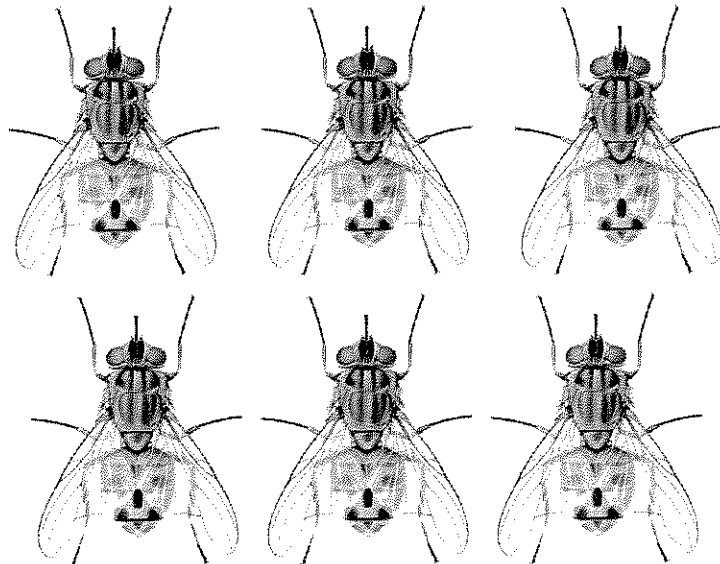




WATER CORPORATION

INVESTIGATION OF THE FACTORS (WEEKS EXPOSED, AMBIENT TEMPERATURE, RAINFALL, MOISTURE, pH AND NH₄) AFFECTING FLY BREEDING IN BIOSOLID CAKE

NOWERGUP REPORT 2002-2003



CENTRE FOR FORENSIC SCIENCE

UNIVERSITY OF WESTERN AUSTRALIA
NEDLANDS WA 6909 TELEPHONE (08) 6488 7288

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Prepared for:

WATER CORPORATION

Nowergup Site
PO Box 7512, Leederville, Western Australia, 6850

By:

Ian Dadour & Sasha Voss

CENTRE FOR FORENSIC SCIENCE

University of Western Australia
Mail Bag M420
35 Stirling Highway, Crawley 6009
PERTH, Western Australia

Cover Photo
Stomoxys calcitrans
Commonly Called the Stable Fly

TABLE OF CONTENTS

<i>TABLE OF CONTENTS</i>	3
1. <i>EXECUTIVE SUMMARY</i>	4
2. <i>ISSUE/BACKGROUND</i>	5
3. <i>INTRODUCTION</i>	7
4. <i>AIMS AND OBJECTIVES</i>	8
5. <i>METHODOLOGY</i>	9
5.1 Study Sites	9
5.2 Experimental Design	9
5.3 Set Up and Sampling	10
5.4 Fly Activity Assessment	11
5.4 Data Analysis	12
6. <i>RESULTS</i>	13
6.1 Fly Breeding in Biosolid Cake	13
6.2 Identification of Factors Influencing Annual Fly Breeding	17
6.3 Factors Influencing Fly Breeding in the First 10 Weeks of Exposure in all Seasons	19
7. <i>DISCUSSION</i>	21
8. <i>CONCLUSIONS AND RECOMMENDATIONS</i>	23
8.1 Conclusions	23
8.2 Recommendations for Best Practice Management	23
8.3 Future Research and Development	24
9. <i>APPENDIX</i>	25
9.1 Primary Nuisance Fly Species	25
10. <i>REFERENCES</i>	27

1. EXECUTIVE SUMMARY

The seasonal pattern of fly breeding in both fresh and aged biosolid cake was established by the exposure of biosolid cake to natural conditions including fly activity over the course of a year. Relationships between fly emergence and six factors (weeks exposed, pH, NH₄, rainfall, temperature and moisture) suspected of facilitating fly breeding were measured. The aim of this study was to investigate fly breeding, with regards to weeks exposed in different seasons. This information will aid in the establishment of a 'best practice' storage procedure for the CBSF.

Four fly species were collected during the study; *Musca domestica* (House Fly), *Stomoxys calcitrans* (Stable Fly), *Fannia canicularis* (Lesser House Fly) and *Muscina stabulans* (False Stable Fly). A total of 5304 flies were identified, however over 97% of these were either *M. domestica* or *S. calcitrans*.

Total fly breeding in biosolid cake occurred all year round with a sharp decline throughout the winter period. The number of emerged flies peaked in the summer months (3163 flies) and only marginally declined during autumn (1545 flies) and spring (532 flies).

Significant positive relationships were observed between the level of fly breeding and ambient temperature ($R^2 = 0.30$, Stepwise Multiple Linear Regression $F_{1,76} = 34.05$, $P < 0.01$), NH₄ content ($R^2 = 0.41$, $F_{1,76} = 52.39$, $P < 0.01$) and weeks exposed ($R^2 = 0.26$, $F_{1,76} = 26.63$, $P < 0.01$). Higher ambient temperatures and a higher NH₄ content of the biosolid cake were associated with increased fly breeding. Similarly, the majority of fly breeding in biosolid cake occurred in the first 10 weeks of exposure to fly activity.

Based on the findings of this investigation it is recommended that the stockpiling of uncovered fresh biosolid cake should be restricted to the months of June through to early October. Throughout the remainder of the year, biosolid cake should be stockpiled in the proposed purpose built biosolid facility and covered for a minimum of 10-12 weeks prior to re-stockpiling either along the Swan Coastal Plain or adjoining agricultural regions. Following this 10-12 week storage duration, biosolid cake may be left uncovered in the purpose built biosolid facility.

2. ISSUE/BACKGROUND

Throughout the agricultural sector, increased fly numbers represent significant economical problems and health issues, especially in relation to the stable fly, *Stomoxys calcitrans* Linnaeus (1758) and the house fly, *Musca domestica* Linnaeus (1758). Several studies have shown that high stable fly densities can result in considerable economic losses throughout rural sectors (Dougherty *et al.*, 1995; Cook *et al.*, 1999). In the United States beef cattle industry, stable flies account for losses of around \$400 million annually (Dougherty *et al.*, 1995). Excessive fly biting and attempts by livestock to dislodge feeding stable flies can cause fatigue, stress, reduced grazing intake and weight loss. In cattle, reduced weight gain can result from infestations of as few as 20 stable flies per animal (Cook *et al.*, 1999). In dairy cattle, heavy infestations (50 flies per animal) can reduce weight gain by 25 percent and milk production by 40-60 percent (Campbell *et al.*, 2001). High numbers of stable and houseflies can also disrupt the efficiency of working animals, such as dogs and horses. In particular, stable flies are vectors of disease carrying organisms and, as such, human health risks may increase in relation to increased fly numbers (Gerozisis & Hadlington, 1995). Predictably, fly breeding in stored manure has become a contentious issue throughout the agricultural sector. As a consequence, the use of new or alternative manure products in agriculture requires the application of both transport and storage guidelines to limit fly breeding.

The Water Corporation produces, transports and temporarily stockpiles approximately 55,000 wet tonnes of biosolid cake annually for direct land application in the agricultural sector. Biosolid cake is a stabilized, organic substance produced by wastewater treatment processes which, in most cases, can be beneficially reused (ARMCANZ, 1995). Once processed, biosolid cake can be used as a low-grade fertilizer in broadacre agriculture, forestry plantations and composting. Biosolid cake offers an alternative to conventional fertilizers while the recycling of waste material provides an effective avenue for its disposal. Dewatered biosolid cake is produced at two facilities in Western Australia; Woodman Point and Beenyup Wastewater Treatment Plants (WWTP). Biosolid cake produced at the Beenyup WWTP has 86% water content, while biosolid cake from Woodman Point WWTP has a lower water content of 82%. A drier pelletised product (3636 wet tonne) also applied to agricultural land, with a water content of only 7%, is produced at Subiaco WWTP.

The extensive transporting and stockpiling of biosolid cake has the potential to facilitate the translocation of different fly species between areas and contribute to an increase in overall fly populations. In light of this concern, the WA Guidelines for Direct Land Application of Biosolids and Biosolid Products (February 2002) considers and addresses these concerns as developed by the Government of Western Australia. As the Water Corporation strictly adheres to these guidelines, it is current policy to avoid recognized stable fly breeding areas such as Gingin, Wanneroo and Kwinana Shires when selecting agricultural reuse locations. In addition, biosolid cake is covered with a securely fitted plastic, roll-on tarpaulin during vehicle transport to limit fly strike on route. Pellets are transported between sites in covered, 2 x 20m³ disposal bins.

Once an agricultural reuse site is selected, a temporary biosolid cake stockpile is established. Uncovered stockpiles are situated in paddocks where the biosolid cake is to be applied. In most cases, application of biosolid cake involves the 'spreading' of between 4 and 8 dry t/ha over the

paddock surface. The biosolid cake is usually incorporated into the topsoil within 36hrs of spreading. During mid-summer incorporation of biosolid cake may be delayed if the soil is considered too dry, thus preventing top soil erosion. Due to the annual nature of farming practices, stockpiles may remain in place for a maximum of eight months. A monitoring program has been established to evaluate fly breeding levels during temporary stockpiling so that appropriate action can be taken in response to excessive fly breeding.

The practice of temporarily stockpiling biosolid cake at agricultural sites has been identified as a potential environmental risk with regards to excessive fly breeding and the associated public health and agricultural/economic concerns posed. In response, a centralised biosolid storage facility (CBSF) will be constructed in the Gillingara shire to service the storage needs of the current agricultural reuse sites. The construction of the facility, capable of housing 40,000 wet tonnes of biosolid cake, has been scheduled for 2005.

The WA Guidelines for Direct Land Application of Biosolids and Biosolid Products (February 2002) stipulate that biosolid cake stockpiles must be covered to limit fly breeding. Research has demonstrated that either full plastic or shade cloth covers reduce fly breeding when used over biosolid material (Nowergup Report, 2000a). Plastic covers act as a barrier to fly entrance and oviposition, protect against rainfall events and hinder the potential dispersal of newly emerged flies. However, the size of the covers required at the CBSF present handling problems and the costs associated with the construction/management of the covers is high. Investigation of potential alternative practices to all-year round covering of stockpiles of biosolid cake would assist in the establishment of a more cost effective and practical 'best practice' storage procedure for the CBSF.

3. INTRODUCTION

Ongoing monitoring of stockpiles of biosolid cake exposed to natural conditions over several years has noted regular seasonal fluctuations in fly breeding in these stockpiles. At farming properties in both the Gillingarra and Yerecoin regions nuisance fly breeding in stockpiles of biosolid cake reaches a peak during the warmer months between October-April and fly breeding is negligible throughout the colder months between June-September. The climatic conditions associated with the winter period are also linked in the literature to a decline in the adult fly populations and hence less breeding activity (Paulin *et al.*, 1998). Therefore the necessity to cover stockpiles of biosolid cake during the winter and cooler parts of the year may be reduced due to this observed seasonality of fly breeding.

In addition, where stockpiles of biosolid cake are exposed to environmental conditions, the chemical state of the biosolid cake can be altered over time. Factors such as the water content, ammonium levels (NH₄) and pH of the biosolid cake can change considerably over time when stockpiled in exposed conditions. Prior monitoring of stockpiled biosolid cake has indicated that the age of the biosolid cake may influence its attractiveness as a fly breeding resource. It appears that biosolid cake aged more than 6 months no longer facilitates fly breeding.

Previous research has identified that the moisture content of the biosolid cake influences both stable and house fly breeding. Low moisture content (<40% moisture) results in a sharp decrease in fly breeding success (Nowergup Report 2000b). The production of a drier biosolid product prior to stockpiling would clearly reduce fly breeding initially, but changes in the moisture content of the biosolid cake are possible during storage. Periods of rainfall may increase the moisture content of the biosolid cake resulting in a potential increase in fly breeding. Observations of 4m³ uncovered biosolid cake have noted that aged biosolid material appears to be hydrophobic (Nowergup Report, 2000b). The dry external crust of the cake acts as a water barrier and rainfall runs off the surface of the biosolid cake to the ground. Investigations into the effect of rainfall events on larger, aged stockpiles has indicated that following an early winter rainfall event, aged biosolid cake appears to remain an unattractive fly breeding resource. However, this may not be the case following a summer rainfall event given the more favorable seasonal conditions and the higher fly activity associated with this period.

Based on research to date, fly breeding appears limited by the age of biosolid cake with respect to seasonal conditions. Understanding the relationship between fly breeding and the changes in the chemical and physical composition of stockpiled biosolid cake over time will be potentially useful in determining how fly breeding can be reduced. Thus, further investigation will aid in the establishment of a 'best practice' storage procedure for the CBSF.

4. AIMS AND OBJECTIVES

1. To identify the factors facilitating fly breeding in stockpiles of biosolid cake. Measured factors include, ambient temperature, NH_4 content, rainfall, moisture content, pH levels, and the age of the biosolid cake.
2. To assess the seasonality of fly breeding in biosolid cake on the Central Coastal region of Perth, Western Australia.
3. To establish the age (in weeks) after which biosolid cake is no longer an attractive resource for fly breeding.
4. To determine if, following long term storage, fly breeding is supported in biosolid cake especially when exposed to repeated rainfall events.

5. METHODOLOGY

5.1 Study Sites

Nowergup Biosolids Facility

Approval was sought from the Department of Environmental Protection and the Health Department of Western Australia to conduct trials involving the biosolid cake at the Nowergup Biosolids Facility. The facility is situated in Nowergup along Wesco Road, 36 km NNW of Perth. The site is leased by the Water Corporation for use of biosolid cake in both the compost and temporary storage areas of the site.

Shenton Park Field Station

Upon completion of the environmental exposure component of the study at the Nowergup facility, the biosolid cake was transferred to a laboratory facility situated at Shenton Park. The Shenton Park Field Station is owned by the University of Western Australia and part leased by the Centre for Forensic Science for consultancy purposes. Biosolid cake was temporarily stored at the Shenton Park Field Station and then following the completion of analysis the biosolid cake was returned to the Nowergup Biosolid Facility.

Subiaco Wastewater Treatment Plant

Biosolid cake sub-samples were analysed by the Water Corporation Subiaco Wastewater Treatment Plant, located on the corner of Brockway Road and Lemnos Street in Subiaco.

5.2 Experimental Design

The seasonal pattern of fly breeding in both fresh and aged biosolid cake was established by the exposure of biosolid cake to climatic conditions and fly activity over the course of one year. In addition, factors suspected of facilitating fly breeding were measured throughout the duration of the study and any relationship observed between fly breeding levels and individual factors was assessed. The following factors were investigated:

- weeks exposed
- ambient temperature,
- rainfall,
- the moisture content of the biosolid cake,
- the pH of the biosolid cake,
- the NH₄ content of the biosolid cake.

5.3 Set Up and Sampling

Fresh biosolid cake was delivered to the Nowergup Treatment Facility from the Woodman Point Wastewater Treatment Plant every two weeks for a period of 12 months commencing on the 8th of October 2002. Set up and sampling regimes were conducted every two weeks such that a total of 26 sampling periods occurred over the duration of the year. At each two week sampling period a series of 60 litre tote boxes measuring 650 x 420 x 280mm were three-quarters filled with biosolid cake using a shovel. Each tote box was fitted with two 50mm drainage holes. Fine mesh was heat moulded over the holes to allow water drainage but prevent fly larvae moving out of the holes. All equipment was cleaned after each sample date to avoid contamination.

On the first sample date (08/10/02), 78 tote boxes were set up in the above manner. Tote boxes were labeled with sample (S1) and replicate numbers (R1-R78). Three tote boxes were immediately removed as controls for the biosolid cake (**Photo 1**). The remaining 75 tote boxes were left exposed to natural conditions and fly activity. Fourteen days later (22/10/02) a fresh delivery of biosolid cake was used to set up an additional 75 tote boxes (S2) (three fewer than the previous sample). Again, three S2 tote boxes were removed as controls for the biosolid cake. A further three tote boxes from the S1 sample period were also removed from the site for examination of fly breeding and chemical analysis. The remaining S1 and S2 boxes were left exposed at the Nowergup site until the next sampling period. This pattern, of setting up fresh biosolid cake in tote boxes (three fewer than the previous sample period) and removing three totes boxes from every sample group, continued every two weeks for a total of 26 sampling periods such that on the final sampling date (S26 – 23/09/03) only three new tote boxes were set up in total. The oldest remaining biosolid cake (S1 totes) was exposed for a total of 50 weeks.

Throughout the duration of the study a weather station located at the Nowergup site recorded hourly precipitation and ambient temperature.

Photo 1: Tote box setup at Nowergup



5.4 Fly Activity Assessment

Tote boxes removed at each sampling period were covered with mesh on site (to prevent further fly strike) and transported by road to the Shenton Park Field Station. A sub-sample of biosolid cake was collected from each set of three tote boxes using a small, clean trowel. This combined sub-sample was then sealed in a clean 500mL wide mouth screw-top plastic jar, labeled with the appropriate sample period and replicate number and delivered to the Subiaco Waste Water Treatment Plant for analysis by the Water Corporation laboratory. Biosolid cake sub-samples were analysed for moisture content, pH and NH₄ levels.

Following sub-sampling the tote boxes were watered and set up at the Shenton Park Field Station. A fly proof mesh cover was fitted over each tote box (**Photo 2**). At the apex of the mesh cover a fly trap was secured at a height of 2 metres. After two weeks, the mesh cover was surrounded with a black plastic bag (punctured with small air-holes to reduce humidity). The function of the black bag was to direct any emerging flies into the fly trap. After six weeks all trapped flies were dead and following collection were identified (based on current taxonomic keys) and counted. All remaining biosolid cake was subsequently returned to the Nowergup discard site.

Photo 2: Fly emergence traps at Shenton Park



5.4 Data Analysis

Total fortnightly fly emergence and the mean of the six measured factors (weeks exposed, pH, NH₄, rainfall, temperature and moisture) was calculated. Due to the skewed nature of the fly emergence data set, the data was transformed using natural logarithm prior to statistical analysis.

Linear Regression analysis was performed to assess the relationship between each of the independent factors and fly breeding levels. The linear statistical significance of each of the relationships was evaluated and the percentage of variance accounted for by each factor determined. Stepwise Multiple Linear Regression analysis was performed to determine the minimum number of factors required to predict total fly breeding. Spearman's Rank Correlations were computed between all factors measured to assess the linear relationship between fly breeding and each of the independent factors and the interrelationships of the independent factors to one another.

The above analysis was performed on data spanning the entire study duration and again using a data set specific to the first 10 weeks of exposure of biosolid cake to fly activity (see below). Significance was determined at the 0.01 level (2-tailed). Analysis was performed using SPSS 12.0 for Windows.

6. RESULTS

6.1 Fly Breeding in Biosolid Cake

Throughout the duration of the study a total of 5304 flies emerged from biosolid cake contained within the tote box replicates. Five fly species were identified from fly traps (**Table 1**)

Table 1: Species and number of flies emerged from biosolid cake.

Fly Species	Total Number of Emerged Flies
<i>Musca domestica</i> (House fly)	3110
<i>Stomoxys calcitrans</i> (Stable fly)	2036
<i>Fannia canicularis</i> (Lesser House fly)	143
<i>Musca stabulans</i> (False Stable fly)	14

All four species have previously been associated with manures used in crop production and with biosolid cake stored at the Nowergup Biosolids Facility (Nowergup Report 2000b, Nowergup Report 2002). In addition, representatives from the family Syrphidae were also identified within fly traps. Previously, this group of flies has only been identified in association with stockpiles of lime amended sludge stored at the Nowergup Biosolids Facility (Dadour *pers com*).

M. domestica followed by *S. calcitrans* were the most dominant species collected in fly traps. **Figure 1** depicts the seasonality of the four fly species breeding in biosolid cake throughout each two week sampling period over the duration of the study. Both *M. domestica* and *S. calcitrans* were observed to breed throughout the year with a seasonal peak throughout summer (03/12/03-25/02/04) and a marked decline in breeding in winter (03/06/03-26/08/03). Sporadic occurrence of *F. canicularis* within fly traps was noted throughout the year, although numbers were comparatively low. In contrast, *M. stabulans* were only observed to breed within biosolid cake throughout November and December. Finally, representatives of the family Syrphidae occurred primarily in September following heavy rainfall but total numbers were negligible.

Fly breeding in biosolid cake occurred all year round with a sharp decline throughout the winter period. The number of emerged flies peaked in the summer months and only marginally declined during autumn (11/03/03-20/05/03) and spring (09/09/03-19/11/03). Variation in fly breeding within each seasonal period is clearly depicted in **Figure 2**. Also evident is a marked trend in fly breeding with respect to weeks exposed which reflects the age of the biosolid cake. Regardless of seasonal variation in fly numbers, the majority of fly breeding occurred within the first 10 weeks of exposure (Table 2). Biosolid cake aged beyond this 10 week period supported low fly breeding.

Table 2: The number and percentage of flies that emerged from biosolid cake during the first 10 weeks of exposure to natural conditions in each season.

SEASON	Number of Flies	Percentage of Total Emergence (%)
Summer	3162	99.9
Autumn	1360	88.0
Winter	23	36.0
Spring	531	99.8

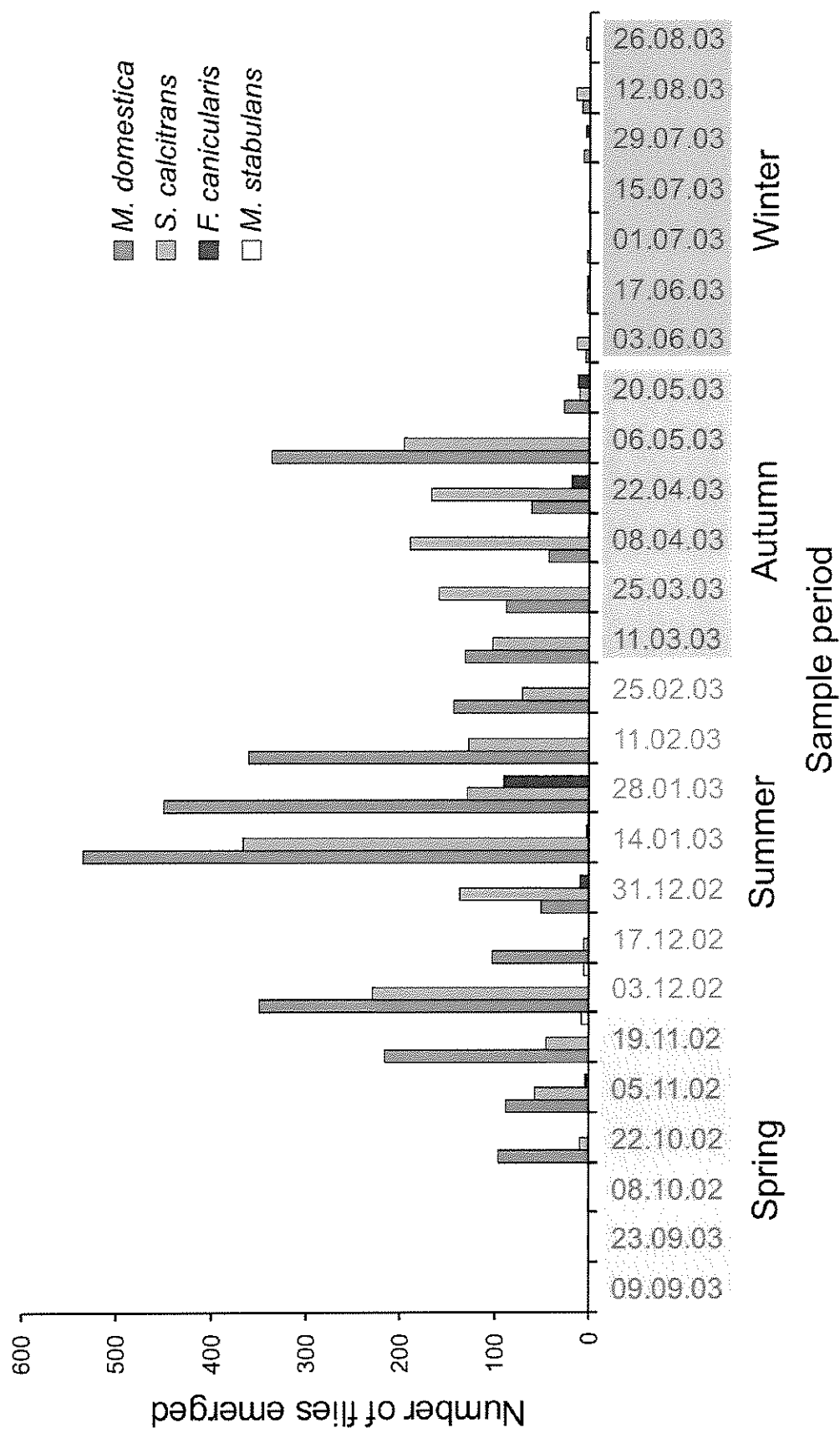


Figure 1: Seasonality of fly breeding in biosolid cake.

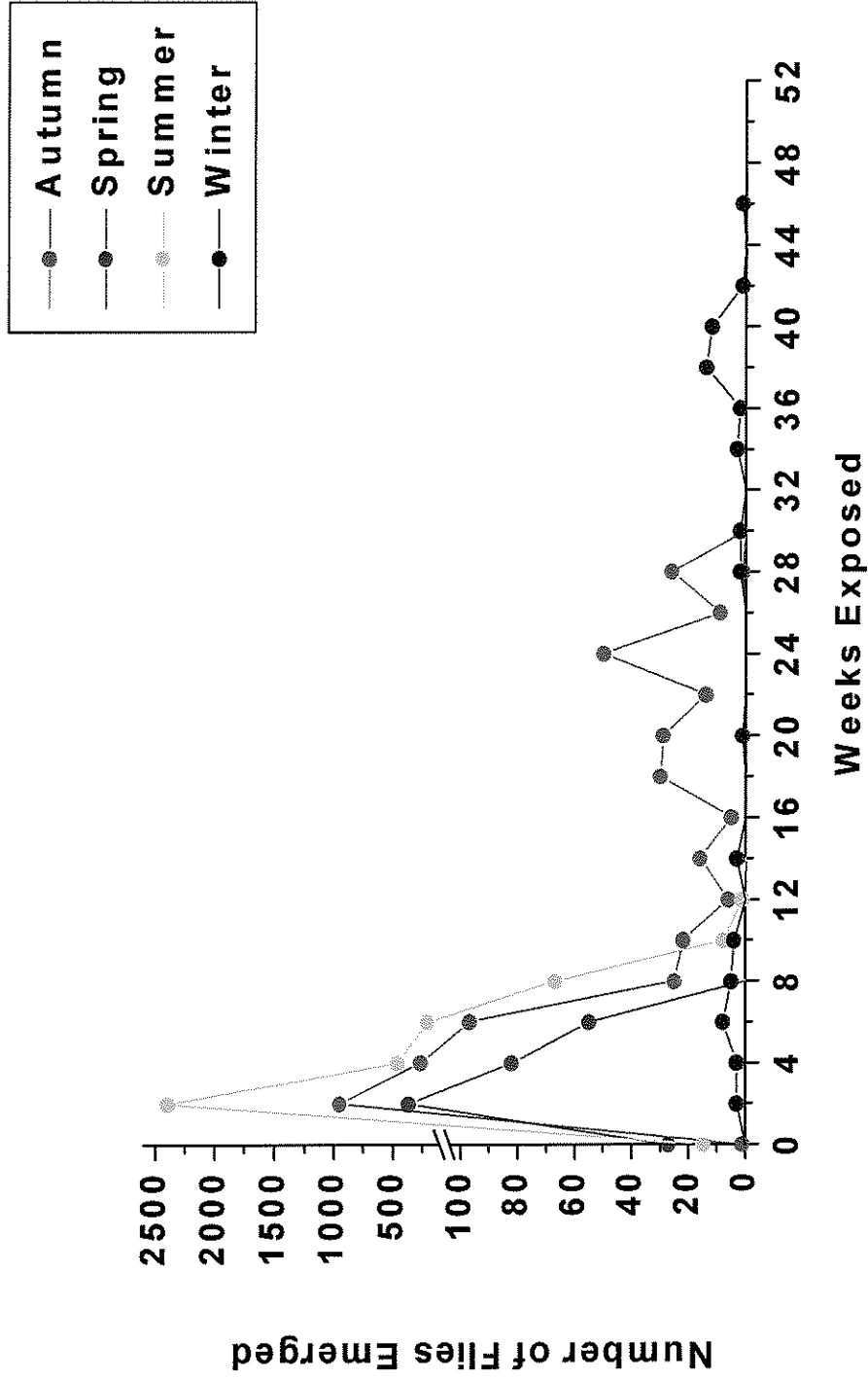


Figure 2: The total number of flies collected from fly traps within each season relative to the age of biosolid cake.

6.2 Identification of Factors Influencing Annual Fly Breeding

Calculation of Spearman's Rank Correlation Coefficient indicated that 3 factors influence fly breeding (weeks exposed, NH₄ and temperature) (Table 3). Linear Regression analyses indicated several factors sequentially influenced fly breeding throughout the duration of the study. Ambient temperature had the greatest influence on fly breeding throughout the year. This was followed by NH₄, the number of weeks exposed to fly activity and to a lesser extent the pH of the biosolid cake (Table 4).

Table 3: Spearman's ranking Correlation Co-efficient for Non-Parametric Step-wise Regression Analysis indicating the relationships between the investigated factors and fly breeding in biosolid cake over 1 year.

Measured Variable	Correlation Coefficient	Significance (2-tailed)	N
Weeks Exposed	- 0.508**	<0.01	78
pH	0.232*	>0.01	78
NH ₄ (ppm)	0.537**	<0.01	78
Rainfall (mm)	- 0.013	>0.01	78
Temperature (°C)	0.568**	<0.01	78
Moisture Content (%)	- 0.058	>0.01	78

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 4: Stepwise Multiple Linear Regression analysis indicating the relationship observed between fly breeding and six factors measured over 1 year.

Investigated Factor	R²	SE	F_{1,76}	P
Ambient Temperature (°C)	0.31	1.85	34.05	<0.01*
NH ₄ Content (ppm)	0.41	1.71	52.39	<0.01*
Weeks Exposed	0.30	1.92	26.63	<0.01*
pH Level	0.09	2.12	7.96	>0.01 NS
Moisture Content (%)	0.00	2.23	0.16	>0.01 NS
Rainfall (mm)	0.05	2.17	4.37	>0.01 NS
Model Summary Predictors: NH ₄ Content and Ambient Temperature	0.51	1.57	39.19	<0.01*

* - Significant Relationship, NS – Not Significant

Ambient Temperature (°C)

Overall a significant positive relationship was observed between the level of fly breeding in biosolid cake and ambient temperature at the Nowergup Treatment Facility ($R^2 = 0.30$, Linear Regression $F_{1,76} = 34.05$, $P < 0.01$). In all seasons except winter the ambient temperature accounted for 30% of the variation in fly breeding. Fly breeding increased with increased ambient temperature (Spearman's Rank Correlation Coefficient = 0.57, $P < 0.01$).

NH₄ (ppm)

A significant positive relationship was observed between fly breeding and the NH₄ content of the biosolid cake ($R^2 = 0.41$, $F_{1,76} = 52.39$, $P < 0.01$). High NH₄ content was associated with a high level of fly breeding such that 41% of the variation in fly breeding was accounted for by the NH₄ content of the biosolid cake (0.54, $P < 0.01$).

Weeks Exposed

A significant negative relationship was observed between the level of fly breeding in biosolid cake and the number of weeks the biosolid cake was exposed to fly activity ($R^2 = 0.26$, $F_{1,76} = 26.63$, $P < 0.01$). Throughout the duration of the study 26% of the variation observed in fly breeding levels was explained by the total number of weeks the biosolid cake was exposed. Fly breeding within the biosolid cake declined as the age of the biosolid cake increased (-0.51, $P < 0.01$).

pH

A significant relationship was observed between fly breeding and the pH of the biosolid cake ($R^2 = 0.08$, $F_{1,76} = 7.96$, $P < 0.05$). Fly breeding was observed to increase with pH to a small degree such that 8% of the variation in fly breeding levels was explained by deviation in the pH of the biosolid cake (0.23, $P > 0.01$).

Moisture Content (%) and Rainfall (mm)

The relationship between the level of fly breeding and the moisture content of the examined biosolid cake was not significant ($R^2 = 0.002$, $F_{1,76} = 0.16$, $P > 0.01$). Similarly, no significant relationship was found between fly breeding levels and rainfall ($R^2 = 0.05$, $F_{1,76} = 4.37$, $P > 0.01$). Based on the calculated correlation coefficient, rainfall was the least important factor influencing fly breeding within biosolid cake (-0.013, $P > 0.01$).

The resulting model presented by analysis of the data using Stepwise Multiple Linear Regression indicated that the minimum set of factors required to predict fly breeding were NH₄ content and ambient temperature. Both factors combined accounted for 51% of the variation observed in fly breeding levels throughout the trial (Stepwise Multiple Linear Regression $R^2 = 0.51$, $F_{1,76} = 39.19$, $P > 0.01$).

6.3 Factors Influencing Fly Breeding in the First 10 Weeks of Exposure in all Seasons

As illustrated in **Figure 2**, graphical examination of emergence data indicated that most fly breeding in biosolid cake occurred in the first 10 weeks of exposure to natural conditions. To examine the effect of the same 6 factors on fly breeding during this period an additional analysis was conducted.

Ambient Temperature (°C)

Within the first 10 weeks, the resulting model presented by analysis of the data using Stepwise Multiple Linear Regression indicated that ambient temperature represented the best predictor of fly breeding. A significant positive relationship was observed between the level of fly breeding and ambient temperature (**Table 5 & 6**). Ambient temperature accounted for 36% of the variation observed in fly breeding in the first 10 weeks of exposure (Stepwise Multiple Linear Regression $R^2 = 0.36$, $F_{1,22} = 12.63$, $P < 0.01$).

Weeks Exposed, NH₄ (ppm) and pH

The relationship between the NH₄ content of biosolid cake and fly breeding was not significant in the first 10 week period of exposure (Linear Regression $R^2 = 0.16$, $F_{1,22} = 4.26$, $P > 0.01$). Similarly, no significant relationship was observed between fly breeding and the age of the biosolid cake within the same period ($R^2 = 0.04$, $F_{1,22} = 0.97$, $P > 0.01$). Lastly, no significant relationship was observed between fly breeding and the pH of the biosolid cake ($R^2 = 0.04$, $F_{1,22} = 0.88$, $P > 0.01$).

Table 5: Stepwise Multiple Linear Regression analysis results indicating the relationship between fly breeding and six factors measured during the first 10 weeks of exposure of biosolid cake to fly activity.

Investigated Factor	R²	SE	F_{1,22}	P
Ambient Temperature (°C)	0.36	1.97	12.63	<0.01*
NH ₄ Content (ppm)	0.16	2.26	4.26	>0.01 NS
Weeks Exposed	0.04	2.42	0.97	>0.01 NS
pH Level	0.04	2.42	0.88	>0.01 NS
Moisture Content (%)	0.08	2.36	2.01	>0.01 NS
Rainfall (mm)	0.29	2.08	8.82	<0.01*
Model Summary				
Predictor: Ambient Temperature	0.36	1.97	12.63	<0.01*

* - Significant Relationship, NS – Not Significant

Moisture Content (%) and Rainfall (mm)

The relationship between fly breeding and the moisture content of the biosolid cake in the first 10 weeks of exposure was not significant ($R^2 = 0.08$, $F_{1,22} = 2.01$, $P > 0.01$). A significant relationship was found between fly breeding levels and rainfall ($R^2 = 0.29$, $F_{1,22} = 8.82$, $P < 0.01$) indicating that limited fly breeding was associated with rainfall events (Spearman's Rank Correlation Coefficient = -0.49 , $P < 0.01$). However, this factor which represents 29% of the variation in fly breeding adds little to the predictive model.

Table 6: Spearman's ranking Correlation Co-efficient for Non-Parametric Step-wise Regression Analysis indicating the relationships between fly breeding in biosolid cake and the 6 factors in the first 10 weeks of exposure.

Measured Variable	Correlation Coefficient	Significance (2-tailed)	N
Weeks Exposed	- 0.192	>0.01	24
pH	0.241	>0.01	24
NH4 (ppm)	0.354	>0.01	24
Rainfall (mm)	- 0.494**	<0.01	24
Temperature (°C)	0.650**	<0.01	24
Moisture Content (%)	- 0.596	>0.01	24

** Correlation is significant and the 0.01 level (2-tailed)

7. DISCUSSION

This study determined the seasonality of nuisance fly breeding in biosolid cake in the central coastal region of Western Australia. Based on the findings of this investigation fly breeding was observed to be most prevalent throughout the summer months of December through February. While not as pronounced, excessive levels of fly breeding were also observed throughout both autumn and spring. The mean of the average ambient temperature observed throughout autumn was higher than that observed in spring (21.2°C and 14.4°C respectively) which accounts for the variation in fly breeding during these seasons. During the winter months of June through August, fly breeding levels were reduced to negligible levels. While nuisance flies appear active throughout three quarters of the year, this activity is predominantly related to ambient temperature. As ambient temperature increases, fly breeding associated with stored biosolid cake increases.

Throughout the year it was also determined that both ambient temperature and the NH_4 content of the biosolid cake were the factors found to influence fly breeding. Given the positive relationship observed between fly breeding and NH_4 content, high concentrations of NH_4 appear to increase the attractiveness of biosolid cake as a fly breeding resource. Reducing the NH_4 content during biosolid cake production may contribute to a decline in fly breeding levels within biosolid cake. Under natural conditions as the biosolid cake ages both the NH_4 and the level of fly breeding declines indicating that it is no longer attractive as a resource (Paulin *et al* 1998).

Other chemical factors investigated included pH and moisture content. While there is some evidence to suggest that biosolid cake associated with a slightly acidic pH are less favorable fly breeding resources, the variation observed in pH, showed no consistent pattern in regard to the age of the biosolid cake. Similarly, while the moisture content of biosolid cake was observed to decline over time, this pattern was impacted on by the effect of rainfall and season. Regardless of variation in the moisture content of the biosolid cake, fly breeding declined and remained low in all seasons following the first 10 weeks in which the biosolid cake was exposed to natural conditions.

Identification of the factors influencing fly breeding in fresh biosolid cake (<10 weeks old) indicated that ambient temperature had the predominant impact of fly breeding levels. The NH_4 content of fresh biosolid cake was no longer found to significantly affect fly breeding. However, this appears to be related to the minimal change observed in NH_4 content over the short 10 week duration. Certainly, the initial NH_4 content of the control samples remained consistent over several weeks before a gradual decline was noted. Furthermore, no significant relationship was identified between fly breeding and the age of the biosolid cake in the first 10 weeks of exposure to fly activity.

A significant negative relationship was observed between rainfall and fly breeding within fresh biosolid cake (<10 weeks old). During the first 10 weeks of exposure to fly activity, in any season, increased rainfall was linked to low fly breeding levels. Given the absence of a relationship between moisture content and fly breeding the observed decline in fly breeding with

rainfall suggests that fly activity is limited in rainfall periods. This is more so during winter when the ambient temperature is cooler.

Previous research (Nowergup Report, 2000b) has indicated that fly breeding is minimized where the moisture content of biosolid cake is less than 40%. Throughout the duration of this study the moisture content of the biosolid cake dropped below 40% on several occasions. However, no relationship was observed between fly breeding and the moisture content of the biosolid cake in either the short (<10 weeks) or long term (>10 weeks). Low moisture content (<40%) was generally observed once the biosolid cake had aged beyond the first 10 weeks. The previous study investigated the impact on fly breeding of reducing the moisture content of biosolid cake. However, moisture content was lowered during production rather than as a result of the gradual loss of water through evaporation over time while exposed to natural conditions. The study duration was relatively short (11 days) and was conducted during autumn (March-April) suggesting that little change in moisture content would have occurred post-storage during the investigation (Nowergup Report, 2000b).

As stated above throughout the duration of this study the moisture content of the biosolid cake declined gradually over time and, in aged biosolid cake (< 10 weeks), a moisture content of less than 40% was observed. However, following periods of rainfall the moisture content of aged biosolid cake increased above this level repeatedly without an associated increase in fly breeding. This suggests that aged biosolid cake is not an attractive fly breeding resource regardless of the moisture content. Rather it appears to be linked to the low NH₄ content associated with aged biosolid cake.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

1. Over the 1 year study ambient temperature was the primary factor influencing fly breeding in biosolid cake. Secondary to this, fly breeding was further influenced by variation in the NH_4 content of the biosolid cake.
2. The seasonality of fly breeding within biosolid cake is primarily driven by the ambient temperature and season. Fly breeding was observed for three quarters of the year with a marked decline in the cooler winter months (June through September).
3. Biosolid cake aged more than 10 weeks does not appear to facilitate excessive fly breeding, regardless of season and temperature variations.
4. The decline in the attractiveness of the biosolid cake as a fly breeding resource appears linked to the associated gradual decline in NH_4 content.
5. The exposure of aged biosolid cake to climatic conditions, such as rainfall events, does not appear to facilitate a reinitiation of fly breeding. Furthermore, corresponding increases in moisture content associated with rainfall events do not appear to facilitate increased fly breeding in aged biosolid cake.

8.2 Recommendations for Best Practice Management

1. Reducing the NH_4 content of the biosolid cake product (< 4000ppm) prior to stockpiling will reduce fly breeding.
2. Stockpiling of fresh biosolid cake throughout the Swan Coastal Plain should be restricted to the months of June through to early October.
3. Throughout the remainder of the year, biosolid cake should be stockpiled in the proposed purpose built biosolid facility and covered for a minimum of 10-12 weeks prior to the establishment of stockpiles throughout the Swan Coastal Plain. Rainfall events do not appear to facilitate a second wave of fly breeding.

8.3 Future Research and Development

While this study has established that aged biosolid cake does not facilitate excessive fly breeding it remains to be determined whether the translocation of aged biosolid cake between sites alters this finding. During translocation the outer crust of the biosolid cake is removed. It may be the properties of the outer layer in aged biosolids that accounts for the reduced attractiveness of the aged product to fly breeding.

1. Conduct a trial to determine if the translocation and re-establishment of aged biosolid cake facilitates a second wave of fly breeding. Stockpiles of various ages should be investigated and the NH_4 content of the core and outer crust should be measured.
2. Conduct a trial investigating the impact of reduced NH_4 content on fly breeding levels in stockpiles of biosolid cake exposed to fly activity over a period of 10 weeks.

9. APPENDIX

9.1 Primary Nuisance Fly Species

The stable fly, *Stomoxys calcitrans*, is abundant throughout the temperate and tropical world and has spread through human activities into the Australasian region. It was first recorded in Australia in 1881 but not in Western Australia until 1912 (Cleland, 1912). *S. calcitrans* is 5 – 7 mm long with a pale spot behind the head, indistinct stripes on the thorax, and seven dark spots on the abdomen. While similar in appearance to the housefly the adult stable fly can be easily distinguished by its biting behavior and the presence of a proboscis (a piercing and sucking mouth part). The proboscis is used to pierce the host's skin to obtain a blood meal (Gerozisis & Hadlington, 1995). Its main hosts are cattle and horses although lesser hosts include man (11% of all bites in USA), dogs, pigs and camels. Bites to domestic stock occur mostly on the lower limbs and belly where animal defense mechanisms such as tail swishing and skin twitching are minimal (Dougherty *et al.*, 1995). The adults feed several times per day, generally in the early morning and late afternoon. Following feeding the fly will move into the shade to digest the meal undisturbed.

Blood meals are obligatory for females before oviposition. A single female can lay between 500 and 600 eggs in her lifetime (22-58 days) creating the potential for rapid population growth. Eggs hatch in 2-5 days and mature to reproductive status in 14-26 days (Gerozisis & Hadlington, 1995). Eggs are commonly deposited in moist and rotting straw (stable litter) especially when mixed with urine or dung (Seddon, 1951). Stable fly larvae have also been recorded in grass clippings (Bull, 1919), rotting vegetation (Roberts, 1952), drifts at the edge of swamps, poultry manure (Dadour, 1994) and associated with horse, kangaroo, wallaby and rabbit droppings (Place, 1915).

Depending on weather conditions, stable flies typically appear in mid-spring, become severe in early summer, and decrease towards the end of summer (Goncalves-Neuza & Veiga, 1994). Monitoring of fly activity during winter indicated that stable flies overwinter as slowly developing larvae in favorable breeding areas (Berkebile *et al.*, 1994). Scant evidence in the literature exists on the stable fly reaching pest proportions (Roberts, 1952).

The cosmopolitan housefly, *Musca domestica*, is associated with man wherever any settlement is made. It was first recorded in Australia in 1849 (Walker, 1849). Adults are similar in size and appearance to stable flies with the exception of the mouth parts. *M. domestica* has sponge-type mouthparts, which are used in the uptake of liquid foods and the regurgitation of saliva to liquefy solid foods for ingestion. The life cycle of the house fly is around 2 – 4 weeks. An adult female can produce between 200 - 500 eggs during her lifetime (Gerozisis & Hadlington, 1995). It breeds in human, horse, cattle, pig, poultry, sheep and kangaroo dung, rotting vegetable matter, kitchen refuse, lawn clippings and carrion (see Pont, 1973 for review). Eggs hatch in less than two days while larval development and pupation time depends largely on temperature (Gerozisis

& Hadlington, 1995). Breeding occurs throughout the year in warmer parts of Australia (Johnston, 1922). Both activity and breeding are strongly influenced by temperature. During colder seasonal conditions houseflies overwinter as larvae although adults can persist indoors given warmer conditions. Winter breeding has been noted indoors (West, 1951).

There are numerous accounts of the house flies' relationship to public health (West, 1951) and it is best known as a vector of bacteria and protozoa that cause enteric disease (Greenberg, 1971). It is found indoors, and although not attracted to man *per se*, alights on food and food preparation areas. Both sexes settle on and around bacteria harboring breeding sites such as animal waste products and then alternate to food sources that are commonly found in and around human habitation. Bacteria is transferred in the process posing a considerable health risk to humans and rural livestock. (Gerozisis & Hadlington, 1995).

Adult flies of both species are strong fliers, and the recorded flight range of 32 km (Bishopp and Laake 1921) is probably far short of the house flies' ability. The flight range of the stable fly is at least 216 km (Hogsette and Ruff 1985). With these capabilities, it is not surprising that both species can locate desirable breeding sites and develop large populations in a relatively short period.

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