results

2.3.1 Captured adult flies from yellow sticky cards (2015)

In total, 2531 calyptrate flies were identified on 210 yellow cards deployed over the 2015 field season. Of the three families targeted, there were 1740 Anthomyiidae, 658 Muscidae and 133 Fannidae. Among 133 Fannidae only 22 (0.86%) specimens were identified as lesser houseflies (*Fannia canicularis*): four flies were captured in the liquid manure treated strip, nine in the mink compost treated strip and nine in the untreated strip.

2.3.2 Molecular identification results

Three specimens that were morphologically identified as *Fannia canicularis* were used to provide molecular confirmation of species identification. A partial COI sequence (650-700bp) for all three specimens produced a 99-100% match with at least one *F. canicularis* sequence in the GeneBank data base; search results are given in Table 2.3.

Table 2.3: The table listing voucher codes of morphologically identified *Fannia canicularis* collected from the mink farm and BLAST search results of their sequences (accession numbers, E-score, Query and Identity of best matching sequences obtained from the Gen Bank database).

Specimens Voucher Codes	Species Name	Base pairs	Best match Accession #	E-Scores	Query	Identity
1B	*Fannia canicularis*	696	HQ979161.1	0	94%	99%
			JX438029.1	0	94%	99%
			KC617820.1	0	94%	99%
			HQ979164.1	0	94%	99%
3B	*Fannia canicularis*	684	HQ979161.1	0	96%	99%
			JX438029.1	0	96%	99%
			KC617820.1	0	96%	99%
			HQ979164.1	0	96%	99%
4B	*Fannia canicularis*	687	HQ979164.1	0	95%	100%
			JQ070056.1	0	91%	100%

2.3.3 SLAM trap and sweep net collection throughout 2015 & 2016

Two *Fannia canicularis* were caught in the SLAM trap over the entire field-season of 2015: one caught sometime between May 22 and June 03, 2015, and the other was caught sometime between June 11 and June 25, 2015.

During 2016 no *F. canicularis* was found in any of the SLAM traps collection. In the sweep net samples, no *F. canicularis* was found.

2.3.4 Trap colour evaluation (2016)

In 2016, no *Fannia canicularis* were caught on any of the sticky cards. However, 84 other *Fannia* species were caught on sticky cards. Results show a significant preference for the yellow colour sticky cards (Wald Chi-square= 53.13, df =1, P <0.0001). The mean number of *Fannia* spp. was 0.73 (se=0.17) in yellow, 0.44 (se=0.13) in blue, and 0.22 (se=0.101) in clear cards. No significant difference in the number of *Fannia* trapped on commercially bought cards (blue and yellow) and the tangle trap coated clear cards (Wald Chi-square= 0.511, df =2, P=0.774). The mean number of *Fannia* spp. was 0.44 at the blue-original card, 0.53 at the blue-tangle foot coated card, 0.30 at the yellow-original, 0.53 at the yellow-tangle foot coated card, and 0.53 at the transparent tangle foot coated card.

The direction of the sticky side of the card was facing had no effect on the number of *Fannia* spp. trapped (Wald Chi-square= 2.000, df =1, P=0.157). The cards those were facing East side had 0.35 flies (Mean), and the cards facing West side had 0.58 flies of the genus *Fannia*.

2.3.5 Soil sampling results for the investigation of the *Fannia canicularis*'s breeding

A total of 44 different insect larvae and three pupae were found among 360 soil samples taken during the 2015 field season: 11 larvae were found in untreated strip, 12 larvae and two pupae were found in liquid manure strip, and there were 21 larvae and one pupa found in mink compost treated strip (see Appendix 2.2 for identified specimens). Among them 23 were identified as Dipterans (20 larvae and three pupae) in either the families of Anthomyiidae, Calliphoridae, Sarcophagidae, Muscidae, and Tipulidae. No larvae of *Fannia canicularis*, a very easy morphology to identify, were found.

2.4 Discussion

In the four months of the 2015 field season, seven trapping sessions with a combined total of 210 sticky cards were deployed for 70 days or 1680 hours and resulted in only 22 *Fannia canicularis* captured. In 2015 I also deployed a SLAM trap at the edge of my experimental plot; the intention was to assess the abundance of *F. canicularis* in the surrounding area of the experimental field. Over the same period that the sticky cards were deployed two *F. canicularis* were captured in the SLAM trap. Therefore, throughout the general field observation while conducting the fieldwork no *F. canicularis* was recorded. Even no *F. canicularis* was captured throughout sweep net sampling. All these results suggest that *F. canicularis* were in low abundance during 2015.

Had replicate strips been set up in this field as well as in nearby fields for 2015, it is hard to imagine that sufficient numbers of *F. canicularis* would have been captured to enable a rigorous statistical examination. These low numbers provided little motivation to expand this initial strip plot assessment to a more powerful replicated design.

Although there was not enough study on the trap color preference of this specific species *Fannia canicularis*, sticky traps have been very effective to capture this species (Steve 1960, Madore and Madore 2010). SLAM traps are also has been frequently used to catch flies (Brown 2005, Skvarla et al. 2016). However, I did consider investigating the effectivity of the sticky trap sampling method: experimenting the trap bias of yellow sticky cards in the following season (2016). A more direct assessment of the yellow cards' utility for trapping *F. canicularis* was

conducted within the same study plot as the 2015 field season. In this experiment, I attempted to compare the effectiveness of sticky cards for three conditions: blue coloured, yellow coloured and colourless cards. As well, for this field season, a second SLAM trap was purchased, and both traps were deployed at two opposite edges of the field plot. For this trapping session, I did not capture any *F. canicularis* on any sticky cards or in any SLAM traps, which is consistent with the low numbers found in 2015. However, there were sufficient numbers of other species of *Fannia* were captured during 2016 to permit an analysis of the effectiveness of colour in attracting members of this genus. Assuming that conclusions for the genus in general provide insights into *F. canicularis* behaviour the results showed that *Fannia* spp. were significantly more often captured on yellow compared to blue or colourless cards. A study by (Goulson et al. 1999) also captured flies from this genus *Fannia* using yellow sticky traps. This result suggests that the low numbers of *F. canicularis* captured in the 2015 season was simply due to their low abundance.

The low abundance of *Fannia canicularis* might be due to the consequence of implementing the liquid manure system in 2014; which removes all build up excrement from under mink cages using mechanical scrapers. Mink faeces are the preferred breeding media of *F. canicularis* Steve (1960); Coffey (1966) reared significant numbers of *F. canicularis* from mink dung. Inspections of fist-sized samples of mink feces from within the barns of Viking Fur Inc. revealed many dozens of *F. canicularis* larvae per sample (communication with Kate Bassett in 2014). For this reason, this species has been recorded as a dominant fly species in many mink farms (Funder and Mourier 1965, Madore and Madore 2010, Prieto et al. 2018). Although, the instalment of this system at Viking Fur Inc. in 2014 minimized the fly nuisance

issues, as not much complaint received from Cavendish residents during that year, the outcome of this study is further confirmed that application of resulting manure will not attract any *F. canicularis*, unlike mink feces.

For breeding *Fannia canicularis* prefer fairly moist feces (Mullens et al. 2001, DuPonte and Larish 2003, Burgess 2012, Ward and Lachance 2015). In addition, Anderson and Poorbaugh (1964) suggest that 35 to 40% moisture provides the best conditions. Lewallen (1954) and Steve (1960) reported that *F. canicularis* may survive moisture levels up to 65%. However, liquid mink manure is 96.6% water and (Appendix 2.1) is, as a consequence unlikely to be an appropriate medium for larval development. However, when applied to a field, the liquid manure would both increase the soil's moisture and nutrient levels, with the possibility of becoming conducive to *F. canicularis* development and, in fact, their larvae have been observed (in small numbers) in the soil of crop fields in Newfoundland and Labrador (communication with Peggy Dixon 2015). The effort that was conducted here, to identify immature stages of *F. canicularis* in the experimental field, did not result in any evidence of breeding in any of the treatment strips. Fly larvae of unidentified species were found, but in very low numbers. It appears that the long-term application of mink compost (solid manure) does not produce a medium that is conducive to fly breeding nor does a single application of liquid mink manure. However, the impact on fly populations with the long-term use of liquid manure is a question that cannot be answered with the field study that I described here.

2.5 Conclusion

Given the low number of *Fannia canicularis* trapped in 2015 (and the complete absence in a single trapping session in 2016), there is little motivation to conduct a further assessment of the relationship between liquid manure and the lesser housefly using a more powerful experimental design. In the short term, it appears that the application of liquid mink manure to the forage field will not positively increase the *F. canicularis* population. It seems reasonable to suggest that this relationship would hold in other parts of our province. However, there is a caveat: this study cannot infer what the long term impact of liquid manure application on the lesser housefly population might be.

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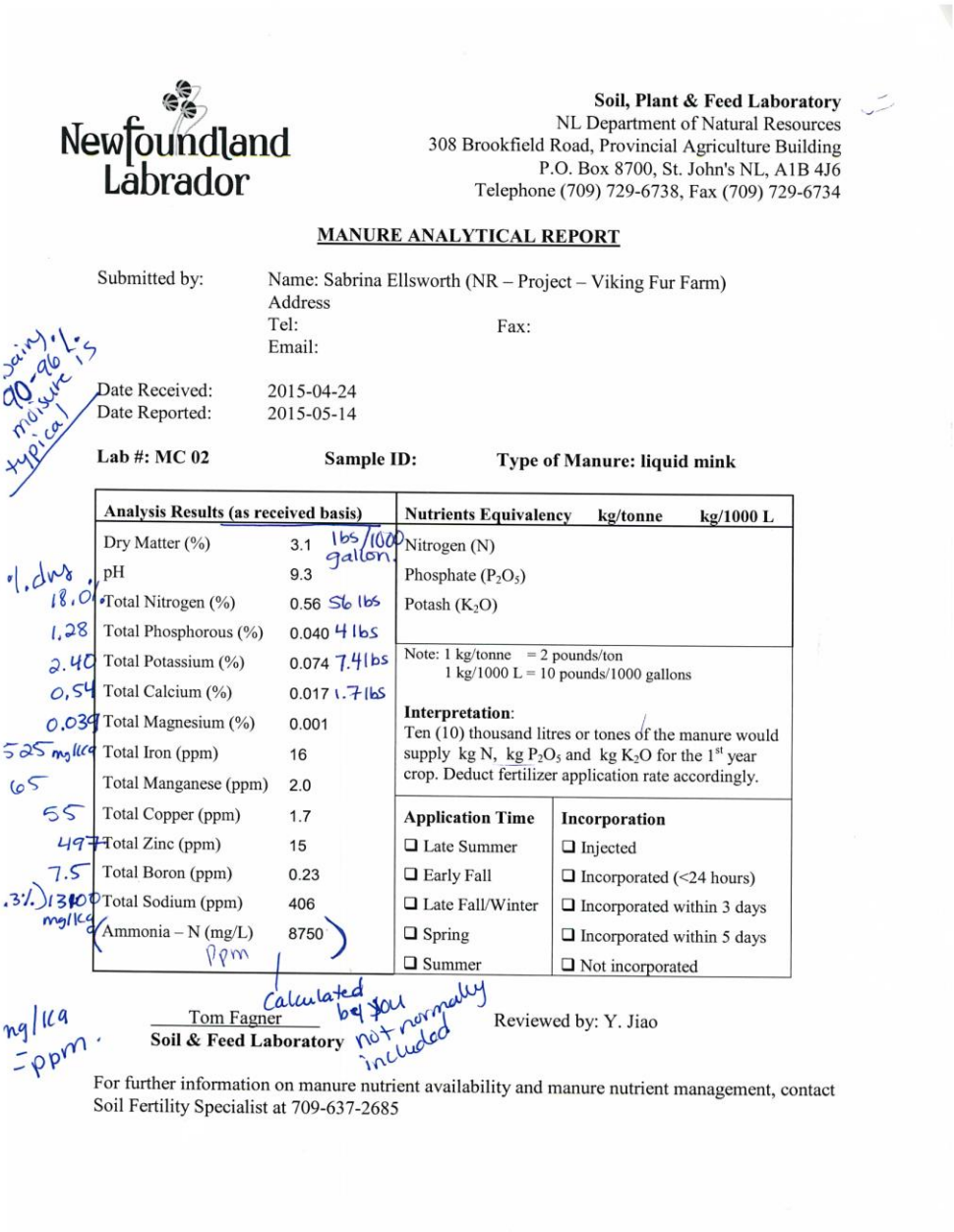
2.7 Appendices

Appendix 2. 1: Analytical report of liquid mink manure.



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MANURE ANALYTICAL REPORT

Submitted by: Name: Sabrina Ellsworth (NR – Project – Viking Fur Farm)
Address
Tel: Fax:
Email:

Date Received: 2015-04-24
Date Reported: 2015-05-14

Lab #: MC 02 **Sample ID:** **Type of Manure: liquid mink**

Analysis Results (as received basis)	
Dry Matter (%)	3.1
pH	9.3
Total Nitrogen (%)	0.56
Total Phosphorous (%)	0.040
Total Potassium (%)	0.074
Total Calcium (%)	0.017
Total Magnesium (%)	0.001
Total Iron (ppm)	16
Total Manganese (ppm)	2.0
Total Copper (ppm)	1.7
Total Zinc (ppm)	15
Total Boron (ppm)	0.23
Total Sodium (ppm)	406
Ammonia – N (mg/L)	8750

Nutrients Equivalency	kg/tonne	kg/1000 L
Nitrogen (N)		
Phosphate (P_2O_5)		
Potash (K_2O)		

Note: 1 kg/tonne = 2 pounds/ton
1 kg/1000 L = 10 pounds/1000 gallons

Interpretation:
Ten (10) thousand litres or tones of the manure would supply kg N, kg P_2O_5 and kg K_2O for the 1st year crop. Deduct fertilizer application rate accordingly.

Application Time	Incorporation
❑ Late Summer	❑ Injected
❑ Early Fall	❑ Incorporated (<24 hours)
❑ Late Fall/Winter	❑ Incorporated within 3 days
❑ Spring	❑ Incorporated within 5 days
❑ Summer	❑ Not incorporated

Tom Fagner
Soil & Feed Laboratory

Reviewed by: Y. Jiao

For further information on manure nutrient availability and manure nutrient management, contact Soil Fertility Specialist at 709-637-2685

Appendix 2. 2: The table listed all specimens collected during soil survey from three experimental strips; those belonging to the order Diptera identified up to the family.

Sampling days	**Untreated strip**	**Liquid manure treated strip**	**Mink compost treated strip**
May, 14	Lepidoptera Wasp larvae	Sarcophagidae (Diptera) Tipulidae (Diptera)	Anthomyiidae (Diptera) Coleoptera (2)
May, 22	Anthomyiidae (Diptera) Coleoptera Tipulidae (Diptera)	Coleoptera (2) Muscidae (Diptera)	Coleoptera Lepidoptera (2) Tipulidae (Diptera)
June, 03	Anthomyiidae (Diptera) (2) Lepidoptera	Anthomyiidae (Diptera) Lepidopteran Coleoptera	Calliphoridae (Diptera) (3) Coleoptera
June, 11	0	Muscidae pupae (1) (Diptera)	Muscidae pupae (1) (Diptera)
June, 25	Coleoptera	Wasp larvae	Coleoptera Sarcophagidae (Diptera)
July, 03	0	Coleoptera	Coleoptera
July, 08	0	0	0
July, 17	Anthomyiidae (Diptera) Lepidoptera	0	Anthomyiidae (Diptera) Calliphoridae (Diptera) Coleoptera Muscidae pupae (Diptera)
July, 24	0	Anthomyiidae (Diptera)	Anthomyiidae (Diptera)
August, 03	0	Coleoptera	Wasp larvae Lepidoptera
August, 12	0	0	0
September, 02	0	Muscidae pupae	0

Appendix 2. 3: Protocol for surveying Egg, larva, pupa or any immature stages of *Fannia canicularis*

Date:

Though not the primary focus of the survey, any larvae, eggs or pupa are routinely found, should be recorded & collected for further investigation under the microscope.

Groups: Each group consisting of 2 persons should work for 1 spot. 1 person can work as a digger and another can work as a sifter.

Materials needed for each person:

Digger: Measuring tape, stakes with flagging tape, spade/shovel (20 cm^2), and gloves.
Sifter: White plastic Tray (1.2 x 1.5 m), knife, sieve, forceps/ tweezers, magnifying glass, labeled vials, 70% alcohol, datasheet, pencils, and gloves.

Responsibility for each person:

Digger: Digger will be responsible for measuring and locating the exact spot following the random numbers provided. Measure the length from the west end of a strip, and the width from the south end of that strip. Mark each spot with a flagging tape attached stake. Before digging visually search the location about 2-3 minutes for the presence of any insects might sit on the surface. Be sure not to overlook any kind of insects present on the soil surface; record the observations. Dig the soil 15 cm deep using the spade and then deposit into the white plastic tray.

Sifter: Break and spread the soil sample into the white plastic tray, separate all the messy parts carefully. Use knife, trowel, sieve or tweezers depending on the type of soil/habitat. Examine carefully logs, plant stems, roots or any vegetative parts with a magnifying glass. Sort out egg, larva, and pupa with a flexible tweezer.

Preserve specimens in a small labeled vial containing 70% alcohol. Use a separate vial for each kind of specimen, and record the material in which you found the larvae. The time limit for each inspection is 20 minutes. Record all the information on the datasheet such as vial number, date and locality, habitat, color, and peculiarities.

Chapter 3. A non-replicated experimental design to address the fly problem in a mink farm: Post-hoc blocking using ACF

3.1 Introduction

The impact of the liquid mink manure application on the lesser housefly (*Fannia canicularis*) population have discussed in chapter 2 which is the main focus of this study. Nevertheless, high numbers of other flies belong to the section calyptratae were caught in yellow sticky cards used to trap *F. canicularis* during 2015's sampling season. Generally, some species of flies in the section calyptratae are highly nuisance and their association are found often with livestock farms as the organic matter provides suitable breeding media for some species (Goulson et al. 2005, Yong et al. 2009). The presence of calyptrate flies in the manure applied experimental field could be a major cause for concern as there might be an association of these flies with liquid manure. Some waste feeding flies e.g. Calliphoridae (Colyer and Hammond 1968) were observed on yellow cards but their number was insufficient for formal analysis, so they were not recorded. I recorded the number of frequently captured calyptrate flies with an intention to analyze the impact of liquid manure application on the general fly population. However, the challenge of analysing the fly data was the absence of replication in the demonstration strip plot. As discussed in chapter 2, a cautious simplest step was chosen to initially monitor the *F.canicularis* attraction to the manure applied field. Generally, this kind of initial approach is sometimes undertaken in agricultural research before implementing a fully replicated experimental design. For example, growing side by side in the field as strips; new crop cultivar

growth can informally be compared (visually). However, replication is a conventional practice in science. Replication enables to separate the true treatment effects from the background noise by controlling experimental error at the scale of strips. So, the experiment without replication usually precludes formal classical statistical analysis, e.g. analysis of variance (Hargrove and Pickering 1992, Scheiner and Gurevitch 2001). Nevertheless, it is not uncommon to find studies that involve no replication; some experiments especially those operating on large scales, challenge replication (Schindler 1987, Frost et al. 1988, Carpenter et al. 1995)**.** For example, examining the consequences of lake acidification on rotifer populations, where acidification of a single lake is a major undertaking, and it may be difficult or sometimes impossible to acidify more than one lake. Such a study would have no replication, and hence sometimes non-replicated experiment is necessary (Scheiner and Gurevitch 2001). In this kind of non-replicated experiment, alternative approach is suggested by Scheiner and Gurevitch (2001) which is making models of the system of interest that allows for replication. One example of alternative approach is getting time series data which is commonly used in different studies where replication is not possible; it involves comparing a series of pre and post treatment measurement on a treatment and a reference system (Stewart-Oaten et al. 1986, Carpenter 1989). Time series are repeated measurements, taken on the same experimental unit through time. In the case of lake acidification instead of acidifying more than one lake, getting a long time series of pre- and posttreatment data from a single acid-treated lake may be feasible. Some studies (Frost et al. 1988, Carpenter et al. 1998) argued that the test of this hypothesis can answer only the question whether a change occurred at the time of the treatment instead of resolving the issue of whether

the change is due to the treatment rather than some other coincidental event. Ultimately, the hypothesis tested here is weaker than the classical hypothesis of no treatment effect.

In a replicated experiment, the experimental units are independent of one another which simplify the statistical models; on the other hand, measurements in time series are usually dependent or auto correlated. Therefore, without replications it is hard to make sure that there is adequate understanding of the experimental error against which treatment effects should be judged.

On this account, I examined the autocorrelation among ten observations (trapped fly numbers) in each strip of my initial demonstration strip plot (see section 2.2.1 in chapter 2) to examine whether observations are independent so that homogenous blocks could be made accordingly. To be effective, blocks need to have a high degree of correlation of observations within blocks, with little or no correlation of observations across blocks. In other words, relatively high variance among blocks, and compared to spatial variance within blocks. Blocking (Post-hoc stratification) that captures spatial patterning allows treatment effects (fixed effects) to be separated from strip effects (random effects). Here the variance partitions into: treatment effect, undesirable strip effect (Block), and the experimental error rather than treatment effect and error; reducing the error variance helps to achieve the integrity and reliability of experimental results. Utilizing this blocking (Post-hoc stratification) approach, I explore the impact of liquid manure application on the fly population and I discuss how I identified appropriate models for the total fly data and each family considering different error structure.

3.2 Materials and Methods

3.2.1 Stratification of strips on the basis of ACF (Post-hoc blocking)

The treatments were completely confounded with strips in the demonstration strip plot which preclude statistical isolation of treatment effects from strip effects. To separate the strip effects from the treatment effects blocking of strips was attempted. To identify homogenous blocks in the strip plot, autocorrelation among ten observations (trapped fly numbers) in each strip was checked using Autocorrelation function (ACF) in SPSS software. ACF was conducted using total adult flies (2531) data collected from yellow sticky cards during 2015, and afterwards separately with three frequently captured fly's families: Anthomyiidae (1740), Muscidae (658), and Fannidae (133). ACF plots are given in Appendix 3.1 A to 3.4 C.

Outputs of ACF showed that observations in a strip were correlated in few lags and then separated through 'zero crossing' (the separation at which autocorrelation value drops to zero and two observations become uncorrelated) at certain lag and became uncorrelated (see ACF plots in Appendix 3.1 A to 3.4 C). Each set of highly correlated observations' lags uncorrelated from neighbouring set of correlated observations' lags by the zero crossing indicates that independent blocks can be made. Although the positon was different, the presence of zero crossing was detected for all the strips with majority of the first zero crossing was occurred at or greater than two trap separations (see Tables 3.1 to 3.12 listing first zero crossing). Therefore, blocking is orthogonal to strips, based on autocorrelation within strips it is assumed that the scale of autocorrelation within strips is similar to that among adjacent strips. Thus, each block was

made by making a group of two adjacent trap locations within each of the three strips (Figure 3.1).

Hence, three strips were assigned to five blocks each contained six trap locations. Each of the five blocks had all three treated strips. Block was used as a random factor to reduce the error variance and to allow a more sensitive test of treatment effects, controlled for block variance.

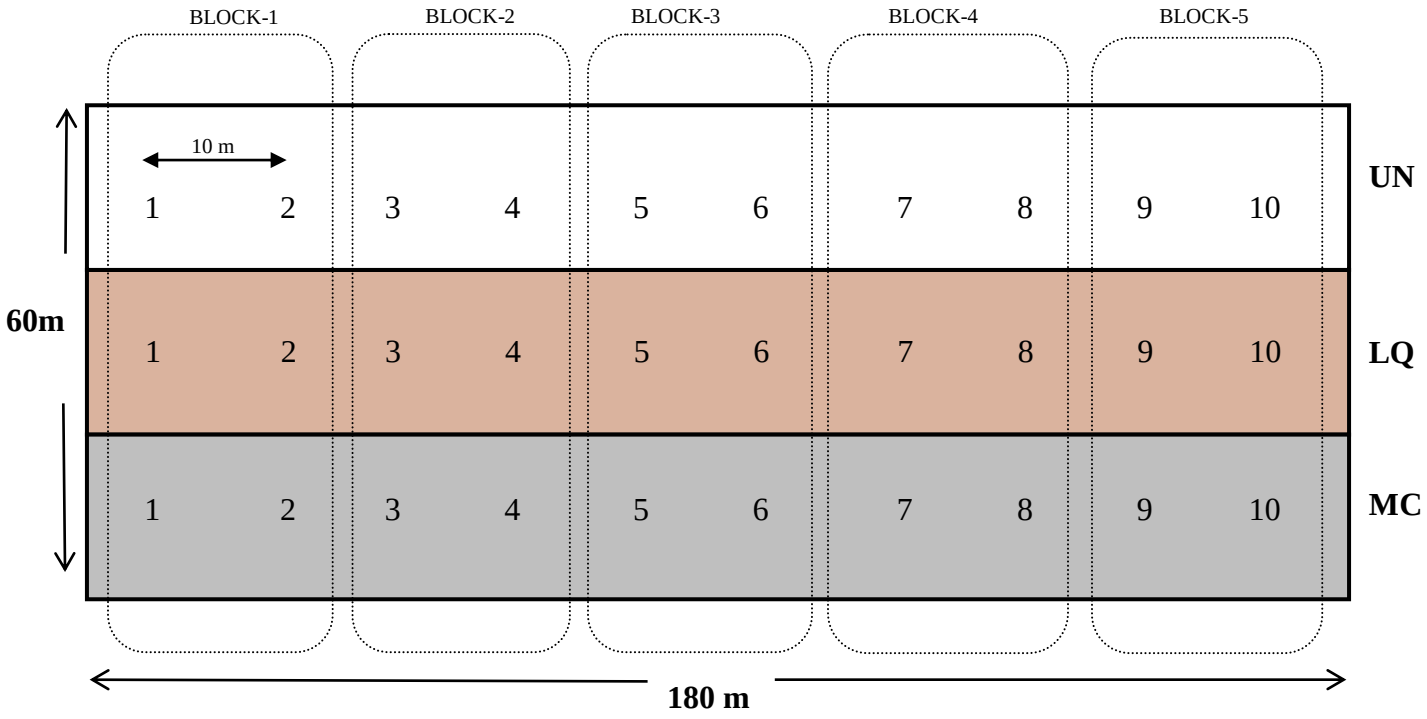

Figure 3. 1: Stratification of three strips (Untreated-UN, Liquid manure treated strip-LQ, Mink compost treated strip-MC) into five blocks (Block 1-5). Numbers 1-10 indicates ten trap locations in each strip, 10 m apart. One block is made of two adjacent trap locations in three strips of demonstration strip plot.

3.2.2 Statistical models

The analysis was done to investigate the impact of liquid manure application on three most frequently captured fly families. For this purpose, the treatment's effect on fly's number of each family during seven sampling periods was analysed by Analysis of variance (ANOVA). The block was included in the model as a categorical random variable.

The model: $No\_F = \beta_o + \beta_T . X_T + \beta_P . X_P + \beta_B . X_B + \beta_{T.P} . X_T . X_P + error$

In this model T indicates treatment (3 categories), P is sampling period (7 categories, fixed effect), and B is block (5 categories, random effect). An interaction term (Treatment*Period) was included to see whether treatment effect depended on sampling period.

The analysis was done separately for total flies (all flies counted together) and for each family: Anthomyiidae, Muscidae and Fannidae.

3.2.3 Error structure

Different potential error structure was investigated to identify an appropriate statistical model for all the data of total flies and flies in each family. First normal error structure (general linear model) was attempted; the assumption of homogeneity was checked by residual versus fit plot, and the normality (distribution of the error terms) was checked by normal probability plot (Q-Q plot). The residuals were neither homogenous nor normal. Afterwards, I investigated non-normal error structures (generalized linear models) by attempting both Poisson distribution and negative binomial models using an identity link. The residuals were neither homogenous nor normal for any of the data of total flies and flies in each family.

According to Hoffman (2004), in case of count data like this study, two of the most common generalized linear models (Poisson and negative binomial model) use the log link function. I re-ran all models using Poisson distribution with log link. Homogeneity and normality was checked with deviance residual versus fit plot and normal probability plot. The residuals were homogenous and normal for all the data of total flies, Anthomyiidae, Muscidae, and Fannidae (Appendix 3.5: Fig. i-viii). After examining different error structure, the best model for all the data of total flies, Anthomyiidae, Muscidae, and Fannidae was selected Poisson distribution with log link.

The Poisson distribution generally assumes no extra dispersion but my data was over dispersed, e.g. the variance (86.12) exceeded its mean number (12. 17). According to Hoffman (2004), in case of extra dispersion data the negative binomial is more appropriate than the Poisson distribution model. Considering this, I checked negative binomial with log link to compare the outcome of both of the Poisson distribution and the negative binomial model. The Poisson model within log link was preferred because the residual fit plot was more homogeneous compared to the negative binomial model within log link for all the analysis of total flies, Anthomyiidae, Muscidae, and Fannidae. Finally, Poisson distribution with log link was used to examine the treatment effects on total flies (all flies counted together) and on the family Anthomyiidae, Muscidae and Fannidae.

3.3 Results

3.3.1 Autocorrelation outcomes

According to the ACF results, fly numbers (observations) in a strip were found correlated over 1-3 lags and then separated by the 'zero crossing' (the point where autocorrelation value crosses zero and two observations become uncorrelated) from their neighboring lags (see Appendix 3.1 A to 3.4 C for all the ACF plots where X-axis shows lags and Y-axis shows ACF value). All the analysis showed first 'zero crossing', most of them were at lags 2-4 implies that all ten observations of fly numbers in each strip were not correlated, they were independent at greater than two lags (two trap separations) in most cases, and greater than four lags in all cases. Tables 3.1 to 3.12 listed positions of all first zero crossing.

In details, addressing the most important strip, LQ strip, analysis with Anthomyiidae shows that the first zero crossing occurred at lags 2-3 during all seven sampling periods with the most common being at lag 2 (Table 3.4). For the Muscidae, the first zero crossing occurred at lags 2-4 with the most common being at lag 2 (Table 3.7). For the Fannidae, first zero crossing occurred at lags 2-4 with the most common being at lag 4 (Table 3.10).

In the MC strip for the Anthomyiidae, the first zero crossing occurred at lags 2-4 during all seven sampling periods with the most common being at lag 3 (Table 3.5). For the Muscidae, the first zero crossing occurred at lags 2-4 with the most common being at lags 2 and 3 (Table 3.7). For the Fannidae, the first zero crossing occurred at lag 2 in all periods (Table 3.11).

In the UN strip for the Anthomyiidae, the first zero crossing occurred at lags 2-4 during all seven sampling periods with the most common being at lag 2 (Table 3.6). For the Muscidae, the first zero crossing occurred at lags 2-4 with the most common being at lags 2 (Table 3.9). For the Fannidae, the first zero crossing occurred at lags 2-3: in two sampling periods the zero crossing occurred at lag 2, and in other two periods zero crossing at lag 3 (Table 3.12).

Table 3.1: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in liquid manure treated strip (LQ) for seven different autocorrelation analysis during seven sampling periods (P1-P7) using total number of flies. See Appendix 3.1.A for ACF plots.

Sampling Periods 1 to 7 (P1-P7)	**First zero crossing**
LQ_Total_P1	at lag 3
LQ_ Total P2	at lag 2
LQ_Total_P3	at lag 2
LQ_Total_P4	at lag 2
LQ_Total_P5	at lag 2
LQ_Total_P6	at lag 4 (negative at lag 1, lag2 and lag 3)
LQ_Total_P7	at lag 4

Table 3.2: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in mink compost treated strip (MC) for seven different autocorrelation analysis during seven sampling periods (P1-P7) using total number of flies. See Appendix 3.1.B for ACF plots.

Sampling Periods 1 to 7 (P1-P7)	**First zero crossing**
MC_Total__P1	at lag 3
MC_Total__P2	at lag 4
MC_Total__P3	at lag 3
MC_Total__P4	at lag 2
MC_Total__P5	at lag 4
MC_Total__P6	at lag 2 (start negative at lag 1)
MC_Total__P7	at lag 3 (negative at lag 1 and lag 2)

Table 3.3: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in untreated strip (UN) for seven autocorrelation analysis during seven sampling periods (P1-P7) using total number of flies. See Appendix 3.1.C for ACF plots.

Sampling periods 1 to 7 (P1-P7)	**First zero crossing**
Un _Total_P1	at lag 3 (negative at lag1 and lag2)
Un _Total_P2	at lag 2
Un _Total_P3	at lag 5
Un _Total_P4	at lag 3 (negative at lag1 and lag2)
Un _Total_P5	at lag 4
Un _Total_P6	at lag 3
Un _Total_P7	at lag 2 (starts negative at lag1)

Table 3.4: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in liquid manure treated strip (LQ) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Anthomyiidae flies. See Appendix 3.2.A for ACF plots.

Sampling periods 1 to 7 (P1-P7)	First zero crossing
LQ_Anth_P1	at lag 3
LQ_Anth_P2	at lag 2
LQ_Anth_P3	at lag 3
LQ_Anth_P4	at lag 2
LQ_Anth_P5	at lag 2
LQ_Anth_P6	at lag 3
LQ_Anth_P7	at lag 2 (starts negative at lag1)

Table 3.5: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in mink compost treated strip (MC) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Anthomyiidae flies. See Appendix 3.2.B for ACF plots.

Sampling periods 1 to 7 (P1-P7)	First zero crossing
MC_Anth_P1	at lag 3
MC_Anth_P2	at lag 4
MC_Anth_P3	at lag 3
MC_Anth_P4	at lag 3 (negative at lag1 and lag2)
MC_Anth_P5	at lag 3
MC_Anth_P6	at lag 3 (negative at lag1 and lag2)
MC_Anth_P7	at lag 2

Table 3.6: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in untreated strip (UN) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Anthomyiidae flies. See Appendix 3.2.C for ACF plots.

Sampling periods 1 to 7 (P1-P7)	First zero crossing
UN_Anth_P1	at lag 2 (starts negative at lag1)
UN_Anth_P2	at lag 2
UN_Anth_P3	at lag 3
UN_Anth_P4	at lag 3 (negative at lag1 and lag2)
UN_Anth_P5	at lag 2 (starts negative at lag1)
UN_Anth_P6	at lag 2 (starts negative at lag1)
UN_Anth_P7	at lag 4 (negative at lag1, lag2, and lag3)

Table 3.7: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in liquid manure treated strip (LQ) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Muscidae flies. See Appendix 3.3.A for ACF plots.

Sampling periods 1 to 7 (P1-P7)	**First zero crossing**
LQ_Mus_P1	at lag 2 (start negative at lag1)
LQ_Mus_P2	at lag 2 (start negative at lag1)
LQ_Mus_P3	at lag 2
LQ_Mus_P4	at lag 2
LQ_Mus_P5	at lag 4 (negative at lag1, lag2 and lag3)
LQ_Mus_P6	at lag 2
LQ_Mus_P7	at lag 4

Table 3.8: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in mink compost treated strip (MC) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Muscidae flies. See Appendix 3.3.B for ACF plots.

Sampling periods 1 to 7 (P1-P7)	**First zero crossing**
MC_Mus__P1	at lag 3
MC_Mus__P2	at lag 3
MC_Mus__P3	at lag 4
MC_Mus__P4	at lag 2
MC_Mus__P5	at lag 2 (start negative at lag 1)
MC_Mus__P6	at lag 3
MC_Mus__P7	at lag 2

Table 3.9: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in untreated strip (UN) for seven autocorrelation analysis during seven sampling periods (P1-P7) using Muscidae flies. See Appendix 3.3.C for ACF plots.

Sampling periods 1 to 7 (P1-P7)	**First zero crossing**
UN_Mus_P1	at lag 2
UN_Mus_P2	at lag 2
UN_Mus_P3	at lag 3
UN_Mus_P4	at lag 2
UN_Mus_P5	at lag 4
UN_Mus_P6	at lag 3
UN_Mus_P7	at lag 2 (start negative at lag1)

Table 3.10: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in liquid manure treated strip (LQ) for autocorrelation analysis using Fannidae flies. There were not enough flies during first, second and 7th collection periods, for this reason analysis was restricted to sampling periods P3 to P6. See Appendix 3.4.A for ACF plots.

Sampling periods 1 to 7 (P3-P6)	**First zero crossing**
LQ_ Fan _P3	at lag 4 (negative at lag 1, lag 2and lag 3)
LQ_ Fan _P4	at lag 3 (negative at lag 1, lag 2)
LQ_ Fan _P5	at lag 2
LQ_ Fan _P6	at lag 4

Table 3.11: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in mink compost treated strip (MC) for autocorrelation analysis during sampling periods P3 to P6 using Fannidae flies. See Appendix 3.4.B for ACF plots.

Sampling periods 1 to 7 (P3-P6)	**First zero crossing**
MC_ Fan __P3	at lag 2 (negative at lag1)
MC_ Fan __P4	at lag 2
MC_ Fan __P5	at lag 2
MC_ Fan __P6	at lag2 (negative at lag1)

Table 3.12: List of first zero crossing (autocorrelation value crosses zero and two observations became uncorrelated) in untreated strip (UN) for autocorrelation analysis during sampling periods P3 to P6 using Fannidae flies. See Appendix 3.4.C for ACF plots.

Sampling periods 1 to 7 (P3-P6)	**First zero crossing**
Un _ Fan_ P3	at lag 3 (negative at lag1 and lag2)
Un _ Fan _P4	at lag 2 (negative at lag1)
Un _ Fan _P5	at lag 2 (negative at lag1)
Un _ Fan _P6	at lag 3 (negative at lag1 and lag2)

3.3.2 Treatment effects on total flies

The analysis show that there was a significant interactive effect of both treatments and collection periods on overall flies (Wald chi square =158.36; df = 12; P <0.0001) which indicates that treatment effects were significantly varied during seven collection periods. During first collection period (P1) before liquid manure was applied, highest number of flies were caught from the mink compost treated (MC) strip. The mean of overall flies was 2.67 (se=0.51) for the strip labelled untreated (UN), while it was slightly higher at 3.17 (se=0.560) for the strip labelled for liquid manure (LQ). And, it was 5.65 (se=0.75) for the strip treated with mink compost (MC) which was the highest in comparison with two other strips (Figure 3.2). During this time only mink compost was being applied.

During both second and third collection periods after the liquid manure application, overall flies were highest in the liquid manure treated (LQ) strip. During period 2 (P2), the mean for the UN strip was 4.95 (se=0.70), for the MC strip it was about two times higher at 9.70 (se=0.98). And, for the LQ strip it was over four times higher at 20.70 (se=1.43) (see Figure 3.2). During period 3 (P3), for the UN strip the mean number was 12.48 (se=1.11). For the MC strip it was 1.8 times higher at 21.10 (se=1.45). And, for the LQ strip it was about 2.5 times higher at 32.79 (se=1.80). During third collection period total fly numbers were overall greater than any other periods (see Figure 3.2).

During the collection periods of P4, P5, and P6, the mean number of overall flies were highest in the mink compost treated (MC) strip (Figure 3.2). Throughout the collection period 4, the UN strip had 6.04 (se=0.77), LQ strip had about the same at 6.24 (se=1.80), and, MC strip had 1.5 times higher at 9.31 (se=0.96) flies. During period 5, the UN strip had 11.49 (se=1.07)

while LQ and MC both strips had about 1.1 times higher at 12.68 (se=1.12) and 13.37 (se=1.15) flies respectively. During period 6, UN strip had 9.74 (se=1.04), LQ strip had 1.6 times higher at 16.14 (se=1.35) and MC strip had more than two times higher at 20.40 (se=1.42) flies.

During the last collection period (P7), mean number of flies was highest in the untreated strip compared to two other strips. For the UN strip it was 13.47 (se=1.16), for the LQ strip it was about slightly lower at 10.40 (se=1.01) and for the MC strip it was also slightly lower at 11.19 (se=1.05).

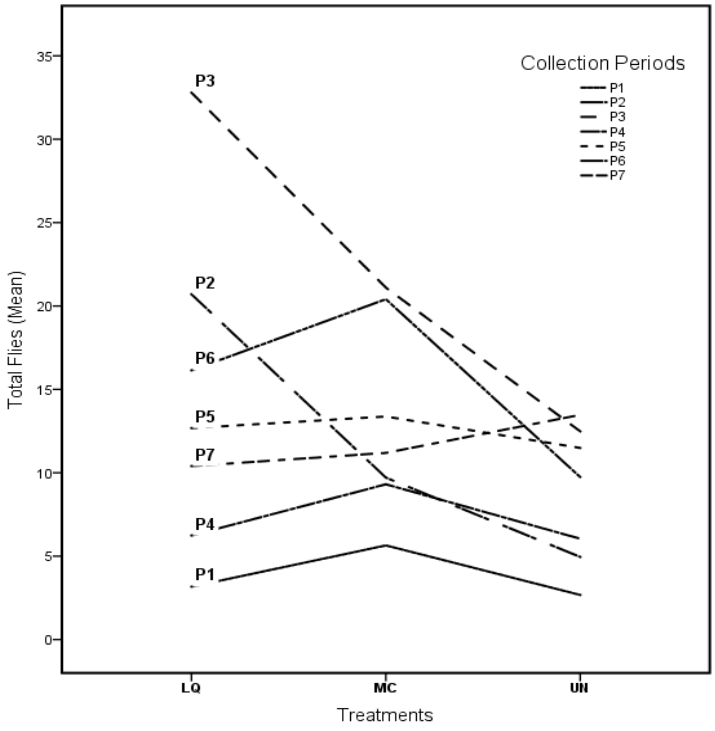

Figure 3. 2: Seven line plots showing the significant interaction effect of three treatments (LQ: liquid manure, MC: mink compost, UN: untreated) and seven collection periods (P1-P7) on total flies (overall flies belongs to Anthomyiidae, Muscidae and Fannidae) ascertained from the Poisson distribution with log link.

3.3.3 Treatment effects on Anthomyiidae

The analysis shows that there was a significant interactive effect of treatment and collection periods (Wald chi square =107.46, df = 12, P<0.0001) on Anthomyiidae flies. During the collection period 1 (P1) before liquid manure was applied, highest number of Anthomyiids were caught from the mink compost treated (MC) strip. The mean of flies for the strip labelled untreated (UN) was 1.81 (se=0.45). For the strip labelled for liquid manure (LQ) it was slightly higher at 2.78 (se=0.52). For the strip labelled for mink compost (MC) it was 4.76 (se=0.69) (Figure 3.3).

During both second and third collection periods after the liquid manure application, Anthomyiids were highest in the liquid manure treated (LQ) strip (Figure 3.3). During period 2 (P2), the mean of Anthomyiidae for the UN strip was 4.76 (se=0.69), for the MC strip it was almost two times higher at 9.12 (se=0.95), and for the LQ strip it was more than four times higher at 19.83 (se=1.40). During period 3 (P3), for the UN strip it was 11.90 (se=1.09), for the MC strip it was almost two times higher at 22.50 (se=1.57) and for the LQ strip it was about three times higher at 35.00 (se=1.97).

During following collection periods (P4, P5, P6 & P7), the mean numbers of Anthomyiids were highest in the mink compost treated (MC) strip (Figure 3.3). During P4, the UN strip had 3. 67 (se=0.60), the LQ strip had almost same at 3.57 (se=0.5) and the MC strip had slightly higher at 5.75 (se=0.75). During P5, the UN strip had 5.85 (se=0.76), the LQ strip had 6.84 (se=0.82) and the MC strip had 7.63 (se=0.87) respectively. During P6, the UN strip had 4.86 (se=0.73), the LQ strip had two times higher at 9.87 (se=1.06) and for the MC strip the mean number was almost three times higher at 14.18 (se=1.19). During P7, for the UN strip the

mean number was 1.69 (se=0.41), for the LQ strip it was slightly lower at 1.29 (se=0.36) and for the MC strip it was 3.13 (se=0.72).

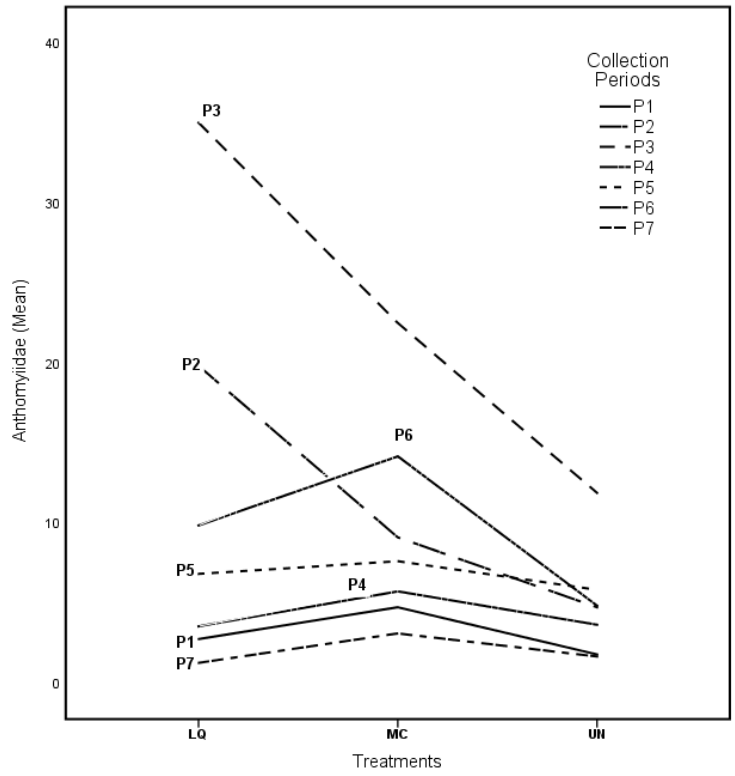

Figure 3. 3: Seven line plots showing the significant interaction effect of treatments (LQ: liquid manure, MC: mink compost, UN: untreated) and seven collection periods (P1-P7) and on Anthomyiidae flies ascertained from the Poisson distribution with log link.

3.3.4 Treatment effects on Muscidae

The analysis shows that there was a significant interactive effect of treatment and collection periods on Muscidae flies (Wald Chi square =21.470, P = 0.044, df = 12) which indicates that the impact of treatments on Muscids varied during seven collection periods.

During first collection period (P1), the mean of Muscids was higher in untreated strip at 1.12 (se=0.35), for the MC strip it was 0.90 (se=0.30) and for the LQ strip it was lower at 0.40 (se=0.2) (Figure 3.4).

During second collection period (P2), after the liquid manure application, highest numbers of Muscids were trapped from the LQ strip (Figure 3.4). For the UN strip the mean was 0.20 (se=0.14), for the MC strip it was about 3 times higher at 0.60 (se= 0.24) and for the LQ strip it was four times higher at 0.80 (se=0.28).

Except second collection periods, during all other collection periods LQ strip had the lowest mean number of Muscids. For example, During P3, the mean of the Muscids was highest at UN strip which is 0.40 (se=0.20), for the LQ and MC strips it was 0.30 (se=0.17) and 0.20 (se=0.14) respectively. During P4, UN had 1.79 (se=0.42), LQ strip had nearly the same at 1.89 (se=0.43) and the MC strip had 2.69 (se=0.51) Muscids. During P5, UN strip had 5.18 (se=0.72), LQ strip had about the same at 4.28 (se=0.69) and the MC strip had slightly lower at 3.59 (se=0.60). During P6, UN strip had 4.28 (se=0.69), LQ and the MC strips had about the same at 5.03 (se=0.75) and 4.28 (se=0.65) respectively. During P7, UN had 11.86 (se=1.09), LQ and MC strips had about the same at 10.30 (se=1.08) and 15.84 (se=1.64) respectively.

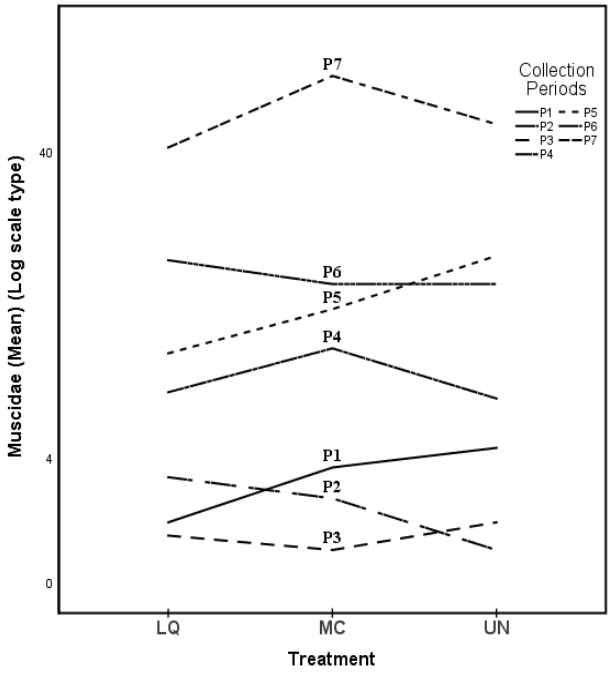

Figure 3. 4: Seven line plots showing the significant interaction effect of three treatments (LQ: liquid manure, MC: mink compost, UN: untreated) and seven collection periods (P1-P7) on flies of the family Muscidae ascertained from the Poisson distribution with log link.

3.3.5 Treatment effects on Fannidae

Only one fly from the family Fannidae was caught during first (P1) and second (P2) collection periods and no flies were caught during last collection period (P7). For this reason, the analysis was restricted to four collection periods (P3, P4, P5, and P6) when a considerable number of flies were collected (Figure 3.5). The analysis shows, there was no significant interactive effect of treatment and collection periods on flies of family Fannidae (Wald chi square = 9.37; df = 6, P = 0.154). On the other hand, main effects were significant. For example, there was a significant effect of treatments on Fannids (Wald chi square = 12.58; df = 2; P = 0.002). The mean number of the Fannids was higher in LQ strip compared to the other two strips; for the UN strip it was 0.41(se=0.11), for the MC strip it was 2.7 times higher at 1.12 flies (se= 0.19), and for the LQ strip it was almost three times higher at 1.15 (se=0.19). However, the t- test analysis shows that there was no significant difference between the LQ (t =3.43; df= 78; P= 0.00097) and MC (t = 3.23; df =78; P= 0.0017) treated strips.

Moreover, the significant effect of collection periods on Fannids (Wald chi square = 17. 84; df = 3; P<0.0001) show that highest number of flies were caught during P5 and lowest were caught during P3 among four collection periods (Figure 3.5). The mean number of flies during P5 was 1.53 (se=0.27) and P3 was 0.36 (se=0.12). The t-test showed P5 had significantly over four times higher flies than P3(t=4.00; df =78; P=0.0001). Furthermore, the second highest number of flies were caught during P6 (Figure 3.5); the mean was 1.03 (se=0.20) significantly more than P3 (t=5.57; df =78; P<0.001). During P4 the mean was 0.75 (se=0.16) significantly (t= 1.99; df =78; P= 0.051) more than P3.

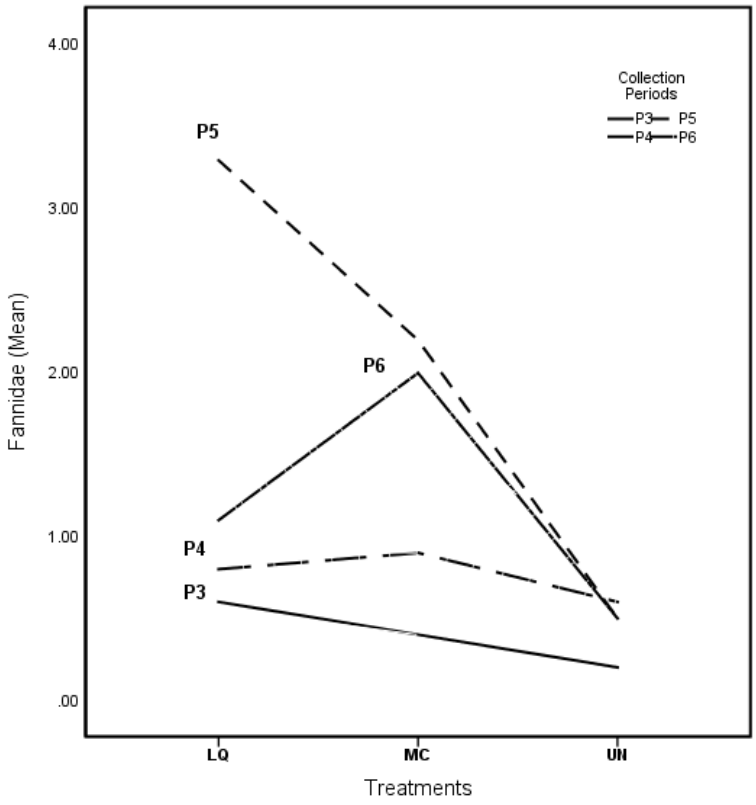

Figure 3. 5: Line plots showing the effect of treatments (LQ: liquid manure, MC: mink compost, UN: untreated) on flies of the family Fannidae obtained from Poisson distribution with log link. (P3-P6 indicates collection periods)

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3.4 Discussion

This chapter represents a blocking approach of a demonstration strip plot by autocorrelation function and analyzing the impact of liquid manure's field application on overall fly population. Given the fact that installation of the liquid manure production system during 2014 minimized the *Fannia canicularis* population (focus group of this study), in 2015 I begin with a basic field experiment with minimum labour and intended to conduct randomized complete block design in the following year. But the first year outcome that is the low number of *F. canicularis* suggested that the effort was sufficient to answer the questions of this project and there was no necessity of conducting more complicated design with more cost. However, the focus was shifted to investigate the frequently captured flies from that treatment treated strip field.

Based on results of this series of experiments and the significant interactive effects, treatment effects on overall flies (total flies) captured during 2015 were significantly varied during all collection periods. For instance, during first collection period (P1: May 14 to 21) a time before liquid manure was applied, total numbers of flies were significantly lowest in all three treatment strips compared to the other collection periods; meanwhile, the highest numbers of flies were caught from the strip treated with mink compost (see Figure 3.2). This is relevant as during that time the mink compost was being applied not only to the mink compost treated strip but to the rest of the southern side of the demonstration plot. However, the flies number immediately increased in liquid manure treated strip after liquid manure application on May 21; in both the second and third collection periods (P2 and P3), overall flies were highest in the liquid manure strip compare to other two strips (Figure 3.2). However, this trend did not

continue; later, from June 25 to July 24, 2015 in all periods of collection, the highest numbers of flies were caught from the mink compost treated strip except the last collection period (P7) when highest was in untreated strip (Figure 3.2). Afterwards, in order to determine if any specific family of flies may have a preference that might cause the striking trend of flies after liquid manure application, investigation was done through separate analysis for each of the fly family.

The significant interactive effect of treatment and collection periods on family Anthomyiidae (highly captured in 2015's collection) shows somewhat same trend as total number of flies. During first collection period, the highest numbers of Anthomyiids (mean 4.76) were trapped from the mink compost treated strip (Figure 3.3) when that particular strip had been applied with mink compost and the other two strips were not yet treated. On the other hand, the highest numbers of Anthomyiids were caught only during the second and third collection periods, from the liquid manure treated strip immediately after the application of liquid manure (Figure 3.3). As a consequence, it can be assumed that the rising trend of total flies immediately after liquid manure application was due to increasing numbers of flies within the family Anthomyiidae during this time. This attraction of Anthomyiidae to animal manure is not unusual. Griffiths and Stewart (2004) documented that some members of the family Anthomyiidae are excrement eaters; they mentioned that Anthomyiidae may eat chicken manure (Figure 3.3). Floate (2011) included Anthomyiidae as a dung feeder in a checklist made for insects that are known to be associated with cattle dung in Canada. The sudden rise of Anthomyiidae flies in liquid manure strip after liquid manure application validates the relationships of Anthomyiidae with liquid manure. Although, this trend did not persist, as following collection periods from June 25 to September 17, 2015 the number of flies remain highest in the mink compost treated

strip comparing to other two strips. Floate (2011) documented that sometimes insects require a physical contact with the dung to assess the suitability of manure and immediately leave if the manure is not suitable. Thus, after liquid manure application some volatile compounds at certain level (Laos et al. 2004, Floate 2011) may attract the Anthomyiidae for short period of time but the attraction does not continue in following time. So it can be assured that the flies of family Anthomyiidae have no preference on liquid mink manure. The application of this manure is unlikely to be a reason to attract Anthomyiids.

Regarding the impact of liquid manure on Muscidae (the second most abundant family), the numbers were slightly higher (mean 0.80) in the strip treated with liquid manure only during second collection period from June 3 to 11 immediately after the liquid manure application (Figure.3.4). Except these, there was no preference of Muscids observed in liquid manure treated strip (Figure.3.4). Overall highest numbers of Muscids were observed during last collection period (Figure.3.4) on September 2 to 17, 2015. Due to the high abundance of Muscidae during this time of the season different weather factors was examined. Many studies report the similar peak activity of Muscids in late summer to early fall (Mullens et al. 1999, Ngoen-klan et al. 2011) and specified the optimal temperature, which is 20–25° (Ngoen-klan et al. 2011, Ma'moun Sh et al. 2017). A study in Ethiopia by Fentie (2004) also documented optimal temperature for some species of *Musca*, which is 23-27^{0}C. He found relatively lower (between 17°C-22 °C) and higher temperatures (between 26°C-31°C) have adverse effects on flies. Considering these reports and the consistent findings in this study (highest numbers of Muscidae in average temperature 16-25°C during September), it can be noted that the optimal temperature for Muscidae appeared to be 23-25°C. Interestingly during the peak season the lowest numbers of

Muscidae were caught from liquid manure treated strip. Although there are some studies documenting association of different species of Muscidae with manure (Farkas et al. 1998, Larrain and Salas 2008, Khan et al. 2012); a study by Laos et al.(2004) documented an association of manure for a species *Muscina stabulans* was due to some volatile compounds and ambient temperatures. Hence, according to the findings of current study, it is suggested that Muscids are not attracted by liquid manure. Certainly, liquid manure application had no large influence upon populations of Muscids in the surrounding area.

In the case of the family Fannidae, interactive effect was not significant ($P = 0.154$). The significant main effects of collection periods this family ($P<0.0001$) provides important information on their seasonal abundance. Although many studies report the activity of different *Fannia* spp. especially *F. canicularis* in spring (Mullens et al. 1999, Mullens et al. 2001, Lewallen 1954, Hall et al. 1972, Steve 1960), in this current study only two flies were caught until mid-June (beginning of sampling periods) when the average temperature was 8 to10°C and 10 to12°C for the first and second sampling periods respectively. To my knowledge there are no studies that specifically reported the activity of Fannidae during the temperature range of 8 to 12°C. It is likely that this absence of Fannidae may be due to relatively colder temperature of Cavendish which might limit the activities of flies during this time. Considerable numbers of flies emerged from mid-June during third collection period (P3) and continued until July; lowest numbers were observed during P3 (Figure 3.5) from June 11 to 25 when the average temperature was 12 to16°C. Fly numbers were significantly increased during the following collection period (P4) on June 25 to July 03 with temperature slightly increased at mean temperature 14-16°C. Although there are limited records of these fly's activity during this temperature (12-16°C), some

Fannia spp. have been recorded having very limited periods of peak adult activities e.g. Canyon flies (*Fannia benjamini* complex) that last only one or two months during late spring and early summer (Mullens and Gerry 2006). The highest numbers of Fannidae were captured in the fifth collection period (P5) from July 03 to 17 compared to all other collection periods (Figure 3.5) with average temperature 16 to 23°C and when most of the days were sunny comparing to overall collection periods. A slight decline was noted in the following collection period (P6) during July 17 to 24 with temperature slightly decreased at an average 12 to 14°C. No Fannidae were caught from September 02 to 17 at the very end of the sampling seasons at almost same temperature (Avg. 16-25°C) as the fifth collection period when highest flies were captured. It is likely that there may be some other environmental factors involved for these fluctuations of fly numbers such as humidity, rainfall, wind speed, gust etc. It would be interesting to investigate details of environmental factors for the seasonal abundance of family Fannidae. Taxonomic details of Fannidae should be studied in greater detail for this purpose as well because individual species of Fannidae may exhibit different patterns of seasonal activity. However, without clear knowledge about the types of genus/species and absence of full season's data it is difficult to comment on the pronounced temperature preference of family Fannidae.

The significant main effect of treatment ($P = 0.002$) shows that the mean number was lowest in the untreated strip, significantly more than two and half times higher in the mink compost treated strip and almost three times higher in the liquid manure treated strip compared to the untreated control strip (Figure 3.5). However, a further t-test was done to examine the difference between two manures which shows that flies in the Liquid manure was not significantly different from the flies in the mink compost.

Liquid mink manure and mink compost contains ammonia and other nitrogenous nutrients (see liquid manure analytical report Appendix 2.1). *Fannia canicularis* are known to be attracted to ammonia as their preferred breeding media (Steve 1960). Therefore, Mohr et al. (2011) reports another species of Fannidae, *Fannia conspicua* Malloch known to be attracted to ammonia; especially after combining with carbon dioxide (ammonium bicarbonate) it has been even shown to attract more of this flies. Similar attraction to ammonia has been reported for other families of Diptera e.g. Tephritidae: fruit flies (Bateman and Morton 1981, Kendra et al. 2005). Due to this attracting capacity, ammonia has been used as a good attractant for trapping wide range of female flies within different family of Diptera e.g. Tephritidae, (Kendra et al. 2005). Ammonia is a primary component of urine and feces (Richards et al. 1975) especially when mink feeds on chickens which are high in ammonia. Floate (2011) reports, the volatile compounds produced during composting process is also responsible for attracting different insects associated with manure. Laos et al. (2004) reports the same with an addition that this attraction was related to minimum and maximum ambient temperatures for *Fannia* sp. There are more than 160 volatile compounds associated with livestock manure (Mackie et al. 1998). The attraction may depend on the concentration and types of a given volatile depending on the types of insects (Floate 2011). Although this current study provides useful indication on the attraction of Fannids to the odour of ammonia in liquid manure and mink compost, it is not therefore pure speculation to claim this outcome because there is a very little information with only 133 Fannids in overall 210 sticky traps throughout 2015's sampling season; in addition, the species level of 111 individuals in family Fannidae is unknown. Further study to species level is needed to evaluate the attraction of *Fannia* spp. in liquid mink manure and mink compost. Overall there was no evidence for that

liquid manure application would be a reason to increase any kind of fly population over the long term.

3.5 Conclusion

In chapter 3, I examined the impact of liquid mink manure on frequently captured fly family (Anthomyiidae, Muscidae, and Fannidae) from the manure treated experimental field. I found substantial rise in the population of Anthomyiidae flies and a slight increase of Muscidae numbers after the application of liquid manure, but did not persist in the following weeks. The family Fannidae were attracted to the liquid manure and mink compost, but only 133 (5.25% of total flies) Fannids were captured overall from 210 sticky traps throughout the sampling season. Out of 133 Fannidae, only 22 were identified as *Fannia canicularis* (Lesser housefly) as this species is the main focus of this study. The outcome was as expected as flies are attracted to the manure, especially immediately after the application of liquid manure to the field. However, the effect of the liquid manure lasted only for a very short time period. Overall, there was no evidence for that liquid manure application would attract any kind of fly population over the long term.

This outcome was quantified on an experimental basis by the novel non-replicated approach introduced in this study. This approach used Post-hoc blocking using spatial autocorrelation to yield a valid error term for statistical analysis of a non-replicated experiment. This approach is applicable in studies where replication is not possible for ethical or practical reasons and in studies where replication is expensive, as in this study.

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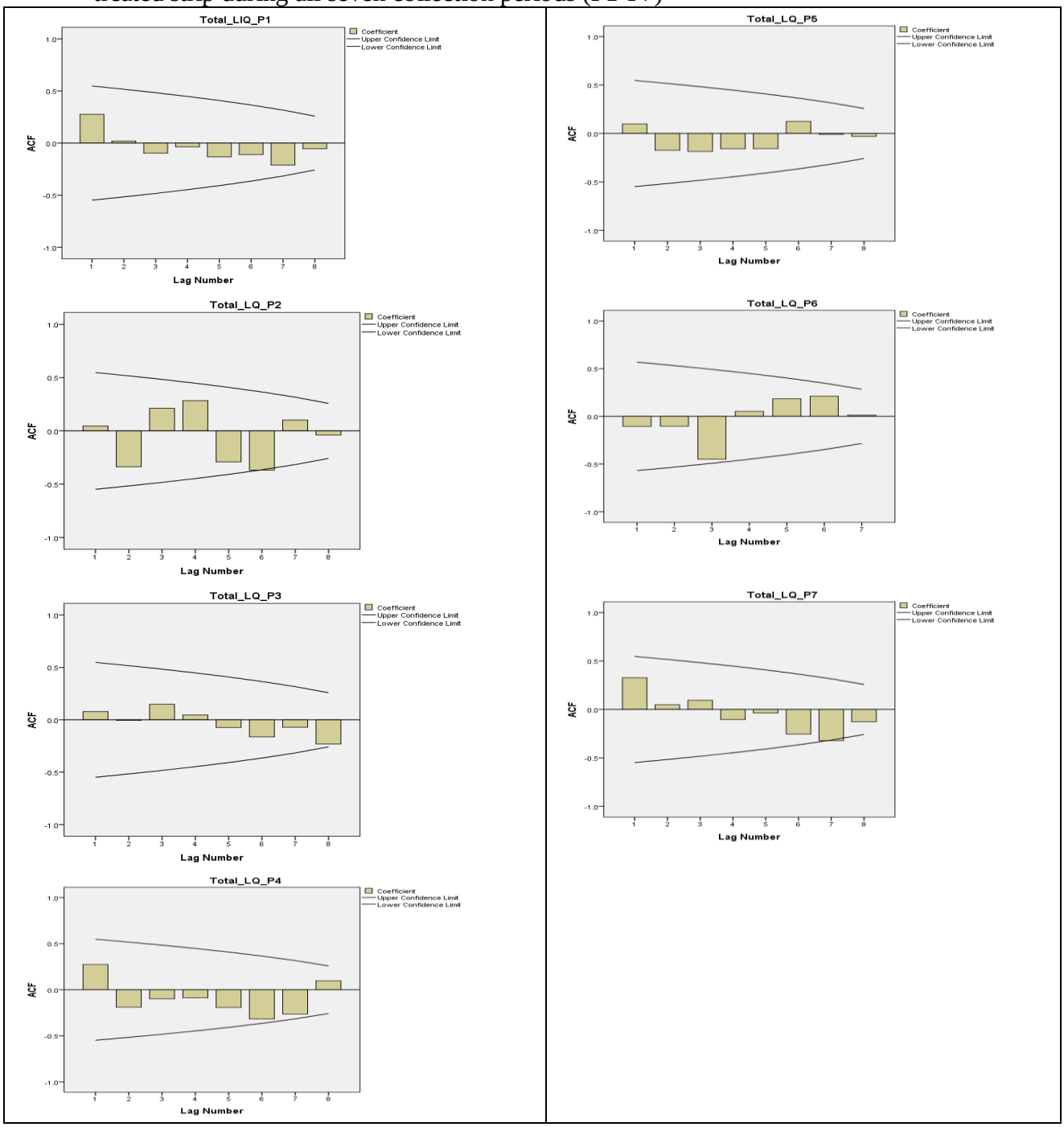
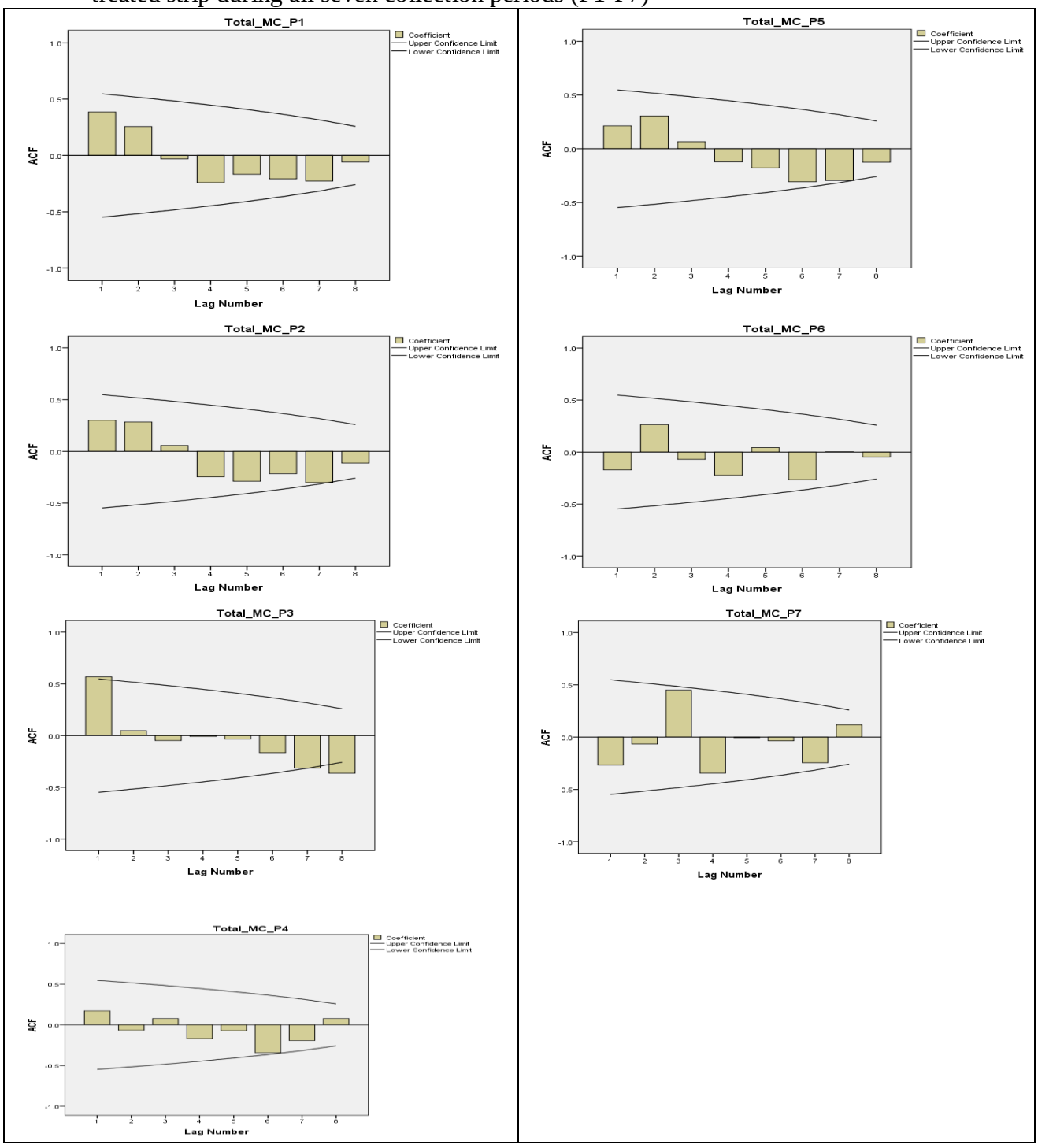
3.7 Appendices

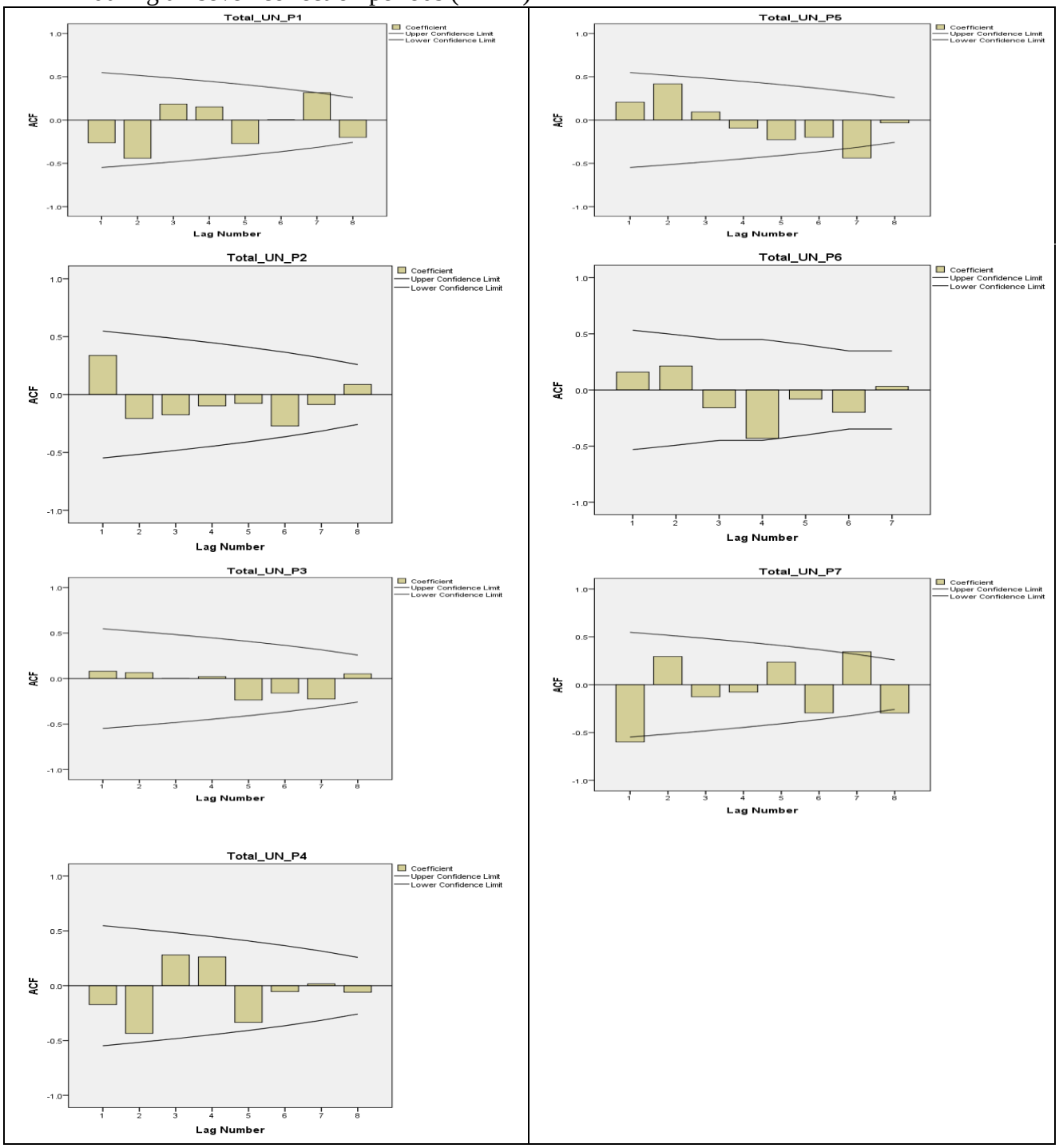
Appendix 3.1. A: Autocorrelation function (ACF) plots of total fly numbers in liquid manure treated strip during all seven collection periods (P1-P7)



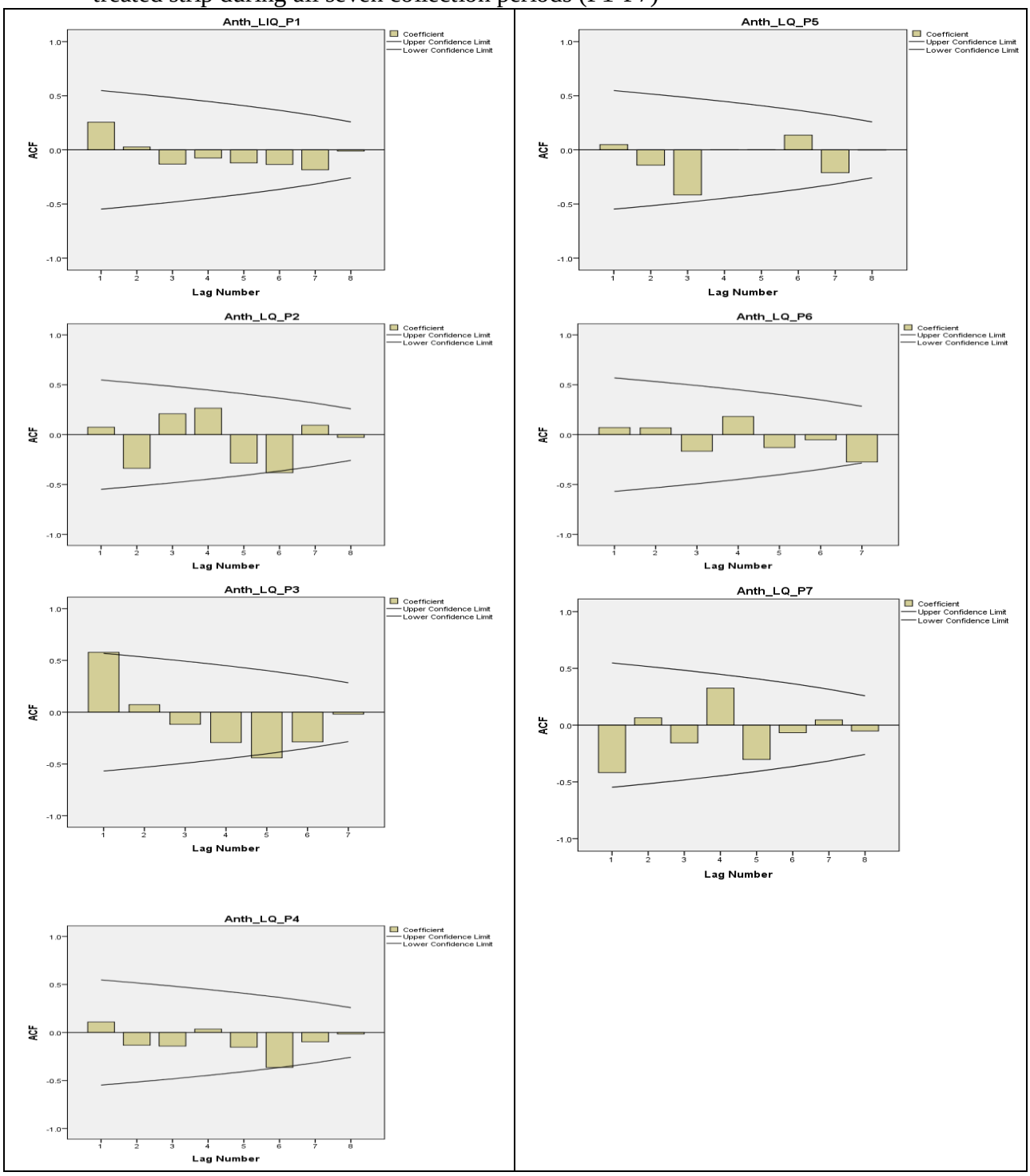
Appendix 3.1. B: Autocorrelation function (ACF) plots of total fly numbers in mink compost treated strip during all seven collection periods (P1-P7)



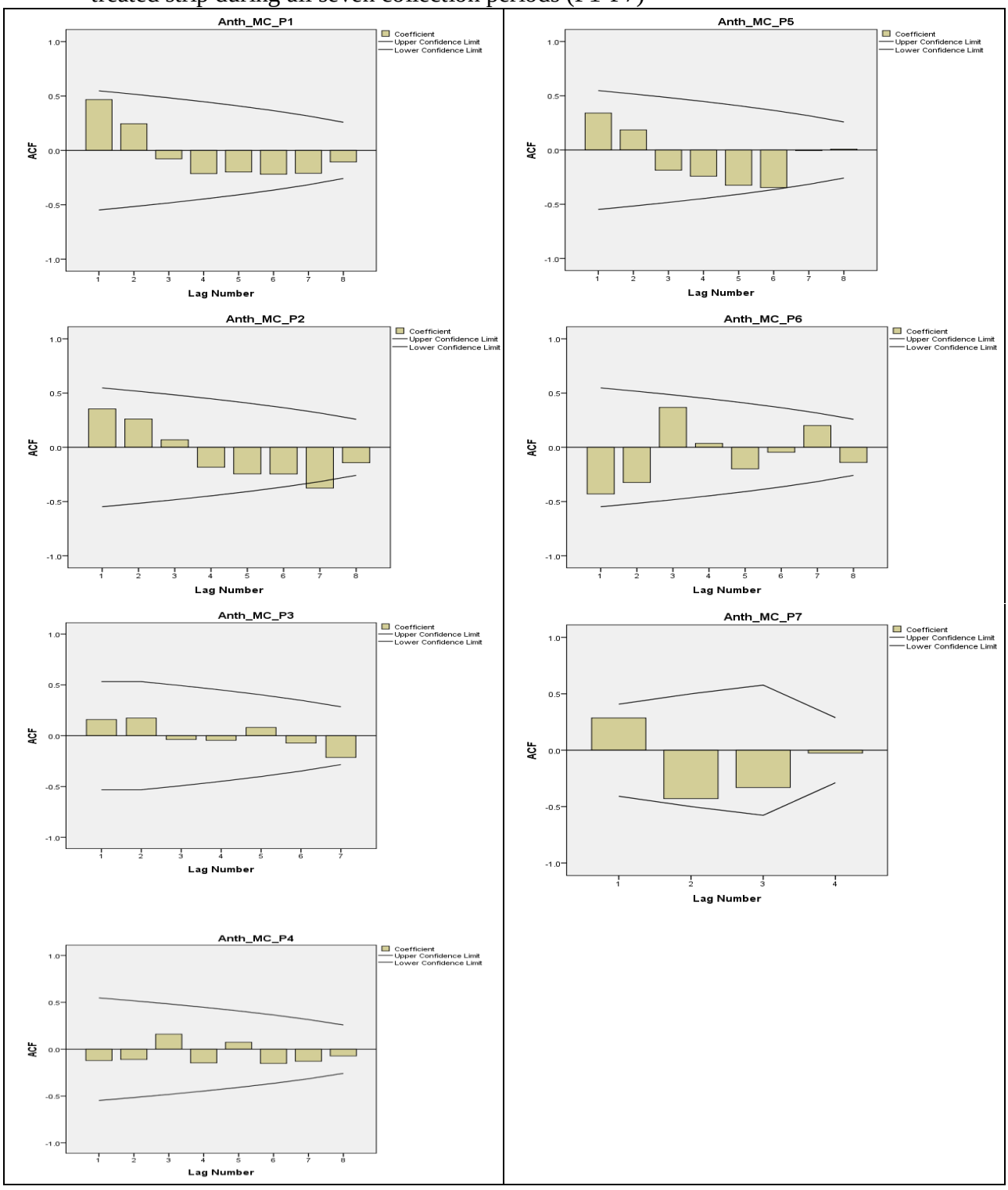
Appendix 3.1. C: Autocorrelation function (ACF) plots of total fly numbers in untreated strip during all seven collection periods (P1-P7)



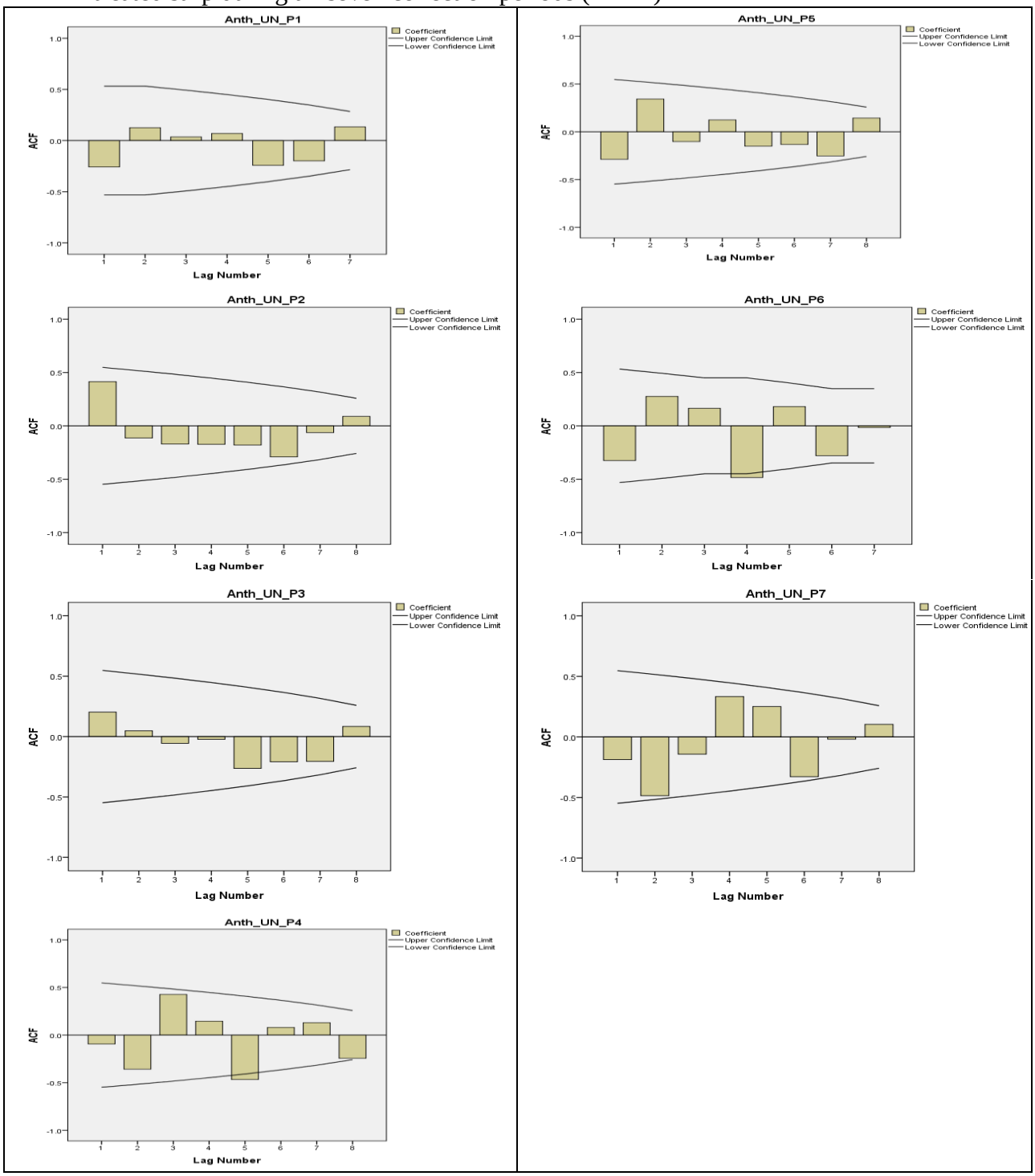
Appendix 3.2. A: Autocorrelation function (ACF) plots of family Anthomyiidae in liquid manure treated strip during all seven collection periods (P1-P7)



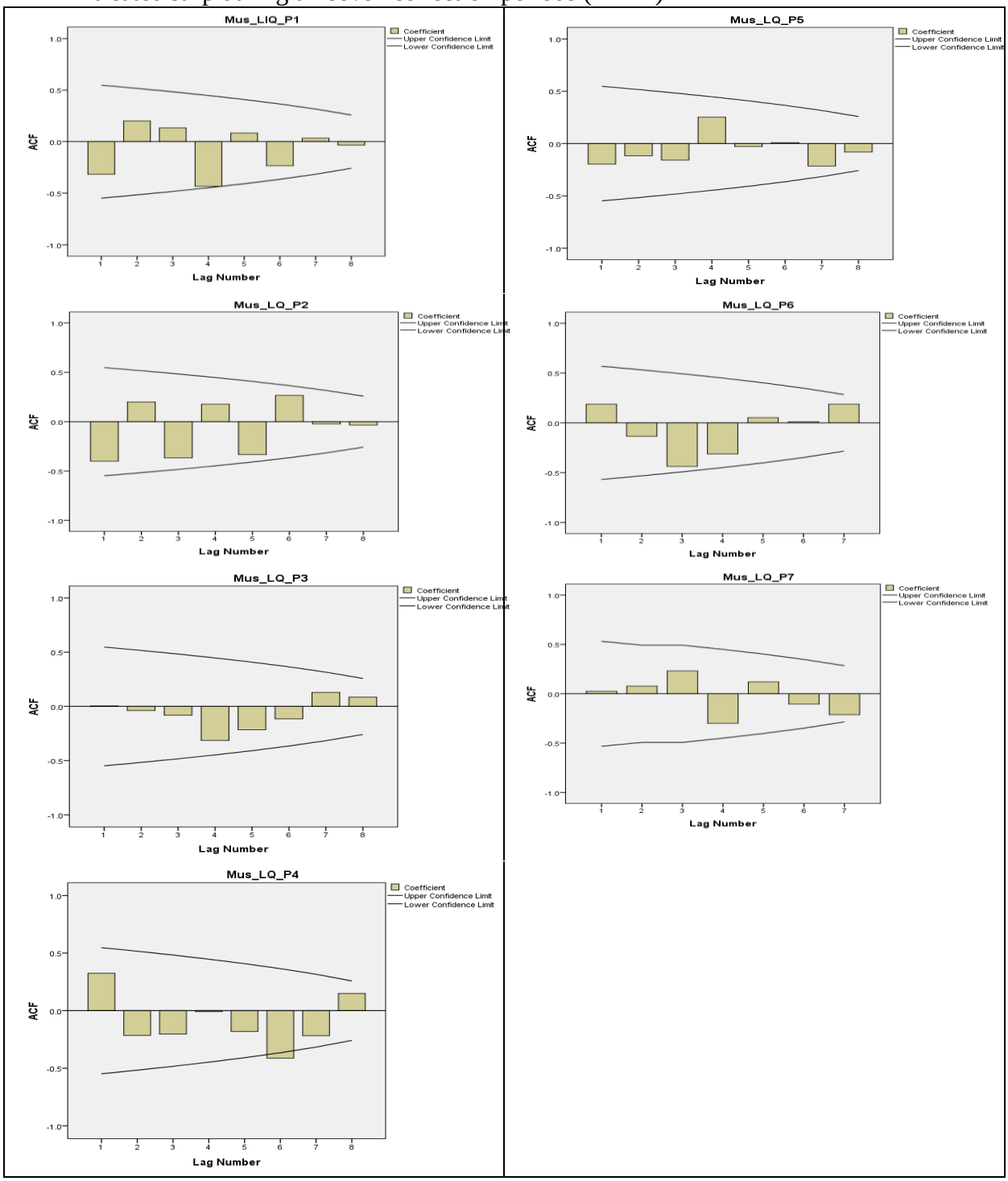
Appendix 3.2. B: Autocorrelation function (ACF) plots of family Anthomyiidae in mink compost treated strip during all seven collection periods (P1-P7)



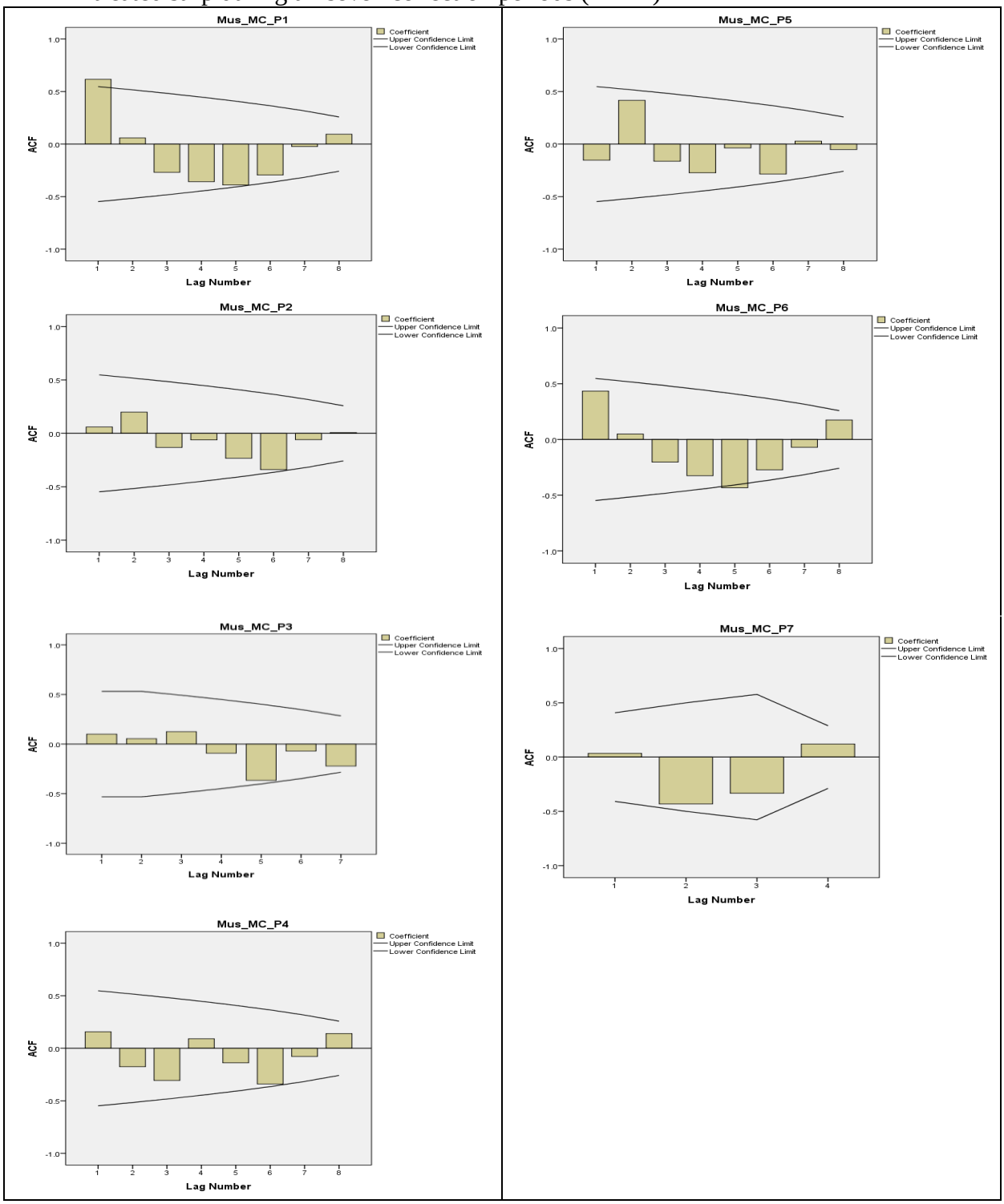
Appendix 3.2. C: Autocorrelation function (ACF) plots of family Anthomyiidae in untreated treated strip during all seven collection periods (P1-P7)



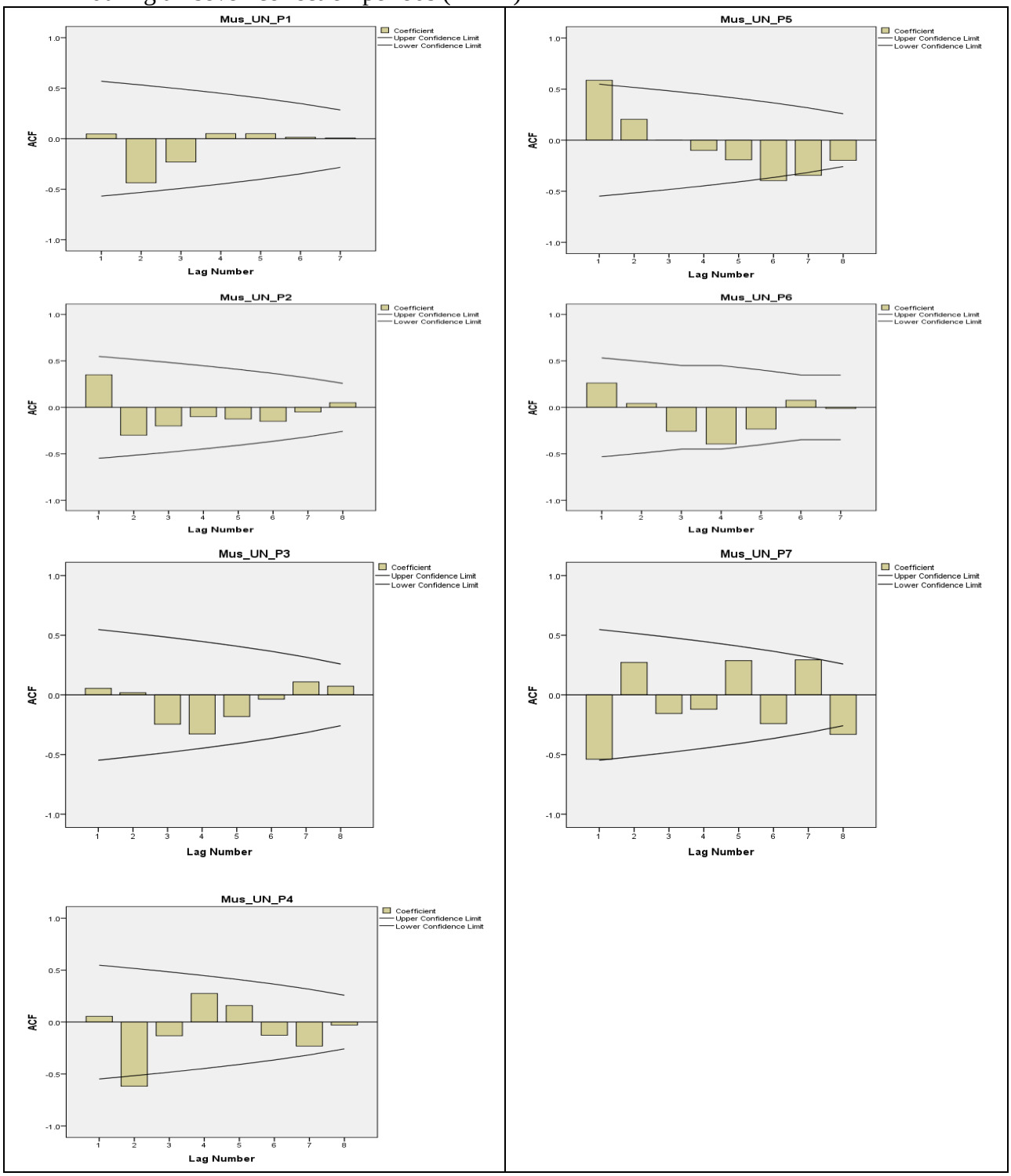
Appendix 3.3. A: Autocorrelation function (ACF) plots of family Muscidae in liquid manure treated strip during all seven collection periods (P1-P7)



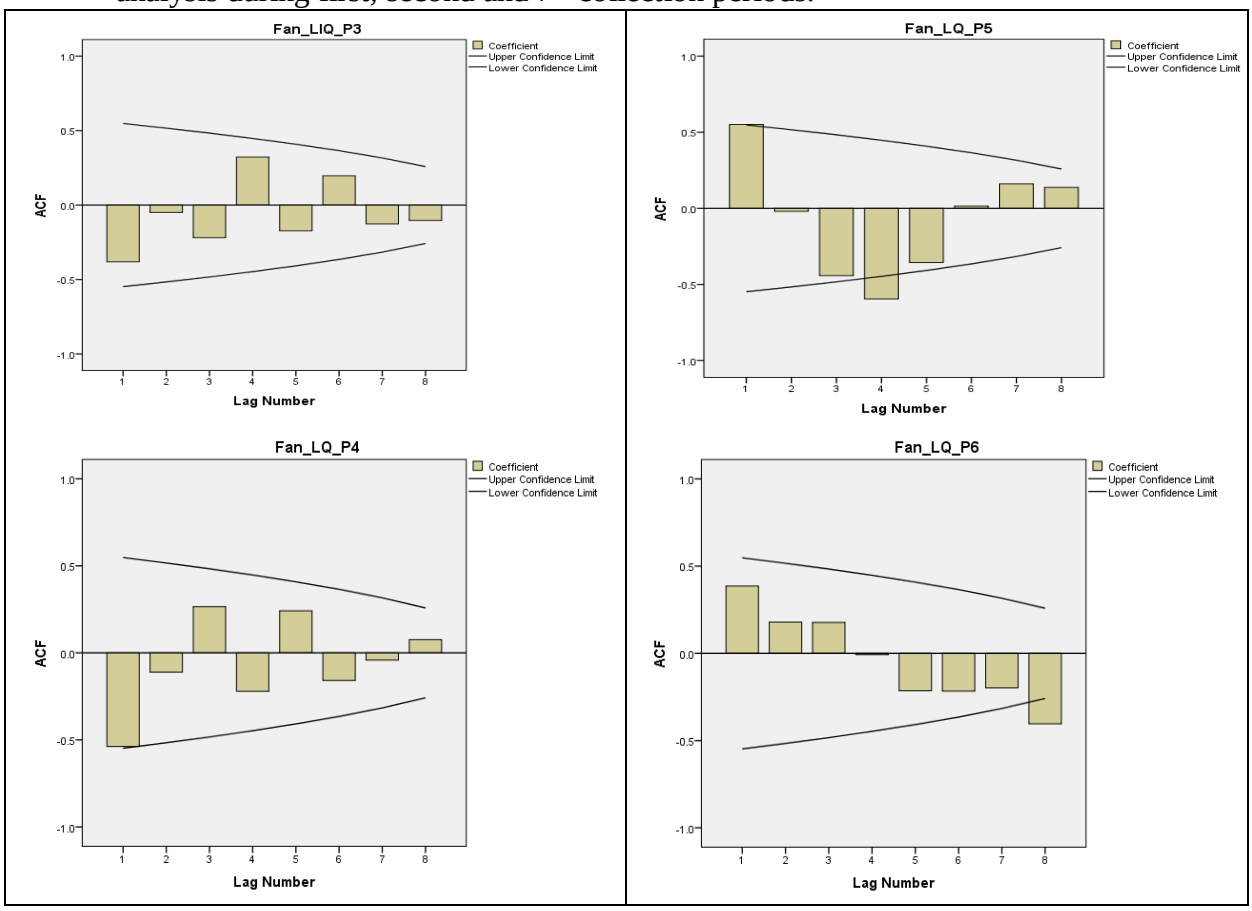
Appendix 3.3. B: Autocorrelation function (ACF) plots of family Muscidae in mink compost treated strip during all seven collection periods (P1-P7)



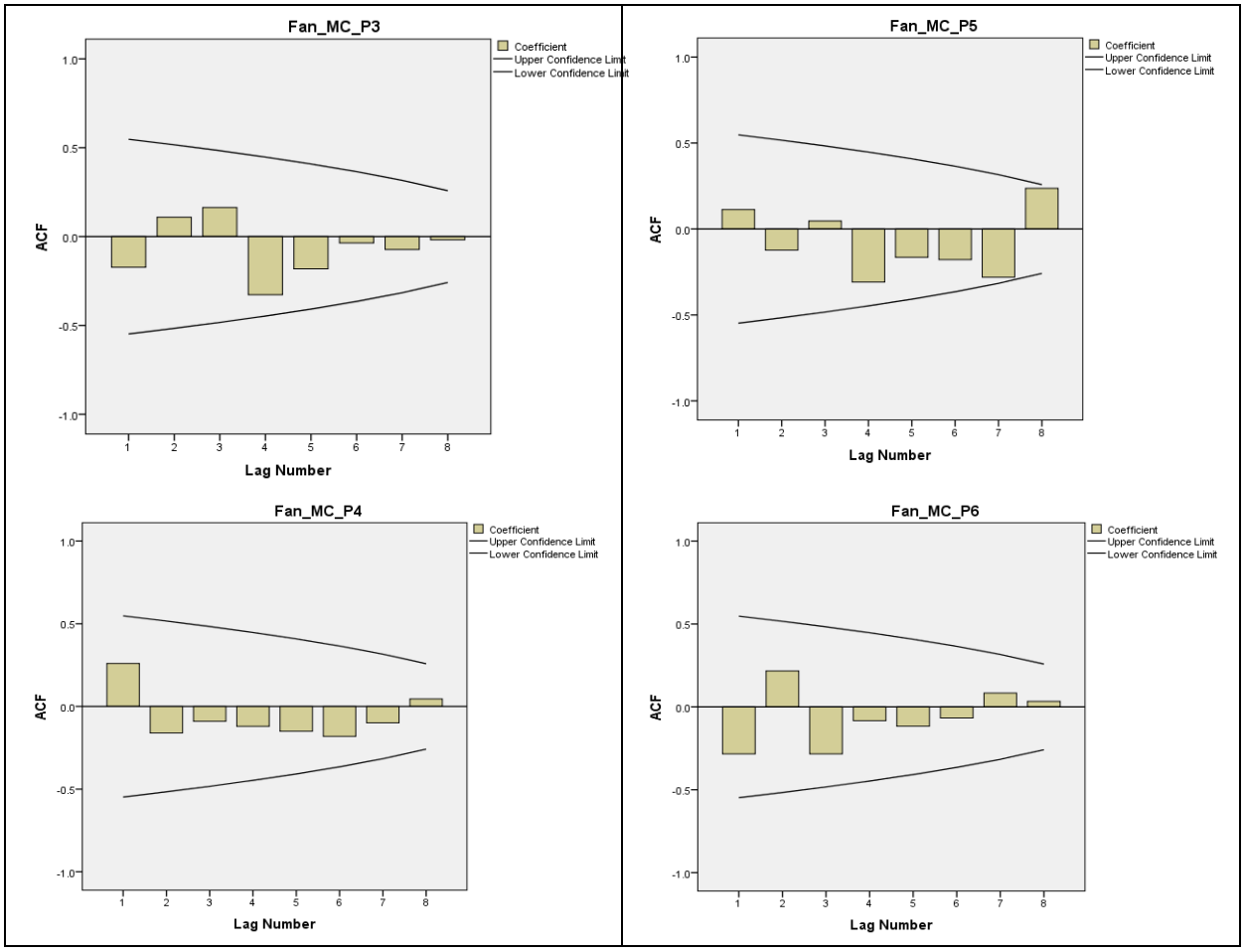
Appendix 3.3. C: Autocorrelation function (ACF) plots of family Muscidae in untreated strip during all seven collection periods (P1-P7)



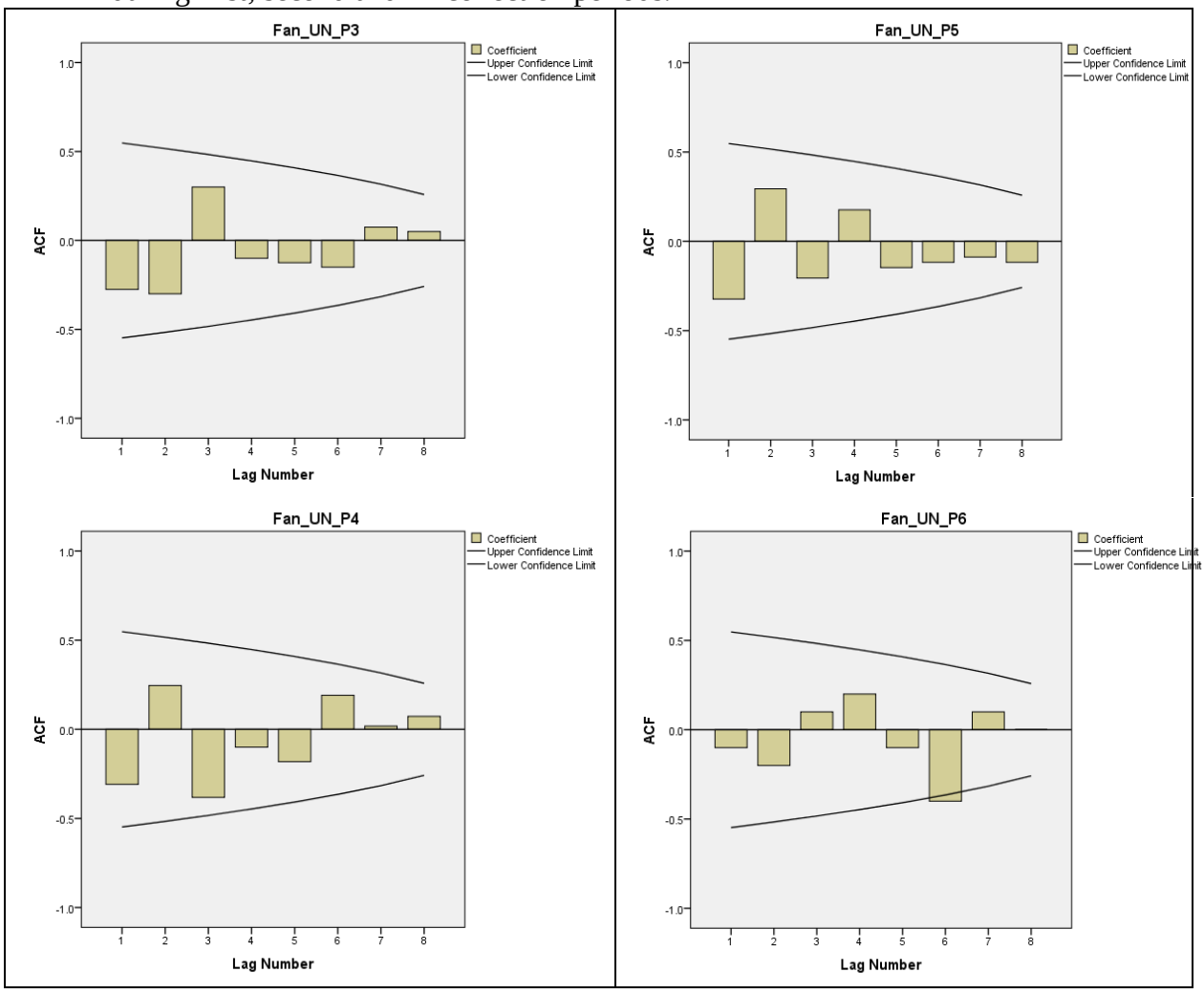
Appendix 3.4. A: Autocorrelation function (ACF) plots of family Fannidae in liquid manure treated strip during collection periods 3 to 6 (P3 to P6). Fly numbers are not sufficient for analysis during first, second and 7th collection periods.



Appendix 3.4. B: Autocorrelation function (ACF) plots of family Fannidae in mink compost treated strip during collection periods 3 to 6 (P3 to P6). Fly numbers are not sufficient for analysis during first, second and 7th collection periods.



Appendix 3.4. C: Autocorrelation function (ACF) plots of family Fannidae in untreated strip during collection periods 3 to 6 (P3 to P6). Fly numbers are not sufficient for analysis during first, second and 7th collection periods.



Appendix 3. 5: Residual vs. fit plot and Q-Q plot for all the analysis, confirm the best models (Poisson distribution within log link) for all the data.

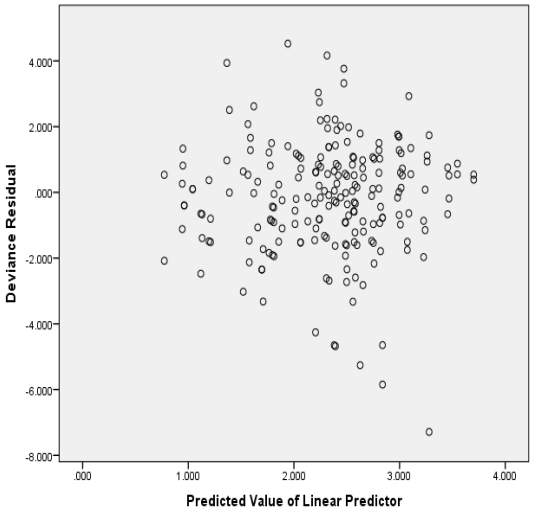


Figure i .Residual vs. fit plot for total number of flies showing the homogenous residuals confirming the Poisson distribution model is appropriate for total number of flies.

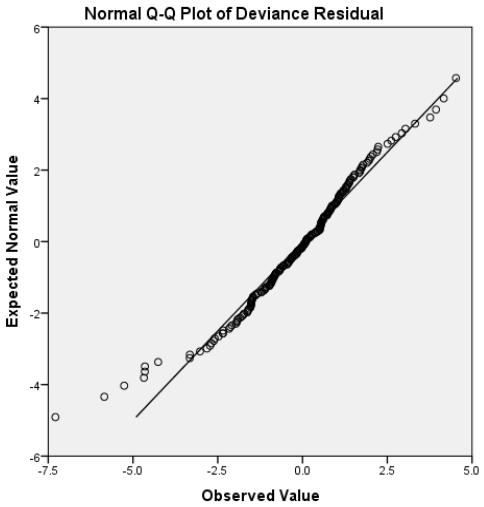


Figure ii. Q-Q plot shows residuals are normally distributed demonstrating the Poisson distribution model is appropriate for total number of flies.

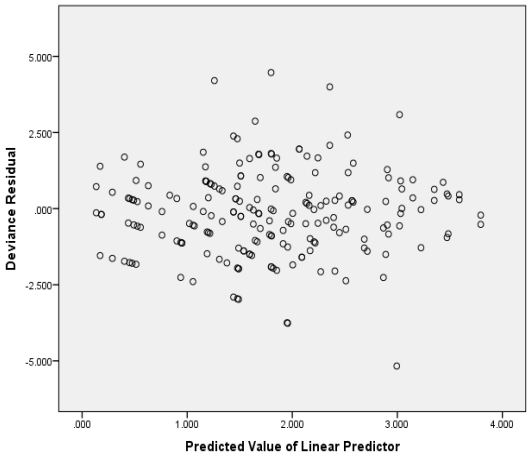


Figure iii. Residual vs. fit plot shows the residuals are homogenous demonstrating the Poisson distribution model is appropriate for family Anthomyiidae.

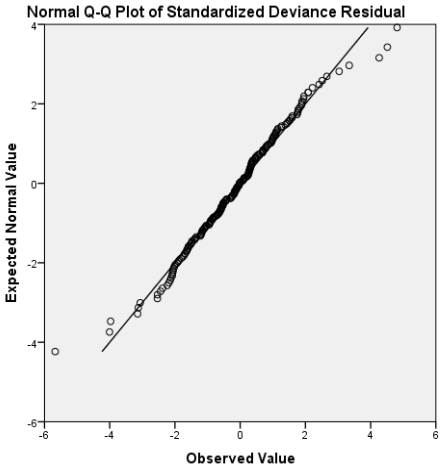


Figure iv. Q-Q plot shows the residuals are normally distributed, demonstrating the Poisson distribution model is appropriate for family Anthomyiidae.

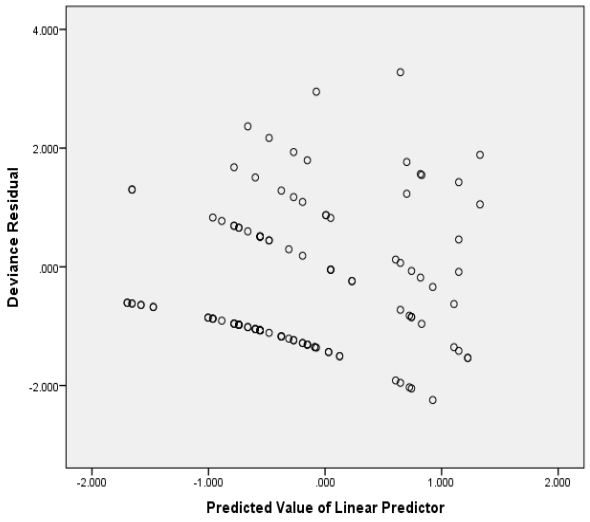


Figure v. Residual vs. fit plot shows residuals are homogeneous demonstrating the Poisson distribution model is appropriate for the family Muscidae.

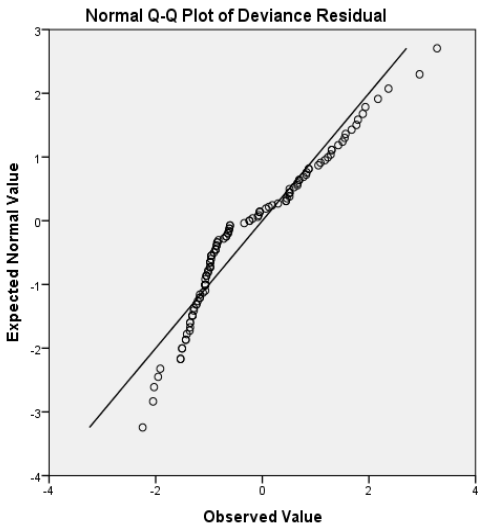


Figure vi. Q-Q plot shows the residuals are normally distributed, demonstrates Poisson distribution model is appropriate for the family Muscidae.

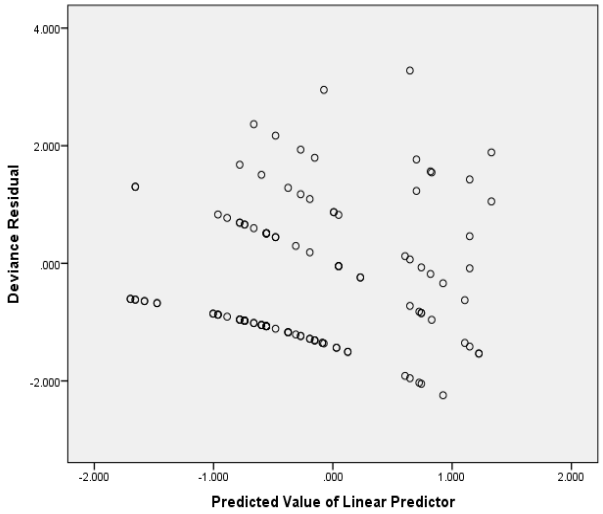


Figure vii. Residual vs. fit plot showing the homogenous residuals confirming the Poisson distribution model is appropriate for the data of family Fannidae.

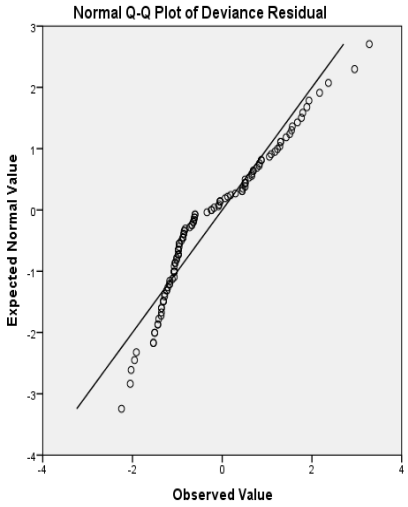


Figure viii. Q-Q plot shows residuals are normally distributed demonstrating the Poisson distribution model is appropriate for the family Fannidae.

Appendix 3. 6: Data of captured flies (*F. canicularis* and *Fannia* spp.) in yellow sticky cards during 2015 collection periods. Numbers 1-10 indicates spots/locations of sticky traps.

Strips	**No. of *F . canicularis***	**No. of *Fannia* spp.**	**Collection Periods**
Un 1	1	0	P1
Un 2	Card missing	Card missing	P1
Un 3	0	0	P1
Un 4	0	0	P1
Un 5	0	0	P1
Un 6	0	0	P1
Un 7	0	0	P1
Un 8	0	0	P1
Un 9	0	0	P1
Un 10	0	0	P1
LQ 1	0	0	P1
LQ 2	0	0	P1
LQ 3	0	0	P1
LQ 4	0	0	P1
LQ 5	0	0	P1
LQ 6	0	0	P1
LQ 7	0	0	P1
LQ 8	0	0	P1
LQ 9	0	0	P1
LQ 10	0	0	P1
MC 1	0	0	P1
MC 2	0	0	P1
MC 3	0	0	P1
MC 4	0	0	P1
MC 5	0	0	P1
MC 6	0	0	P1
MC 7	0	0	P1
MC 8	0	0	P1

MC 9	0	0	P1
MC 10	0	0	P1
Un 1	0	0	P2
Un 2	0	0	P2
Un 3	0	0	P2
Un 4	0	0	P2
Un 5	0	0	P2
Un 6	0	0	P2
Un 7	0	0	P2
Un 8	0	0	P2
Un 9	0	0	P2
Un 10	0	0	P2
LQ 1	0	0	P2
LQ 2	0	0	P2
LQ 3	0	1	P2
LQ 4	0	0	P2
LQ 5	0	0	P2
LQ 6	0	0	P2
LQ 7	0	0	P2
LQ 8	0	0	P2
LQ 9	0	0	P2
LQ 10	0	0	P2
MC 1	0	0	P2
MC 2	0	0	P2
MC 3	0	0	P2
MC 4	0	0	P2
MC 5	0	0	P2
MC 6	0	0	P2
MC 7	0	0	P2
MC 8	0	0	P2
MC 9	0	0	P2

MC 10	0	0	P2
Un 1	0	0	P3
Un 2	0	0	P3
Un 3	0	0	P3
Un 4	1	0	P3
Un 5	0	0	P3
Un 6	0	0	P3
Un 7	1	0	P3
Un 8	0	0	P3
Un 9	0	0	P3
Un 10	0	0	P3
LQ 1	0	0	P3
LQ 2	0	3	P3
LQ 3	0	0	P3
LQ 4	0	0	P3
LQ 5	0	0	P3
LQ 6	2	0	P3
LQ 7	0	0	P3
LQ 8	1	0	P3
LQ 9	0	0	P3
LQ 10	Card missing	Card missing	P3
MC 1	0	0	P3
MC 2	0	1	P3
MC 3	0	1	P3
MC 4	0	0	P3
MC 5	1	1	P3
MC 6	0	0	P3
MC 7	0	0	P3
MC 8	0	0	P3
MC 9	Card missing	Card missing	P3
MC 10	0	0	P3

Un 1	1	0	P4
Un 2	0	1	P4
Un 3	1	0	P4
Un 4	0	0	P4
Un 5	0	0	P4
Un 6	0	0	P4
Un 7	0	1	P4
Un 8	0	0	P4
Un 9	1	1	P4
Un 10	0	0	P4
LQ 1	0	0	P4
LQ 2	1	1	P4
LQ 3	0	0	P4
LQ 4	0	2	P4
LQ 5	0	0	P4
LQ 6	0	0	P4
LQ 7	0	3	P4
LQ 8	0	0	P4
LQ 9	0	0	P4
LQ 10	0	1	P4
MC 1	0	0	P4
MC 2	0	5	P4
MC 3	2	1	P4
MC 4	0	0	P4
MC 5	0	0	P4
MC 6	0	0	P4
MC 7	0	0	P4
MC 8	0	0	P4
MC 9	0	0	P4
MC 10	1	0	P4
Un 1	0	0	P5

Un 2	1	2	P5
Un 3	0	0	P5
Un 4	1	0	P5
Un 5	0	0	P5
Un 6	1	0	P5
Un 7	0	0	P5
Un 8	0	0	P5
Un 9	0	0	P5
Un 10	0	0	P5
LQ 1	0	1	P5
LQ 2	0	1	P5
LQ 3	0	4	P5
LQ 4	0	6	P5
LQ 5	0	6	P5
LQ 6	0	8	P5
LQ 7	0	3	P5
LQ 8	0	1	P5
LQ 9	0	1	P5
LQ 10	0	2	P5
MC 1	0	5	P5
MC 2	2	0	P5
MC 3	1	0	P5
MC 4	0	1	P5
MC 5	0	0	P5
MC 6	1	1	P5
MC 7	0	2	P5
MC 8	0	0	P5
MC 9	0	5	P5
MC 10	1	3	P5
Un 1	0	0	P6
Un 2	0	1	P6

Un 3	0	0	P6
Un 4	Card missing	Card missing	P6
Un 5	0	0	P6
Un 6	0	1	P6
Un 7	0	1	P6
Un 8	0	0	P6
Un 9	0	1	P6
Un 10	0	1	P6
LQ 1	Card missing	Card missing	P6
LQ 2	0	0	P6
LQ 3	0	1	P6
LQ 4	0	1	P6
LQ 5	0	1	P6
LQ 6	0	1	P6
LQ 7	0	2	P6
LQ 8	0	1	P6
LQ 9	0	2	P6
LQ 10	0	2	P6
MC 1	0	1	P6
MC 2	0	0	P6
MC 3	0	2	P6
MC 4	0	0	P6
MC 5	0	5	P6
MC 6	0	1	P6
MC 7	0	8	P6
MC 8	0	1	P6
MC 9	0	0	P6
MC 10	0	2	P6
Un 1	0	0	P7
Un 2	0	0	P7
Un 3	0	0	P7

Un 4	0	0	P7
Un 5	0	0	P7
Un 6	0	0	P7
Un 7	0	0	P7
Un 8	0	0	P7
Un 9	0	0	P7
Un 10	0	0	P7
LQ 1	0	0	P7
LQ 2	0	0	P7
LQ 3	0	0	P7
LQ 4	0	0	P7
LQ 5	0	0	P7
LQ 6	0	0	P7
LQ 7	0	0	P7
LQ 8	0	0	P7
LQ 9	0	0	P7
LQ 10	0	0	P7
MC 1	0	0	P7
MC 2	0	0	P7
MC 3	0	0	P7
MC 4	0	0	P7
MC 5	0	0	P7
MC 6	0	0	P7
MC 7	0	0	P7
MC 8	0	0	P7
MC 9	0	0	P7
MC 10	0	0	P7