Developing a risk prediction model for the seasonality of *Lucilia* spp. in New Zealand

A thesis presented in partial fulfilment of the requirements
for the degree of

Doctor of Philosophy
in
Veterinary Science

at Massey University, Manawatū, New Zealand.

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2023

MASSEY UNIVERSITY
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Note for Examiners
Explanation of COVID-19 Impacts

Thank you for taking the time to examine this thesis, which has been undertaken during the Covid-19 pandemic. The New Zealand Government's response to Covid-19 includes a system of Alert Levels which have impacted upon researchers. Our University's pandemic plan applied the Government's expectations to our research environment to ensure the health and safety of our researchers, however, research was impacted by restrictions and disruptions, as outlined below.

For a six-week period from March 26 to April 27 2020, New Zealand was placed under very strict lockdown conditions (Level 4 – Lockdown), with students and staff unable to physically access University facilities, unless they were involved in essential research related to Covid-19. All field work ceased and data collection with humans was restricted to online methods, if appropriate. The restrictions were partially lifted on April 27, but students and staff were not generally allowed back into University facilities until May 13.

Ongoing disruptions have also been encountered for some students due to uncertainties over the potential for future Covid-19-related restrictions on activities, and a Covid-19 cluster outbreak based in Auckland in New Zealand on 12 August 2020 led to the imposition of rolling Level 2 (Reduce) and Level 3 (Restrict) conditions until 23 September 2020. Auckland campus based students remained on Level 2 until 7 October 2020. This Alert Level system continues to be utilised throughout 2021.

These changing Alert Levels have meant that some research students had experimental, clinical, laboratory, field work, and/or data collection or analysis interrupted, and consequently may have had to adjust their research plans. For some students, the impacts of Covid-19 stretched far beyond the lockdown period in April/May 2020, as they may have had to significantly revise their research plans.

Overseas travel is not permitted by the University and restrictions have been placed on the New Zealand borders which are closed to non-New Zealand citizens and permanent residents. This meant that international students who were based offshore at the time of lockdown, were unable to return to New Zealand. A small number of offshore students were provided permission to return to New Zealand in early 2021. Many students have also suffered from anxiety and stress-related issues, and have had financial impacts, meaning their research progress has been significantly delayed.

This form, as completed by the supervisor and student, outlines the extent that the research has been affected by Covid-19 conditions.

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Please consider the factors listed below in your assessment of the work.

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Date: 10-May-22
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The principal aim of this PhD was to develop a prediction model for flystrike of sheep in New Zealand. These studies aimed to examine the seasonality of the two main dipteran species, L. cuprina and L. sericata that cause flystrike in New Zealand. From September 2018 until June 2019, weekly sampling of specimens on eight farms across the North and South Islands were undertaken. This was planned to occur the following year also.

However, Covid-19 travel restrictions impeded this. The first stage of the 2019/2020 study from September until December 2019 was conducted successfully. However, during the period from March until June 2020 travel restrictions were imposed during this time resulting in no data being collected aside from one farm. Therefore, Chapters Seven and Eight were not as planned due to missing data.

Experiments had been planned to examine the dependencies of L. sericata and L. cuprina in their life cycle to various indices of temperatures, humidities and decreasing photoperiods. However, during the March to June period of 2020 colonies of L. sericata and L. cuprina in a temperature-controlled room could not be maintained. The data from these studies would have been used to create a prediction model that would have been compared to the observed data sets in 2018/2019 and 2019/2020.

The overall impact of Covid-19 changed the focus of the analysis to a more epidemiological approach in Chapters Seven and Eight. A further chapter was added to complete the thesis which examined the molecular analysis of L. cuprina and L. sericata.

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Abstract

Flystrike of sheep in New Zealand is principally caused by *Lucilia cuprina* and *Lucilia sericata*. A series of studies were conducted to develop models to describe and predict the seasonal occurrence of these *Lucilia* spp. in New Zealand.

Dipterans were collected on a weekly basis on eight farms across New Zealand over three fly seasons (2018/2019, 2019/2020, 2020/2021) using the LuciTrap® with a Stickytrap attachment (*Lucilia* spp. n = 10,559). Covid 19 travel restrictions restricted the collection of samples during the 2019/2020 and 2020/2021 seasons. Dipterans were initially identified using morphological characteristics, with further validation using the nuclear 28s rRNA and the mitochondrial ND4 gene. The morphological identification had an accuracy of 71 % for *L. cuprina* and 55 % for *L. sericata*, compared to the molecular method ($p < 0.05$). Consequently, the counts of both species were combined for modelling purposes.

The seasonality of *Lucilia* spp. adult flies span from early October until late May with variability of weeks duration between farms and three weeks between seasons for individual farms. A hurdle model was used to describe the occurrence of *Lucilia* spp. from the 2018/2019 season ($p < 0.05$). Significant variables include soil temperature, rainfall, maximum temperature and photoperiod with lag times of one to seven weeks.

A second model used the 2018/2019 seasonal data to predict the start of the 2019/2020 season using a mixed-effects logistic regression model using weather data from the closest Virtual Climate Station. 10 cm soil temperature and Soil Moisture Deficit Index predicted the start of the season within two weeks of the observed season ($p < 0.05$).

Four trap and bait combinations were compared to help choose a supplementary on-farm technique to confirm model predictions. A negative binomial model fitted for *Lucilia* spp. catch data found no difference between LuciTrap® combined with LuciLures and the Western Australian Trap combined with sheep liver preserved in 30 % sodium sulphide ($p > 0.05$). While the other two trap and bait combinations were significantly worse for catching *Lucilia* spp. ($p < 0.05$).

These models should provide information to allow farmers to make more informed decisions for flystrike control.

Acknowledgements

I feel the need to use the phrase ‘it takes a village’ when I am reflecting on the work which is outlined in this thesis as I have worked with a number of people across New Zealand over the past number of years. I would firstly like to thank each of my supervisors: Professor William Pomroy, Professor Paul Kenyon, Dr Kristene Gedye and Associate Professor Kevin Lawrence for their help and support over the last number of years. These have been considerably challenging with Covid 19 and the difficult circumstances we have been working through.

Secondly, I would like to thank the New Zealand Merino Company for funding this research and Monica Schwass for her continuous support in the coordination of this project. This project would not be possible without the support of the farm managers who allowed me to put up weather stations and collect samples on their farms. In addition, I would like to thank Cam and Emma Laurie for their help in collecting fly samples. I would like to thank Caitlin Jackson for her help in collecting fly samples as well.

I would like to thank Massey University for awarding me the Vice-Chancellor Doctoral Scholarship. I would also like to thank the School of Veterinary Science for the numerous funding awards for research and travel which I have been awarded over the past number of years. In the parasitology laboratory, the help and support from Barbera Adlington, Ann Tunnicliffe, Dr. Ian Scott, and Frances Miller have been immense whether it was helping me with my microscope, collecting samples and maintaining the insectary. I would also like to thank Anne Tunnicliffe for her help collecting samples on the farms. Tony Russell for his help in making the Western Australian fly traps and giving me a lend of equipment for fieldwork. In the epidemiology laboratory where I did my molecular work, I would like to thank Niluka Velethanthiri, Lynn Rodgers, Sayani Ghosh, Dr. Julie Collins-Emerson, Dr. Shahista Nisa, Dr. Mathew Knox and Dr. Anne Midwinter for their help and for answering my endless questions. Neville Haack for his support and assistance with the setting up of the insectary. In the ecology department, Tracy Harris for her help with the microscope and taking photos of the fly samples.

I am extremely fortunate to have had a fantastic group of office mates during my PhD: Natasha, Jimmy, Emanuelle, Gayani, Cindy, Juliana, James, Jacques, Bo, Andrea, Dinisha, Thanh and Mandefort. I also want to thank my father, sister, and family for their support throughout this journey.

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Chapter One. Review of Literature

1.1 Introduction

Cutaneous myiasis or flystrike has been defined as an “infestation of the living tissue of live vertebrates by larvae of flies from the arthropod order Diptera that feed for varying periods of time on the hosts’ living tissue or bodily substances” (Zumpt, 1965). Flystrike in sheep can cause significant damage to the fleece, weight loss and if undetected can lead to death (Broadmeadow et al., 1983, Raadsma and Baker, 1983, Heath et al., 1987, Hall, 1997). Flystrike can occur on the head, body, tail, breech, pizzle, poll and feet of a sheep (Miller, 1939, MacFarlane, 1941, Heath and Bishop, 1995, Pickering, 2013). However, cases of flystrike are primarily found around the breech and on, or near, the withers (Heath and Bishop, 1995, Wardhaugh, 2001). Flystrike is both a welfare and an animal production issue (Tenquist and Wright, 1976, Plant, 2006). The cost to remedy these issues in New Zealand has been estimated to cost $60.2 million annually of which $34 million is spent on chemicals (B+LNZ Genetics, 2014). A recent survey suggests that cases of flystrike are being detected in regions in New Zealand where it was not previously an issue (Corner-Thomas et al., 2017).

In New Zealand flystrike or cutaneous myiasis is predominantly caused by two species: *Lucilia cuprina* (Diptera: Calliphoridae; Wiedemann, 1830) and *Lucilia sericata* (Diptera: Calliphoridae; Meigen, 1826), while *Calliphora stygia* (Diptera: Calliphoridae; Fabricius, 1782) is noted in a relatively few cases (Bishop et al., 1991, Heath, 1994, Heath and Bishop, 1995, Cole and Heath, 1999, Heath and Leathwick, 2001, Heath and Bishop, 2006). Each of these three species is known to be able to initiate flystrike on sheep with intact skin and is generally considered to be primary strike flies. Other species such as *Chrysomya rufifacies* can then invade these wounds as secondary strike flies. These three primary strike flies follow the general dipteran life cycle. This cycle includes adult flies which emerge from pupae in the soil and each female can lay up to 200 – 300 eggs; these eggs will pass through three larval stages, then drop off the animal and pupate in the soil (Zumpt, 1965, Wall et al., 1992a, Wardhaugh et al., 2001).

Until the arrival of *L. cuprina* in the late 1970s to New Zealand, the flystrike season was somewhat predictable occurring from November until late March and losses due to flystrike were generally minimal if some precautions were followed (Tenquist and

Wright, 1976, Dear, 1986). With a predictable season, farmers were able to shear their sheep at an opportune time and apply insecticides to provide extended durations of protection (Tenquist and Wright, 1976, Dear, 1986). However, the arrival of *L. cuprina* lengthened the flystrike season from late September until May and increased the levels of flystrike compared to before (Bishop et al., 1991, Heath and Bishop, 1995, Cole and Heath, 1999, Heath and Leathwick, 2001, Heath and Bishop, 2006). Consequent to this, the exact start and end of the flystrike season in New Zealand are not well defined and does not allow for sheep farmers in New Zealand to make clear informed management decisions which would allow for the appropriate timing of measures to mitigate against this disease.

There are several measures that farmers may take to reduce instances of flystrike. These include the timing of physical activities such as shearing, dagging and the appropriate timing and usage of chemicals to sheep to provide protection (Levot, 1995, Savage, 1998, Cole and Heath, 1999, Cranston et al., 2017). These measures are most effective from both an animal welfare and economic perspective when a clear understanding of the timing of the seasonality of the fly species which may cause flystrike is known.

A flystrike model, if made available to New Zealand farmers, would be highly advantageous in the control of cutaneous myiasis, much like those currently used in the United Kingdom for *L. sericata* (Wall et al., 2000, Wall et al., 2002) and in Australia for *L. cuprina* (Wardhaugh et al., 2007, Horton and Hogan, 2010). Such models assist farmers to make strategic decisions on the management of their sheep by providing a level of risk for flystrike on a weekly basis. They are combined with recommendations in England to provide guidelines to reduce cases of flystrike by using flytraps as part of an integrated pest management strategy (French et al., 1995). The model in the United Kingdom is provided on a subscription basis to farmers on the following website https://alerts.nadis.org.uk/ and the Australian model is also on a subscription basis available on https://askbill.com.au/home. These models use temperature and humidity data to predict the start, end, and severity of the flystrike season (Wall et al., 2000, Wall et al., 2002, Wardhaugh et al., 2007, Horton and Hogan, 2010). The United Kingdom model integrates the temperature-dependent life cycle of *L. sericata* in the model to predict the start, end and severity of the flystrike season (Wall, 1993, Wall et al., 1993b, Fenton et al., 1998, Wall et al., 2000). In contrast, the Australian model is purely a probability model based on the likelihood of a strike occurring and the severity within the flock. If a flystrike model for primary strike dipterans (*L. cuprina, L. sericata* and *C. stygia*) could be developed for New Zealand it would allow sheep farmers to make more accurate strategic decisions to manage flystrike.

Before the creation of a model for New Zealand to predict the seasonality of the main fly species which cause flystrike several questions need to be addressed. These questions pertain to understanding the seasonality of these species under New Zealand conditions and whether there is a difference in the length of the season across the country which is most likely attributed to climatic variation. Once these key criteria are addressed, a prediction model could be constructed that would allow farmers to make key decisions at appropriate times during the season. It is also of importance to establish an effective combination of flytrap and bait that may be used to monitor populations of *Lucilia* species on farms in New Zealand.

1.1.2 Aims

Therefore, the following objectives shall be addressed in this thesis:

i. An evaluation of the morphological and molecular methods used to identify *Lucilia* spp. in New Zealand (Chapter Two)

ii. A determination of the most efficient trap and bait combination to monitor populations of *Lucilia* spp. on New Zealand farms (Chapter Three)

iii. Determine the start, the end, and the peak of the season for *Lucilia* spp. across New Zealand (Chapter Four)

iv. Create a prediction model for *Lucilia* spp. across New Zealand based on seasonal climate variation using an onsite weather station and using the closest Virtual Climate Station (Chapters Five and Six).

1.2. Biology

1.2.1 Flystrike in Sheep

1.2.1.1 The historical seasonal occurrence of flystrike in New Zealand

Before the arrival of *L. cuprina* to New Zealand, two major trends were observed with flystrike and sheep. In spring, crutch strike predominated whereas in autumn body strike predominated (MacFarlane, 1941, Murray, 1956). The hypothesis developed to explain this trend was that adult sheep were generally shorn during November/December. By autumn, the fleece had grown to a sufficient length that it would retain moisture and thus be conducive to being flystruck (MacFarlane, 1938, MacFarlane, 1941). Flies preferentially seek a location to lay eggs that has high humidity, a low light intensity and preferably with a nutrient source so that emerging larvae have a better chance of survival whilst initiating the strike lesion.

Since the arrival of *L. cuprina*, this trend has appeared to have changed (Table 1.1); where breech strike has become the predominant area for strike on sheep in New Zealand (Heath and Bishop, 1995, Pickering, 2013). In addition, this new species has increased the amount of flystrike found in New Zealand and lengthened the season by three months (Heath and Bishop, 2006). This has changed from the earlier findings of MacFarlane (1941), where cases of body strike were seen to be more prevalent, especially caused by *C. stygia* (= *Calliphora laemica*, Diptera, Calliphoridae; White, 1843); although this is breed-specific (Table 1.1). It has not been noted in New Zealand publications as to which specific area of the body on sheep *L. sericata* primarily targets.

1.2.1.2 The occurrence of flystrike on sheep in Australia and the United Kingdom

There is a breed effect on the occurrence of flystrike in sheep, especially the wool type on those sheep. In Australia, where fine wool breeds such as Merino predominate, breech strike is still seen to be the most common type of strike (Watts et al., 1979, Wardhaugh and Morton, 1990). Body strike, whilst less common, is still a problem and is seen to be associated with periods of heavy rain across the season and is therefore unpredictable

(Wardhaugh and Morton, 1990, Wardhaugh et al., 2007). Moisture on a sheep’s body and wet weather are seen as key factors for outbreaks of body strike (Wardhaugh et al., 2001).

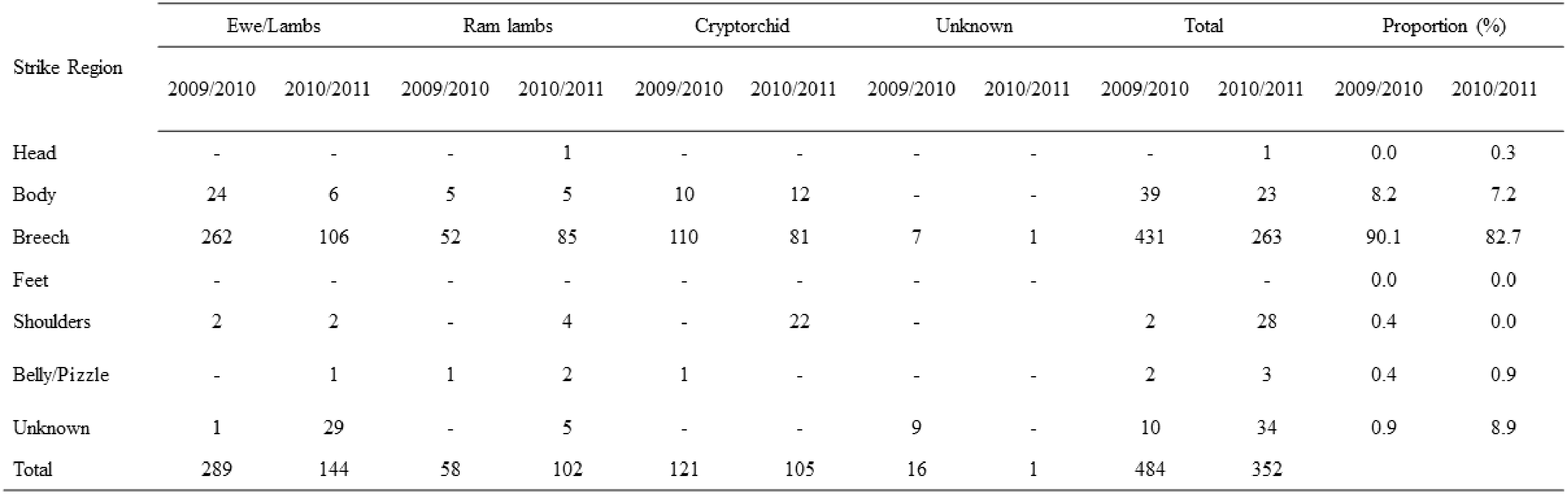
In the United Kingdom, where coarser wool type breeds predominate, breech strike is also the most prevalent form of strike with body strike reported to be infrequent. This is especially for lambs which are more susceptible to this type of strike than older sheep (French et al., 1995, Broughan and Wall, 2007). Another observation relates to the effect of altitude. In Scotland, *L. sericata* was found to be absent in cases of flystrike at altitudes above 200 metres and was replaced by alternative species such as *Lucilia caesar* (Diptera: Calliphoridae; Linnaeus, 1758) and *Protophormia terraenovae* (Diptera: Calliphoridae; Robineau-Desvoidy, 1830) (MacLeod, 1943a, MacLeod, 1943b, Morris, 1997, Morris and Titchener, 1997).

In New Zealand, coarser wooled breeds of sheep are found throughout the country, with Romney and Romney-cross sheep being the most prevalent (Cranston et al., 2017). Finer wooled Merino sheep are found in the high country regions in Central Otago on the South Island (Cranston et al., 2017). Merino sheep are noted to have higher rates of body strike in New Zealand compared to other breeds of sheep (Table 1.1). Breech strike is the most common form of flystrike that occurs in New Zealand as Romney-type sheep are the most prevalent breeds found throughout the country (Table 1.1, Table 1.2; (Scobie and O'Connell, 2010).

Table 1.1 The relative distribution of the type of strike on Merino/comeback and half-bred, crossbred, and purebred English sheep (Macfarlane 1938) and the location of strike on sheep in New Zealand from 1985 – 1995 (Heath and Bishop, 1995).

	Macfarlane, 1938		Heath and Bishop, 1995
Region	Merino and Comeback	Half-bred, crossbred, and purebred English	Not breed specific
Head	3.7%	0%	7.1%
Body	50.4%	13.9%	9.2%
Breech	26.9%	82.1%	81.2%
Abdomen			2.5%
Udder	0%%	1.8%	
Foot	0.9%	1.6%	

Table 1.2 Occurrence of strike on 11 farms on the North and South Island of New Zealand, 2009/2010 & 2010/2011 from Pickering 2013.



Strike Region	Ewe/Lambs		Ram lambs		Cryptorchid		Unknown		Total		Proportion (%)	
	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011
Head	-	-	-	1	-	-	-	-	-	1	0.0	0.3
Body	24	6	5	5	10	12	-	-	39	23	8.2	7.2
Breech	262	106	52	85	110	81	7	1	431	263	90.1	82.7
Feet	-	-	-	-	-	-	-	-		-	0.0	0.0
Shoulders	2	2	-	4	-	22	-		2	28	0.4	0.0
Belly/Pizzle	-	1	1	2	1	-	-	-	2	3	0.4	0.9
Unknown	1	29	-	5	-	-	9	-	10	34	0.9	8.9
Total	289	144	58	102	121	105	16	1	484	352		

1.1.1.3 Factors that make sheep attractive to primary strike flies

Females typically oviposit on sheep due to some pre-disposing factor that makes that particular area of the sheep attractive (Wardhaugh, 2001). Factors on the breech of a sheep such as wrinkles, urine staining, dags, fleece moisture can make a sheep more susceptible to being struck (Greeff et al., 2018). Whereas the body, shoulder, chest, belly and feet of sheep are less prone to strike as they are generally drier although footrot lesions between the claws of the feet are commonly struck (Heath and Bishop, 1995). These parts of the body can become more susceptible to becoming flystruck after periods of sustained heavy rain which can lead to an increase in moisture; these are seen as key causes for outbreaks of body strike (Wardhaugh et al., 2001, Wall et al., 2002). Wounds from shearing, dog bites or kea (*Nestor notabilis*, New Zealand alpine parrot) can also leave sheep susceptible to being struck (Reid et al., 2019); however instances of myiasis from these causes are more serendipitous in nature.

Bacteria such as *Dermatophilus congolensis* and *Pseudomonas aeruginosa* can also predispose sheep to the possibility of body and breech strike (Gherardi et al., 1983). Dermatophilosis occurs after persistently heavy rain, which moistens the area allowing the bacteria to colonise skin resulting in dermatitis with serous exudate. The resulting dried serous exudate consolidates in between wool fibres and causes lesions of lumpy wool to form (Wabacha et al., 2007). This provides an ideal nutrient source for the first instar and the scabs also tend to insulate the skin surface, maintain a higher humidity, and offer some protection from direct sunlight. *Pseudomonas* dermatitis occurs when the fleece of a sheep is moist for a prolonged period and results in an erosive skin lesion with serous exudate. This then offers the same advantages to the flies as the serous exudate with *Dermatophilus*.

Flies are attracted to sheep by their odour and will fly for some distance upwind following such odours. Typically, these odours are associated with lesions on the skin of sheep and thus are suitable sites for oviposition. Odour has also been identified as an additional factor to explain why certain sheep within a flock are more prone to strike than others (Ashworth and Wall, 1994, Brodie et al., 2014, Brodie et al., 2016, Greeff et al., 2018). The following Volatile Organic Compounds (VOC) have been noted to attract *Lucila sericata*; dimethyl sulphide, dimethyl disulphide, dimethyl trisulfide, indole, 1-butanol, phenol, hydrogen sulphide, ethanethiol (Brodie et al., 2014, Brodie et al., 2016, Verheggen et al., 2017). The odours of some bacteria including *Pseudomonas* *aeruginosa*, *Bacillus subtilis*, *Proteus mirabilis* and *Enterobacter cloacae* which have all been isolated from the wool of sheep have been shown to be attractive to female *L. cuprina* (Emmens and Murray, 1982). Phenylacetic acid is another VOC, which attracts gravid females whilst males do not respond to this chemical (Liu et al., 2016). Phenylacetic acid is found in the urine of sheep and may also play a part in attracting females to the hindquarters of sheep associated with urine splash onto wool (Martin, 1973).

Thus, reviewing these factors, the occurrence of flystrike is more than just about identifying the seasonal factors which control fly abundance. Nevertheless, identifying key dates such as the start and end of the fly activity season remains the most important determination of flystrike in New Zealand. Predicting fly numbers within the season are likely to be less helpful but still worthy of understanding.

1.2.2 General Biology of Calliphoridae

1.2.1.1 Introduction

Three species of dipterans are the primary causes of flystrike in New Zealand: *L. cuprina*, *L. sericata*, *C. stygia* (Murray, 1956, Tenquist and Wright, 1976, Dymock et al., 1990, Heath and Leathwick, 2001). *Lucilia cuprina* has been documented to be the most frequent cause of flystrike in New Zealand, with *L. sericata* and *C. stygia* being less prevalent (Bishop et al., 1991, Heath and Bishop, 1995, Heath and Bishop, 2006).

Dipterans including those in the family Calliphoridae have a developmental cycle that moves through the following stages: egg – 1st instar – 2nd instar – 3rd instar – pupa – adult (see Figure 1.1). An exception to this cycle has been noted in *Calliphora stygia*, where they have the ability to lay 1st instar larvae rather than lay eggs when temperatures are above 21 °C (Roberts, 1977). The length of time that development will take from egg to the adult stage varies from 11 to 55 days according to temperature for *L. cuprina*, *L. sericata* and *C. stygia* (see Tables 1.3, 1.4, 1.5). A key feature of all their life cycles is how they overwinter. This generally involves some form of adaptation but commonly involves some form of diapause at a juvenile stage of the life cycle, typically the prepupal stage.

It has been found that the majority of prepupal larvae of *L. sericata* overwinter in the top 10 cm of soil (Pitts and Wall, 2005). This has also been found to be relatively similar in Australia, where *L. cuprina* were found to overwinter at soil depths of between 1 – 7.5 cm (Wardhaugh and Dallawitz, unpublished; cited by Wardhaugh, 2001). The pupae of *L. cuprina* were found at shallower depths (~1 cm) during the summer months and this was attributed to higher soil temperatures (Foster et al., 1975); although this trend has not been investigated in *L. sericata*. In addition, the pupation depth of *L. sericata* has been found to decrease in highly compacted soils (Ullyett, 1950, Cammack et al., 2010).

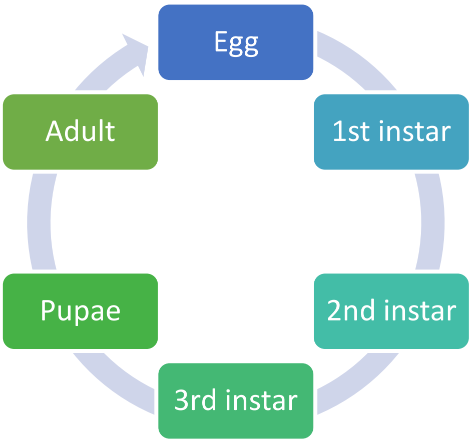


Figure 1.1 The life cycle of Calliphoridae.

2.2.2.2 Calliphora stygia

Figure 1.2 Lateral view of *Calliphora stygia.*

Calliphora stygia is a native species of Australia that was first recorded in northern New Zealand in 1841 (Dear 1986). This species is thought to have been introduced into the South Island in the 1880s and steadily established itself across New Zealand (Kurahashi, 1971), although a map of abundance indicates it rarely moves further south than Nelson and Marlborough (Heath cited in Dear 1986). The adults are present from spring until autumn (Dear, 1986, Dymock et al., 1990, Dymock et al., 1991, Dymock and Forgie, 1995). However, it has been suggested it is adapted to low temperatures and may appear during winter (Miller, 1939, Zumpt, 1965, Kurahashi, 1971). This species is also found throughout Australasia (Miller, 1939, Zumpt, 1965, Kurahashi, 1971, Dear, 1986). It lays eggs on carrion as well as live animals (Zumpt, 1965). The females lay eggs at lower temperatures whilst at temperatures of >21 °C they may lay 1st instar larvae (Miller, 1939, Zumpt, 1965, Kurahashi, 1971, Roberts, 1977, Dear, 1986). The exact cause of this behaviour change are unknown.

1.2.2.3 *Lucilia sericata*

Figure 1.3 Lateral view of *Lucilia sericata.*

Lucilia sericata is of Palearctic origin. It is the primary cause of flystrike in England (Davis, 1934, MacLeod, 1943a, Cragg, 1955, Wall et al., 1992a), Scotland (Morris and Titchener, 1997, Milne et al., 2007, Tongue et al., 2017) Hungary (Farkas et al., 1997), some parts of South-Eastern Australia (Plant et al., 1999) and in South Africa (Scholtz et al., 2000). *Lucilia sericata* arrived in New Zealand during the late 1800s which is sometime after the first arrival of sheep into this country (Miller, 1939). The seasonal occurrence of adult flies of *L. sericata* in New Zealand is from mid to late November until late March (MacFarlane, 1938, Miller, 1939, MacFarlane, 1941, Murray, 1956, Dear, 1986). Surveys and reports up until approximately 1980 noted it as the primary cause of flystrike in New Zealand (Miller, 1939, Murray, 1956, Dear, 1986, Dymock et al., 1991, Atkinson and Leathwick, 1995, Dymock and Forgie, 1995). However, this changed with the arrival of the arrival of *L. cuprina* to New Zealand in the late 1970s (Dymock et al., 1990, Dymock and Forgie, 1995, Heath and Bishop, 2006).

Lucilia sericata is reported to be found throughout the North Island. Whereas, in the South Island, it is not reported to be found as far south as Central Otago, Southland and Fiordland (Tenquist and Wright, 1976). Thus, in these regions, flystrike was seen to be a minimal issue in 1976 (Tenquist and Wright, 1976).

1.2.2.4 *Lucilia cuprina*

Figure 1.4 Lateral view of *Lucilia cuprina.*

Lucilia cuprina likely arrived in New Zealand in the late 1970s but was only confirmed to be present in 1984 (Bishop et al., 1991, Heath and Bishop, 2006). Following its identification in the North Island, it then spread throughout New Zealand until identified in Southland in 1999 (Figure 1.4). One possible explanation, but not proven, for its arrival is that it arrived on ships carrying live shipments of sheep to the Middle East which brought a part-load from Australia, then sailed to New Zealand over only a few days to load more sheep which would have been a convenient time for the larvae to develop into adult flies. However, the actual method the fly used to arrive in New Zealand remains unproven. The fly did likely did come from Australia as the first reports on isolates from New Zealand demonstrated the presence of genes associated with diazinon organophosphate resistance which were common in *L. cuprina* in Australia at that time (Wilson and Heath, 1994, Gleeson et al., 1994, McKenzie, 1994, Wilson et al., 1999). Previously, *L. cuprina* was detected in imported goods from Australia and Asia during the 1960s but failed to become established (Dear, 1986, Bishop, 1995). *Lucilia cuprina* has been reported as the primary cause of flystrike to sheep in Australia (Mackerras, 1904, Waterhouse, 1947, Johnstone, 1954, Ryan, 1954, Watts et al., 1979), South Africa (Smit, 1928, Whitnall, 1931, Monning and Chilliers, 1944, Ullyett, 1950, Howell et al., 1978, Scholtz et al., 2000, Scholtz et al., 2011) and now in New Zealand (Heath and Bishop, 2006).

Lucilia cuprina has had three effects on the occurrence of flystrike since it arrived in New Zealand. Firstly, by lengthening the flystrike season, secondly, by increasing the incidence of flystrike, and thirdly, by increasing the number of geographical locations where flystrike occurs (Tenquist and Wright, 1976, Heath, 1994, Heath and Bishop, 1995, Heath and Bishop, 2006, Corner-Thomas et al., 2017).

The seasonality of *L. cuprina* was determined by farmers sending in larvae from cases of flystrike from across New Zealand and the species present were then identified morphologically (Heath and Bishop, 1995). In this report, it was noted that the average season of *L. cuprina* in the North Island was from September until May and in the South Island was a much shorter season from February until May. In a subsequent publication, Heath and Bishop (2006), stated that the average season on the North Island was from November to May; while in the South Island was from October until May. However, a difference in the length of the season between sites and regions in New Zealand could not be identified. This was largely due to the self-referral and self-selection bias in the data sets gathered which would not allow for a statistical interpretation.

Several studies have been conducted to assess the seasonality of *L. cuprina* by using flytraps with a variety of baits including at Limestone Downs at Port Waikato (Dymock et al., 1990, Dymock et al., 1991), several other locations in the Auckland region (Dymock and Forgie, 1995) and in the Manawatu (Cottam et al., 1998). All of these studies suggested that *L. sericata* has a longer season than *L. cuprina* (Dymock et al., 1990, Dymock et al., 1991, Dymock and Forgie, 1993, Dymock and Forgie, 1995, Cottam et al., 1998).

The status of *L. cuprina* as one or two separate species has been under debate for some years and this has confused the early information on this species. It was originally described as a single species with a distribution that included much of the tropics (Zumpt, 1965). More recently it has been proposed that there are two distinct subspecies of *L. cuprina*: *L. cuprina cuprina* originating from Asia and *L. cuprina dorsalis* originating from Africa (Waterhouse and Paramonov, 1950, Foster et al., 1975, Kitching, 1981, Norris, 1990, Stevens and Wall, 1996, Stevens et al., 2002, Wallman et al., 2005, Williams and Villet, 2014). *Lucilia cuprina dorsalis* has been noted to be more closely related to *L. sericata* on the basis of their geographical location and general similarity of character (Waterhouse and Paramonov, 1950).

Identification of these two sub-species was originally based solely on the colouration of the thorax; where *L. c. cuprina* was described as having a general colouration of olive green and *L. c. dorsalis* has a metallic coppery green (Ullyett, 1950, Waterhouse and Paramonov, 1950). The colouration of the forefemora was also noted to be an additional feature to separate *L. sericata* from *L. cuprina* (Wallman, 2001a). There have been further claims in regards to *L. c. dorsalis* being a rural species while *L. c. cuprina* is deemed to be a species found in an urban setting (Norris, 1990). This urban-rural divide theory is largely based on morphological identification based on colouration (Norris, 1990). However, this criteria for separation based on colouration is difficult to utilise as *Lucilia* species have been noted to change colouration as they age (Szpila, K. per comm). In addition, the use of colouration to separate the subspecies of *L. cuprina* has been regarded as being inconsistent and not recommended (Ullyett, 1950, Waterhouse and Paramonov, 1950). To date, no actual morphological key has been published to separate *L. c. cuprina* from *L. c.dorsalis*.

It has been suggested that only *L. c. dorsalis* exists in New Zealand based primarily on the notion of the rarity of *L. c. cuprina* in Australia (Bishop, 1995, Heath and Bishop, 2006). This is further supported by resistance to diazinon being detected in flies recovered in New Zealand which is common in Australia (McKenzie and Purvis, 1984, McKenzie, 1984, McKenzie, 1994, Wilson and Heath, 1994, Gleeson et al., 1994, Wilson et al., 1999). The morphological criteria described above were used to identify introductions into New Zealand before modern molecular techniques were available. These indicate there were multiple interceptions of *L. c. cuprina* from Western Samoa, Hong Kong, Australia, Pulecheawa, China and Hong Kong at the New Zealand border from the late

1960s, whereas *L. c. dorsalis* interceptions were recorded from Zambia, Australia and Ethiopia over the same period (Bishop, 1995). This would suggest that there could have been multiple origins and repeated introductions of *L. cuprina* to New Zealand (Bishop, 1995). The use of molecular methods would provide more clarity about these earlier interceptions in New Zealand. In addition, it would provide greater certainty of the identification of the subspecies as there is a lack of a distinct morphological characteristic to separate these two subspecies.

1.2.2.5 Hybrid species of *Lucilia cuprina*/*Lucilia sericata*

The occurrence of a hybrid species of *L. cuprina*/*L. sericata* raises a further scenario. A hybrid species has been confirmed using molecular markers in Hawaii (Stevens and Wall, 1996, Stevens and Wall, 2001, Stevens et al., 2002, Wells et al., 2007), Australia (Wallman et al. 2005, Nelson et al. 2012), Asia (Harvey et al. 2008, Wells et al. 2012), South Africa (Tourle et al., 2009) and North America (Debry et al., 2010). Hybrids have been found to possess typical *L. cuprina* nuclear sequences but have an *L. sericata* mitochondrial gene (Stevens et al., 2002, Wallman et al., 2005, Tourle et al., 2009, Debry et al., 2010). It has not been confirmed if there are hybrid species of *L. cuprina*/*L. sericata* in New Zealand.

Laboratory experiments have indicated that *L. cuprina* and *L. sericata* actively interbreed and the hybrids display an *L. cuprina*-like morphology (Ullyett 1945, Waterhouse 1950). Variations in larval and pupal development timings may also be observed compared to the parental species (Martínez-Sánchez et al., 2007). A hybrid species will typically produce fewer eggs than the parental species and the males are sterile resulting in reduced overall fitness (Simovich et al., 1991). To date investigations into hybrids of *L. cuprina*/*L. sericata* has not been successful in maintaining successful colonies and the reasons for this are not fully understood (Haack, per comm.).

1.2.2.5.1 Secondary strike species

Secondary strike/myiasis is caused by flies opportunistically laying eggs on already wounded or struck areas (Norris, 1959a, Monzu, 1979, Hall, 1997). Secondary flystrike can cause a rapid extension of the strike wound which can lead to more severe illness or death of the struck sheep (Norris, 1959a, Monzu, 1979, Hall, 1997). Several species have been recorded on already-struck sheep and were classified as secondary species (Norris, 1959a). Such secondary species include those which aren't able to initiate a strike-through intact skin but can utilise the nutrients available in an existing strike wound. Reports of secondary strike are seemingly rare in the literature and interspecies competition is poorly understood (Ullyett, 1950, Hall, 1997). Preventative measures targeted for secondary strike are not seen to be productive nor do they have an economic value, over and above that achieved by control of primary strike flies (Tellam and Bowles, 1997). Therefore, preventative measures in New Zealand should be concentrated on the primary strike flies i.e. *L. cuprina*, *L. sericata* and *C. stygia*.

1.2.2.5.2 *Chrysomya rufifacies*

Figure 1.5 Lateral view of *Chrysomya rufifacies*.

Chrysomya rufifacies are seen to be the most important secondary sheep strike species (Mackerras, 1936, Ullyett, 1950, Zumpt, 1965, Tenquist, 1977, Kitching, 1981, Baumgartner, 1993). It differs from other secondary strike species in that it is a facultative myiasis fly, which is dependent on the presence of other larvae on a wound (Mackerras, 1936, Ullyett, 1950, Zumpt, 1965, Tenquist, 1977). Its larvae can outcompete other species as they become predatory at the second and third instar stage where they prey on larvae of other species (Smit, 1931, Ullyett, 1950, Norris, 1959b, Zumpt, 1965, Wells and Greenberg, 1992, Omar et al., 1994a, Faria et al., 1999, Madeira, 2001, Watson and Carlton, 2005, Rosati and VanLaerhoven, 2007, Yang and Shiao, 2012). This results in species that initiated a strike disappearing, giving the impression that *C. rufifacies* was the initiator of the strike (Fuller, 1934, Mackerras, 1936, Ullyett, 1950, Norris, 1959b, Baumgartner, 1993, Omar et al., 1994a, Omar et al., 1994b). This behaviour has resulted in some authors stating incorrectly that it is a primary cause of flystrike in New Zealand and Australia (O'Flynn and Moorhouse, 1979, Vogt, 1988, Atkinson and Leathwick, 1995, Heath and Bishop, 1995, Dymock and Forgie, 1995, Morris, 2005, Heath and Bishop, 2006).

1.2.2.6 Overwintering phases of *Lucilia cuprina*, *Lucilia sericata* & *Calliphora stygia*

Dipteran flies are typically most active when temperatures are at their seasonal peak and later cease activity in the winter months. They overwinter in a prepupal stage during these colder months, due to changes in climate namely photoperiod and temperature (Tauber et al., 1986, Danks, 2007). This change is observed in the female fly which will lay eggs that will then develop to a prepupal stage and overwinter (Danks, 1987). The mechanisms for the induction of this overwintering phase during these colder months vary between species.

There is considerable confusion and conflict in the literature by several authors in regards to the specific definitions of diapause with some splitting it into obligatory diapause and facultative diapause. In addition, some insects avoid diapause and utilise quiescence to proceed through unsuitable environmental conditions.. The source of this confusion about diapause has been due to a lack of consensus regarding the terminology used by different authors and the suggestion of overly complex classification systems for modes of dormancy (Tyshchenko, 1966, Mansingh, 1971). Reviews of the confusion and discussion of literature regarding the terminology of insect dormancy have been clarified by two influential reviews of the topic (Tauber et al., 1986, Danks, 1987). Most agree that diapause is a defined physiological state and the features of diapause vary across the Class Insecta.

For the sake of clarity in this thesis, the terminology for the following phrases shall be used to refer to the different modes of dormancy as outlined by Kostal (Kostal, 2006). They are the following: obligatory diapause, facultative diapause and quiescence. It should be noted that there is still considerable debate surrounding the existence of these subdivisions of diapause in the literature (R. Wall per comm). Kostal (2006) defines several phases during diapause. This author further divides the initiation phase for diapause into two states. Obligatory diapause refers to initiation happening without any external environmental triggers, whereas facultative diapause requires environmental stimuli to initiate the diapause process and thus can vary over time and place. In this review, Kostal (2006) notes that the understanding of termination of diapause is incomplete but this author does not indicate any differences in requirements for obligatory and facultative diapause in this regard.

The obligatory diapause is characterised by a change in environmental conditions that affect the adult female fly and this change induces a cessation in development at a certain larval stage where it then overwinters (Vinogradova and Zinovjeva, 1972, Danks, 1987, Saunders, 1987, Danks, 2007). It is understood that specific stimuli such as photoperiod and/or temperature may induce this obligatory diapause state which persists overwinter (Tauber and Tauber, 1970, Danks, 1987, Tougeron, 2019). Obligatory diapause has been noted to be initiated a generation early during favourable environmental conditions and ahead of adverse conditions (Kostal, 2006, Harsimran Kaur et al., 2017). In some species, the response to decreasing photoperiod has been noted to be gradual rather than a strict sudden threshold (Tauber et al., 1986). In addition to the effect of photoperiod, the effect of low temperatures results in lower pupal mass. Thus, temperature conditions at the end of the season are likely to influence the size of the population for the following year or season (Abarca, 2019).

Facultative diapause, which is governed by a response to adverse environmental factors, results in a female fly laying eggs which develop to the prepupal stage and overwinter (Schneiderman and Horwitz, 1958, Kostal, 2006). Facultative diapause may be flexibly induced depending on environmental cues including photoperiod but may be enhanced by cooler temperatures which could vary from year to year (Kostal, 2006). This means that the annual number of generations could be variable from year to year if environmental conditions are favourable (Numata and Shintani, 2022). Photoperiod has been noted as the main environmental variation to govern most species that have been closely studied such as *Ades albopictus* (Pumpuni, 1989, Benedict et al., 2007, Lacour et al., 2015), *Melonoplus sanguinipes* (Fielding, 2007) and *Wyeomyia smithii* (Bradshaw et al., 2000). A further trend with these species noted by each of the previous authors is that this phenotypic plasticity allows these species to adapt to novel environments.

Quiescence differs further from obligate and facultative diapause as the development is a direct response to a change in temperatures that are too low for development to continue; also there is an absence of maternal signalling for this change to occur (Kostal, 2006, Danks, 2007). Instead, quiescence is a cessation of growth or development directly induced by the prevailing environmental conditions namely temperature within the thermal tolerance of a species (Shelford, 1929, Mansingh, 1971). If climatic conditions change during winter with increasing temperatures for at least a period of time, which is above a species' lower developmental threshold, then this would allow for the organism to recommence and continue in its development (Kostal, 2006).

The influence of a seasonal overwintering pattern in response to environmental conditions will affect a species' distribution during a season and therefore influence the size of the niche it occupies (Hutchinson, 1957, Tougeron, 2019). This would result in niche differentiation between species which undergo, for example, either facultative diapause or quiescence (Bruno et al., 2003, Pearman et al., 2008). This would therefore allow species undergoing these types of overwintering phases to exploit resources in the absence of interspecific competition from other species (Bruno et al., 2003, Thomson et al., 2010). In further contrast, a species which undergoes obligatory diapause may occupy a narrower stricter niche governed by specific set of environmental conditions where it would be expected to be present each seasonal year, although in the presence of other competitors (Kostal, 2006, Harsimran Kaur et al., 2017).

1.2.2.6.1 *Lucilia cuprina*

The method of overwintering of *L. cuprina* is unclear and misunderstood. Two contrasting claims on the overwintering phase of *L. cuprina* have been put forward: a quiescence stage (Ash and Greenberg, 1975a, Ash and Greenberg, 1975b, Foster et al., 1975, Kitching, 1977, Foster and Helman, 1979, Kitching, 1981) and a facultative diapause stage (Dallawitz and Wardhaugh, 1984, McLeod, 1997, McLeod, 2001). A quiescence stage could imply that *L. cuprina* would suffer high losses of overwintering larvae in cooler regions which may be reinvaded from warmer regions each year; this has been suggested as a mechanism for the range expansion of *L. cuprina* in Indiana in the United States of America (Owings and Picard, 2018). Whereas studies conducted in the southwest of Australia have suggested that a change in maternal photoperiod induces a developmental arrest in the prepupal stage (Dallawitz and Wardhaugh, 1984, McLeod, 1997, McLeod, 2001). In addition, these Australian studies have also found the asynchronous emergence of *L. cuprina* the following spring (Dallawitz and Wardhaugh, 1984, McLeod, 1997, McLeod, 2001). To date there is also no clear understanding of the triggers for re-activation after diapause for this species.

Thus, based on reports and studies to date, the method of overwintering of *L. cuprina* is ambiguous with multiple conflicting interpretations. Much of the literature regarding the overwintering of *L. cuprina* is based on observations from field data. There is a distinct lack of laboratory studies in controlled conditions such as a plant growth chamber that would allow for the establishment of the actual mechanism of overwintering for *L. cuprina*. Understanding this phenomenon is important when considering the development of models to describe fly abundance for this species.

1.2.2.6.2 *Lucilia sericata*

Lucilia sericata has an obligatory diapause stage whereby a decrease in photoperiod induces a female fly to lay eggs which overwinter in a prepupal stage (Davis, 1934, Cragg and Cole, 1952, Fraiser and Smith, 1963a, Zumpt, 1965, Ring, 1972). The maternal induction of diapause for *L. sericata* has been confirmed (Cragg and Cole, 1952). The climatic variables which induce diapause in *L. sericata* have been described in separate studies.

The induction of diapause has been attributed to a decrease in temperature and a decrease in photoperiod. It was found that *L. sericata* entered diapause at 15.8 °C in Canada as part of a developmental study of larval stages kept at constant temperatures (Anderson, 2000). It should be noted that the adults were maintained in cages outside, and the study was conducted when daylength was shortening which may have affected the results. Also, the environmental temperatures under which the adult flies were under were not noted nor was the decrease in photoperiod. It is not understood if there were specific temperature thresholds that would induce diapause or if this threshold may differ due to biogeographical realms.

The influence of maternal photoperiod on the induction of diapause in *L. sericata* has also been described (Tachibana and Numata, 2004); whereby the instances of diapause increased below 13 hours of daylight per day. It has not been investigated if the induction of diapause is similar in *L. sericata* in other parts of the world.

Termination of diapause has been reported to be due to an increase in soil temperature (Wall et al., 1992a). Calculation of the lower developmental threshold for *L. sericata* from laboratory developmental data has been estimated by an extrapolation of the x-intercept of linear regression (Wall et al., 1992a, Grassberger and Reiter, 2001, Cervantes et al., 2018). The lower developmental threshold for *L. sericata* typically ranges from 8.3 - 11.3 °C (Greenberg, 1991, Wall et al. 1992, Grassberger and Reiter, 2001, Gosselin et al., 2010 and Cervantes et al., 2018) (Table 4.6). This lower developmental threshold is typically estimated due to the low success rate of experiments at low temperatures and further discussion of these methods can be found in following section 1.2.3.3 (Cervantes et al., 2018).

1.2.2.6.3 *Calliphora stygia*

Calliphora stygia has been shown not to respond to a change in photoperiod (Roberts, 1977, Roberts et al., 1983). Moreso, *C. stygia* exhibits a quiescence overwintering habit with a base temperature of 5 °C (Roberts, 1977, Roberts et al., 1983). As illustrated in Figure 1.6, the lifecycle of *C. stygia* increases in length due to decreasing temperatures in the colder months from May until September with a shorter lifecycle observed in the other warmer months during the year. Consequently, periods of warmer weather that are above the trigger temperature could result in the presence of adult *C. stygia*. It was also proposed that this species favours a synchronous emergence at dawn as this is the period of the day with the highest relative humidity (Roberts, 1977, Roberts et al., 1983).

Currently, there is no knowledge of when the season of *C. stygia* in New Zealand starts and ends. Previous fly trapping studies have begun in early September and this species was immediately present in traps and ceased while it was still being caught in May (Dymock et al., 1990, Dymock et al., 1991). Similarly, Heath and Bishop (2006) found that this species was present all year round in both the North and South Islands. It could also be suggested that variation of its seasonal pattern could occur due to warmer or colder climates where it may not be present during winter (Heath and Bishop, 1995). Such observations are all consistent with this species following a quiescence overwintering habit.

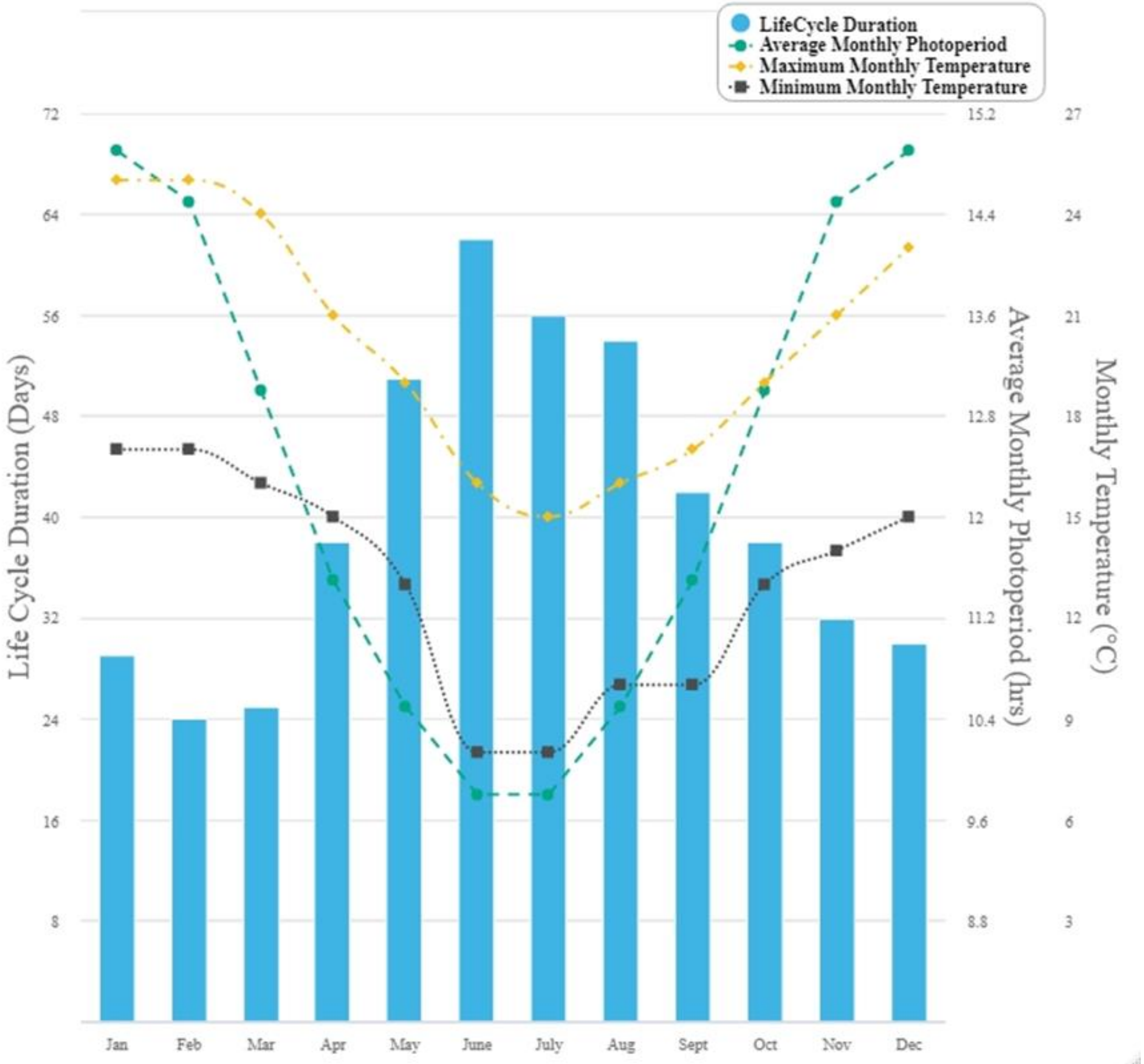


Figure 1.6 The average monthly life cycle duration of *Calliphora stygia* was maintained in field conditions in the Auckland compared to average monthly temperatures, maximum and minimum temperatures, and average monthly photoperiod 1974 – 1976 taken from Roberts (1977).

1.2.3 Developmental studies on Dipterans

1.2.3.1 Differences in Methodologies of Developmental studies

There is a lack of standardised developmental studies at a constant temperature which hampers direct comparison between *L. sericata* and *L. cuprina* and is thus further limited by geographic differences (Greenberg, 1991, Tarone and Foran, 2006, Amendt et al., 2007, Tarone et al., 2011) see Tables 1.3, 1.4, 1.5). Comparisons between studies outlined in Tables 1.3, 1.4, and 1.5 are further compromised due to differences in the methodologies utilised. Firstly, by whether specimens are monitored in terms of hours (Grassberger and Reiter, 2001, Kotze et al., 2015) or by days (Nishida, 1986). Secondly, if the studies begin recording the time of development from the time an egg is freshly laid (Roe, 2014, Bambaradeniya et al., 2018), or when the first instar emerges (Anderson, 2000, Kotze et al., 2015, Sa et al., 2016). Thirdly, by the identification, or not, of the wandering/post-feeding/pre-pupal stage as a stage of development. The justification for the change to this stage varies between studies by either the cessation of feeding by the larvae and movement away from this substrate (Grassberger and Reiter, 2001, Roe, 2014), or by the colouration of the larvae (Anderson, 2000, Cervantes et al., 2018). This change from a character-based morphological identification of each larval stage to one based on movement is not a reproducible method between studies. In some studies, the prepupal stage is identified when the puparium is white and when the pseudocephalon is completely withdrawn in *L. sericata* and *L. cuprina* (Barros-Cordeiro et al., 2016, Karabey and Sert, 2018). The use of this method is arguably a more accurate, verifiable, and reproducible method (Cepeda-Palacios and Scholl, 2000, Barros-Cordeiro and Pujol-Luz, 2010, Defilippo et al., 2013, Barros-Cordeiro et al., 2016, Salazar-Souza et al., 2018). Fourthly, comparisons are limited, by the choice of tissue substrate used in the developmental studies. Differences in the type of tissue used to grow maggots has been noted to affect the length of the larvae (Kaneshrajah and Turner, 2004, Clark et al., 2005, El-Moaty and Kheirallah, 2013). However, this does not cause a significant difference in developmental timings (Bernhardt et al., 2017).

1.2.3.2 Maggot Masses

One important factor that developmental studies at constant temperature cannot address is that of maggot masses which can develop on flystruck sheep (Greenberg, 1991, Byrd and Butler, 1997, Rivers et al., 2011). A maggot mass is essentially a large aggregation of second and third instar larvae. The general rule is that the greater the number of larvae in a mass, the higher the temperature of the mass (Slone and Gruner, 2007, Heaton et al., 2014). A maggot mass is capable of generating heat, which can exceed lethal temperatures that would generally be observed in a developmental study, and this temperature can also be higher than the ambient temperature (Campobasso et al., 2001, Richards et al., 2009a, Charabidze et al., 2011, Kotze et al., 2016). However, larvae can regulate at these excessive temperatures by continuous movement around the periphery and centre of the mass (Heaton, 2014, Heaton et al., 2014). Speculative theories on how larvae survive these temperatures, whilst continuing to develop, include larvae moving from the inner to the cooler outer realms of a maggot mass, as mentioned above, the size of the maggot mass and its compaction or due to environmental factors which may cool a maggot mass. However, these theories are speculative rather than experimentally proven (Huntington et al., 2007, George et al., 2013, Johnson and Wallman, 2014, Ody et al., 2017). Regardless, whilst maggot masses may influence fly numbers during a season, they should not influence the overwintering phase.

1.2.3.3 Developmental data of *Lucilia cuprina*, *Lucilia sericata* & *Calliphora stygia*

Developmental data for *L. cuprina, L. sericata* and *C. stygia* show that there are considerable differences in developmental timing across the world where the same temperatures are utilised (Tables 1.3, 1.4 and 1.5). There are currently no published developmental data at constant temperature for *L. cuprina* or *L. sericata* for New Zealand conditions, nor are there any suitable data sets from Australia. Experiments at constant temperature were conducted for *C. stygia* in New Zealand, which showed a minimum developmental temperature of 10°C and a maximum temperature of 30°C (Roberts, 1977). Developmental studies for *L. cuprina*, *L. sericata*, *C. stygia* and *C. rufifacies* were undertaken by the Entomology Group within Ag-Research in the 1990s (pers. comm. D. Cole and N. Haack former employees) but unfortunately, the data produced from these studies were not published. There are also unpublished developmental data sets from Australia for *L. cuprina* from New South Wales and Flinders Island (Wardhaugh, 2001).

Developmental data for *C. stygia* in Australia has been partially developed (Kinnear et al., 1968, O'Flynn, 1983, Williams and Richardson, 1984), (see Table 1.3). For the development of a flystrike model for New Zealand, developmental data at constant temperature for *L. cuprina*, *L. sericata* and *C. stygia* will ideally be required to mimic the ranges of temperatures likely to be experienced. Developmental data could then be used to mimic these insects' response to increasing seasonal temperatures.

The developmental times for *L. cuprina* and *L. sericata* differ from various regions from egg to adult stages (Table 1.3, 1.4). It can be observed in the data in these tables that the total developmental time for *L. cuprina* has been found to vary from country to country, where for example at 30°C it varies between 333 hours in India (Sa et al., 2016), 264 hours in Japan (Nishida, 1986) and 226 hours in South Africa (Kotze et al., 2015). Similarly, for *L. sericata*, the same pattern has been found where at 25°C development time varies between 297 hours in Austria (Grassberger and Reiter, 2001), 370.1 hours in the USA (Roe, 2014) and 401 hours in Ecuador (Pruna et al., 2019). Given the variation of up to 100 hours or around 4 days can be found at the same temperatures for both species, it further necessitates data for both species from the New Zealand bioregion.

Table 1.3 Previously published developmental studies on *Lucilia cuprina*.

Temperature °C	Developmental stage (hrs)						Total development time (hrs)	Region	Reference
	Egg	1st instar	2nd instar	3rd instar	Post feeding	Pupa			
9°C	Eggs did not hatch							Australia	(O'Flynn, 1983)
10°C	Eggs hatched							Australia	(Vogt and Woodburn, 1980)
10°C	Eggs hatched no adult emergence							Australia	(Dallawitz, 1984)
15°C	45	48	48	-	-	600		Australia	(O'Flynn, 1983)
15°C	-	48	96	312	768		1,224	Japan	(Nishida, 1986)
15°C	Development completed							Australia	(Dallawitz, 1984)
18°C		46	121	220		360	747	South Africa	(Kotze et al., 2015)
19°C	29.39	-	-	-	-	338.64	727.92	USA	(Ash and Greenberg, 1975a)
20°C	-	22	23	23	149	410	627	India	(Sa et al., 2016)
20°C	24.40	32.20	23.40	223.3		33.10	638.40	Sri Lanka	(Bambaradeniya et al., 2018)
20°C	26	60 - 72				312 - 336		Australia	(O'Flynn, 1983)
20°C	-	24	24	120	264		408	Japan	(Nishida, 1986)
21°C	-	31	78	125		220	463	South Africa	(Kotze et al., 2015)
24°C	-	26	57	105		162	350	South Africa	(Kotze et al., 2015)
25°C	-	18	26	23	128	336	531	India	(Sa et al., 2016)
25°C	18.0	20.30	14.00	138.0		207	397.30	Sri Lanka	(Bambaradeniya et al., 2018)
25°C	13 - 19	-	-	-		-	-	Australia	(O'Flynn, 1983)
25°C		48	96	216	120		480	Japan	(Nishida, 1986)
27°C	14.40	16.40	17.00	67.30		173.30	288.40	Sri Lanka	(Bambaradeniya et al., 2018)
27°C	13.08	-	-	-		143.04	450.96	USA	(Ash and Greenberg, 1975a)
27°C	-	20.5	46	75		112	253.5	South Africa	(Kotze et al., 2015)
28°C	-	12	12			168		Australia	(O'Flynn, 1983)
30°C	-	16.30	23	21.30	83.30	193	333	India	(Sa et al., 2016)
30°C	-	16	42	70		98	226	South Africa	(Kotze et al., 2015)
30°C	-	24	24	120	96		264	Japan	(Nishida, 1986)
33°C	-	14.5	36	65		85	200.5	South Africa	(Kotze et al., 2015)
34°C	-	-	-	-	-	144	-	Australia	(O'Flynn, 1983)
35°C	-	14	34	14.30	81.30	144	287	India	(Sa et al., 2016)
35°C	-	24	24	144	168		360	Japan	(Nishida, 1986)
35°C	9.69	-	-	-	-	120.24	249.6	USA	(Ash and Greenberg, 1975a)
38°C	6	12.30	7.30	165		216	407	Sri Lanka	(Bambaradeniya et al., 2018)
40°C	-	16.30	33.30	14.30	71.30	122	267	India	(Sa et al., 2016)
40°C	Eggs hatched							USA	(Melvin, 1934)
42°C	Eggs did not hatch							Australia	(Dallawitz, 1984)
42.5°C	Eggs did not hatch							Australia	(Vogt and Woodburn, 1980)
42.5°C	Eggs did not hatch							USA	(Melvin, 1934)

Table 1.4 Previously published developmental studies for *Lucilia sericata.*

Temperature °C	Developmental stage (hrs)						Total development time (hrs)	Region	Reference
	Egg	1st instar	2nd instar	3rd instar	Post feeding	Pupa			
8°C	No development							Ecuador	(Pruna et al., 2019)
9°C	Minimum development time							Russia	(Marchenko, 2001)
10°C	71	-	-	-	-	865.3	2904.0	France	(Cervantes et al., 2018)
10°C	102	No development						Ecuador	(Pruna et al., 2019)
10.4°C	155	162.3	134.2	232.3	696.3	2418.3	3643.4	USA	(Roe, 2014)
11°C			1014.48			2484	3498.48	Russia	(Marchenko, 2001)
12°C	70.2	196.3	-	-	-	773.8	1488.6	France	(Cervantes et al., 2018)
12°C			966.168			1656	2622.168	Russia	(Marchenko, 2001)
12.7°C	32.3	103.7	94.4	200.8	1539.3	920.4	2890.9	USA	(Roe, 2014)
13°C			917.856			1243.2	2161.056	Russia	(Marchenko, 2001)
14°C			869.544			993.6	1863.144	Russia	(Marchenko, 2001)
15°C	31	56	70	115	340	No emergence	No data	Austria	(Grassberger and Reiter, 2001)
15°C	46.2	144.0	-	-	-	521.1	937.7	France	(Cervantes et al., 2018)
15°C			821.256			828	1649.256	Russia	(Marchenko, 2001)
15°C	Development completed							USA	(Melvin, 1934)
15.1°C	51.7	85.9	41.5	134.7	440.2	954.5	1708.5	USA	(Roe, 2014)
15.8°C	-	40.6	94.3	135.7	233.7	382.3	775.0	Canada	(Anderson, 2000)
16°C			772.944			71.4	1483.344	Russia	(Marchenko, 2001)
17°C	28	39	54	79	200	442	842	Austria	(Grassberger and Reiter, 2001)
17°C			724.632			621.6	1346.232	Russia	(Marchenko, 2001)
17.5°C	45.6	39.8	51.2	100.6	89.2	434.4	760.8	USA	(Roe, 2014)
18°C	40.0	84.1	-	-	-	345.1	652.3	France	(Cervantes et al., 2018)
18°C	36	36	55	276		374	778	Ecuador	(Pruna et al., 2019)
18°C			676.32			552	1228.32	Russia	(Marchenko, 2001)
19°C	24	27	42	60	118	293	564	Austria	(Grassberger and Reiter, 2001)
19°C	28.83	-	-	-	-	728.4	1293.8	USA	(Ash and Greenberg, 1975)
19°C			628.008			496.8	1124.808	Russia	(Marchenko, 2001)
20°C	22	24	35	53	108	209	451	Austria	(Grassberger and Reiter, 2001)
20°C	579.696					451.2	1030.896	Russia	(Marchenko, 2001)
20.1°C	28.0	29.9	34.2	97.2	27.7	336.4	553.4	USA	(Roe, 2014)
20.7°C	-	20.9	52.3	78.3	127.9	245.7	486.2	Canada	(Anderson, 2000)
21°C	19	23	29	47	103	158	379	Austria	(Grassberger and Reiter, 2001)
21°C			531.384			415.2	946.584	Russia	(Marchenko, 2001)
22°C	17	19	26	46	94	137	339	Austria	(Grassberger and Reiter, 2001)
22°C	19.2	26.4	46.56	84	-	157.2	333.36	Columbia	(Rueda et al., 2010)
22°C			483.072			381.6	864.672	Russia	(Marchenko, 2001)
22.5°C	22.1	23.6	31.8	63.6	61.3	222.2	424.6	USA	(Roe, 2014)
23°C			434.736			355.2	789.936	Russia	(Marchenko, 2001)
23.3°C	-	21	45	77	145	264	468.5	Canada	(Anderson, 2000)
24°C	15.8	42.3	-	-	-	185.5	328.7	France	(Cervantes et al., 2018)
24°C			386.472			331.2	717.672	Russia	(Marchenko, 2001)
25°C	14	16	19	36	87	125	297	Austria	(Grassberger and Reiter, 2001)
25°C	16.9	21.5	23.9	49.0	56.4	202.4	370.1	USA	(Roe, 2014)
25°C	24	12	36	122		206	401	Ecuador	(Pruna et al., 2019)
25°C			338.16			309.6	647.76	Russia	(Marchenko, 2001)
26°C			289.848			292.8	582.648	Russia	(Marchenko, 2001)
26.67°C	18	20	12	40	90	168	288	USA	(Kamal, 1958)
27°C	13.86	-	-	-	-	164.4	324.5	Austria	(Ash and Greenberg, 1975)
27°C			241.536			276	517.536	Russia	(Marchenko, 2001)
27.5°C	11.8	19.0	15.6	77.8	33.3	186.9	344.4	USA	(Roe, 2014)
28°C	11	11	16	30	87	120	275	Austria	(Grassberger and Reiter, 2001)
28°C			193.224			261.6	454.824	Russia	(Marchenko, 2001)
29°C			144.912			249.6	394.512	Russia	(Marchenko, 2001)
30°C	10	10	15	27	87	119	268	Austria	(Grassberger and Reiter, 2001)
30°C	11.4	13.2	17.0	45.1	52.6	157.9	297.2	USA	(Roe, 2014)

30°C	12	12	24	86		154	288	Ecuador	(Pruna et al., 2019)
30°C	11.2	28.8	-	-	-	143.0	268.1	France	(Cervantes et al., 2018)
30°C	96.6					237.6	334.2	Russia	(Marchenko, 2001)
32.5°C	9.7	9.5	16.4	48.9	72.3	151.6	308.4	USA	(Roe, 2014)
34°C	8.5	9.5	12	27	82	120	259	Austria	(Grassberger and Reiter, 2001)
35°C	8.45	-	-	-	-	241.7	270.24	USA	(Ash and Greenberg, 1975)
35°C	24	12	12	163		161	372	Ecuador	(Pruna et al., 2019)
37°C	12	12	24	No development				Ecuador	(Pruna et al., 2019)
37.2°C	Development completed							USA	(Melvin, 1934)
40°C	Eggs did not hatch							USA	(Melvin, 1934)

Table 1.5 Previously published developmental studies on *Calliphora stygia.*

Temperature	Developmental stage (hrs)						Total development time (hrs)	Region	Reference
	Egg	1st instar	2nd instar	3rd instar	Post feeding	Pupa			
5°C	Minimum development temperature							Australia	(O'Flynn, 1983)
9°C	72	120	96			888 - 960		Australia	(O'Flynn, 1983)
10°C	Minimum development temperature							Australia	(Williams and Richardson, 1984)
	Minimum development temperature							New Zealand	(Roberts, 1977)
15°C	-	288 - 336				456	-	Australia	(O'Flynn, 1983)
20°C	24	72		-	-	312 - 366	-	Australia	(O'Flynn, 1983)
25°C	11	20	24	-	-	240	-	Australia	(O'Flynn, 1983)
28°C	-	-	-	-	-	216	-	Australia	(O'Flynn, 1983)
30°C	Maximum development time							New Zealand	(Roberts, 1977)-
34°C	Maximum development time							Australia	(O'Flynn, 1983)
35°C	Maximum development time							Australia	(Williams and Richardson, 1984)

1.2.4 Trapping Methods

1.2.4.1 Introduction

A variety of trapping methods can be utilised to trap necrophagous flies including traps with chemical lures or animal tissues as bait, light traps, and serendipitous netting (see details below). When choosing a trapping method, the following should be considered: the suitability of the environment/sample site and the length of sampling time. These points dictate the type of bait to be used, which can either be a chemical or an animal tissue. Flytraps have been used as experimental tools to study the flies themselves but have also been proposed to eliminate flies from an environment and consequently decrease the risk of flystrike.

1.2.4.2 Bait

The use of animal tissues is the traditional bait utilised for attracting calliphorid flies and a wide variety of necrophagous dipteran families have been utilised in different studies. Common materials used are pig liver, sheep liver, pig muscle, pig kidney and squid (Hwang and Turner, 2005, Baz et al., 2007, Farinha et al., 2014).

The use of baited flytraps without the use of chemicals has been studied by several researchers in South America and Portugal (Table 1.6). The following combinations of baits have been trialled against one another as outlined in Table 1.6. No single combination of non-chemical bait has been identified to be better than the other for flies in the family Calliphoridae, nor has there been a trial that contrasts the performance of non-chemical baits versus chemical baits.

Table 1.6 Combinations of non-chemical baits used in previous field trials for Calliphoridae.

Bait	Publication
70g of Pork muscle, pork liver, commercial cat food	(Farinha et al., 2014)
150g of rotten bananas with yeast and 150g of rotten squid	(Dufek et al., 2016)
200g of rotten cow liver and 200g of dog faeces	(Mulieri et al., 2015)
200g of bone meal, cow liver and rotten chicken viscera	(Vilte et al., 2020)
300g of bovine liver	(Azevedo and Kruger, 2013)
50g of chicken liver	(Barbosa et al., 2020)
Sardines, Shrimp, beef liver with banana	(D'Almeida and Fraga, 2007)
12g of sardines, beef liver or chicken gizzards	Moretti Tde and Godoy (2013)
20g of chicken viscera	(Serbino and Godoy, 2007)
100g of chicken liver, sardine, and pork	(Vasconcelos et al., 2015)

Numerous combinations of offal bait with or without additional chemicals have been trialled in New Zealand using a modified version of the Western Australian Trap (Dymock and Forgie, 1995). These authors contrasted various combinations of baits and concluded that no single bait type was the most effective to capture *Lucilia* spp. wind-orientated version of the Western Australian Trap using a bait of 200 ml of minced liver and 1.5 % sodium sulphide solution absorbed in a 3 cm pad was also trialled at two heights of 1.5 metres and 0.65 metres (Dymock et al., 1991). Significantly more *C. stygia*, *C. rufifacies* and *L. sericata* were caught with the higher trap whereas for *L. cuprina* no difference in catch was found between the two heights (Dymock et al., 1991). Further trials to contrast the results of this study with baits without chemicals were not conducted. Neither trap nor any bait combination was found to be successful in suppressing primary strike fly populations nor were they found to be able to actively target all primary strike flies in New Zealand. To date, the debate continues as to the ideal bait for attracting *L. cuprina*, *L. sericata* and/or *C. stygia*.

1.2.4.3 Chemical lures

A wide variety of different chemical lures have been trialled to attract flies. The most frequently used chemical lure is sodium sulphide solution, which is commonly added to animal tissue such as pig liver. Sodium sulphide keeps the offal tissue moist, attractive for a longer period and also gives off a strong odour that attracts blowflies (Hwang and Turner, 2005, Kavazos and Wallman, 2012, Harvey et al., 2019). This bait has been used with a wide variety of concentrations and volumes ranging from 1.5 % in 125 ml of water with 250 g of the liver (Harvey et al., 2019); to 5 g of the liver in 10 ml of 30 % sodium sulphide (Hwang and Turner, 2005); there does not appear to be a set concentration of sodium sulphide required for it to be effective. However, sodium sulphide is not approved for usage on farms in New Zealand by the Agricultural compounds and Veterinary Medicines (ACVM) due to the potential negative effect on on-farm animals and potentially causing hypoxia in water bodies (Government, 1997).

The effectiveness of odour-emitting funnel traps using a combination of three lures, dimethyl trisulphide, mercaptoethanol and o-cresol was shown to be effective in reducing the impact of *Calliphora vicina* on stockfish in Norway (Aak et al., 2011). While by placing stickytraps above containers baited with the synthetic attractants of sodium sulphide, 2-mercaptoethanol and butyric acid, were more selective and had higher catches of *L. cuprina* than those baited with a liver in a sodium sulphide solution (Urech et al., 2004, Urech et al., 2009). These same three chemicals have subsequently been used in the commercial LuciTrap® (Urech et al., 2004, Urech et al., 2009); where the chemicals are within a container and flies are required to enter holes into the trap. Most chemical lures have the advantage that they can remain attractive for up to six months, compared to animal tissues which may need to be replaced/replenished on a weekly basis (Weidner et al., 2015, Weidner et al., 2017). The chemical lures are typically in a solution that slowly vaporises over time. A negative aspect of chemical lures is that they have Health and Safety implications, especially when used on-farm, where farm staff may come into contact with dangerous chemicals with significant health warnings which would also require appropriate protective wear.

1.2.4.4 Colour of Trap

Colour is not a significant factor for attracting blowflies compared to odour (Wall and Smith, 1996, Wall and Fisher, 2001). Although blowflies can differentiate between colours, no preference for a particular colour has been found (Fukushi, 1989, Wall et al., 1992b, Pickens et al., 1994, Mello et al., 2009). However, it is known that brightly coloured traps stand out as a visual cue, providing a contrast from the background (Wall et al., 1992b, Aak et al., 2011). Both the LuciTrap® (Urech et al., 2004, Urech et al., 2009) and Western Australian Trap (Cole, 1996) are coloured yellow for this very reason. It has been noted that the catches in the LuciTrap® decrease with age and this could be due to colour fading of the traps or due to staining by the chemical lures and dead flies (Horton et al., 2001b, Scholtz, 2003).

1.2.4.5 Trap design

Many different designs of fly traps have been developed over the last 120 years. The following section reviews a small number of the more common designs. In brief, a trap needs to attract flies and then capture them by some means to prevent escape.

1.2.4.5.1 Bottle Trap

The most popular method of sampling and one that is used worldwide is a bottle trap (Hwang and Turner, 2005, Farinha et al., 2014). Many variations have been described but they all generally follow the same principles. The trap typically consists of two, two-litre plastic bottles cut and reassembled into an upper and lower bait chamber. The upper chamber are punctured with < 1 mm holes to provide ventilation but prevent flies from escaping. The lower chamber has X shaped veins along the side of the container to allow the entry of flies but as they protrude inwards it does not allow for the escape of the fly. In the lower chamber, bait is placed in a plastic dish and held in place by either selotape or by wire. The wire is wound around the top of the trap to allow it to be attached to various substrates. Some of the possible variations include painting the inside of the trap black or not using the lower chamber of the trap (Castro et al., 2010, Greco et al., 2014, Cavallari et al., 2014, Farinha et al., 2014). Numerous studies have utilised these traps over a 24-hour sampling period (Castro et al., 2010, Farinha et al., 2014, Olea et al., 2017, Moré et al., 2018). The bait used is typically animal tissue when the target is for calliphorids. However, there are subsequent problems with the bait drying out and the number of non-target species that are also attracted, resulting in the prolonged processing of the catch (Brett, 2017).

1.2.4.5.2 Western Australian Trap

Numerous versions of the Western Australian Trap have been utilised since it was first developed in Western Australia (Newman and Clark, 1926). The original design was constructed using cutdown kerosene tins with a wire gauze top and a second kerosene tin underneath to hold the bait. Flies would enter the lower chamber through the horizontal slits attracted by the bait; as they would be attracted by the light, they would then move into the upper chamber where they would be captured. In the original reports of field use, the Western Australian Trap was typically baited with beef mince and sodium sulphide (Mackerras et al., 1936, Vogt and Havenstein, 1974). When originally described it was shown to be more successful than a South African-designed trap which was also used at that time (Newman and Clark, 1926).

A further alteration to the design was made by using an aluminium insect screen sitting on top of a steel stand (Williams, 1984). Using this combination it was found to trap a large number of Calliphoridae in a short time frame (Williams, 1984). Limitations of using this trap include the significant cost of its construction (Williams, 1984) and the amount of maintenance involved with processing the recovered samples (Ward and Farrell, 2000). A variation in the design allows the trap to self-orientate itself toward the wind (Vogt et al., 1985a, Dymock et al., 1990, Dymock et al., 1991).

1.2.4.5.3 Western Australian Fly Trap – New Zealand Version

This trap is a plastic version of the galvanized iron Western Australian Trap and has been used extensively in New Zealand (Cole, 1996). While this trap has been successful at trapping flies, no data is available comparing it with other trap methods or the populations of flies caught (Cole, 1996). The trap resembles the plastic bottle trap and largely follows the same principles with the bait being in a lower chamber leading to an upper trap chamber through a narrow opening through which flies cannot return. This barrier is a wire mesh with a 9 mm diameter opening. The outside of the two 3.2 litre Click Clack®

chambers are painted bright yellow and the inside of the trap is painted black, but the top of the upper trap chamber is left clear with no paint inside or outside. The lower chamber has six 20 mm holes in its sides to allow the entry of flies. Bait is placed in the lower chamber on the plastic lid. Flies are attracted to the bait and enter the lower chamber of the trap. As the inner chamber is painted black, flies naturally move towards the light coming in from the top of the upper chamber and remain in the trap.

1.2.4.5.4 LuciTrap®

The LuciTrap® was released commercially in 1995 as an alternative to the use of other fly traps using an offal bait (Urech et al., 2001a). The primary target for the LuciTrap® was to attract gravid female *L. cuprina* using three chemical lures: Sodium Sulphide, 2-Mercaptoethanol and Butyric acid (Urech et al., 2001a). The LuciTrap® has been used both as a monitoring tool and as a means to reduce populations of Calliphoridae on farms in Australia (Urech et al., 1993, Urech et al., 1996, Urech et al., 1998, Urech et al., 2001b, Horton et al., 2001a), South Africa (Scholtz et al., 2000, Scholtz et al., 2011) and Indonesia (Urech et al., 2014). To date, this trapping method has not been used to monitor *L. cuprina* in New Zealand.

There have been concerns about some aspects of this trap’s design (Hall et al., 2003). Previous pilot studies using the LuciTrap® in Spain and Hungary did not catch *L. sericata*; it was thought that either the trap itself was not catching the fly or the fly itself was not attracted by the chemicals in the trap (Hall et al., 2003). As a modification, Stickytraps were placed on the lids of the LuciTraps and within the plastic container of the trap (Hall et al., 2003). They found that the trap itself did not catch any *L. sericata* which differed from earlier results in Tasmania, Australia (Horton et al., 2001a) and South Africa (Scholtz et al., 2000, Scholtz et al., 2011). However, the Stickytraps on top of the LuciTraps caught more *L. sericata* than the actual LuciTrap® itself (Hutchinson, 2000, Hall et al., 2003). It was also found that there was a significant difference in size between adult *L. sericata* caught within the trap and those caught on the Stickytrap on top of the trap. Hall et al. (2003), suggested that larger flies could not enter the trap. Furthermore, far more flies were found to be attracted to the presence of the lures, than those caught in the traps. Based on these findings, it was recommended the use of a stickytrap attachment with a LuciTrap in the Southern Hemisphere could be used to augment the catch from the LuciTrap (Hall et al., 2003), especially when considering *L. sericata*.

Further alterations were made to the LuciTrap® in Indonesia (Urech et al., 2014). Where a bin lid was erected above the lid of the trap to reduce the amount of rain getting into the trap. Droplets of water would collect in the openings on the lid occluding these entry ways for flies. Originally it was designed to be used in dry Australian conditions and therefore, it may not be as suitable for New Zealand's wetter weather conditions, without a lid .

1.2.4.5.5 Stickytrap

The use of a stickytrap is a popular method of catching flies as it is a passive and cheap methodology (Devi and Roy, 2017). Polyisobutylene is the typical sticky substance used on these traps (Murphy, 1985). The limitation of this method is that it will catch non-target flies as well Aak et al. (2011). It has not been shown that utilizing a StickyTrap is a more effective in actively targeting a specific species of fly than a trap (Hall et al., 2003, Urech et al., 2004).

1.2.4.5.6 Water-based traps

The use of water-based traps such as the Rescue Trap®, Red Top Flycatcher®, the Buzz Disposable Flycatch® and Easy Trap® have been used to collect Calliphoridae (Richards et al., 2009b, Harvey et al., 2010). The methodology is simple and just requires adding water to the dry bait and later sieving the contents of the trap. The issue with these traps is that the bait itself can dry out and additional water may be required to keep the trap attractive. Another issue is that captured flies left in the water can degrade and they may become unidentifiable (R. Wall per comm). Water-based traps have not been evaluated against other trap types.

1.2.4.5.7 Modified butterfly trap

This trapping method was successfully used in Iran to sample Calliphoridae, Sarcophagidae, Muscidae and Fanidae (Akbarzadeh et al., 2012). This trap is a polyethene container with four round openings on the side of the container. Each opening was equipped with a funnel with a 1 cm diameter to prevent the escape of the flies. Each trap was baited with 20-30 grams of sheep offal. This trap has not been compared to other trap types outlined previously, nor has this trap been demonstrated to be effective in catching either *L. cuprina* or *L. sericata*.

1.2.4.5.8 Modified pitfall traps

Another trap method is the modified pitfall trap baited by squid which has been successfully used in surveys to trap various species of Calliphoridae (Baz et al., 2007, Martín-Vega and Baz, 2013). The efficiency of a modified pitfall trap has not been compared to another trap type. Furthermore, the likelihood of this trap being used on a farm is limited as the traps may cause injury to animals due to these traps being partly buried. In addition, there is a high potential that traps could be lost due to interference by animals.

1.3 Identification methods for dipterans

1.3.1 Introduction

The accurate identification of the correct species is paramount for research including, but not limited to, an estimation of the minimum time since death in a medicolegal sense and for modelling seasonal population occurrences of primary strike flies (Wall et al., 1992a, Fenton, 1998, Fenton et al., 1998, Wall et al., 2000, Grassberger and Reiter, 2001, Grassberger and Reiter, 2002, Wall et al., 2002, Wang et al., 2016, Wang et al., 2019). Numerous keys, checklists and seasonal records have been published for Calliphoridae from Australasia (Dear, 1986, Wallman, 2001a, Wallman, 2001b), North America (Whitworth, 2006), South America (Barros de Carvalho and De Mello-Patiu, 2008, Gonzalez et al., 2017), Europe (Szpila, 2010, Szpila, 2012, Szpila et al., 2013), Asia (Yang et al., 2014), Africa (Williams and Villet, 2014) and the Middle East (Akbarzadeh et al., 2015)

Some species of Calliphoridae and other families of forensic interest are difficult to separate purely on a morphological basis. For example, within the *Lucilia* genus such as *Lucilia caesar* compared to *Lucilia illustris* (Diptera: Calliphoridae; Meigen, 1826) and between many Sarcophagidae species (Szpila, 2012, Meiklejohn et al., 2013a, Meiklejohn et al., 2013c). In some instances, with these species, the genitalia of flies may have to be extracted, examined, and compared (Meiklejohn et al., 2013a, Meiklejohn et al., 2013c). In such cases, molecular techniques are best used to support morphological identification as identification using the latter can be subjective (Stevens et al., 2002, Meiklejohn et al., 2011, Meiklejohn et al., 2013b, Sonet et al., 2013, Schoofs et al., 2018). Molecular methods may also be used to aid in the identification of a damaged specimen. Recent literature has also indicated the merit of using wings as an alternative morphological measure to identify Calliphoridae and to estimate the size of a fly (Hall et al., 2014, Sontigun et al., 2017, Grzywacz et al., 2017, Macleod et al., 2018, Szpila et al., 2019) and Muscidae (Grzywacz et al., 2017).

This section of the literature review will focus on the morphological and molecular methods used to identify and differentiate *L. cuprina* and *L. sericata* which are the focus of this thesis.

1.3.2 Morphological Identification

The identification and separation of *L. cuprina* and *L. sericata* have been greatly simplified through identification keys by various authors (Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018). Anatomical structures such as the number of paravertical setae on the back of the head, the number and location of hairs on the humeral callus and notopleuron are characters that can be used to separate *L. cuprina* and *L. sericata* (Akbarzadeh et al., 2015, Lutz et al., 2018); further clarification for this can be found in Chapter Two, where a graphical morphological key displays these characters. Williams and Villet (2014) reported that it was possible to separate female *L. sericata, L. cuprina* and a hybrid species between these two species, using only morphological characters based on the results of a discriminant function analysis. Stevens et al. (2002) had earlier examined and compared the genitalia of *L. cuprina* and the hybrid and found that they were indistinguishable from each other excluding this feature.

It has been claimed that differentiation of the two subspecies of *L. cuprina*: *L. c. cuprina* and *L. c. dorsalis,* can be undertaken based on colouration (Waterhouse and Paramonov, 1950) with *L. c. cuprina* being olive bronze whereas *L. c. dorsalis* has a more *Lucilia*-type colouration of bright green (Waterhouse and Paramonov, 1950). However, the use of colouration is generally dependent on the quality of preservation of the sample, how a sample was stored and was noted as being an inconsistent character (Waterhouse and Paramonov, 1950, Ullyett, 1950). Differentiation of subspecies of *L. cuprina* has not been included in morphological identification keys of adults in the family Calliphoridae described by others (Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018). Given the unsuitability of using a reliable morphological method to separate subspecies of *L. cuprina* and hybrid species of *L. sericata*/*L. cuprina* from *L. cuprina*; a suitable molecular method should be used instead.

1.3.3 Molecular techniques

Several techniques have been used historically to identify and distinguish various species of Calliphoridae. A molecular technique is especially helpful to support one's morphological identification, especially where there are species that are difficult to distinguish, for example, *L. caesar*/*L. illustris*, *L. cuprina*/*L. sericata*. Molecular techniques are also useful for the identification of damaged specimens or for immature specimens which may lack the features that would allow them to be identified by the standard dichotomous identification keys. The key to any molecular identification is knowledge of the DNA sequence from either the genome or the mitochondria. The core component of identifying DNA sequences is through either Sanger sequencing or next-generation sequencing.

1.3.3.1 Sanger Sequencing

Sanger sequencing utilizes dideoxynucleoside triphosphates (ddNTPs) to terminate a reaction at the 3' end at a specific point during the amplification of a nucleotide sequence (Sanger et al., 1977a, Sanger et al., 1977b). Sequencing is done in a single direction; thus, it is recommended to perform Sanger sequencing in the opposing directions on the same region to confirm the result. Sanger sequencing is a cost-effective method of sequencing when one is looking at a single area of a genome with a base pair size of >800 – 1,000 base pairs and limited sample size (Crossley et al., 2020). A critical aspect of Sanger sequencing is *a priori* knowledge of the target sequence to be able to design primers.

1.3.3.2 Next-Generation Sequencing

More recent technology has moved towards next-generation sequencing (NGS) where multiple fragments can be run simultaneously. The advantage of this method is the degree of sensitivity and resolution to detect possible mutations in large sequences in a single session (Hert et al., 2008, Hu et al., 2021). This methodology is best used if one wishes to sequence an entire genome in a short amount of time. In addition, NGS requires no prior knowledge of the target sequence. However, given the high costs of this method per sample in comparison to a single Sanger sequencing PCR, it is not typically performed for studies with large populations when only looking at one part of the genome.

The advantages of NGS over Sanger sequencing largely depend on the time and cost. Sanger sequencing is advantageous when one is targeting single genes or short sequences of a small number of samples (~96 samples). Next-Generation Sequencing is a more worthwhile method to implement when one wishes to target several genes or whole genomes in multiple samples simultaneously. The obvious drawback of using NGS compared to Sanger sequencing is the raw computational power that is required to do complex data analysis compared to the relatively simple analysis required for a single sequence with Sanger sequencing. When choosing which sequencing method to use in consideration of the biological question being answered and the financial cost to do so.

1.3.3.3 Sequences of *Lucilia cuprina* and *Lucilia sericata* from New Zealand

There are relatively few studies that have molecularly identified flies in the family Calliphoridae including *Lucilia* species in New Zealand; Table 1.7 outlines the number of nuclear and mitochondrial sequences that have been uploaded to NCBI (National Centre for Biotechnology Information). The first study to do so intended to identify which subspecies of *L. cuprina* were present in New Zealand using two molecular methods (Gleeson and Sarre, 1997). The first method used Restriction Fragment Length Polymorphism (RFLP) analysis to compare the whole mitochondrial genome of ten *L. cuprina* from nine locations across New Zealand (Dargaville, Kaiwaka, Papakura, Gisborne, Bulls, Levin, Masterton, Blenheim and Waiau) to samples from four sites in Australia (Gleeson and Sarre, 1997). It was found that there was almost no variation between the samples from both countries based on 51 restriction sites (Gleeson and Sarre, 1997). The second method undertaken compared a short sequence of 472 base pairs of the mitochondrial gene, CO1 (Cytochrome c oxidase I), of two specimens from each of the following locations in New Zealand (Blenheim, Masterton, Dargaville) to two individual samples collected each from South Africa, Malaysia and Australia (Gleeson and Sarre, 1997). With the small number of samples investigated using phylogenetic analysis, it was still possible to conclude that there had been multiple introductions of *L. cuprina* into New Zealand (Gleeson and Sarre, 1997). However, there was a variability between the specimens which did not allow for the subspecies of *L. cuprina* to be distinguished (Gleeson and Sarre, 1997).

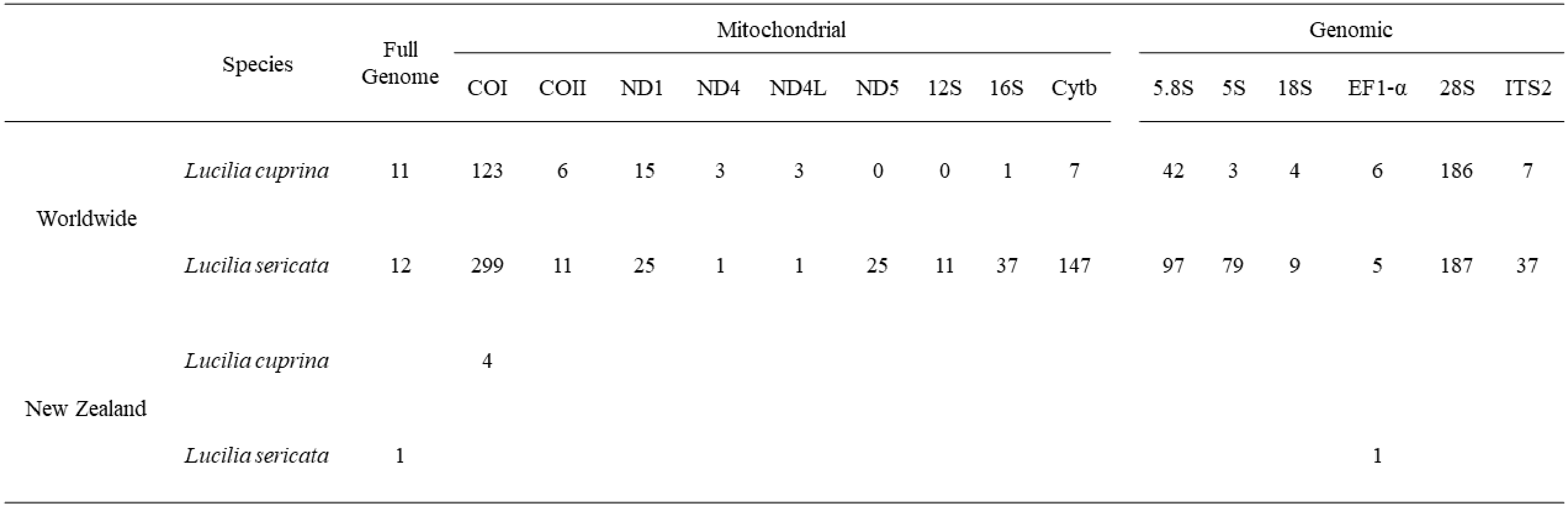
Two other studies used the 12s rRNA gene (a 322 base pair fragment) to distinguish *L. cuprina* and *L. sericata* from New Zealand to several specimens from other countries worldwide (Stevens and Wall, 1996, Stevens and Wall, 1997). It was found that the *L. sericata* were all identified using this gene. However, for *L. cuprina*, the majority were identical including those from New Zealand except for a sample from Senegal which differed by one base pair and a sample from Townsville, Australia which differed by two base pairs. More recently, the first full mitochondrial genome for *L. sericata* from New Zealand has also been added to NCBI (Palevich et al., 2021) and this now allows for a wider comparison of the phylogenomic diversity within the *Lucilia* and wider Calliphoridae family to be undertaken in the future.

1.3.3.4 Sequences of *Lucilia cuprina* and *Lucilia sericata* worldwide

Given the ease of isolation, the copy number, the use of ubiquitous primers, the conservation of its sequence and the conformity of its structure across several species; Cytochrome Oxidase subunit 1 gene (CO1) has been a region within the mitochondria that has been used in numerous studies (Hebert et al., 2003). CO1 has been noted to be highly effective for identifying many different species such as Green Turtles (Tikochinski et al., 2020), birds (Johnsen et al., 2010), zooplankton (Carroll et al., 2019), bullfrogs (Dejean et al., 2012) and a wide variety of species of fish (Ward et al., 2005). It is also notable that the CO1 region is the most utilised gene when reporting for *Lucilia* species on NCBI (National Centre for Biotechnology Information) (Table 1.7). NCBI is a database maintained currently by the National Institutes of Health (NIH) in the United States of America, which allows for the open-access of bioinformatic information to the wider scientific community and the general public (Benson et al., 2010).

However, CO1 has been found to lack the intraspecific variation required to distinguish closely related species within the *Lucilia* genus (Wells et al., 2007, Sonet et al., 2012, Shayya et al., 2018, Sandoval-Arias et al., 2020).

Table 1.7 The number of nucleotide sequences currently uploaded to the NCBI website as of 24th of March 2022 for both *Lucilia cuprina* and *Lucilia sericata* from New Zealand only and worldwide.



	Species	Full Genome	Mitochondrial									Genomic					
			COI	COII	ND1	ND4	ND4L	ND5	12S	16S	Cytb	5.8S	5S	18S	EF1-α	28S	ITS2
Worldwide	*Lucilia cuprina*	11	123	6	15	3	3	0	0	1	7	42	3	4	6	186	7
	Lucilia sericata	12	299	11	25	1	1	25	11	37	147	97	79	9	5	187	37
New Zealand	*Lucilia cuprina*		4														
	Lucilia sericata	1													1		

More recent studies have sequenced the entire mitochondria of several *Lucilia* species: *Lucilia porphyrina* (Diptera: Calliphoridae; Walker, 1850) (NC_019637.1, NC_053672.1, NC_019637.1) (Nelson et al., 2012), *L. illustris* (NC_028056.1) (Schoofs et al, direct submission to NCBI), *L. illustris* (MT584139.1) (Leerhoei et al, direct submission to NCBI), *L. illustris* (KM657109.1, KM657110.1) (Schoofs et al., 2018), *L. caesar* (KM657111.1 - KM657113.1) (Schoofs et al., 2018), *Lucilia coerulveiviridis* (Diptera: Calliphoridae; Macquart, 1855) (NC_029486.1) (Junqueira et al. direct submission to NCBI), and *Lucilia shenyangensis* (Diptera: Calliphoridae; Fan, 1965) (MW446947) (MW446947) (Chen et al., 2021). These studies have offered a greater insight into different regions of the mitochondrial genome that may be targeted to differentiate between a variety of *Lucilia* species, for example, a number of the subunits of the NADH dehydrogenase (Complex I) region of the mitochondria have been examined and compared (Table 1.7).

The NADH dehydrogenase subunit 4 (ND4), NADH dehydrogenase subunit 5 (ND5) and NADH dehydrogenase subunit 6 (ND6) have been noted in recent publications to be highly variable between species within the *Lucilia* genus. It was noted that the ND5 and ND6 contained the most species-specific single nucleotide polymorphisms (SNP) overall within the family Calliphoridae including between *L. cuprina* and *L. sericata* (Nelson et al., 2012, Schoofs et al., 2018). The ND4 region has been found to be able to separate the subspecies of *L. cuprina* and *L. sericata* (Wallman et al., 2005, Nelson et al., 2012). In various mosquito species, the ND4 region is used to identify differing haplotypes (Cameron et al., 2010, Twerdochlib et al., 2012, Lopes et al., 2021) and to detect resistance to some insecticides (Aguirre-Obando et al., 2015, Ding et al., 2020). A similar measure could be done with *Lucilia* species worldwide, but there is a limited number of sequences currently available on NCBI. It should be noted that there is a variation of SNPs within the family Calliphoridae and species-specific markers may have to be developed to show these differences (Nelson et al., 2012, Schoofs et al., 2018).

1.3.3.5 Hybrid species of *Lucilia cuprina* and *Lucilia sericata* worldwide

Mitochondrial (mtDNA) is inherited maternally. However, relying on just a mitochondrial marker as a means to confirm morphological identification may prove to be misleading as hybrid species may exhibit intermediary characteristics between the two species (Figure 1.7). Nuclear markers are inherited from both parents, even when the morphological characteristics of one parental species may be dominant, but the hybrid may possess mtDNA from only the maternal line. Thus, the combined use of mtDNA and nuclear DNA (nDNA) will allow for the identification of a hybrid species.

If active hybridization was occurring then one would expect to find a mixture of *L. sericata* and *L. cuprina* haplotypes (i.e., F2, Figure 1.7). However, field studies have not found an active hybrid. Instead, several studies have assumed the existence of hybrid haplotypes based on a limited number of sequences due to its possession of an *L. cuprina* nDNA sequence and its similarity to the *L. sericata* mitochondrial gene (i.e. F1, Figure 1.7) (Stevens et al., 2002, Wallman et al., 2005, Wells et al., 2007, Harvey et al., 2008, Tourle et al., 2009, Debry et al., 2010). It is proposed an *L. cuprina* / *L. sericata* hybrid may occur based on the following diagram with *L. cuprina* being the dominant phenotype (Figure 1.7).

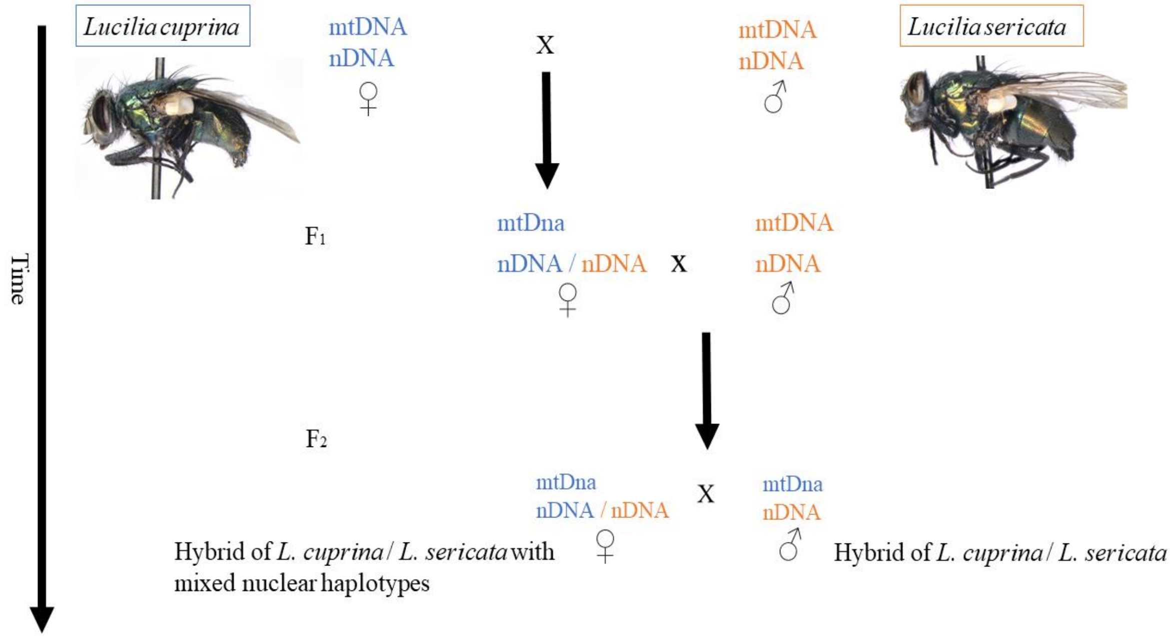


Figure 1.7 The expected hybrid species formation over time from the parental generation, F1 to F2. Where the F2 generation would form from mating with the F1 generation i.e., the initial hybrid formed from the two separate species.

As outlined in Table 1.8, several nuclear and mitochondrial genes have been used to separate these two species. The following publications which reported a combination of nuclear and mitochondrial genes also attempted to separate these two species from the hybrid (Stevens et al., 2002, Wallman et al., 2005, Wells et al., 2007, Harvey et al., 2008, Tourle et al., 2009, McDonagh and Stevens, 2011, Williams and Villet, 2013). In each of the publications, the majority of the sequences reported are less than 1,500 base pairs in length to diagnose the existence of a hybrid species (Table 1.8). It is notable from the literature which investigates the hybrid species of *L. cuprina / L. sericata* that there are no complete sequences of the full genome or the mitochondrial genome. If these were available it would allow for greater certainty of the existence of a hybrid species and comparisons to be made to sequences of other *Lucilia* species (Debry et al., 2010).

Table 1.8 The following summarizes the nuclear and mitochondrial genes used to distinguish *Lucilia cuprina* and *Lucilia sericata*. The range in length of the base pairs for each gene is also specified.

Authors	Mitochondrial					Nuclear			Protein	Full Genome
	COI	COII	ND4	ND4L	16s	28s	ITS-2	EF1-α	Per	
Stevens et al. 2002	1,538	690-710				2,153-2189				
Wallman et al. 2005	822	638	1551-1568							
Wells et al. 2007	1545									
Harvey et al. 2008	1167									
Tourle et al., 2009	439					678				
Debry et al., 2010										
Williams and Villet, 2013	576					654			722	
Marinho et al., 2012	752				513-538	1,006	321-336			
McDonagh and Stevens, 2011	1,538	690-710						1,044-1,264		
Gleeson and Sarre, 1997	472									
Nelson et al., 2012										14,340-14,830

1.4 Methods to combat flystrike

1.4.1 Physical methods

Shearing gives sheep up to thirty days of protection from flystrike as any eggs deposited on the skin will desiccate (Tellam and Bowles, 1997, Wardhaugh et al., 2007) although shearing wounds themselves may be prone to becoming struck. Shearing is typically undertaken in New Zealand in conjunction with other events on a farm i.e., weaning and is not focused on preventing flystrike necessarily. The timing of shearing varies from farm to farm depending on the breed of sheep, the type of shearing regime preferred (shearing once a year, twice a year or every eight months) and climatic conditions. Crutching is the removal of moist wool around and below the tail and between the hind legs which is often damp and has accumulated faecal debris attached (Tellam and Bowles, 1997). Crutching prevents wool from being soaked with urine or the region from being soiled by diarrhoea, both of which would attract flies to this region of the body (Watts et al., 1978, French et al., 1996). Sheep are crutched throughout the year for various reasons such as pre-lambing, pre-shearing or when sheep are observed to be dirty by farmers. If undertaken to prevent flystrike it will only influence strike occurring in this location (i.e., breech strike) and not strike on other parts of the body such as the withers, although it may reduce the olfactory signal making the sheep less attractive to flies.

Mulesing is the removal of skin bearing wool around the breech of a sheep and reduces the risk of dags accumulating and reduces the threat of breech strike (Horton, 2013). This practice was seen to be ideal for breeds of sheep which are prone to wrinkles and produce greasier wool such as merinos, especially in Australia (Hatcher and Preston, 2015, Mortimer et al., 2017, Horton et al., 2018). The practice of mulesing sheep has been banned in New Zealand since the 1st of October 2018 under the Animal Welfare (Care and Procedures) Regulations (Industries, 2018).

Tail docking (removal of tail) is performed on young lambs to prevent the accumulation of faecal matter and urine staining around the breech to reduce the risk of flystrike (Riches, 1941, Riches, 1942, Fisher et al., 2004). Studies in New Zealand have shown that tail length has little effect on the formation of dags (Pomroy et al., 1997, Scobie et al., 1999); although the impact of tail length on flystrike was not measured during either of these studies. Residual tail length post-docking, with merino sheep in Australia, has been reported to influence strike, where a medium-length tail reduced breech strike more than shorter tails (Riches, 1941, Riches, 1942). Selection for shorter tail length has been shown to be possible with no reported negative impact on body weight or spine length (Greeff et al., 2015, Scobie and O'Connell, 2002). Current New Zealand welfare guidelines prohibit tails to be docked any shorter than the end of the caudal folds. The description associated with this guideline indicates this should allow sufficient tail to cover the anus and vulva in ewes and is consistent with the desired length for subsequent minimisation of dag accumulation and hence flystrike occurring.

Active monitoring of sheep for cases of flystrike by farmers is a labour-intensive method of managing flystrike. Sheep can also be assessed if they are flystruck based on behavioural changes and observation of the struck area itself (Grant et al., 2019). Few farmers in New Zealand cull fly struck sheep from their flock as a long term method of managing this disease; thereby removing the genetic predisposition for flystrike in their flock (Pickering, 2013, Burrows, 2018). This long-term strategy may not be appealing to some farmers who may prefer the application of chemicals and integrated pest management systems (see later section) as a more feasible way of managing this disease (Scholtz, Cole and Heath, 1999).

Removal of carcasses from farmland is an important measure as it removes a region where further populations of necrophagous flies may develop (Cole and Heath, 1999). This is especially important during spring, where ewes and lambs may perish due to disease or a cold spell of weather. The recommended best practice in New Zealand is to place carcasses in offal pits although many farmers still simply dispose of carcasses out of sight in gullies. Pastula and Merritt (2013) suggested that a cut-off point at one metre exists with buried carrion, where calliphorids may not be able to colonize the buried carrion. This criterion should be used as a rule of thumb for the minimum depth of an offal pit on farms.

The use of fly traps has been proposed as an additional methodology to reduce the population of flies on a farm (see section 1.2.4 for further details). In some areas such as Queensland, Australia where temperatures allow for *L. cuprina* to be active all year round, traps would need to be used intensively throughout the year (O'Sullivan et al., 1984). Whereas in higher latitudes, where flystrike is more seasonal; one would only need to focus on those time periods when adult flies are active. In temperate climates such as

England, traps used early in the fly season reduced the emerging fly population and reduced cases of flystrike over the entire season (French et al. 1995). It could be suggested that this would be a suitable management strategy to use in countries with similar climates such as New Zealand. However, in New Zealand, fly trapping was not found to significantly ($p > 0.05$) reduce cases of flystrike (Heath and Leathwick, 2001). Similarly, in a ten-year study in New South Wales, Australia, where flytraps were used to control *L. cuprina*, it was found there was no effect on the population nor did it reduce cases of flystrike (McLeod and McLeod, 2001). Smit (1926) stated that a flytrap should only be used as a secondary measure in temperate countries to manage flystrike; this view is still correct almost 100 years later given that flytraps have limited success in being able to control flystrike and the population of primary strike flies.

1.4.2 Chemical methods

Chemicals are a popular method of controlling flystrike at a flock level. They are typically applied as a preventive for flystrike or in reaction to the appearance of flystrike in a flock. Chemicals can be applied by pour-on’s, hand jetting, automatic jetting races, shower dips and plunge dips (West et al., 2018). Each has its own advantages and disadvantages, but these will not be discussed in detail as they are outside the scope of this review.

A wide range of chemicals have been and are still utilised including pyrethroids (including high cis-cypermethrin, alpha-cypermethrin and deltamethrin), organophosphates (such as diazinon), benzoylphenylureas (such as triflumuron and triflumuron), spinosads (such as spinosyn A and D) and a variety of insect growth inhibitors such as cyromazine and dicyclanil (Wall et al., 1993b, Heath and Bishop, 1995, Levot, 1995, Horton, 2015). Pyrethroids and organophosphates are used to offer protection from flystrike for a set period ranging from four to twelve weeks, while insect growth inhibitors are effective at targeting larval stages with longer periods of persistence and withholding periods (Savage, 1998, Bowen et al., 1999, Lonsdale et al., 2000, Levot and Sales, 2004, Wardhaugh, 2005). More recently, commercial preparations of combinations of chemicals for preventing flystrike have become available.

In order to slow the development of resistance, it is generally recommended in practice that farmers use a combination of chemicals over the course of a season rather than just using a single one (Waghorn et al., 2013). *L. cuprina* and *L. sericata* have recently been shown to have a low resistance to cyromazine, dicyclanil and triflumuron (Levot and Sales, 2004, Waghorn et al., 2013, Baker et al., 2014). The long-term viability of using these chemicals to manage these species is debatable as more resistant blowflies are likely to emerge. Resistance to organophosphates has previously been found in *L. cuprina* (Wilson and Heath, 1994, Gleeson et al., 1994, Whyard et al., 1994, Levot, 1995, Wilson et al., 1999, Chen et al., 2001, Sandeman et al., 2014). Indeed, this resistance was one of the early indications that the *L. cuprina* flies originally found in New Zealand had likely come from Australia where such organophosphates resistance is common, rather than elsewhere in the world.

In Australia, trials showed that the application of insecticides to sheep early in the season was highly effective in reducing the incidences of flystrike associated with emerging populations of *L. cuprina*. However, although this approach was effective it was considered likely to lead to an increase in dependence on chemicals as a management tool (McKenzie and Anderson, 1990). These authors further recommended that these measures would be more effective if they were applied on a district or county level, rather than just on individual farms. This contrasts with the control of most other diseases where the individual farmer can take measures for their farm and the boundary fence will prevent introductions from elsewhere.

Similarly, there is also currently a dependence on the use of chemicals as a management tool for flystrike in New Zealand (Cole and Heath, 1999, Waghorn et al., 2013). In New Zealand and Australia, the regulations on withholding periods differ. In Australia, the Australian Pesticides and Veterinary Medicines Authority regularly update the appropriate withholding periods intervals for all approved chemicals. In New Zealand, there are compulsory withholding periods for certain chemicals before the meat can be consumed which is governed by the Ministry for Primary Industries and there are voluntary withholding periods before wool can be shorn.

Judging the timing of chemical use is where an effective model to predict fly numbers would be useful. Waiting until early cases of flystrike are seen on farm implies some sheep will suffer from clinical flystrike yet if used too early then some of the protection period offered by the chemical will be lost.

1.4.3 Some Alternative methods

1.4.3.1 Dung beetles

Faecal matter is a source of protein for female flies. Consequently, a reduction in the amount of dung would be beneficial and could potentially reduce population sizes (Dymock, 1993). The introduction of various species of dung beetles to Australia has been associated with a reduction in the populations of flies (Hughes et al., 1978, Ridsdill-Smith, 1981, Ridisdill-Smith and Hayles, 1990). A range of dung beetle species have recently been introduced to New Zealand in 2020, but as of yet their potential impact on fly populations is not known (https//dungbeetles.co.nz).

1.4.3.2 Genetic control of populations of *Lucilia cuprina*

The release of sterile males which carried eye mutations intending to induce a population collapse and the possible eradication of *L. cuprina* has been proposed as an economically sound control method (Foster et al., 1988). It was initially pursued in Australia in the 1970s and trials were conducted (Williams et al., 1974, Vogt et al., 1985b). This programme was developed to the extent that several field trials during the 1980s were conducted in the Shoalhaven River Valley in New South Wales, Flinders Island and further trials on the wider Furneaux Island group in the Bass Strait between Victoria and Tasmania (Foster et al., 1993). However, this methodology of population control of *L. cuprina* was not pursued further due to the high financial cost of constructing a mass-rearing facility for a national programme (Foster et al., 1993). Its potential was reviewed for use in New Zealand, but no field trials were carried out to test its potential up until this current time (Scott et al., 2004, Scott, 2014, Yan and Scott, 2015).

1.5 Weather and Models

1.5.1 Weather in New Zealand

New Zealand has variations in climatic conditions ranging from Northern New Zealand being a sub-tropical climate to the Southern South Island being temperate. Due to the prevailing westerly winds, the western side of the North and South Islands have higher amounts of rainfall and lower temperatures (Table 1.9). Mountain chains that extend the length of both main islands provide a rain shadow effect to the eastern side with reduced rainfall and increased temperatures. The climate of New Zealand has been divided into 12 groupings by the National Institute of Water and Atmospheric Research (NIWA) (Table 1.9). How these variations affect the distribution and development of *Lucilia* flies is unknown; it is clearly important from the perspective of flystrike control.

Table 1.9 Climatic variations across New Zealand by location and Zone by monthly averages for the period 1971 - 2000. Source NIWA.

Climatic Zones	Location	Annual Rainfall	Wet days	Total Sunshine	Mean Temperature
		mm	>= 1.0 mm	hours	°C
Northern New Zealand	Kaitaia	1334	134	2070	15.7
	Whangarei	1490	132	1973	15.5
	Auckland	1240	137	2060	15.1
Central North Island	Tauranga	1198	111	2260	14.5
	Hamilton	1190	129	2009	13.7
	Taupo	1102	116	1965	11.9
	Rotorua	1401	117	2117	12.8
South -Western North Island	New Plymouth	1432	138	2182	13.7
	Palmerston North	967	121	1733	13.3
	Wanganui	882	115	2043	14.0
	Wellington	1249	123	2065	12.8
Eastern North Island	Napier	803	91	2188	14.5
	Gisbourne	1051	110	2180	14.3
	Masterton	979	130	1915	12.7
Northern South Island	Nelson	970	94	2405	12.6
	Blenheim	655	76	2409	12.9
Western South Island	Westport	2274	169	1838	12.6
	Hokitika	2875	171	1860	11.7
	Milford Sound	6749	186	1800*	10.3
Eastern South Island	Kaikoura	844	86	2090	12.4
	Christchurch	648	85	2100	12.1
	Timaru	573	81	1826	11.2
Inland South Island	Lake Tekapo	600	78	2180	8.8
	Queensland	913	100	1921	10.7
	Alexandra	360	66	2025	10.8
	Manapouri	1164	129	1700*	9.3
Mount Cook	Mount Cook	4293	161	1532	8.8
Southern New Zealand	Dunedin	812	124	1585	11.0
	Invercargill	1112	158	1614	9.9
Chatham Islands	Catham Islands	855	133	1415	11.4

1.5.1.1 Global climate cycles which affect the climate of New Zealand

The climate of New Zealand is affected by three climate oscillations: Interdecadal Pacific Oscillation (IPO), Indian Ocean Dipole, El-Niño Southern Oscillation, and the Southern Annular Mode. The IPO has been found to increase or decrease the frequency and strength of El Niño and La Niña climatic trends over a 20-30-year period in New Zealand (Zhang et al. 1997).

An El Niño phase of the IPO in New Zealand is characterised by having south-westerly winds. Consequently, dry conditions generally occur on the east coast that may lead to drought and on the west coast wetter conditions generally occur (Salinger and Mullan, 1999). In contrast, La Niña is characterised by north-easterly winds that increase rainfall in the north-east of the North Island and reduce rainfall in the southern half of the South Island (Hay et al., 1993). A higher frequency of El Niño phases has been observed over the past two decades and there is considerable debate about whether the intensity of such conditions is affected by anthropogenic climate change (Cai et al., 2014, Wang et al., 2017, Freund et al., 2019). The magnitude and onset of both the El Niño and La Niña phases in the IPO can be forecasted with accuracy from six to seventeen months in advance (Meng et al., 2018, Park et al., 2018, Ham et al., 2019, Dijkstra et al., 2019). Such future predictions for when an El Niño phase will occur and further when the peak of these events will occur have been used to forecast the occurrence of other diseases such as dengue fever, cholera, malaria and chikungunya in various places in the world (Anyamba et al., 2019).

Pickering (2013) conducted a study over two years in the North and South Islands recording the location of flystrike (breech, body, shoulders, head, belly and pizzle in male sheep) on 10 Romney flocks. The first year of the study was during an El Niño phase whilst the second year exhibited a La Niña phase weather pattern (Pickering, 2013). It was observed that during the El Niño summer there was a peak in flystrike occurrence from January until March whereas during the La Niña phase the peak was from February until April (Pickering, 2013). No further inferences were made by Pickering (2013), regarding the effects of climate on instances of flystrike as the focus of the work was largely on the genetic parameters that made Romney sheep susceptible to sheep,

1.5.1.2 The Southern Annual mode

The Southern Annual Mode (SAM) refers to the non-seasonal set of westerly winds which encircle the Antarctic circle. SAM has three phases: positive, negative, and neutral. It affects the patterns of wind, temperature and rainfall in New Zealand (Gordon, 1986). During the positive phase, it reduces the amount of rainfall and results in higher than normal temperatures on the west of the North and South Islands (Renwick and Thompson, 2006). During a negative phase, the opposite is observed with lower temperatures and increased rainfall along the west of both islands (Renwick and Thompson, 2006). It is unknown how a positive or negative phase of SAM would affect incidences of flystrike in New Zealand.

1.5.1.3 Current climate change predictions for New Zealand

Under current climate change predictions for New Zealand, mean temperatures are expected to increase by 0.7 – 1 °C by 2040 and by 0.7 – 3 °C by 2090 (Anon, 2016). It is also likely to influence the patterns of the IPO and SAM. Precipitation is predicted to increase in the west and south; but decrease in the north and east of New Zealand (Anon, 2016). Extreme precipitation events and drought are likely to become more common under current projections across New Zealand (Anon, 2016). These potential changes will have effects on the flystrike season, where a mean temperature increase of 0.2 – 2 ℃ is predicted (Mullan et al., 2008). It could also lead to the flystrike season increasing in length. In addition, changes in rainfall will influence fleece dampness etc., which together with an increase in temperature could lead to additional generations of primary strike flies over the course of the season and higher populations of flies throughout the season.

1.5.2 Models

1.5.2.1 *Lucilia sericata* model for flystrike prediction in the United Kingdom

Models for predicting the seasonality of *L. sericata* and forecasting the instances of flystrike on sheep currently used in the United Kingdom are biological in design, including climatic factors that cause changes to the body of the sheep. *Lucilia sericata* is the primary cause of flystrike in the United Kingdom (Davis, 1934, Cragg, 1955, French et al., 1995). The current model in use estimates the start and end of the season according to a degree day model based on the threshold temperature of the various life stages of *L. sericata* (Wall et al., 1992a). The relative risk of flystrike occurring is based on the number of susceptible lambs, fleece humidity, wool length, worm burden score, faecal contamination index and rainfall (Wall et al., 2000, Wall et al., 2002). Numerous versions have been developed to predict the seasonality of *L. sericata* utilising several model methodologies including; deterministic (Wall et al., 1992a, Wall et al., 1992b, Wall et al., 1993b, Wall et al., 1993a), stochastic (Fenton et al., 1997, Fenton, 1998, Fenton et al., 1998) and Markov chain models (Wall et al. 2002). In the following sections, each model will be described individually and then critiqued.

1.5.2.1.1 Deterministic model

The initial model developed for *L. sericata* was a deterministic model melding the use of field data with experimental data, at a constant temperature of the various life cycle stages of the blowfly. The start of the season is estimated based on accumulated degree days from the first day of spring, when mean temperatures exceed a lower developmental threshold of 9.2 °C (Wall et al., 1992a). A lower developmental threshold of 9.2 °C was based on an extrapolation of a linear regression to estimate developmental zero, from experiments conducted at constant temperatures which examined the various life stages of *L. sericata* (Wall et al., 1992a). The data used for this model was based on the occurrence of *L. sericata* on one farm on the outskirts of Bristol in the South-West of the United Kingdom (Wall et al., 1992a, Wall et al., 1992b). The model divides the life cycle of the blowfly into stages and identifies the effect of temperature on each stage independently (Wall et al., 1992a). The model was constructed based on laboratory experiments compared to those observations in the field. Firstly, each adult female will produce 200 eggs on three occasions during its lifecycle with 15 % of eggs reaching the first, 7 % reaching the 2nd and 2.5 % reaching a third instar stage (Wall et al., 1993a, Wall et al., 1993b). Secondly, egg development will take 10 – 12 hours and the larval stages shall take 2.5 days to complete; therefore, the model assumes a 3-day constant development time to the 3rd instar due to a sheeps' skin temperature being within a range of 28 – 34 °C (Cragg, 1955, Wall et al., 1992a). The wandering and pupal stages are then subject to the daily temperatures of the soil (Wall et al., 1993a, Wall et al., 1993b). The initial population was allowed to be 10 individuals with half being females and they were assumed to emerge on the 1st of May; by the 120th day, any eggs will develop through to a wandering stage and then pause development as it enters diapause (Wall et al., 1993a, Wall et al., 1993b). The model predicts four generations to appear throughout the season with a fifth to overwinter and not emerge until the subsequent spring. It was found that the deterministic model predicted that all of the emergent flies appeared at the same time which did not agree with the observed field data (Wall et al., 1993a, Wall et al., 1993b).

1.5.2.1.2 A comparison to later models using Monte Carlo simulations

A later series of publications compared the deterministic model to a stochastic model using a Monte Carlo simulation technique (Wall et al., 1993b, Wall et al., 1993a, Fenton and Wall, 1997, Fenton et al., 1998). The use of a stochastic model introduces a natural increase in the population of *Lucilia sericata* over the course of a season and therefore, may increase the precision of the model output. Development at each life cycle stage has been assigned a Weibull parameter to reflect the variability in degree-days experienced at each stage of the life cycle. A Weibull function allows for an equivalence to be made between the mean and median (Weibull, 1951). Pupal development in the soil was changed to reflect the diurnal temperature patterns with the further use of developmental fractals rather than the use of mean temperatures that may underestimate the degree days and emergence patterns (Fenton, 1998). Developmental fractals may be calculated based on the preceding formula, whereby it would reflect the diurnal temperature pattern throughout the day (French et al., 1994, Fenton et al., 1997, Fenton et al., 1998). Emergence can be predicted over several days as pupae are subject to fluctuating temperatures rather than mean temperature and all individuals may not develop at the same time to the preceding stages. In addition, in an environment these fractal temperatures would vary from one on-farm location to another location to another. These variables were then simulated using a Monte-Carlo model based on the averaged output of the model after 40 runs.

A further Monte Carlo model was developed to predict instances of flystrike in England by introducing factors that might influence the likelihood of sheep actually getting struck. Where hypothetical climatic variables of rainfall and mean temperature were used to predict the numbers of *L. sericata* with an emergent adult population of ten individuals with half being female and half male (Fenton et al., 1998). Using a logistic regression model with an assumed flock of sheep consisting of 100 ewes and 100 lambs born in mid-spring (Fenton et al., 1998). Factors were added to the model which made sheep more susceptible to being flystruck. The amount of faecal soiling in the breech area is based on historical partners of pasture helminth abundance (Fenton et al., 1998). The humidity of a fleece of a sheep is based on the amount of rain that had fallen on any one day; where humidities greater than 80 % are assumed to be suitable for the development of eggs (Fenton et al., 1998). The model showed that the first cases of flystrike occurred on ewes. The number of strikes on lambs increased due to the increase in faecal soiling and fleece growth whereas, for ewes, the rate of flystrike was constant throughout the season (Fenton et al., 1998). It was found that instances of flystrike on sheep were limited by the low population of *L. sericata* at the start of the season (Fenton et al., 1998). The opposite was found once the population of flies were at their highest later in the season, as the amount of susceptible sheep limited the number of strikes (Fenton et al., 1998). Therefore, it was concluded that management strategies regarding the reduction of cases of flystrike should aim to suppress the early emergent fly population (Fenton et al., 1998); thus reducing the relative risk of flystrike (French et al., 1994, French et al., 1995).

1.5.2.1.3 The current United Kingdom model for *Lucilia sericata*

Models are currently being utilised as a flystrike prediction tool in the United Kingdom (Wall et al., 2000, Wall et al., 2002) and are available publically as a subscription service https://alerts.nadis.org.uk/. The model is essentially the Fenton (1998) model but with a larger assumed population of 10,000 ewes and lambs with seasonal patterns that may make them more susceptible to flystrike. The model assumes with 1,000 adult female *L.*

sericata to emerge in the spring. Factors in the model are rainfall, baseline fleece humidity, worm burden score, fleece humidity index, fleece humidity, wool length score, the number of susceptible lambs, drying and a faecal contamination index. The mean date of shearing was also added to the model. These parameters were compared to field data from 370 farms.

1.5.2.1.4 Limitations in the United Kingdom models

There is a potential constraint whereby *L. sericata* is assumed to have a lower developmental threshold which is the same across the entire United Kingdom. This may not necessarily be the case as evidenced in Scotland (MacLeod, 1943a, Morris, 1997, Morris and Titchener, 1997), where *L. sericata* is replaced at an altitude of 200 metres by *L. caesar* and *P. terraenovae*. This would suggest that there may be a difference in the lower developmental thresholds of strains of *L. sericata* across the United Kingdom. For example in Paris, France, Cervantes et al. (2018), found a lower developmental threshold ranging from 8.3 – 8.9°C which depended on the mathematical model used. In contrast, using a *L. sericata* colony in Brussels, Belgium, Gosselin et al. (2010), found a lower developmental threshold of 9.5°C. It is an accepted limitation with developmental data for these species that their accuracy may vary within biogeographical ranges due to the amount of time it takes to conduct these experiments (Amendt et al., 2007).

It was also noted that the accuracy of the model in the United Kingdom varied from region to region due to the use of a single weather station per region rather than a variety of weather stations in a region to describe the predicted flystrike incidence (Wall et al., 2002). This results in the predictions in the early season having a lag of two to four weeks behind the observed pattern of flystrike cases. In addition, the model currently used in the United Kingdom utilises mean air temperature instead of soil temperature, which was used in the original models (Wall et al., 1993a, Wall et al., 1993b, Wall et al., 2002). Soil temperature is not available from the national weather stations in the United Kingdom and therefore is not in the current model. A measure to overcome this would be to examine the seasonality of *L. sericata* together with instances of flystrike across different regions in the United Kingdom to illustrate these climatic differences and to show the differences in emergence timings.

There is a further potential issue with the use of temperature to predict instances of flystrike. Ody et al. (2017) found that *L. sericata* did not lay eggs below 17.5℃, this suggests that the oviposition by the adult fly is temperature-dependent. This temperature is much higher than the lower developmental threshold of 9.2℃ for the pupae (Wall et al., 1992a). The temperature was not included by either Wall et al. (2000), (2002) as a variable to predict oviposition on sheep by *L. sericata*. Moreso correlations were made between the fly population and instances of flystrike on sheep. Given the results from Ody et al. (2017), further work should examine whether these trends for temperature are valid for instances of flystrike in the United Kingdom and whether this would increase the accuracy of the predictions.

The prediction of the end of the season with each of the models for the United Kingdom assumes that the larvae from ovipositing females will all enter a diapause state after a certain date (Wall et al., 1993b, Wall et al., 1993a, Fenton and Wall, 1997, Fenton et al., 1997, Wall et al., 2002). More recent work by Tachibana and Numata (2004) has shown that a decrease in the photoperiod below 13 hours of daylight induces an increase in the rates of diapause in *L. sericata*. Further work by Anderson (2000), indicated that a decrease in temperature together with a change in photoperiod induced a diapause state. Adding these elements to the existing model may increase the accuracy of the forecast of the end of the season for *L. sericata* in the United Kingdom.

To date these various United Kingdom models have not been tested in New Zealand.

1.5.2.2 *Lucila cuprina* models for flystrike in Australia

A small number of models have been developed to describe the occurrence of this fly and flystrike. The models have more recently been incorporated into commercially available predictive models such as “FlyBoss” http://flyboss.com.au/ and “AskBill” https://askbill.com.au/home. The actual models incorporated into these two commercial programmes are unpublished but is derived from earlier models in Australia as described below.

The earliest model is that described by (Vogt and Bedo, 2001). This was presented at the Flystrike & Lice IPM Control Strategies (FLICS) conference in 2001 and at that time Dr. W.G. Vogt was deceased and the model was presented by Wardaugh on his behalf (Wardhaugh et al., 2001); it was commented that this may not be the latest version developed by the senior author. This model was for just fly numbers and did not include any risk factors for flystrike on sheep. In this model, the concept of "arbitrary development units" (ADU) was proposed and a total of 100ADU was necessary from oviposition to the eclosion of female flies from pupation. The development rate of immature stages is a nonlinear rate function $R=\exp(-6.18+0.30T-0.0043T^2)$. The egg and developing larval stages are on the animal and relatively constant and account for 31.5ADU and is of a predictable and reasonably constant length. A further 16ADU was required after the L3 had left the sheep by the prepupal stage assuming the temperature is above the minimum of 16 °C for pupation to occur. Having achieved the pupation stage a further 52.5 ADU was then required for females to reach eclosion and 47.5ADU for males. There is no apparent restriction on pupation occurring between temperatures of -1.5°C to 42.5°C once it has commenced. The authors describe female development in day-degrees (DD) above a threshold of 8°C with 57DD required for the first ovarian cycle to be completed and eggs laid from the time they are laid as eggs. During the prepupal stage, all accrued development is negated if >12 mm rain falls within the preceding 24 hours as this requires the larvae then move to find a drier site that interferes with the commencement of pupation.

A later model was subsequently published which uses a pasture growth index as a proxy for fly numbers as well as the risk factors for sheep to become flystruck (Wardhaugh et al., 2007). It is a statistical model which is a Poisson regression using climatic weather patterns of temperature, rainfall and humidity with husbandry practices that may reduce the possibility of sheep being flystruck, Table 1.10 (Wardhaugh et al., 2007). This model has been used in Australia to predict flystrike across the country and has been validated using Australian weather data (Horton and Hogan, 2010).

Table 1.10 Climatic factors used to describe instances of flystrike on sheep by *Lucilia cuprina* (Wardhaugh et al., 2007).

Climatic factors		Husbandry			
Temperature	Rainfall/Humidity	Chemicals	Wool length	Worm Burden	Faecal Contamination
No incidences of flystrike below <12.7 ℃	In a week, the number of days with no rainfall and the number of days with rainfall greater than 5mm	100% protection period depending on the chemical class used	Number of days since shearing	None	Dung quality – according to average mass of puparium of larvae found in pasture

The need to estimate the number of flies has been dismissed in this model due to it being time-consuming and technically demanding and sought to identify 'substitutes' for fly abundance (Wardhaugh et al., 2007). Yet in an earlier publication, Wardhaugh and Morton (1990) stated that the type of strike (i.e. body or breech strike) caused by *L. cuprina* was due to the size of the fly population. Population growth of *L. cuprina* was measured by using a pasture growth index as a proxy for various weather and environmental parameters (Wardhaugh et al., 2007). The index was originally proposed by Fitzpatrick and Nix (1970) using three indices including Light Index (LI), Temperature Index (TI) and Moisture Index (MI) where each is given a value between 0 and 1. Their index is then defined by the equation GI= LI X TI X MI. This was then further used by Sands and Hughes (1976) to model the development of the Australian Bush Fly *Musca vetustissima*. This detailed version of the Pasture Growth Index (PGI) model was then used by Wardhaugh et al. (2007) to model the occurrence of flystrike. Such a pasture growth index would not only include factors that act towards fly abundance but also sheep being more susceptible to flystrike, such as damp fleeces. On examination of this pasture growth model, it was apparent it followed a 'broken stick' pattern with a straight-line GI <0.21 and another straight line for GI>0.21. When analysed separately, temperature followed a similar 'broken stick' model with an inflexion at 12.4°C. Interestingly, Wardhaugh et al. (2007) also noted that cases of flystrike were not noted below 12.7°C.

However, as this is for flystrike it is difficult to extricate the effects of fly numbers *per se* from the more complex model for flystrike.

In Australia, *L. cuprina* has differing seasonality depending on location and climatic conditions (Foster et al., 1975, Monzu, 1978, McLeod, 1997, De Cat et al., 2012). The model by Wardhaugh et al. (2007) does not seek to predict a season for *L. cuprina* and assumes the risk of flystrike throughout the year i.e. it makes no attempt to predict the re-appearance of adult flies after the winter. Pasture growth of *Trifolium subterraneum* is used as a proxy for the population dynamics of *L. cuprina* using a temperature threshold of 5 °C above which degree days are accumulated with an onset of winter dormancy below this temperature (Sands and Hughes, 1976, Wardhaugh et al., 2007). In Northern Territory, Queensland and Western Australia, *L. cuprina* is present all year round thus a model based on the risk of flystrike due to climatic conditions is suitable (Urech et al., 2009). For Victoria, New South Wales, South Australia and Tasmania this model is not applicable; as *L. cuprina* overwinters in a prepupal stage with emergence the following spring (Foster et al., 1975, Monzu, 1978, Dallawitz and Wardhaugh, 1984, McKenzie, 1990, McKenzie, 1994, Horton et al., 2001a). Clearly, the Wardhaugh et al. (2007) model is more suitable for the parts of Australia which have a population of *L. cuprina* all year round and can predict strike risk based on weather conditions. In areas where overwintering in diapause of some form occurs, a model that can predict the start and end of the season may be more accurate (McLeod, 1997, McLeod, 2001, De Cat, 2007, De Cat et al., 2012).

Two attempts have been made to develop a predictive model to describe the overwintering emergence of *L. cuprina* in New South Wales and Victoria by exceeding a lower developmental threshold temperature in the soil (McLeod, 1997, McLeod, 2001, De Cat, 2007, De Cat et al., 2012). De Cat (2007), (2012) showed that the median emergence of *L. cuprina* was largely consistent over the two-year timeline of the study. In contrast, McLeod (1997), (2001) found that over three years the spring emergence of *L. cuprina* was variable and influenced by the temperatures experienced over winter. McLeod (1997), (2001) attempted to create a valid predictive model but was hampered by a lack of developmental data of *L. cuprina* at low temperatures and instead used data from wandering larvae of *L. sericata.* This introduces an unknown factor into the discussion and a fundamental error to the predictive value of these models. Nevertheless, three different models to describe the spring emergence of *L cuprina* were compared (McLeod, 1997, 2001). These were a linear degree-day model and two nonlinear functions and all three gave similar results. De Cat (2007), (2012) did not formulate a model *per se* but described that recommencing development showed a requirement for increasing soil temperatures (measured at 5 cm depth) of 1.5°C over four days and soil temperatures remaining above 11 °C for at least 7 consecutive days. There was no attempt by De Cat (2007), (2012) to compare their results to the model described by McLeod (1997), (2001). Nevertheless, the authors both commented that these studies highlighted the need for more detailed studies on the immature stages of *L. cuprina*.

None of these Australian derived models for *L. cuprina* have been tested using data from New Zealand studies.

1.5.3 Summary

There are two separate climatic conditions for when the season of *L. cuprina* and *L. sericata* begins which is dictated by soil temperature at 10 cm and when instances of flystrike may occur dictated by ambient temperature. The types of models previously outlined above are dependent on the developmental data of their respective species (McLeod, 1997, McLeod, 2001, Wall et al., 2002, De Cat, 2007, De Cat et al., 2012). As outlined in Section 1.2.3, there are no suitable data sets for either *L. cuprina* or *L. sericata* for New Zealand conditions to make these types of models, nor has the minimum temperature been defined at which strains of *L. cuprina* or *L. sericata* from New Zealand will oviposit. It is unknown if the criterion used in the United Kingdom or Australia are suitable to predict the start and end of the flystrike season for both *L. sericata* and *L. cuprina* in New Zealand.

Chapter Two. The molecular and morphological identification of *Lucilia cuprina* and *Lucilia sericata* from eight sites in New Zealand

2.1 Introduction

There are two species of *Lucilia* currently found in New Zealand: *L. cuprina* and *L. sericata*. Both species are important from a veterinary perspective as they can initiate flystrike in sheep (Ullyett, 1950, Heath and Bishop, 2006). Flystrike has been defined as the infestation of the living tissue of live vertebrates by larvae of flies from the arthropod order Diptera that feed for varying periods of time on the hosts' living tissue or bodily substances (Zumpt, 1965). Flystrike has been estimated to affect between 3 – 5 % of the 26 million sheep currently in New Zealand (Corner-Thomas et al., 2017).

Lucilia sericata is considered to have arrived in New Zealand in the late 1880s from Europe (Miller, 1939). The second species, *L. cuprina*, only arrived in New Zealand in the late 1970s (Heath and Bishop, 1995, Heath and Bishop, 2006). There are two subspecies of *L. cuprina*: *L. c. cuprina* found in much of Asia and the Northern tropical regions of Australia and *L. c. dorsalis* originating from Africa and is the dominant *L. cuprina* subspecies found in Australia (Stevens and Wall, 1996, Stevens et al., 2002, Wallman et al., 2005, Williams and Villet, 2014).

There have been multiple interceptions of *L. cuprina* at the New Zealand border and it was considered that *L. c. dorsalis* was the species found around sheep in New Zealand (Bishop, 1995). Resistance to diazinon was observed in *L. cuprina* soon after the original identification of flies from sheep in New Zealand (Wilson and Heath, 1994, Wilson et al., 1996, Wilson et al., 1999, Chen et al., 2001, Sandeman et al., 2014). As *L. c. dorsalis* was said to be the main subspecies of *L. cuprina* found in Australia where it was also resistant to diazinon, this led researchers in New Zealand to believe that *L. cuprina* originated solely from Australia (Heath and Bishop, 2006). However, Gleeson and Sarre (1997) suggested that there were multiple introductions of *L. cuprina* from different countries into New Zealand which may have included both subspecies. This was based on a comparison of the mitochondrial gene, Cytochrome c oxidase subunit I (CO1), from multiple samples across New Zealand against samples from Australia, South Africa, and Malaysia.

Identification of *L. cuprina* and *L. sericata* has traditionally been achieved using morphological keys (Akbarzadeh et al., 2015, Lutz et al., 2018) which allows the separation of these two similar species. These keys utilise the number of hairs on the paravertical setae, the number of hairs on the posterior slope of the humeral callus, the number of hairs on the edge of the notopleuron and the number of setulae below each inner vertical seta in the central occipital region to distinguish between *L. cuprina* and *L. sericata*. A combination of these characteristics is best employed to identify and distinguish these species as there can be a variation in the number of hairs in the metasernal area which may lead cause misidentification. Since the development of molecular techniques, several different genes have been used to identify and distinguish between *L. cuprina* and *L. sericata* (summarized in Table 1.8).

It was originally suggested that general colouration of the fly was sufficient to distinguish between the subspecies of *L. cuprina* (Bishop, 1995). However, general colouration is not a reliable character as a means to differentiate between species as factors such as exposure to sunlight or the preservation of a specimen can alter the colouration (Ullyett, 1950, Waterhouse and Paramonov, 1950). Given the inconsistency of colour as a characteristic to distinguish these species, it is not included in published morphological keys to distinguish flies in the family Calliphoridae (Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018).

The use of morphological identification for hybrid species of *L. cuprina*/*L. sericata* has been fraught with the difficulty of the methodology. Laboratory experiments indicated that *L. cuprina* and *L. sericata* interbreed and the hybrids display an *L. cuprina*-like morphology (Ullyett 1945, Waterhouse 1950). Males of the hybrid species have been found to be morphologically identical to male *L. cuprina* aside from dissection and comparison of the genitalia (Stevens et al., 2002). By comparison, the females can be distinguished using the number of hairs found on the scutellum, notopleuron and humeral callus (Williams and Villet, 2014). Both methods are of high difficulty and highly dependent on the skill of the entomologist. Both studies used a small sample size, and neither is feasible for common practice with a large sample size. Hence, molecular methods have been used to confirm the presence of hybrid species.

Hybrid species will inherit nuclear DNA from both parents while they will only inherit mitochondrial DNA (mtDNA) from the maternal parent. Hybrid species can differ from the parental species as they may have a reduced fitness by producing fewer eggs or producing sterile males (Price et al., 2001, Reed and Markow, 2004). Introgressive hybridization may also occur between the parental species and the hybrid species which could result in the transfer of positively selected alleles such as those for pesticide resistance.

Interbreeding between *L. cuprina* and *L. sericata* resulting in hybrid species has been noted in the following countries: United States (Stevens and Wall, 1996), Australia (Wallman et al., 2005), Asia (Wells et al., 2007, Harvey et al., 2008) and South Africa (Tourle et al., 2009). This has not yet been noted in New Zealand. Each of these studies has used a combination of nuclear and mitochondrial genes to distinguish the hybrid from the parental species.

The majority of the publications regarding hybrid species of *L. cuprina*/*L. sericata* have based this upon the mitochondrial gene Cytochrome c oxidase I (COI) using sequences of around or less 1,500 base pairs (Stevens et al., 2002, Wallman et al., 2005, Wells et al., 2007, Tourle et al., 2009, Harvey et al., 2008, Williams and Villet, 2013, Marinho et al., 2012, McDonagh and Stevens, 2011, Gleeson and Sarre, 1997). However, studies which utilised both the COI and Cytochrome c oxidase II (COII) genes found there was a lack of intraspecific variation to distinguish between closely related species/subspecies/hybrids within the *Lucilia* genus (Wells et al., 2007, Sandoval-Arias et al., 2020). Wallman et al. (2005) demonstrated the use of the alternative mitochondrial genes NADH dehydrogenase subunit 4 (ND4) and NADH-ubiquinone oxidoreductase chain 4L (ND4L) as genes that were effective in being able to separate *L. cuprina*, *L. sericata* and the subspecies of *L. cuprina*. In the study performed by Nelson et al. (2012), the entire genome of eight *L. cuprina* and four *L. sericata* flies were sequenced and it was confirmed that ND4 could be used to separate the two subspecies of *L. cuprina* as well as separate them from *L, sericata*. ND4 has also been used in mosquito species such as *Aedes aegypti* to identify haplotypes and determine geographic phylogenetic patterns (Moore et al. 2013).

Several dissenting views have been presented on the status of hybrid species of *L. cuprina* and *L. sericata*. It has been suggested that perhaps *L. c. cuprina* may be a hybrid of *L. sericata* and *L. c. dorsalis*; therefore they should be regarded as separate species (Wallman et al., 2005). This separation has been suggested to be due to the geographical proximity of these species in the African and North American continents (Norris, 1990, Harvey et al., 2008). Others have stated that the hybrid species is an intermediary between *L. cuprina* and *L. sericata* i.e., and F_1 (Figure 1.7) (Stevens and Wall, 1996, Stevens and Wall, 1997, Stevens et al., 2002, Wells et al., 2007, Nelson et al., 2007). However, there is no evidence to suggest that a functioning stable hybrid of both species is currently found nor is there a full genomic sequence of a hybrid currently published (Nelson et al., 2012) (See Table 1.8 in the literature review).

The research described in this chapter aimed to assess the accuracy of the morphological identification of *L. cuprina* and *L. sericata* results by utilizing molecular analysis of nuclear and mitochondrial genes. The methodology used here will be used to validate the method used to define the seasonality of these species in Chapter Four and the later modelling chapters in this thesis. By analysing and sequencing a nuclear and a mitochondrial gene, we also aimed to confirm which subspecies of *L. cuprina* are currently found in New Zealand. In addition, this would allow an investigation as to whether there is a hybrid species of *L. cuprina*/*L. sericata* currently present in New Zealand.

2.2 Methods

2.2.1 Specimen Collection

A total of 5,778 specimens of *L. cuprina* and 2,775 *L. sericata* were collected from eight sites distributed across New Zealand beginning in September 2018 until June 2019 as described later in Section 4.2. The approximate location of each farm is outlined in Table 2.1 and Figure 2.1.

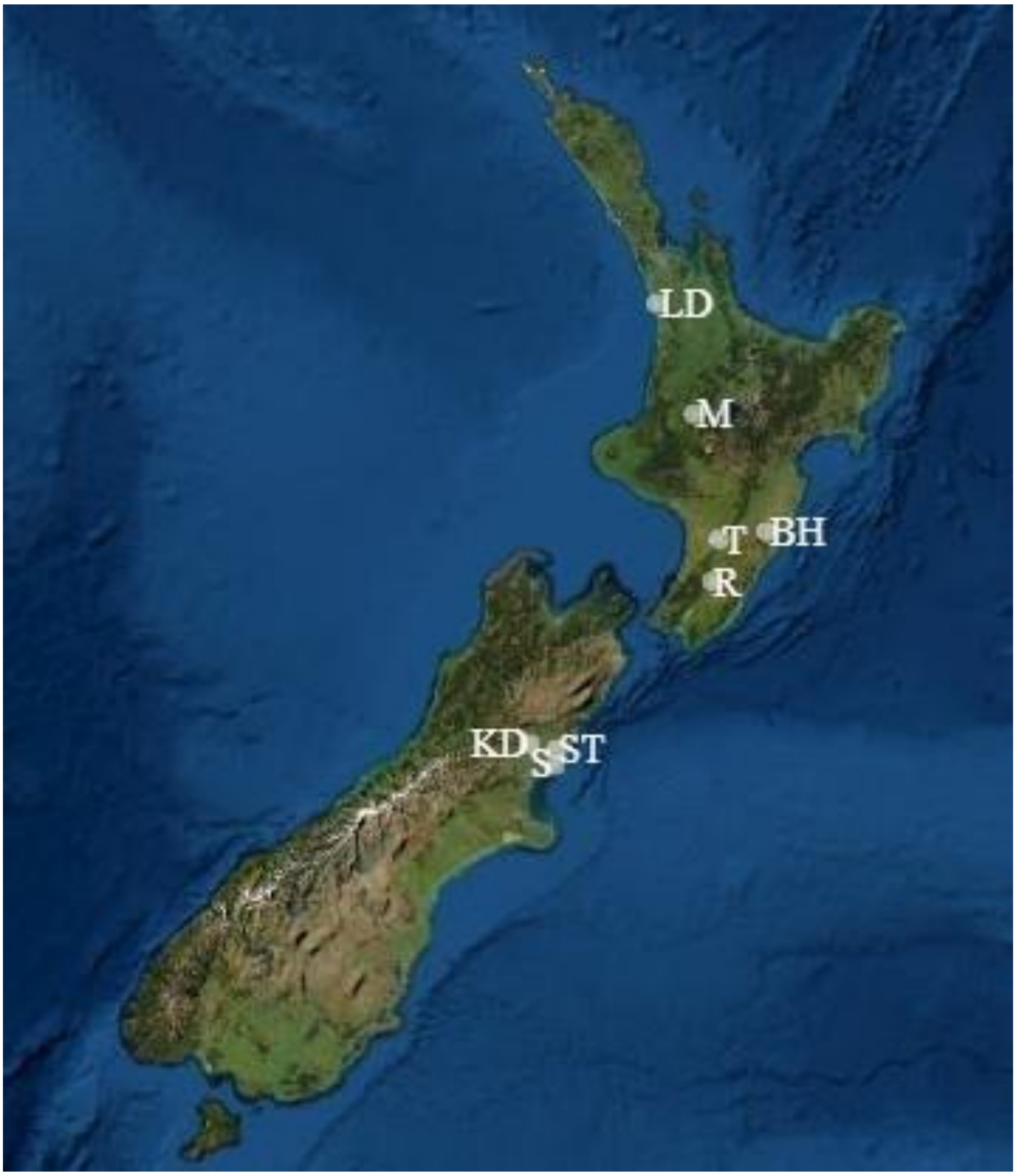


Figure 2.1 A map of New Zealand indicating the location of the eight farms where samples were collected. Each farm is indicated by an acronym to preserve its anonymity.

Table 2.1 The location, latitude, longitude, altitude, size of farm and the breed of ewes on each farm enrolled in the study. The exact location of these farms has been redacted due to privacy.

Farm	Region	Latitude[a]	Longitude[a]	Altitude (metres)	Size of farm	Breed of sheep
“T”	Manawatu	-40.#####	175.#####	80 - 250	476ha	A
“R”	Wairarapa	-40.#####	175.#####	180 - 250	686ha	A
“BH”	Hawkes Bay	-40.#####	176.#####	100 – 420	1,300ha	B
“M”	Ruapehu	-38.#####	175.#####	280 - 450	1,750ha	A
“LD”	Waikato	-37.#####	174.#####	0 - 200	1,500ha	A
“KD”	Canterbury	-42.#####	172.#####	280 - 450	3,219ha	A
“ST”	Canterbury	-42.#####	173.#####	160 - 280	1,800ha	A
“S”	Canterbury	-42.#####	173.#####	0 - 100	3,000ha	A

A Coarse-woolded, dual-purpose Romney

B Perendale

C Ultra fine merino sheep

a The exact latitude and longitude for each farm has not been provided due to privacy.

2.2.2 Morphological Identification

Upon collection, these samples were stored in 70% alcohol after collection and left at room temperature. Only intact specimens were selected for this comparative study. They were initially identified utilising a Leika EZ4 Stereo Microscope with a zoom range from 0.8x – 3.5x (Leica Microsystems GmbHErnst-Leitz-Straße 17-37, 35578 Wetzlar, Germany). Six morphological characteristics as outlined in (Table 2.2), were chosen from an examination of the literature as key characteristics to separate adults of *L. sericata* from *L. cuprina* (Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018). In the literature, several characteristics were noted to be specific to males and females and any characteristics related to the comparison of genitalia were not considered further for this study (Stevens et al., 2002, Williams and Villet, 2014).

Although the intention was to use a random sample of flies by farm and month, irregular catches meant a convenience sample of the best specimens available over the ten months of the collection was used instead. Approximately four flies, two identified morphologically as *L. cuprina* and two as *L. sericata* were selected each month from each farm over the collection period giving a total of 197 samples (See Appendix A, Table A.1

for a full list of samples, locations, and the dates of the collection). After morphological identification, these samples were then dried on a paper towel, placed on an insect pin, and stored in an entomological box at room temperature.

2.2.3 Morphological Re-examination after Molecular Identification on Key Samples

To further assess the accuracy of the morphological keys a small number of flies were selected after being classified utilizing molecular tools to carefully reassess the morphological markers using enhanced magnification. For this a total of 12 dried pinned specimens of *L. cuprina* and *L. sericata* were chosen whose identification had been confirmed molecularly using the 28s rRNA region and the mitochondrial ND4 gene. The number of samples to distinguish characteristics is considered adequate based on previous studies (Stevens et al., 2002, Williams and Villet, 2014). Photographs of each of the specimens were taken using an Olympus SZX7 (Olympus Corporation, 34-3 Hirai, Hinode-cho, Nishitama-gun, Tokyo 190-0182, Japan). To increase the depth of field of the images, multiple images were taken at different focus distances with a focus stacking method utilizing a pyramid smoothing function was used to combine the final image using the software Helicon Focus (199b Moskovsky Avenue, 61082, Kharkiv, Ukraine). Characters used in this key were compiled from the following morphological keys and are outlined in Table 2.2 (Waterhouse and Paramonov, 1950, Dear, 1986, Holloway, 1991, Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018).

Table 2.2 Characteristics examined to identify and distinguish *Lucilia cuprina* from *Lucilia sericata* including references for traits.

Characteristic	*Lucilia cuprina*	*Lucilia sericata*
Number of paravertical setae on the back of the head [a]	0-2	2-8
the number and location of hairs on the humeral callus and notopleuron [b]	6-8	0-4
Metasternal area [c]	Bare	Setose
Colour of fore femora [d]	Metallic blue to black	Metallic green blue
Bristles on scutulum [e]	Length of dorsal bristles smaller or equal in length to lateral hairs	Length of lateral hairs are longer than dorsal hairs
Width of the frontal stripe [f]	Frontal stripe as wide as parafrontal plate	Frontal stripe twice as wide as the parafrontal plate

a (Waterhouse and Paramonov, 1950, Dear, 1986, Holloway, 1991, Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018)

b (Waterhouse and Paramonov, 1950, Dear, 1986, Holloway, 1991, Wallman, 2001a, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018)

c (Wallman, 2001a, Akbarzadeh et al., 2015, Lutz et al., 2018)

d (Waterhouse and Paramonov, 1950, Dear, 1986, Holloway, 1991, Wallman, 2001a)

e (Waterhouse and Paramonov, 1950)

f (Waterhouse and Paramonov, 1950, Holloway, 1991)

2.2.3 Morphological key to identify *Lucilia cuprina, Lucilia sericata* and *Chrysomya rufifacies*

The following key was used to morphologically identify flies with the *Lucilia* genus and *C. rufifacies*. These are read and followed as per normal usage by moving to the next feature listed at the right-hand side following observation of the feature. Figures 2.2 – 2.7 show photographs to illustrate these morphological features.

1. Stem vein of wing bare on the dorsal surface (Figure 2.2 A); greater ampulla without stuff erect hairs (Figure 2.2 B)

……………………………..……………………………………………………...2

2. Stem vein haired on the dorsal surface (Figure 2.3 A); greater ampulla with stiff erect hairs (Figure 2.3 B)

………………………………………………………………….……………….…3

3. Lower calypters with hairs on dorsal surface; thorax non-metallic dark and dusted (Figure 2.2 B)

Lower calypters without hairs on the dorsal surface, thorax bright metallic green, sometimes bluish or copper (Figure 2.2 B, 1.4, 1.5)

……………………………………………………………………………………....4

4. Stiff effect hairs on greater ampulla (Figure 2.3 B); dorsal surface of lower calypters have dense hairs (Figure 2.3 B); anterior spiracles of thorax pale yellow cream or white (Figure 2.4); genae orange or black (Figure 2.4).

……………………………………………………*Chrysomya rufifacies* (Figure 1.5)

Katatergite bare or pubescent (Figure 2.5), basicosta bright yellow (Figure 2.6 B) *Lucilia* species

……………………………………………………………………………………....5

5. The posterior slope of humeral callus with 6 – 8 hairs (Figure 2.6); surface of notopleuron between notopleuron seta and edge of notopleuron with 8 – 16 hairs (Figure 2.6).

……………………………………………………… *Lucilia sericata* (Figure 1.3)

The surface of notopleuron between last notopleural seta and edge of notopleuron with only 2 – 5 hairs; metasternal area bare Figure 2.7); metasternal area bare (Figure 2.7).

……………………………………………………… *Lucilia cuprina* (Figure 1.4)

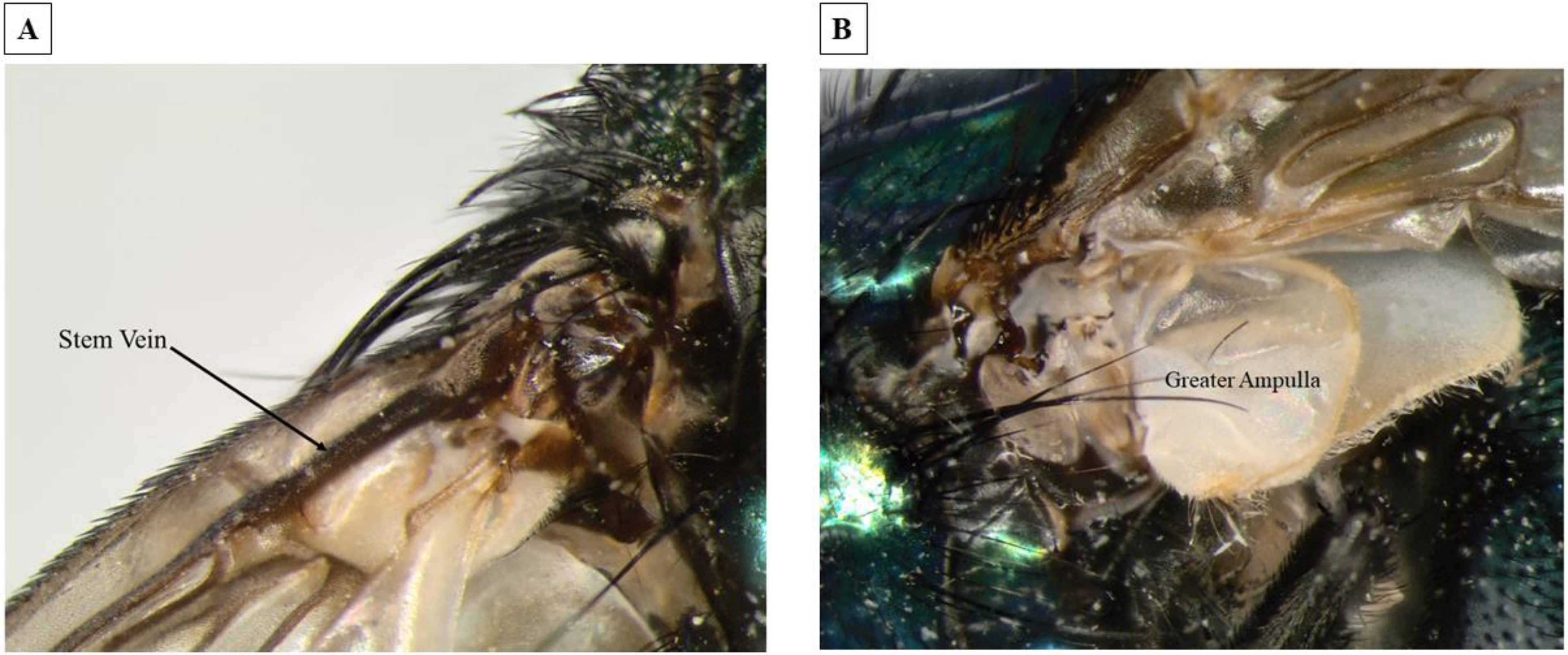


Figure 2.2 *Lucilia cuprina* basal part of the wing with stem vein indicated (A) and greater ampulla indicated from the anterior part of the abdomen (B).

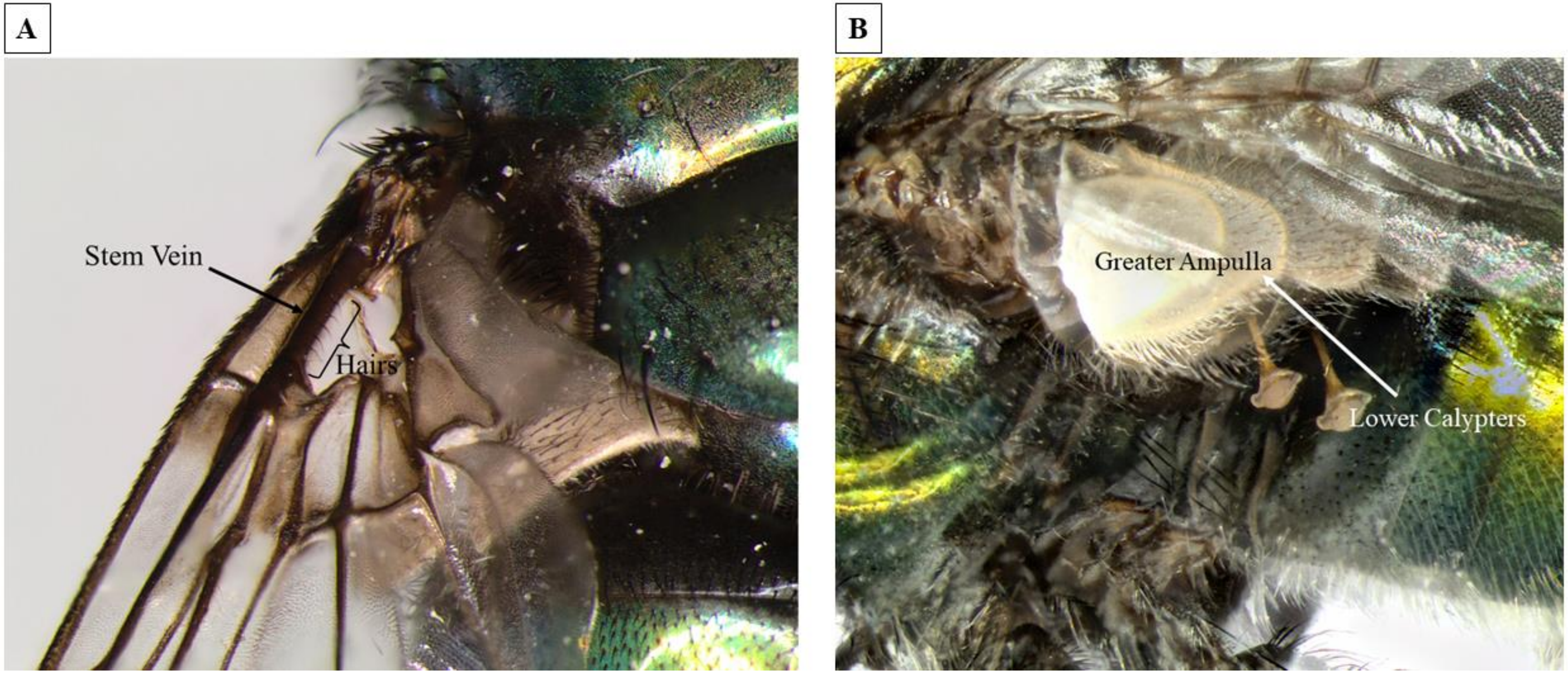


Figure 2.3 *Chrysomya rufifacies* basal part of the wing with stem vein indicated stem vein (A) and greater ampulla/ lower calypters indicated from the anterior part of the abdomen (B) outlined.

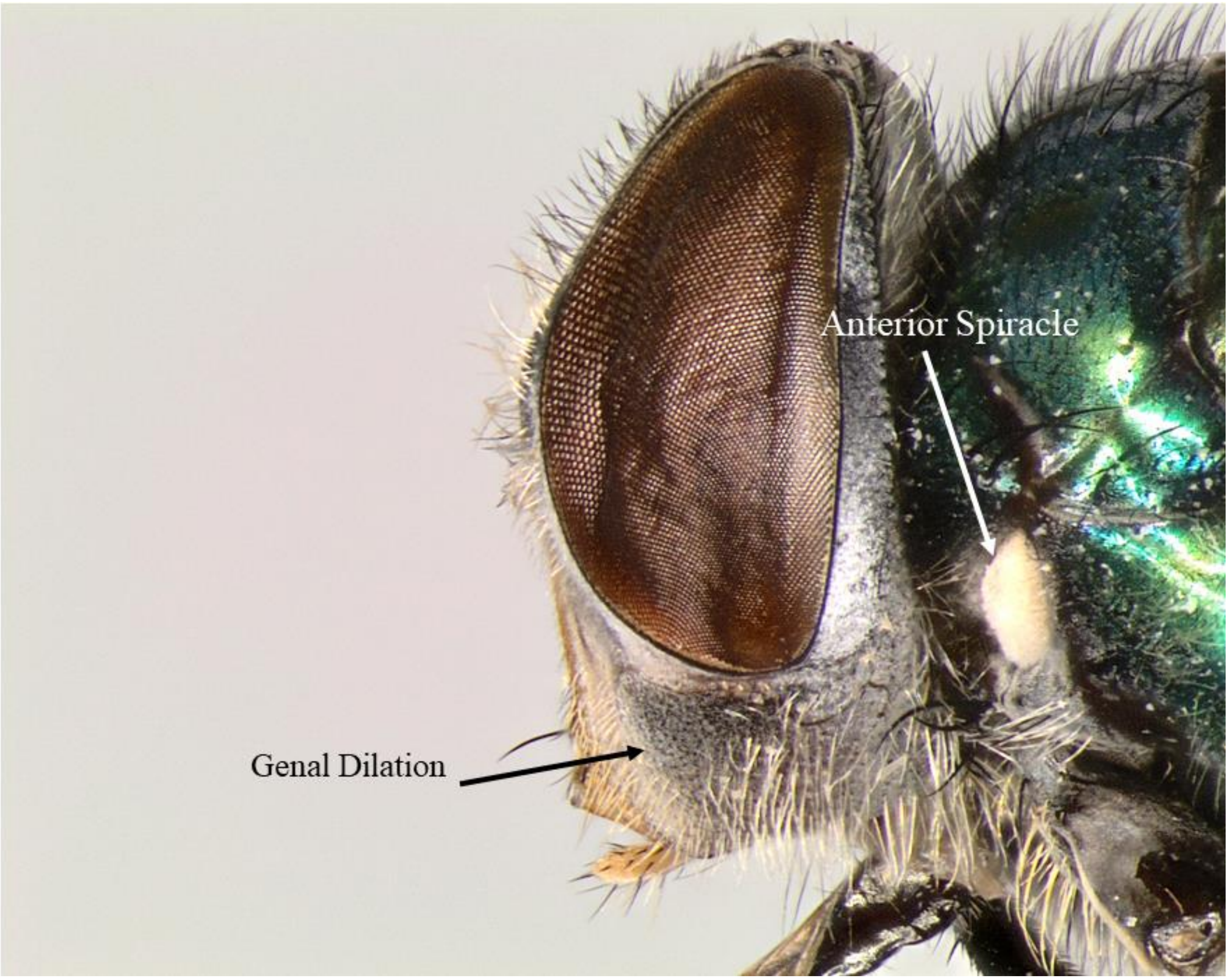


Figure 2.4 *Chrysomya rufifacies* lateral view of genal dilation and anterior spiracle.

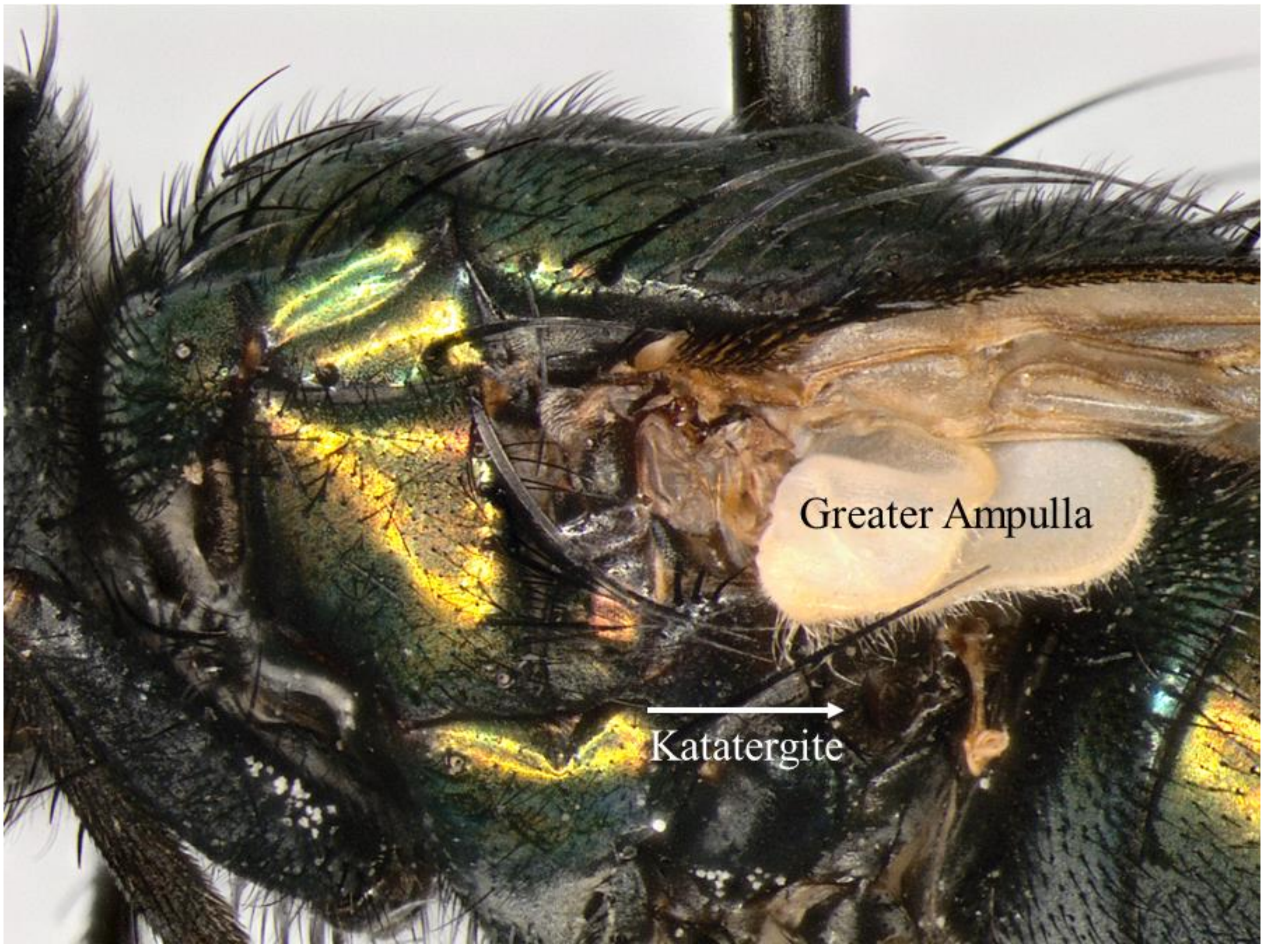


Figure 2.5 *Lucilia cuprina* anterior part of abdomen with greater ampulla and katatergite indicated.

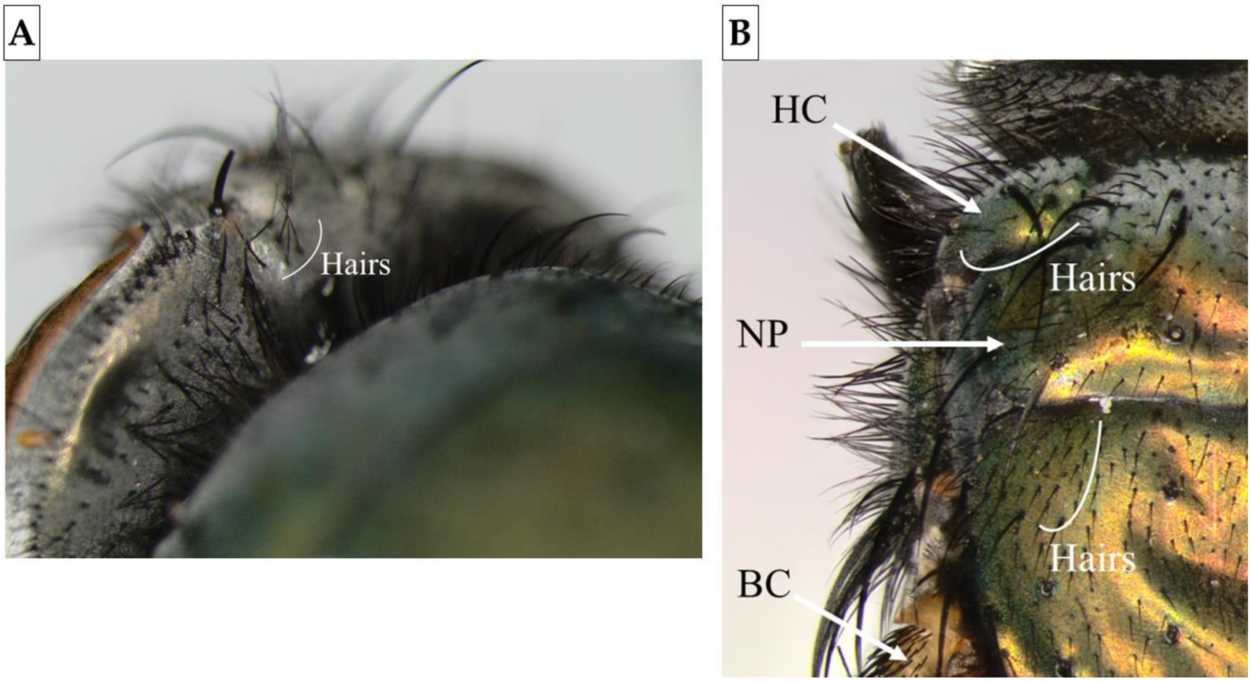


Figure 2.6 *Lucilia sericata* posterior view of head (A) and posterior view of the thorax (B). Humeral callus (HC), notopleuron (NP) and Basicosta (BC).

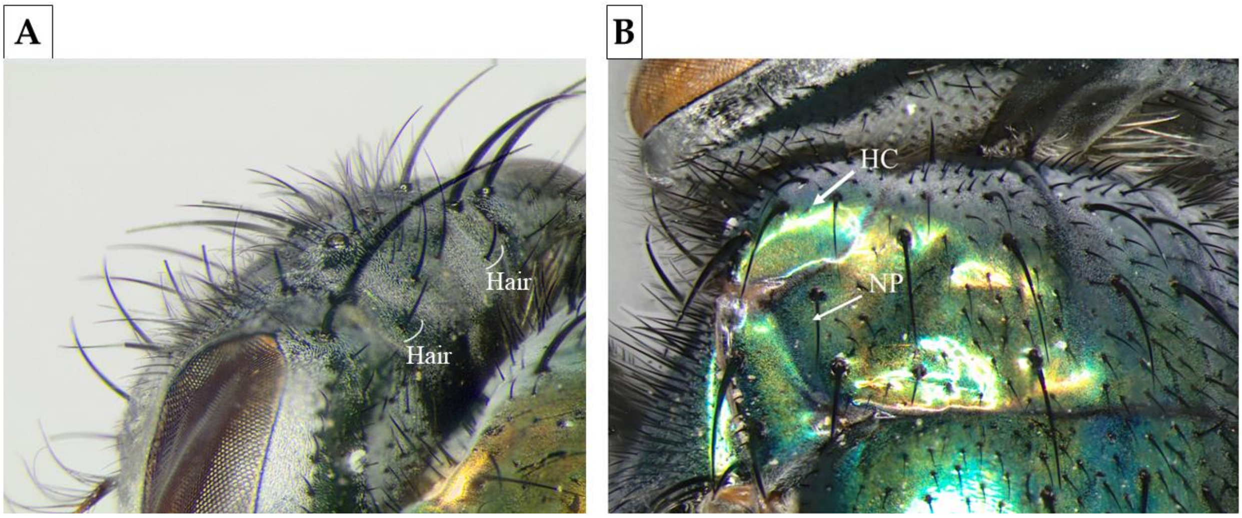


Figure 2.7 *Lucilia cuprina* posterior view of head (A) and posterior view of the thorax (B). Humeral callus (HC) and notopleuron (NP).

2.2.4 Molecular Identification

2.2.4.1 DNA Extraction

Two hind legs were removed from each fly using forceps and placed into a 1.5 ml microcentrifuge tube. The legs were then crushed using a micro-pestle for approximately 30 seconds. The remaining portion of the fly was then retained for later reference.

2.2.4.2 Salting out protocol

A salting-out protocol was utilised, where two rear legs were removed with forceps and crushed for approximately 30 seconds in a 1.5 µl Eppendorf container (Sunnucks and Hales, 1996). The specimens were then incubated overnight at 55 °C on a heat block with 600 µl of TNES buffer (50 mM Tris, 400 mM NaCl, 20 mM EDTA and 0.5 % v/v of SDS) with 10 µl of Proteinase K (20 mg/mL) (Ambion, Thermofisher, Waltham, Massachusetts, United States). They were then placed on a heat block at 100°C for five minutes to ensure the removal of all traces of Proteinase K from the sample. This was then precipitated with 85 µl of 5 M NaCl and shaken for 15 seconds. After which, the sample was then precipitated in 1 volume of 100 % ethanol and left overnight at –20 °C. The following day, the sample was centrifuged at 18,400 rcf for 5 minutes and the supernatant was poured out. The wash step was then repeated where 1 volume of 70 % ethanol was added to the sample and again centrifuged at 18,400 rcf for 5 minutes. The 70 % ethanol was then removed, and the sample was left for one hour to air dry at room temperature. Twenty microlitres of nuclease-free water were added to the air-dried DNA and this was left overnight at 4°C to resuspend the DNA.

2.2.4.3 PCR method

Two DNA regions were amplified by PCR and later sequenced: firstly, the 28s rRNA region in the nuclear genome and secondly for a subset of these, the mitochondrial ND4 gene (Table 2.3). Nuclear and mitochondrial sequences were run as a concentrate, a 1:10 dilution (2 µl: 18 µl nuclease-free water) and a 1:100 dilution (2 µl: 198 µl nuclease-free water) to determine the best dilution range. If no amplicons were observed, then the sample was rerun again with the same concentrate. If no amplicons were observed again, then the sample was rerun with an additional 1:200 (4 µl : 796 µl nuclease-free water) concentrate of the sample. A total reaction volume of 20 µl was prepared to consist of 2 µl of template DNA, 1x Solis Biodyne HotFirePol (Solis Biodyne, Estonia), 0.2 µM of each primer (Integrated DNA Technologies (IDT), IA, USA), all made to the final volume with nuclease-free water.

2.2.4.4 Primers used

The following primers were used to assess the 28s rRNA in the nuclear genome and the mitochondrial ND4 gene of *L. cuprina* and *L. sericata* (Table 2.3).

Table 2.3 List of nuclear and mitochondrial primers used in this study.

Region	Primer name	Sequence	Source
28s rRNA, nuclear	28 Forward_seq	5’ – GAG GGA AAG TTG AA AGA AC – 3’	(Stevens et al., 2002)
28s rRNA, nuclear	28 Reverse	5’ – GTT AGA CTC CTT GGT CCG TG – 3’	(Stevens et al., 2002)
28s rRNA, nuclear	28 Forward	5’ – CCC CCT GAA TTT AAG CAT AT – 3’	(Stevens et al., 2002)
28s rRNA, nuclear	28 Reverse_seq	5’ – CTC TCT ATT CAG AGT TCT TTT C – 3’	(Stevens et al., 2002)
ND4 mtDNA, mitochondrial	M432_R	5’ – TAA AAG AAA TCA ATG TAA AA – 3’	(Wallman et al., 2005)
ND4 mtDNA, mitochondrial	M378_F_MOD	5’ – CAA CCT GAA CGT TTG CAA GC – 3’	Designed in this study
ND4 mtDNA, mitochondrial	ND4_R2	5’ – TCT CGA ACA GAA CCT CCT CT – 3’	(Wallman et al., 2005)
ND4 mtDNA, mitochondrial	ND4_R1	5’ – TCT TCG TCT TCC TGT TCG TTC A – 3’	(Wallman et al., 2005)
ND4 mtDNA, mitochondrial	ND4_F	5’ – AAC CTG AAC GGT TGC AAG CG – 3’	(Wallman et al., 2005)
ND4 mtDNA, mitochondrial	M430_R Mod	5’ – GAA ACA GGA GCT TCA ACA T – 3’	Designed in this study

2.2.4.5 PCR Protocol

A touchdown program was carried out using an Eppendorf Mastercycler, Nexus GX2 (Hamburg, Germany) with an initial hold at 95 °C for 15 minutes, 10 cycles of 95°C for 30 seconds, then 60°C for 30 seconds with the temperature decreasing 1°C each subsequent cycle and finally 72°C for 30 seconds; then 40 cycles of 95°C for 30 seconds, 50°C for 30 seconds and then at 72°C for 1 minute and then elongated for 7 minutes at 72°C, the reaction was then held at 10°C. Samples were stored at 4°C until visualization. The same method was used for 28s rRNA and ND4 gene.

To assess the PCR results, the products were run on 1% agarose gels (Bioline, London, United Kingdom), in 1 X TAE (Tris-acetate-EDTA) (40mM Tris, 20mM Acetate and 1mM EDTA) stained with 5mM of Redsafe (iNtRON, Seongnam, South Korea). Agarose gels were run in 1 X TAE at 85V for 50 minutes. The size of PCR products was determined in comparison with a 100 bp ladder (Hyperladder, 100 bp, Bioline, London, United Kingdom). If amplicons of the appropriate size were observed; they were cut out using a scalpel and placed in a 1.5ml microcentrifuge container with 50µl of an elution buffer (5mM, Tris/HCl, pH 8.5); this was then left overnight at 4°C. Eluted DNA was submitted to Massey Genome Service (Massey University, Palmerston North, New Zealand) for bidirectional Sanger sequencing with appropriate primers.

2.2.5 Sequence analysis

2.2.5.1 28s rRNA

Sequences were visually assessed for quality. Using the software Geneious (10.2.6, 2018, BioMatters, Auckland, New Zealand), the consensus sequences were then pairwise aligned using the Geneious ‘Map to Reference” function with the default measures. The published reference sequences of 28s of *L. cuprina*, (AJ417709.1), and *L. sericata,* (FJ650535.1), were used to confirm the morphological identification of either species.

2.2.5.2 ND4

The sequences were first assessed visually, a consensus sequence was then generated of *L. cuprina* ND4 sequences which were pairwise aligned using the Geneious ‘Map to

Reference' function with the default measures to the following sequences of both subspecies of *L. cuprina* NC_019573 and JX913745 - JX913753 (Nelson et al., 2012). These sequences were also compared to the following *L. sericata* sequences: JX913754 - JX913757 (Nelson et al., 2012); this was to assess whether these sequences were hybrid species of *L. cuprina*/*L. sericata*. These published sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). All sequences were trimmed to 221 base pairs in length, to retain the maximum number of high-quality bases within the sequence length. These *L. cuprina* sequences were also translated to the protein using the insect mitochondrial code. These sequences were compared on three forward and three reverse reading frames to the *L. cuprina* subspecies sequences and *L. sericata* sequences using the insect mtDNA code. Phylogenetic analysis and tree construction was performed using Geneious (version 10.2.6). The support for each node was estimated using a bootstrap analysis with 100,000 replicates.

2.2.6 Statistical methods

A Chi-Squared Test was performed to see if there was a difference between the molecular and morphological identification methods. The overall totals of correctly and cumulative incorrectly identified species were used in this test (Table 2.4).

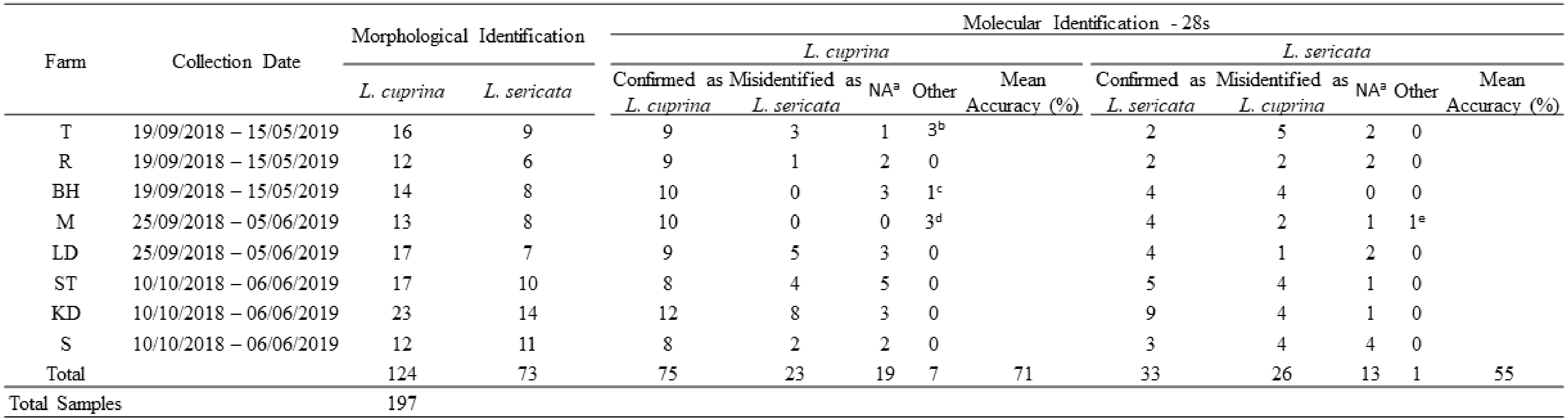
2.3 Results

A total of 197 samples were used in this study. However, 32 samples could not be successfully sequenced, leaving, a total of 165 samples that had the 28s rRNA region of the nuclear genome successfully sequenced. All combinations of primers were trialled using the touchdown cycle and consistent results were obtained using the 28s_Fseq + 28s_R primer combination (Table 2.4). Two specimens were identified as *Pales pavida* and six were identified as being *Chrysomya rufifacies* (Table 2.4). The morphological identification of *L. cuprina* was confirmed in 75 specimens and *L. sericata* for 33 specimens (Table 2.4). However, 26 specimens originally identified morphologically as *L. cuprina* were shown to have been misidentified and were confirmed as being *L. sericata* (Table 2.4). In addition, 23 specimens originally identified morphologically as *L. sericata* were also shown to have been misidentified and were confirmed as *L. cuprina* specimens. Overall, the accuracy of morphological identification of *L. cuprina* was 71 % and for *L. sericata* was 55 %. All *L. cuprina* and *L. sericata* sequences were found to be identical to the 28s reference sequences. No difference was found between farms for the 28s sequences for both species.

For the ND4 region, a total of 100 *L. cuprina* sequences were used that had been identified as this species from the 28s rRNA primer (Table 2.5). The ND4 primers were run using the touchdown cycle with all possible combinations of primers with these *L. cuprina* samples (Table 2.5). Positive amplicons were produced with the M378_F and M430_R_mod (Table 2.5). A total of 70 sequences were successfully sequenced whilst 30 were not (Table 2.5). Each of these sequences was found to align to previously published *L. cuprina* sequences (Table 2.5). None of the mitochondrial *L. cuprina* sequences was found to align to previously published *L. sericata* sequences. This indicates there were no hybrid flies were characterised.

The Chi-Squared test found there was a significant difference between the molecular and morphological identification for both species ($p<0.05$). The morphological identification of these species was then rechecked based on the confirmed molecular identification. The source of the error was attributed to a misidentification based on the initial morphological characters and not with the published keys. No single character was identified which led to the incorrect identification of these species.

Table 2.4 A summary of the comparison of morphological and molecular identification using the nuclear 28s rRNA gene of *Lucilia cuprina* and *Lucilia sericata.* The accuracy of the morphological identification of both species is also outlined.



Farm	Collection Date	Morphological Identification		Molecular Identification - 28s									
				L. cuprina					*L. sericata*				
		L. cuprina	*L. sericata*	Confirmed as *L. cuprina*	Misidentified as *L. sericata*	NA[a]	Other	Mean Accuracy (%)	Confirmed as *L. sericata*	Misidentified as *L. cuprina*	NA[a]	Other	Mean Accuracy (%)
T	19/09/2018 – 15/05/2019	16	9	9	3	1	3[b]		2	5	2	0	
R	19/09/2018 – 15/05/2019	12	6	9	1	2	0		2	2	2	0	
BH	19/09/2018 – 15/05/2019	14	8	10	0	3	1[c]		4	4	0	0	
M	25/09/2018 – 05/06/2019	13	8	10	0	0	3[d]		4	2	1	1[e]	
LD	25/09/2018 – 05/06/2019	17	7	9	5	3	0		4	1	2	0	
ST	10/10/2018 – 06/06/2019	17	10	8	4	5	0		5	4	1	0	
KD	10/10/2018 – 06/06/2019	23	14	12	8	3	0		9	4	1	0	
S	10/10/2018 – 06/06/2019	12	11	8	2	2	0		3	4	4	0	
Total		124	73	75	23	19	7	71	33	26	13	1	55
Total Samples		197											

[a] Sequencing or PCR unsuccessful

[b] *Pales pavida* 2, *Chrysomya rufifacies* 1

[c] *Pales pavida* 1

[d] *Chrysomya rufifacies* 3

[e] *Chrysomya rufifacies* 1

Table 2.5 Outlines the sequenced *Lucilia cuprina* samples using the mitochondrial dehydrogenase subunit 4 (ND4) gene

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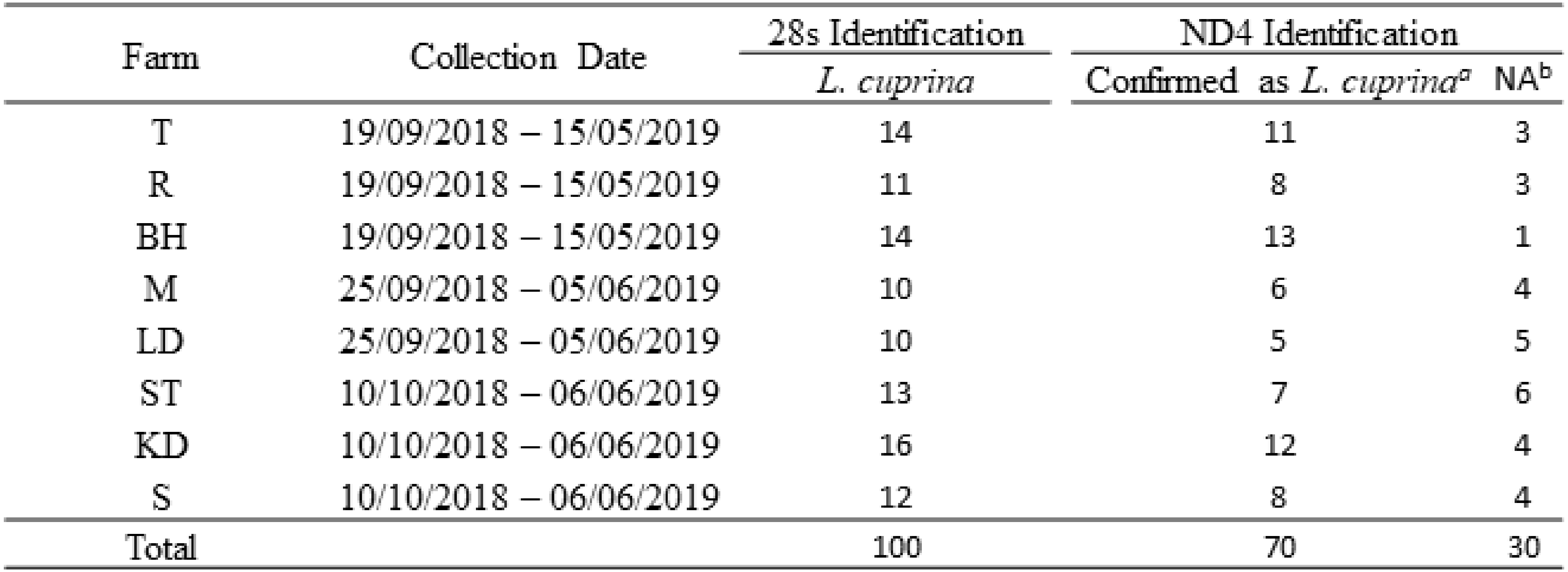


Farm	Collection Date	28s Identification	ND4 Identification	
		L. cuprina	Confirmed as *L. cuprina*[a]	NA[b]
T	19/09/2018 – 15/05/2019	14	11	3
R	19/09/2018 – 15/05/2019	11	8	3
BH	19/09/2018 – 15/05/2019	14	13	1
M	25/09/2018 – 05/06/2019	10	6	4
LD	25/09/2018 – 05/06/2019	10	5	5
ST	10/10/2018 – 06/06/2019	13	7	6
KD	10/10/2018 – 06/06/2019	16	12	4
S	10/10/2018 – 06/06/2019	12	8	4
Total		100	70	30

[a.] defined on the basis of polymorphisms at position 6 (A/T), position 21 (T/C) position 63 (A/G), position 66 (C/T), position 69 (A/G), position 142 (A/G), position 150 (T/C), position 213 (C/T) and position 216 (A/C) compared to published sequences of *Lucilia cuprina* (JX913746.1, JX913752.1) and *Lucilia sericata* (JX913754.1). See Figure 2.10.

[b.] Sequencing or PCR unsuccessful

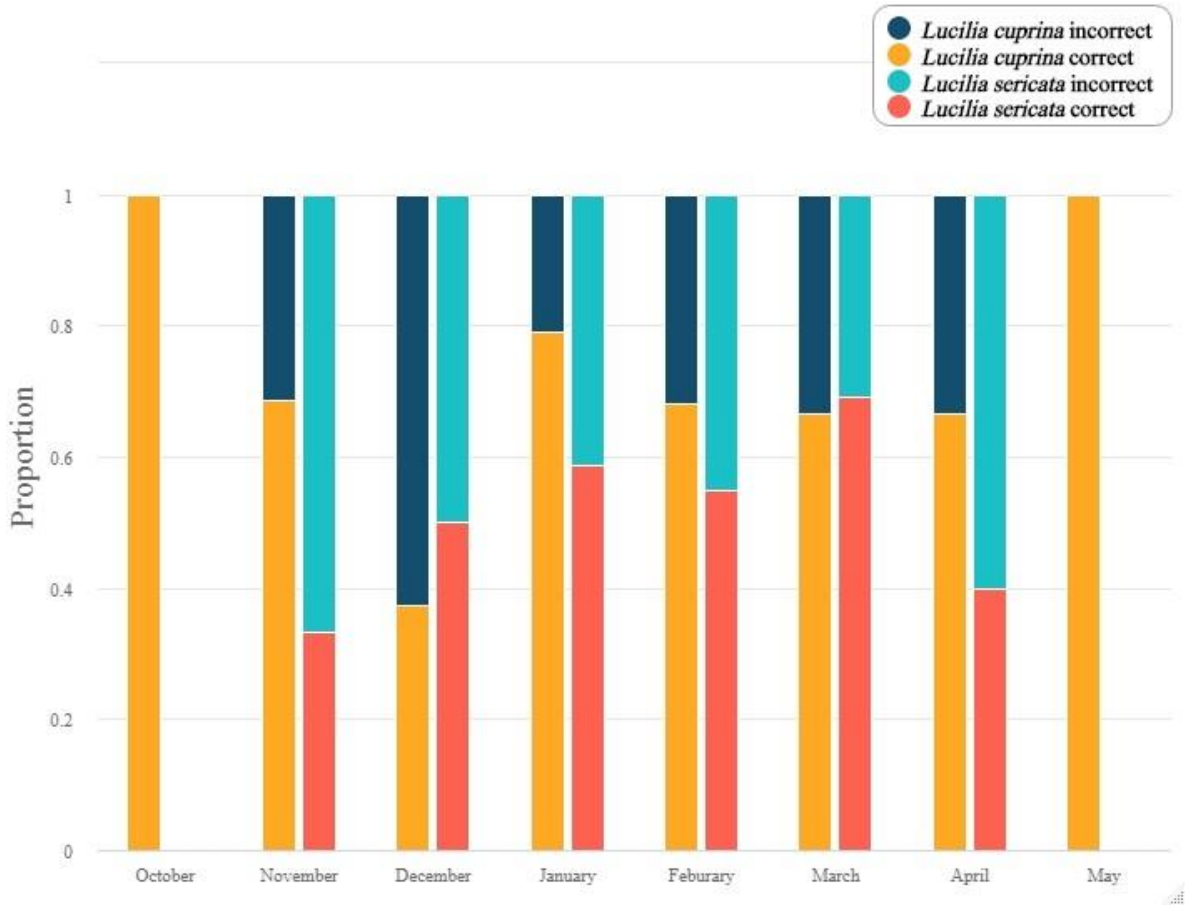


Figure 2.8 The proportion of *Lucilia cuprina* and *Lucilia sericata* were identified per month across eight farms in New Zealand using the nuclear 28s rRNA gene. Samples from all farms are coagulated per month.

The accurate morphological identification of *L. cuprina* and *L. sericata* was found to decrease per month (Figure 2.8). The highest number of misidentifications occurred during February and March when most samples were collected (Figure 2.8). No morphological misidentifications occurred during the months of October and May (Figure 2.8). *Lucilia cuprina* was found to be the sole species present during the months of October and May on each farm (Figure 2.8). While both species were present from November until April on each farm (Figure 2.8).

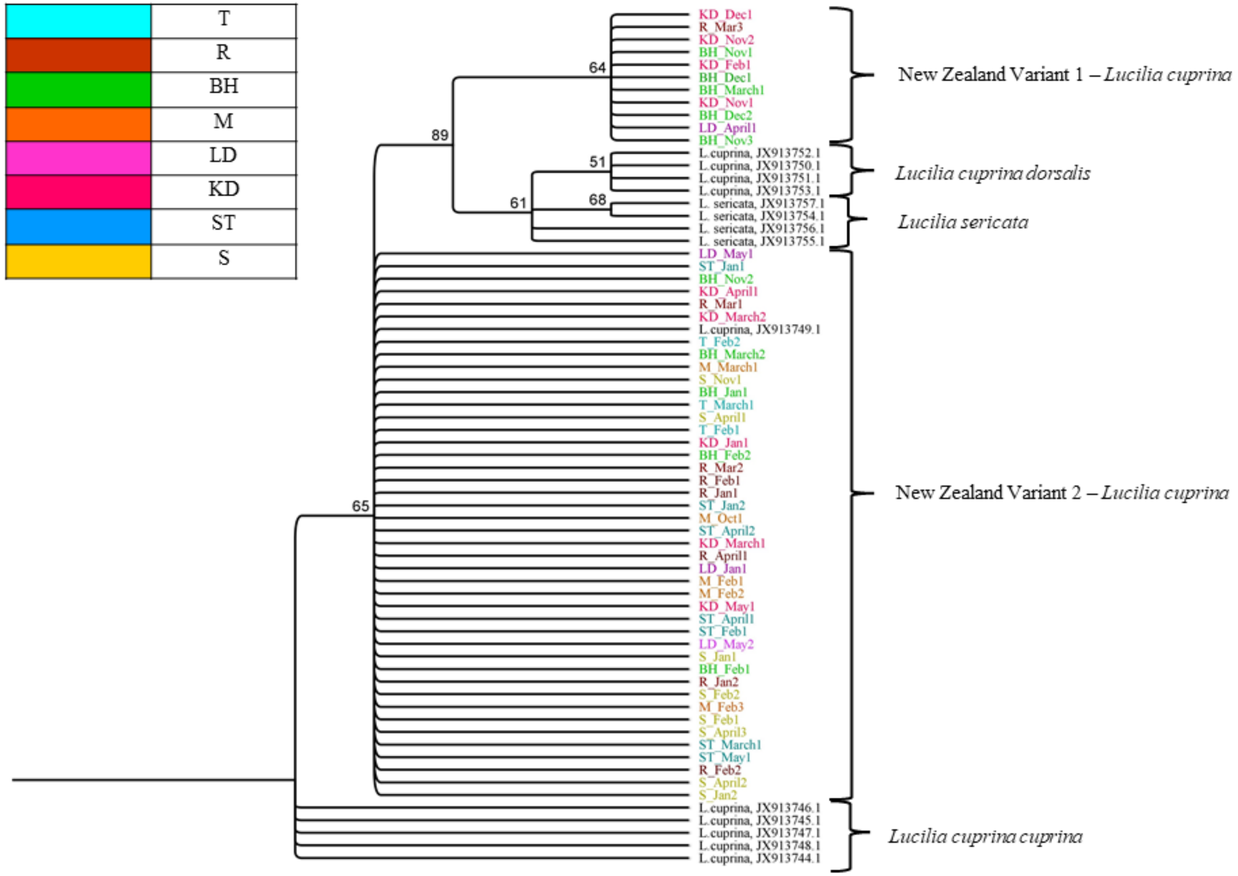


Figure 2.9 A phylogenetic tree of the two New Zealand variants of *Lucilia cuprina* based on 221 base pair regions of the mitochondrial ND4 region compared with publicly available sequences of *Lucilia cuprina* and *Lucilia sericata* from NCBI. The NCBI accession numbers for each of these sequences are shown. Phylogenetic analysis and tree construction were performed using Geneious (version 10.2.6). The nodal support was determined using a bootstrap analysis with 100,000 replicates in Geneious (version 10.2.6), and a consensus tree with a 50 % threshold was generated from the bootstrapped data.

The phylogenetic tree outlined in Figure 2.9 was developed from the analysis of 67 sequences of the mitochondrial ND4 gene from the confirmed *L. cuprina* samples in this study and the nine *L. cuprina* and *L. sericata* sequences obtained from NCBI. The phylogenetic tree in Figure 2.9 shows three groups within the *Lucilia cuprina* species. There is strong support for the first group being comprised of *L. c. dorsalis* and a potential New Zealand variant (New Zealand Variant 1) of *L. cuprina*. *L. sericata* would appear to sit within this same group. A second group consists of just the second New Zealand variant (New Zealand Variant 2) and the third group comprises *L. c. cuprina* using sequences from NCBI. There was no particular trend where one variant was found on only one farm nor was there a seasonal trend identified of one subspecies being found earlier or later in the season than the other (See Table A.1 in Appendix A). Further work is required to confirm if this separation is correct within the *Lucilia* genus given the length of these sequences.

There were no morphological characteristics identified as part of this study to support the separation of these two variants to these groupings. The two New Zealand variants of *L. cuprina* can be characterised based on the polymorphisms in the ND4 gene at position 6 (A/T), position 21 (T/C) position 63 (A/G), position 66 (C/T), position 69 (A/G), position 142 (A/G), position 150 (T/C), position 213 (C/T) and position 216 (A/C) (Figure 2.10).

Sequences were translated using the insect mtDNA code and when translated in the first frame reversed this then aligned to published sequences (AFV08423.1, AFV08501.1 and AFV08527.1) (Figure 2.10). All polymorphisms observed conferred a synonymous substitution except the mutation at position 142 (Figure 2.10), creating a non-synonymous substitution and changing the protein sequence from alanine to valine.

The two *L. cuprina* (*L. c. dorsalis* and *L. c. cuprina*) genotypes and New Zealand Variant 2 of *L. cuprina* have valine in the same region with identical protein sequences (Figure 2.11). The Alanine mutation was found to be prevalent in 11/70 specimens. Whereas the Valine mutation was found in a significant (*p* ≤0.05) proportion of the specimens (59/70) according to a t-test.

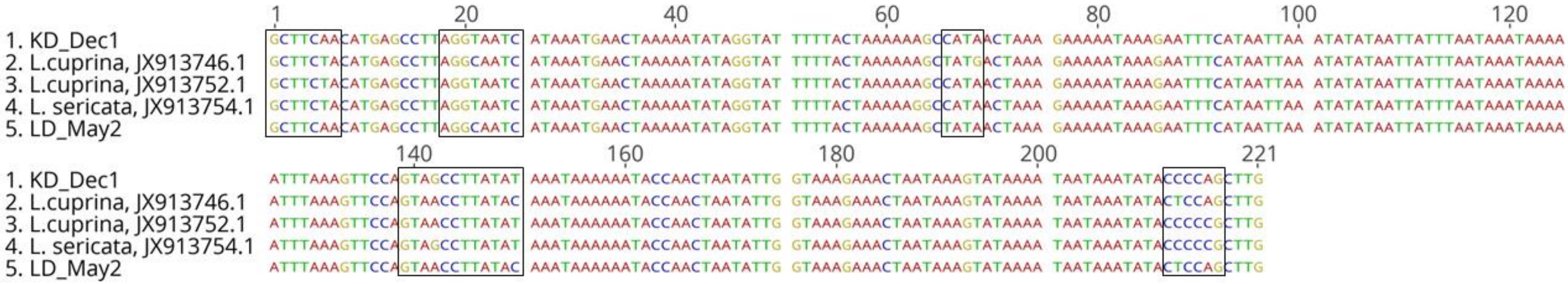


Figure 2.10 Mutations were identified on the mitochondrial dehydrogenase subunit 4 (ND4) gene in nine separate positions for the two New Zealand variant *Lucilia cuprina* sequences compared to previously published sequences of *Lucilia cuprina* (JX913746.1, JX913752.1) and *Lucilia sericata* (JX913754.1).

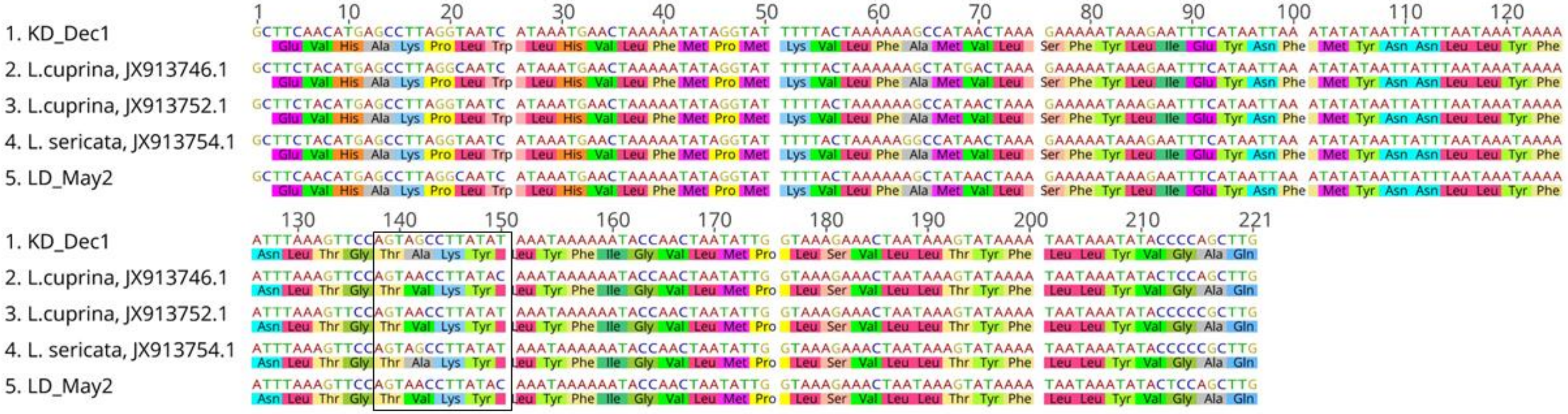


Figure 2.11 Translation of proteins in frame 1 in reverse of the mitochondrial dehydrogenase subunit 4 (ND4) sequences of the two New Zealand variants of *Lucilia cuprina* compared to previously published sequences of *Lucilia cuprina* (JX913746.1, JX913752.1) and *Lucilia sericata* (JX913754.1).

2.4 Discussion

The key aim of this study was to assess morphological and molecular methodologies that may be used to distinguish *L. cuprina* and *L. sericata*. It was found that the accuracy of the morphological identification was not consistent for these species when compared to the results from sequencing the 28s rRNA region. Using the ND4 gene for confirmed specimens of *L. cuprina* it was apparent there were two genotypes of this species in New Zealand and neither exactly matched with published sequences of *L. c. dorsalis* or *L. c. cuprina*. There was also no evidence found for the existence of a hybrid species of *L. cuprina*/*L. sericata* in New Zealand.

2.4.1 Limitations of Morphological Identification

The results of this study demonstrate the limitations of morphological identification of *Lucilia* species. It is noted that there is considerable variation in the number paravertical setulae between each species hence why a combination of characters should be employed as suggested by other authors (Akbarzadeh et al., 2015, Lutz et al., 2018). Despite using a combination of these characters, misidentifications of *L. cuprina* and *L. sericata* clearly occurred. However, a re-examination of these samples found the morphological keys to be accurate.

The influence of a seasonal sample of these species may also affect the size of the specimens at the start and the end of the season. Therefore, the quality of the samples may have impacted the accuracy of the morphological identification. For example, the highest number of misidentifications of *L. sericata* took place in November and April which in New Zealand would represent the start and the end of the season for this species (Miller, 1939, Dear, 1986, Heath and Bishop, 1995, Heath and Bishop, 2006). As the traps were only emptied once a week which could have led to damage or post-mortem changes which could have made morphological identification less accurate. However, the sampling method tried to overcome this by selecting only well-preserved specimens.

Difficulty in morphologically identifying other *Lucilia* species such as *L. caesar*/*L. illustris* has previously been noted (Velasquez et al., 2010, Szpila, 2012). As evidenced in this study, there is difficulty in consistently identifying these species using morphological identification alone, especially when examining large numbers of flies (including bycatch) caught during field studies. This was shown by a comparison of the two identification methods using a chi-squared test. It would be recommended that a proportion of samples should be verified via a suitable molecular marker for future studies. It would be recommended that in future studies a proportion of samples should be verified by a second experienced person, which was not done in this study and later via a suitable molecular marker to ensure accurate identification.

2.4.2 Variants of *Lucilia cuprina* from New Zealand

The results of this study using the ND4 gene suggest that there are three genotype groupings within the *Lucilia* genus: the first comprising *L. c. dorsalis*, *L. sericata* (both from NCBI sequences) and New Zealand Variant 1 of *L. cuprina*, the second grouping containing New Zealand Variant 2 of *L. cuprina* and the third comprising *L. c. cuprina* (from NCBI sequences). There does not appear to be a particular regional trend of a certain genotype, nor does it appear to be a certain genotype being found earlier or later in the season based on the samples used in this study. Furthermore, based on the morphological identification of these species, there is no evidence to suggest that there are any particular morphological features that are unique to New Zealand in the *Lucilia* genus compared to those previously described (Wallman, 2001a, Akbarzadeh et al., 2015, Lutz et al., 2018). There was also no support that there is a hybrid species of *L. cuprina*/*L. sericata* in New Zealand based on the samples from this study. If this was the case one would have expected to have a match with the mitochondrial region of the *L. sericata* region much like Stevens et al. (2002). In this study, all mitochondrial region sequences of confirmed *L. cuprina* by 28s rRNA also aligned to *L. cuprina.*

2.4.3 Protein structure

A non-synonymous substitution was found in the *L. cuprina* mitochondrial ND4 region from this region compared to previously published sequences. Upon a translation of the ND4 region, a mutation of a single alanine to a valine was found in both *L. cuprina* variants unique to New Zealand. It could be investigated further if the *L. cuprina* variant present in New Zealand without this valine protein substitution has a competitive advantage or is it found in higher proportions than the other with the mutation. It is not expected that this substitution will induce a change in function due to the relative similarity in the structure, both are non-polar and hydrophobic amino acids.

2.4.4 Limitations of the study

Currently, there is considerable uncertainty regarding which subspecies of *L. cuprina* are currently found in New Zealand. In addition, there have been no previous studies which have investigated the geographical spread of the subspecies within New Zealand. It was more pertinent to conduct a larger study of samples with fewer markers than a small survey with a large number of samples. The latter method would be more suitable in later studies, as it can be shown that both subspecies have been identified in New Zealand, or at least at the border. In addition, Tourle et al. (2009) noted that only a small proportion of *L. cuprina* samples were hybrid species as part of a study in South Africa, it is arguable that by using a smaller sample size; some of the variability from these samples would have been missed. Furthermore, it was more time and cost-efficient to use a PCR method to assess which subspecies of *L. cuprina* are in New Zealand and if there is a hybrid species of *L. cuprina*/*L. sericata* in New Zealand.

Genetic mutations in the ND4 region have been documented in mosquito species such as *Aedes agypti* (Lopes et al., 2021) and *Anopheles sinensis* (Ding et al., 2020) inducing resistance to pyrethroids. This indicates the importance of the mtDNA ND4 gene for the potential development of pesticide resistance. Resistance to insecticide chemicals such as organophosphates (Wilson and Heath, 1994, Wilson et al., 1996), diazinon (Levot, 1995) and insect growth regulators (Levot and Sales, 2004) have been noted in *L. cuprina*. These studies infer resistance to certain insecticides, but they do not offer sufficient detail on a molecular basis. Knowing which regions of the genome of *L. cuprina* where resistance may occur or develop over time is important as it will allow for the development of new insecticides (Anstead et al., 2015). There are no reports of known insecticide resistance from New Zealand in *L. cuprina* (except for organophosphates). However, the presence of insecticide resistance in other species associated with the mtDNA ND4 indicates that further study into the mutations observed in this research are warranted. These investigations could be targeted insecticide resistance tests and full mtDNA sequencing. It is also a notable issue that there are only ND4 sequences of *L. cuprina* from Australia currently available which greatly limits comparisons that could be made to sequences worldwide. Further studies could proactively monitor the resistance of *Lucila* species across a country and actively communicate if there is any growing resistance to any chemicals used to combat flystrike to farmers.

2.4.5 Further Work

The improvement of the method of this study should consider what questions to address for future work whether they are for a quick method for identification of specimens or for a more nuanced approach. A molecular method to rapidly identify *L. cuprina* and *L. sericata* should be implemented as part of a routine assessment of the population given the prevalence of subspecies and variants of *L. cuprina*. This is especially important where many specimens may need to be identified and assurances can be made that this would be consistent Sanger sequencing may be the gold standard, but it also takes around a week for the full process to be performed. It could also be considered that a more rapid method such as quantitative (qPCR) and loop-mediated isothermal amplification (LAMP) could be implemented. These methods have the advantage of being completed within an hour or less, whereas Sanger sequencing takes much longer. These methods are commonly implemented to detect mosquitoes in a cost-effective manner (Thabet et al., 2022) and as part of wider vector surveillance programs for various diseases carried by mosquitos (Fynmore et al., 2021, Dieme et al., 2022).

From a more phylogenetic perspective, targeted studies using methods such as shotgun sequencing, next-generation sequencing, microsatellites, or full genome sequencing should be implemented to identify if there are variants of *L. cuprina* which are currently present in New Zealand. In addition, similar studies could be conducted to confirm the existence of a hybrid species of *L. cuprina*/*L. sericata*.

2.5 Conclusion

The results of this study identify the limitations in accurately identifying *L. cuprina* and *L. sericata* using morphological methods alone and reinforces the need to use a molecular identification method. There was no evidence to suggest that there are any morphological features that are unique to New Zealand in the *Lucilia* genus which differ compared to those previously described. There is no evidence to suggest that there is a hybrid species of *L. cuprina*/*L. sericata* present in New Zealand. The results of this study suggest that there are three genotype groupings in the ND4 gene within the *Lucilia* genus: the first comprising *L. c. dorsalis*, *L. sericata* and the New Zealand Variant 1 of *L. cuprina*, the second grouping containing New Zealand Variant 2 of *L. cuprina* and the third comprising *L. c. cuprina* found in New Zealand. This is based on the presence of nine mutations in the ND4 genome of *L. cuprina*. A non-synonymous substitution of a single alanine to a valine has been found in one of the variants of the *L. cuprina* clades from the samples collected in New Zealand and its origin is unclear.

A consequence of the finding of inaccuracy of morphological identification in this study has meant that for all subsequent chapters in this thesis no attempt will be made to differentiate between *L. cuprina* and *L. sericata* and these will just be considered as *Lucilia* spp.

Chapter Three. A field evaluation of the LuciTrap and the Western Australian Trap with three different bait types for catching *Lucilia* spp.

3.1 Introduction

Flystrike or cutaneous myiasis is a disease caused by certain species of blowflies that lay eggs on the skin surface of sheep with larvae that subsequently parasitise the skin surface. If left untreated, a sheep can be subject to repeated oviposition; this can result in distress to the animal and can lead to the death of the animal. In New Zealand, three species of Diptera can cause flystrike in the absence of open wounds: *L. cuprina* and *L. sericata* are regarded as the main species, while *C. stygia* is associated with only a few cases (Heath and Bishop, 1995, Heath and Bishop, 2006).

Flytraps can be utilised on sheep farms for two main purposes. The first use is to reduce the populations of *Lucilia* spp. and, thus, the incidence of flystrike on sheep (French and Morgan, 1996, Heath and Leathwick, 2001). The second use is as a surveillance tool, enabling farmers to recognise when myiasis-inducing flies are present on their farms, i.e., to signal the start of a new flystrike season and make informed strategic management decisions to control flystrike (Heath and Leathwick, 2001). It was shown that, in the United Kingdom, the deployment of flytraps in early spring reduced the emerging population of *L. sericata* and reduced the annual flystrike levels (French and Morgan, 1996). However, it is shown that the use of flytraps did not reduce the number of cases of flystrike on a farm in New Zealand during the course of a season (Heath and Leathwick, 2001).

Numerous types of flytraps have been trialled and used on farms, including bait bins (Anderson et al., 1990), the Western Australian Trap (Newman and Clark, 1926, Vogt and Havenstein, 1974, Cole, 1996), a wind-orientated Western Australian Trap (Vogt et al., 1985a, Cole, 1996, Dymock et al., 1991, Azevedo and Kruger, 2013), water-based traps', such as the such as the Rescue Trap ®, Red Top Flycatcher ®, the Buzz Disposable Flycatch ® and Easy trap ® (Richards et al., 2009b, Harvey et al., 2010) and the LuciTrap® (Urech et al., 1998, Urech et al., 1996, Urech et al., 2004, Urech et al., 2009). The LuciTrap® includes three chemical lures (LuciLures®), which are held in plastic containers within the body of the LuciTrap®. The LuciLure® containers have open cotton wicks to dispense chemicals and last for up to six months.

Although there have been some contrasting results, overall, the LuciTrap® has been shown to be the most effective trap for *L. cuprina* in Australia (Urech et al., 2009) and South Africa (Scholtz et al., 2000). Although the effectiveness of the LuciTrap® for *L. cuprina* in New Zealand is currently unknown, it appears to be the most appropriate method for trapping *L. cuprina* due to the specificity of its trapping design. Two important features of the trap design are the attractiveness and persistence of the chemical lures and the size of the holes in the lid, which restrict the entry of non-target flies (Urech et al., 1994). In contrast, there have been differing reports of the success of the LuciTrap® for catching *L. sericata*, performing poorly in Hungary (Hall et al., 2003) compared to South Africa (Scholtz et al., 2000) and Australia (Urech et al., 2009, Horton et al., 2001a). In the Hungarian study, it was theorised that the holes in the lid of the LuciTrap were too small for *L. sericata* to enter the trap itself, as flies were observed around the periphery of the LuciTrap® but not inside the trap itself (Hall et al., 2003). Alternative lures and not the three LuciLures® from the LuciTrap® were used in the Hungarian study, which may account for the differences found in that study compared to those in South Africa and Australia.

The Western Australian Trap design has been widely used in several studies in Australia to trap *L. cuprina* and *L. sericata* using sheep liver and sodium sulphide as bait (Vogt and Havenstein, 1974, Vogt et al., 1983). More recently a modified version of the Western Australian Trap constructed from easily obtained plastic containers has been used extensively in studies across New Zealand (Cole, 1996). The overall efficiency of the Western Australian Trap is unknown, although previous studies using this trap have reported that it is effective in trapping all target species (Cole, 1996).

Sheep liver in 30 % sodium sulphide has historically been used as bait to successfully attract necrophagous flies. The addition of sodium sulphide prevents desiccation of the liver itself, as well as acting as a chemical attractant. However, one of the major drawbacks of this bait is the need to replace it on a fortnightly basis (Hwang and Turner, 2005). Furthermore, there are health and safety concerns about the use of sodium sulphide in New Zealand at present and access to the chemical for farmers to self-dispense is restricted, owing to the dangers it poses to people and the wider environment

(Government, 1997). It is unknown if substituting the liver in sodium sulphide with an alternative offal bait or with the LuciLures in the Western Australian Trap could be as effective in attracting and monitoring a variety of necrophagous flies, although the use of LuciLures® themselves have some restrictions. One alternative bait is squid, which was found to be effective in attracting *L. sericata* and other blowflies (Baz et al., 2007). Further potential benefits of squid are that it does not require the addition of chemicals and is readily available through fishing tackle outlets.

However, the effectiveness of squid for monitoring both *L. sericata* and *L. cuprina* when placed in the Western Australian Trap is unknown under New Zealand conditions. Therefore, this study aimed to contrast the effectiveness and specificity of the catch of LuciTrap with its lures and the Western Australian Trap using three types of lures (LuciLures, squid and sheep liver with 30 % sodium sulphide). The overall aim of this study was to provide farmers with a trap and bait combination that may be used to monitor the populations of these species and, therefore, be used to actively manage flystrike on farms.

3.2 Methods

This was an incomplete factorial experimental design in which two types of traps: the LuciTrap® (Bugs for Bugs, Toowoomba, Queensland, Australia) and the Western Australia Trap as modified by (Cole, 1996) and three types of baits (LuciLure®, Bugs for Bugs, Toowoomba, Queensland, Australia) comprising three separate chemical lures (LuciLure® A—120 g/L sodium sulphide—80 mL; LuciLure® B—1055 g/L 2-mercaptoethanol at 95 %, 47 g/L indole—60 mL at 5 %; and LuciLure® C—960 g/L butanoic acid, 60 mL held in separate plastic containers with cotton wicks), sheep liver (50 g in 100 mL of 30 % sodium sulphide) and squid (50 g) were used.

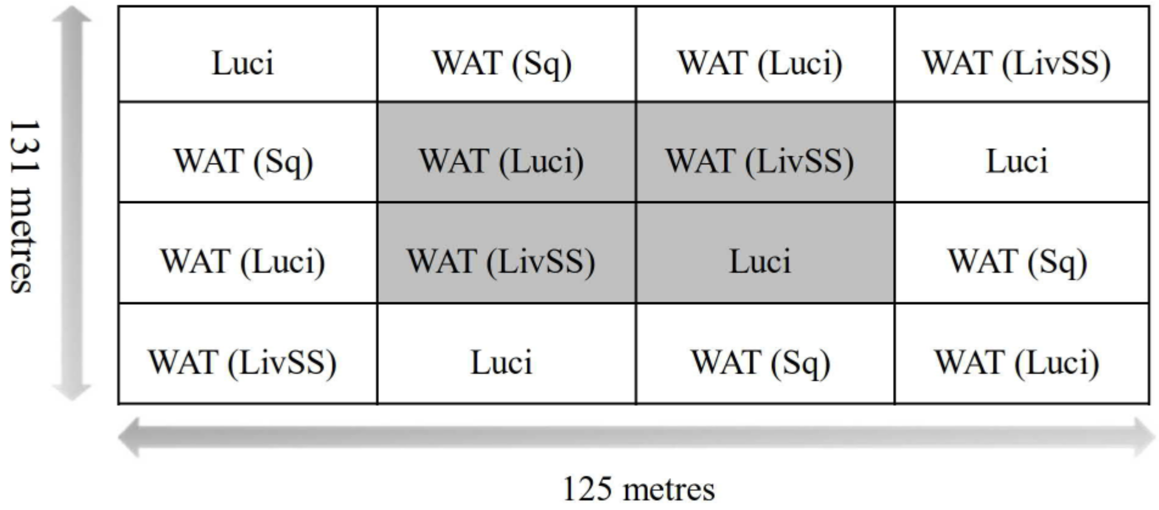
Therefore, the four treatments compared in this study were: (i) LuciTrap® with its three LuciLure® baits (Luci), (ii) Western Australian Trap with squid (WAT (Sq)), (iii) Western Australian Trap with the three LuciLure® baits (WAT (Luci)) and (iv) the Western Australian Trap with sheep liver and 30% sodium sulphide (WAT (LivSS)) as presented in Table 3.1.

Table 3.1 Summary of the trap and bait combinations used in this study.

Trap Type	Bait Type	Acronym
LuciTrap®	LuciLure® baits *	Luci
Western Australian Trap	50 g of squid	WAT (Sq)
Western Australian Trap	LuciLure® baits *	WAT (Luci)
Western Australian Trap	50 g of sheep liver in 100 mL of 30% sodium sulphide	WAT (LivSS)

* LuciLures® include lures A, B and C (Urech et al., 1998, Urech et al., 1996, Urech et al., 1994, Urech et al., 2004, Urech et al., 2009).

The study was conducted on Massey University's Keebles Sheep Farm approximately 5 km from Palmerston North at Massey University in New Zealand (40°23'30.1””S latitude, 175°36'18.3"E longitude). Throughout the study, sheep were grazing in the paddocks adjacent to where the traps were located. A total of sixteen traps and bait combinations, with four replications of each treatment. The traps were placed 1.5 m above ground and arranged in a four-by-four orthogonal Latin square array with each equally spaced (Figure 3.1). The study was conducted over six weeks in mid to late summer (42 days from the 18th of January until the 8th of March 2019), and each trap remained in the same position throughout the study.



Luci	WAT (Sq)	WAT (Luci)	WAT (LivSS)
WAT (Sq)	WAT (Luci)	WAT (LivSS)	Luci
WAT (Luci)	WAT (LivSS)	Luci	WAT (Sq)
WAT (LivSS)	Luci	WAT (Sq)	WAT (Luci)

Figure 3.1 Outline of the four-by-four orthogonal Latin square array study design. LuciTrap® with LuciLure® A, B and C (Luci); Western Australian Trap with squid (WAT (Sq)), Western Australian Trap with LuciLure® A, B and C (WAT (Luci)); and Western Australian Trap with sheep liver and sodium sulphide (WAT (LivSS)). Grey shading designates the inner portion, while the white background designates the outer portion of the trapping study design.

Weather data was sourced from Palmerston North Airport (40°19'15.6"S latitude, 175°37'05.7"E longitude) managed by the National Meteorological Service (https://cliflo.niwa.co.nz/, accessed on 1 October 2019) approximately 8 km from the study site.

A modified version of the Western Australian Trap as previously described (Cole, 1996) was utilised in this study, with the following alterations. The trap was constructed from two 3.2 L clear polystyrene plastic domestic storage containers (Click Clack, Innova Products Limited, Palmerston North, New Zealand) sitting upside down on top of each other. Eight 10 mm diameter holes were made around the lower chamber of the trap. A metal gauze was fashioned into a bowl shape with its convex side facing upwards and then glued to the inside of the upper chamber about 200 mm from its lower margin to facilitate the upper sections sitting on the lower section. A 10 mm diameter metal tube was placed in the centre of the gauze for flies to pass through (Figure 3.2). The inner and outer layers of both chambers were first painted with a waterborne surface sealer; then, the outer surface was painted bright yellow and the inside painted black (Figure 3.3). The top of the upper chamber was left unpainted, as flies would enter the trap and naturally move towards a light source, thus, remaining in the trap.

Figure 3.2 The unpainted version of the Western Australian Trap, showing the internal design.

Figure 3.3 Western Australian Trap in a wire basket at the study site.

For the treatments, WAT (Sq) and WAT (LivSS), the squid and the 50 g of sheep liver in 100 mL of 30 % sodium sulphide, respectively, were placed in a 250 mL plastic container with a plastic lid punctured with 1–2 mm holes in the lower chamber (Figure 3.3). The LuciTrap® lures in the WAT (Luci) were taped together in an upright position in the lower chamber. Each Western Australian trap was placed in a metal wire basket to keep it upright throughout the study and to prevent any obstruction of the entry holes (Figure 3.3). The LuciTraps' were bolted to a secure post.

All traps were sampled on a weekly basis, and the flies collected were preserved in 70 % alcohol. The day of collection was the first day of the collection week. The 50 g of sheep liver in 100 mL of 30 % sodium sulphide and squid were individually replaced on a weekly basis. The LuciLure® baits were not changed in the LuciTrap nor the Western Australian Trap for the six-week duration of the study.

3.2.1 Identification

Collected flies were identified using a stereomicroscope. Muscidae and Sarcophagidae were identified to the family level (Zumpt, 1965, Domínguez and Pont, 2001, Oosterbroek, 2006, Szpila, 2012, Meiklejohn et al., 2013a, Meiklejohn et al., 2013c, Grzywacz et al., 2017), whilst all Calliphoridae were identified to species level using published keys (Dear, 1986, Holloway, 1991, Wallman, 2001a, Szpila, 2010, Akbarzadeh et al., 2015, Lutz et al., 2018). *Lucilia* spp. were identified to genus level only (Dear, 1986, Wallman, 2001a).

3.2.2 Statistical Analysis

The distribution of trapped flies was presumed to be from a Poisson process. However, exploratory plots also showed that the data for *Lucilia* spp. were zero-inflated. Consequently, the following series of nested models were tested to identify the best fit for the data: Poisson, Quasi-Poisson, negative binomial, zero-inflated Poisson, zero-inflated Quasi-Poisson and zero-inflated negative binomial, using the *glmmTMB* package (Brooks et al., 2017) in R (Team, 2020).

Two count models were fitted to the data for the target species, the first was the mean model of the mean catch per week for each trap type:

Equation 1.

$$\ln(\text{target species catch}) = b_0 + b_1x_1 + \ldots + b_kx_k,$$

And the second was a rate model, which was the mean model adjusted for the total catch per week for each target species and trap type:

Equation 2.

$$\ln\,(\text{target species catch}) = b_0 + b_1x_1 + \ldots + b_kx_k + \ln\,(\text{total catch})$$

where $\ln\,(\text{total catch})$ in Equation 2 is referred to as the offset and the beta coefficient is constrained to equal 1, so that the estimated rate is the true rate. The rate estimated using this model is the number of target species caught per 100 total flies caught.

These two models provide different information, the first model identifies which trap and bait combination caught the most target species flies per se, whilst the second indicates which trap and bait combination was more discriminating towards the target species. A more discriminating trap could potentially make it far easier for the farmer to observe the target species when they first appeared in the season. In the models, each of the traps was compared to the WAT (LivSS).

The week of the collection was entered as a continuous variable a priori into all the models. In addition, individual weather variables per week (the mean temperature, minimum temperature, maximum temperature, total rainfall, and mean wind speed) were tested and retained if at $p < 0.05$. However, due to the potential of temperature variables being co-linear to each other, only one temperature variable was tested in the model at a time. Variables were removed using a backward selection method, and interactions between weather variables and trap type were also tested in the model.

Each model was compared using Akaike's information criterion (AIC) and Bayesian information criterion (BIC) (Burnham and Anderson, 2016). The model with the lowest AIC and/or BIC was the best fitting model for the data. The *emmeans* package (Lenth et al., 2018) was used to compare the estimated mean catch and the rate of catch for each trap type for *Lucilia* spp. A Tukey's comparison was then made to compare trap types using $p < 0.05$. The simulated residuals of the resulting models were then compared to the observed data using the *DHARMa* package (Hartig, 2020) to assess the fit of the models.

The mean daily temperature/week was calculated over seven days, and the rainfall was calculated as the total rainfall per week. The maximum and minimum temperatures were taken as the maximum and minimum temperatures recorded at any time over the course of the entire week. The mean wind speed was calculated as the mean speed over seven days.

To assess the potential influence of a location of a trap and bait combination in the Latin square, each trap was designated as being either an inner or an outer trap (Figure 3.1). The catch from each location, whether inner or outer, was added together for *Lucilia* spp. and by-catch, respectively. The by-catch was designated to be all other non-target species, i.e., not *Lucilia* spp. or *C. stygia*. A one-way ANOVA was then performed to assess whether there was a significant difference between fly catches in the inner and outer traps of the design.

3.3 Results

A total of 22,616 flies were collected over the six weeks (Table 3.2). This included the following species of Calliphoridae: *Lucilia* spp., *C. stygia*, *Calliphora quadrimaculata* (Diptera: Calliphoridae; Swederus, 1787), *C. rufifacies* and *Chrysomya megacephala* (Diptera: Calliphoridae; Fabricus, 1794). The two most common calliphorid species caught were *Lucilia* spp. comprising 2.4 %, respectively, of the total number of flies caught as shown in Table 3.2. The most numerous dipteran families in the bycatch were Sarcophagidae with 80.7 % and Muscidae with 16.6 % of the total as shown in Table 3.2.

As indicated earlier, *Lucilia* spp. and *C. stygia* were the three target species. However, a large disparity in the results for each of these species was found. For *Lucilia* spp., there were many individual traps each week with null catches: *Lucilia* spp. (27/96, 28 %), while for *C. stygia*, a total of only three specimens were caught over the entire six-week study, across all trap types. As a result, no further inferences could be made regarding *C. stygia* due to the low sample size.

Table 3.2 The total number and species of flies caught over six weeks by LuciTrap[a]; WAT (Sq)[b]; WAT (Luci)[c]; and WAT (LivSS)[d] trap types.

Order	Family	Species	LuciTrap [a]	WAT(Sq) [b]	WAT (Luci) [c]	WAT (LivSS) [d]	Total
		Lucilia spp.	109	19	186	235	549
		Calliphora stygia	0	0	2	1	3
		Calliphora quadrimaculata	0	1	0	1	2
		Calliphora vicina	1	0	0	0	1
		Chrysomya rufifacies	0	1	0	9	10
		Chrysomya megacephala	0	0	0	2	2
	Fanniidae		7	2	2	0	11
	Sarcophagidae		1502	3559	7224	5928	18,213
	Muscidae		326	850	1401	1170	3747
	Polleniidae		23	5	2	4	34
	Staphylinidae		0	0	2	0	2
	Unknown		4	2	0	1	7
Hymenoptera	Vespidae		0	0	0	1	1
Total			1974	4438	8823	7381	22,616

[a] LuciTrap with LuciLure A, B and C.

[b] Western Australian Trap with squid.

[c] Western Australian Trap with LuciLure A, B and C.

[d] Western Australian Trap with sodium sulphide and sheep liver.

An overall comparison of the unadjusted catch, based on the confidence intervals, indicates the WAT (Luci) caught more bycatch than LuciTrap® (Table 3.3). All other treatments did not differ for by-catch. WAT (Sq) caught less *Lucilia* spp. than WAT Luci and WAT (LivSS), but it did not differ from LuciTrap. WAT (LivSS), WAT (Luci) and LuciTrap did not differ in their weekly catch of *Lucilia* spp. There was no effect ($p >$ 0.05) on the location of a trap on the inner and outer realms of the Latin square array for *Lucilia* spp. and by-catch.

Table 3.3 The mean (95 % confidence intervals, (CI)) unadjusted weekly catch of *Lucilia* spp. and by-catch in LuciTrap [a]; WAT (Sq) [b]; WAT (Luci) [c]; and WAT (LivSS) [d] trap types.

Trap	*Lucilia* spp.	By-Catch [e]
LuciTrap [a]	18.2 (3.3–33.1, 95% CI)	310.8 (26.2–595.5, 95% CI)
WAT (Sq) [b]	3.2 (0.2–3.2, 95% CI)	736.5 (82.8–1390.2, 95% CI)
WAT (Luci) [c]	31.0 (1.5–63.5, 95% CI)	1439.5 (605.8–2273.2, 95% CI)
WAT (LivSS) [d]	39.2 (29.0–49.3, 95% CI)	1191.0 (93.2–2288.8, 95% CI)

[a] LuciTrap with LuciLure A, B and C.
[b] Western Australian Trap with squid.
[c] Western Australian Trap with LuciLure A, B and C.
[d] Western Australian Trap with Sodium Sulphide and sheep liver.
[e] By-catch included all non-target species.

The general trend indicates that the catch of *Lucilia* spp. steadily increased until the fourth week of the study (Figure 3.4). By the third week of the study, the catch from the Luci, WAT (Luci) and WAT (LivSS) treatments increased for *Lucilia* spp., whereas there was a consistent low catch rate from the WAT (Sq) over the course of the entire study (Figures 3.4). Most of the by-catch was in the second week and then declined towards the end of the study overall trap types (Figure 3.5).

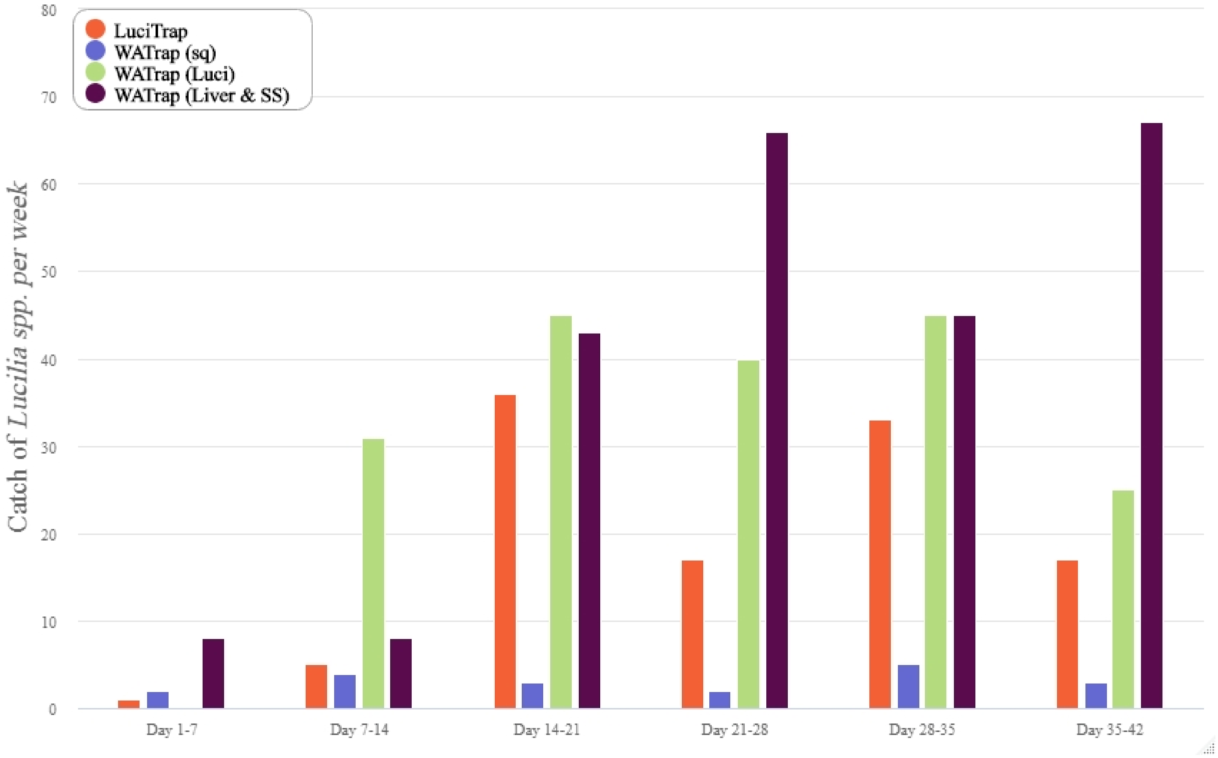


Figure 3.4 Weekly catch of *Lucilia* spp. using the four different trap types: LuciTrap with LuciLures A, B and C, (LuciTrap); Western Australian Trap with squid, (WAT (Sq)); Western Australian Trap with LuciLures A, B and C, (WAT (Luci)); and Western Australian Trap with Sodium Sulphide and sheep liver, (WAT (LivSS)).

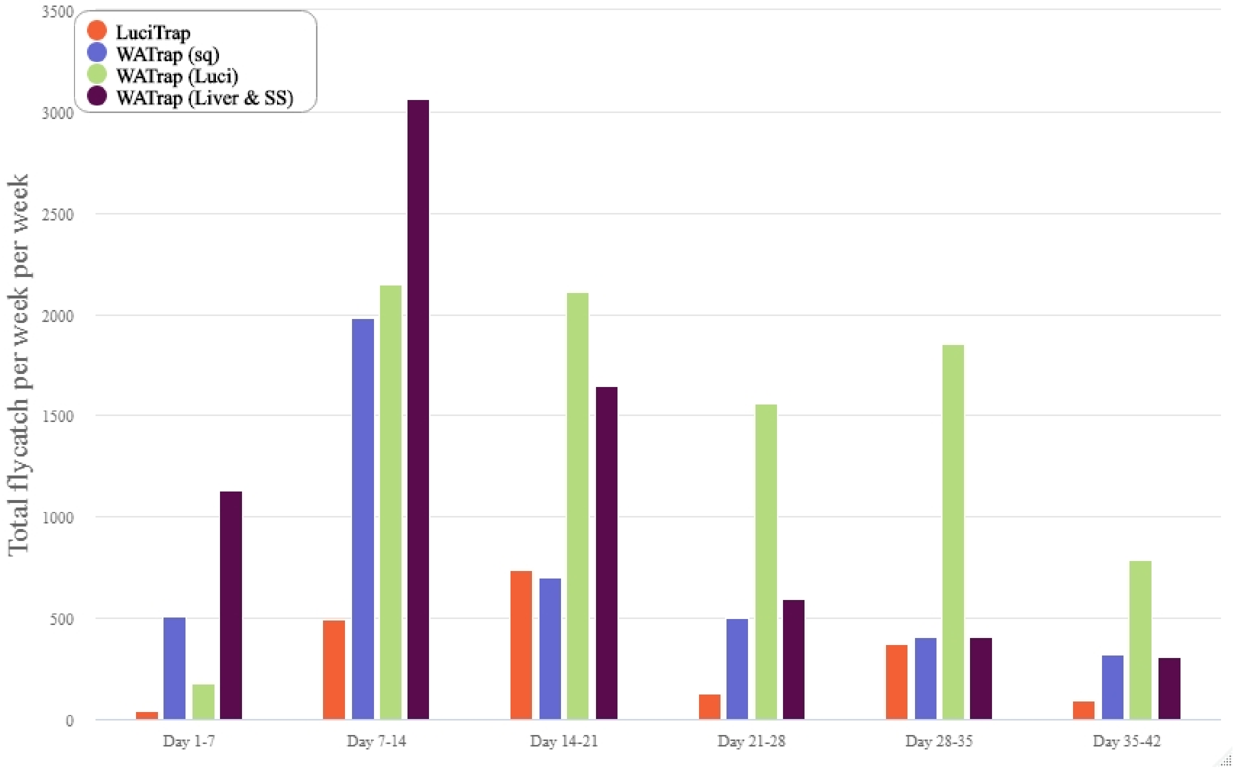


Figure 3.5 Weekly bycatch with each of the four different trap types: LuciTrap with LuciLures A, B and C, (LuciTrap); Western Australian Trap with squid, (WAT (Sq)); Western Australian Trap with LuciLures A, B and C, (WAT (Luci)); and Western Australian Trap with Sodium Sulphide and sheep liver, (WAT (LivSS)).

3.3.1 Weather data

The weather data illustrated in Figure 3.6 shows little variability in temperature over the course of the six-week trial period. Rainfall occurred sporadically throughout the study; a total of 42.9 mm fell; with 20.7 mm of this falling in the final week (Figure 3.6). The maximum temperature for the study period was 28 °C occurring in the second week of the study. The mean and minimum temperatures decreased over the latter three weeks of the study (Figure 3.6). Windspeed was highest in the first week of the study and sporadically decreased throughout the study (Figure 3.7).

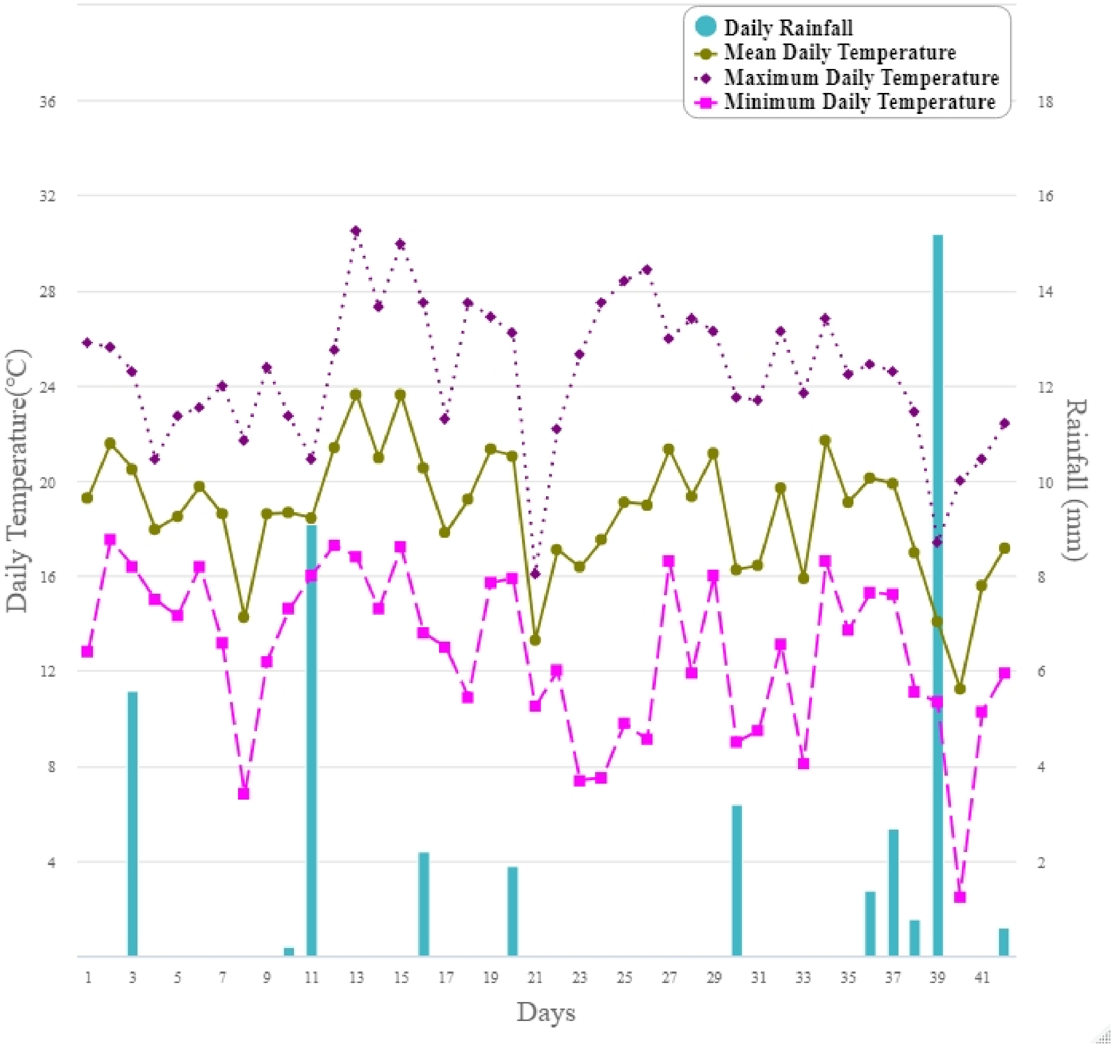


Figure 3.6 The mean, maximum and minimum temperature (°C) and rainfall (mm) over 42 days (18 January – 1 March 2019) from Palmerston North Airport (40°19'15.6"S latitude, 175°37'05.7"E longitude) managed by the National Meteorological Service (https://cliflo.niwa.co.nz/, accessed on 1 October 2019) approximately 8 km from the study site.

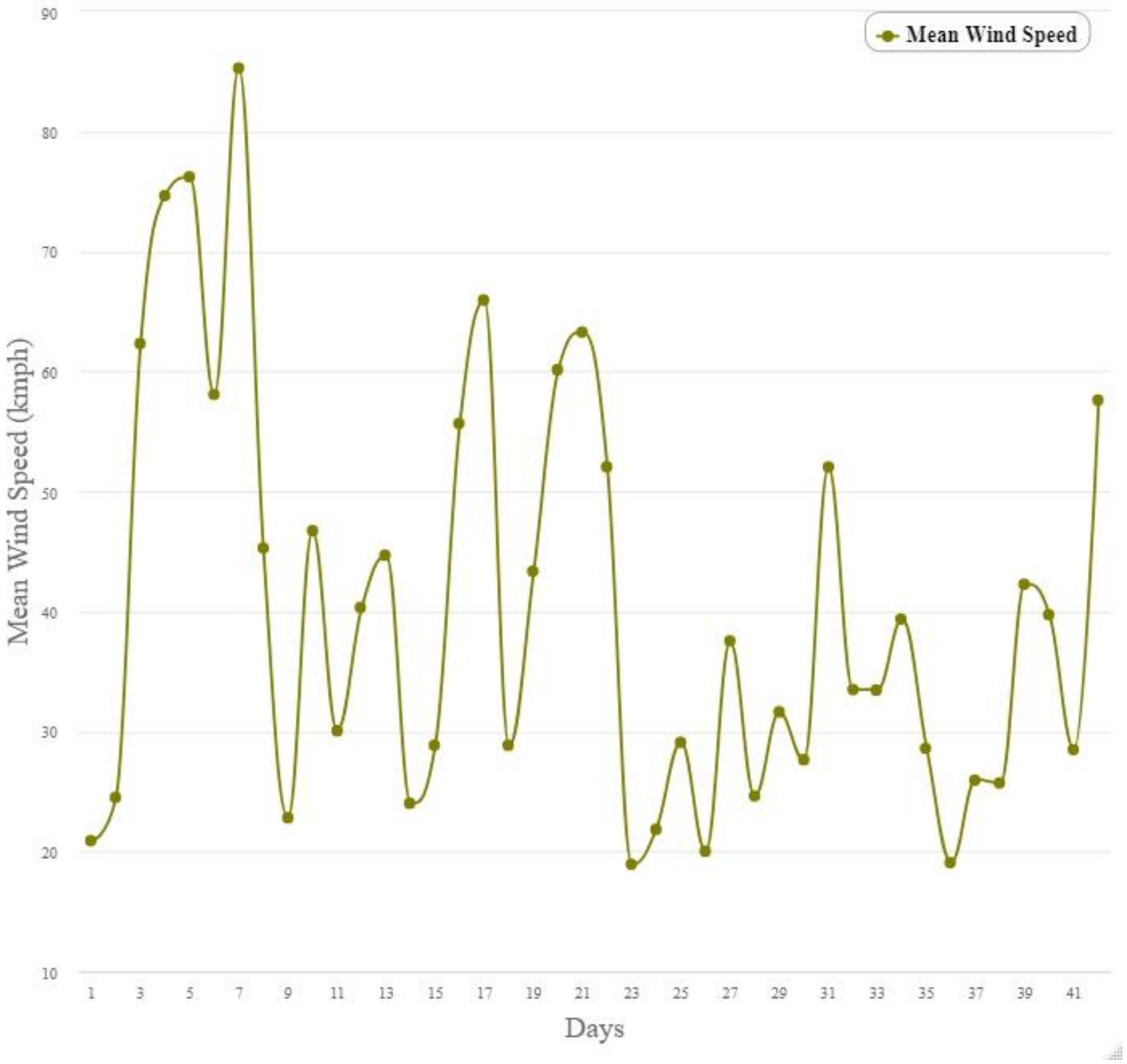


Figure 3.7 The mean wind speed per day over 42 days (18 January–1 March 2019) from Palmerston North Airport (40°19'15.6"S latitude, 175°37'05.7"E longitude) managed by the National Meteorological Service (https://cliflo.niwa.co.nz/, accessed on 1 October 2019) approximately 8 km from the study site.

3.3.2 Results of mean model for *Lucilia* spp.

The best-fitting model to describe the mean catch of *Lucilia* spp. was a negative binomial model with an R^2 of 0.36. In addition to the trap type (Table 3.4), the maximum temperature was found to be the only weather parameter that was able to significantly ($p < 0.01$) explain the catch of *Lucilia* spp. The maximum temperature was found to be positively ($p < 0.05$) related to the catch of *Lucilia* spp. A Q-Q plot and simulated residuals plotted against observed data both were found to be normally distributed (plot shown in Appendix section B.1.1). For *Lucilia* spp., the highest mean weekly catches were in the WAT (LivSS) and WAT (Luci) with no difference between them ($p > 0.05$) (Figure 3.8). LuciTrap caught fewer ($p < 0.05$) than the WAT (LivSS), but no difference ($p > 0.05$) was found between the LuciTrap and the WAT (Luci). There was no difference ($p > 0.05$) between LuciTrap and WAT (Sq) (Figure 3.8).

Table 3.4 Summary of the model coefficients, confidence intervals (CI) and *p* values for each predictor of mean catch of *Lucilia* spp. The mean catch model includes the catch of the target species and the catch of all species in each trap per week.

Predictors	Co-Efficients	CI	*p* Value
Intercept	−5.29	−8.78 – −1.79	0.003
WAT (LivSS) [a]	reference	-	-
LuciTrap [b]	−0.88	−1.40 – −0.37	0.001
WAT (Sq) [c]	-1.86	-2.53 – -1.20	<0.001
WAT (Luci) [d]	-0.28	-0.72 – 0.17	0.224
Week [e]	0.36	0.22 – 0.50	<0.001
Maximum Temperature [f]	0.25	0.12 – 0.38	<0.001

[a] Western Australian Trap with sodium sulphide and sheep liver.
[b] LuciTrap with LuciLure A, B and C.
[c] Western Australian Trap with squid.
[d] Western Australian Trap with LuciLure A, B and C.
[e] Week was defined as the week of catch.
[f] Maximum Temperature was designated as the maximum temperature each week.

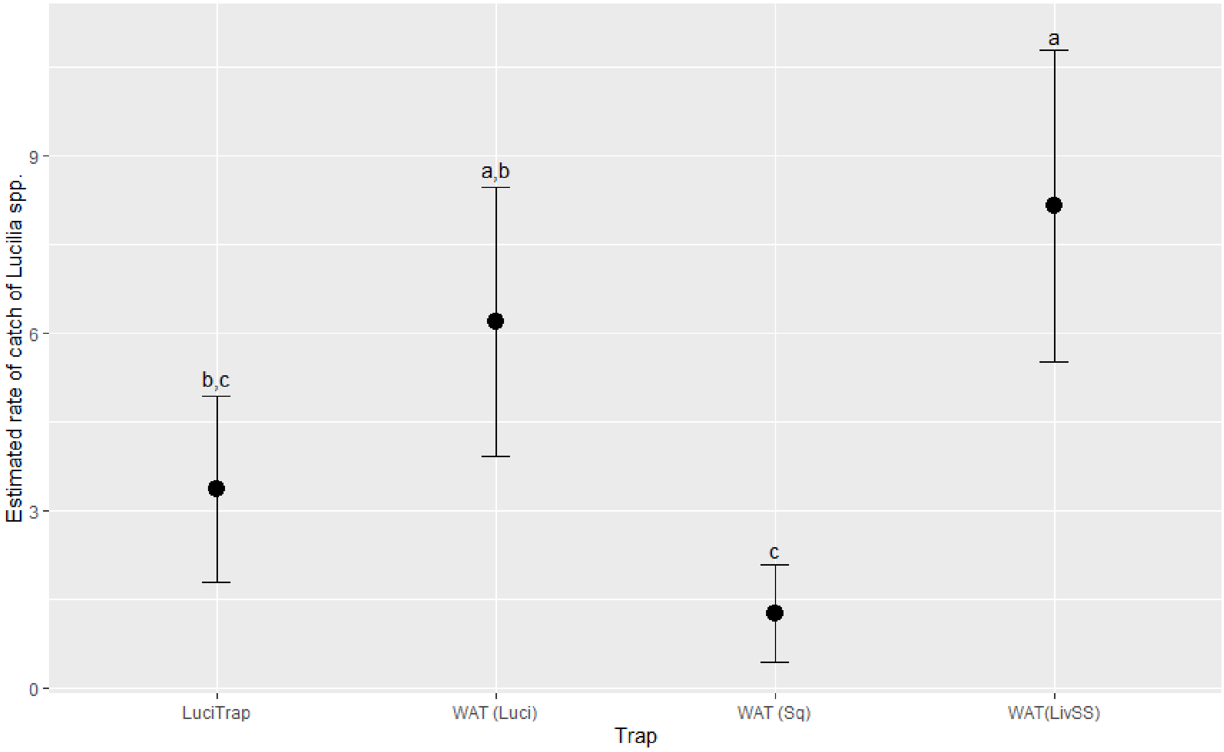


Figure 3.8 The estimated mean catch per week of *Lucilia* spp. by each trap type adjusting for the effect of maximum temperature. Trap types with differing letters (a, b, and c), within target species, are significantly different based on a Tukey comparison of least square means ± standard error ($p < 0.05$). LuciTrap with LuciLure A, B and C (LuciTrap); Western Australian Trap with squid (WAT (Sq)), Western Australian Trap with LuciLure A, B and C (WAT (Luci)); and Western Australian Trap with sheep liver and sodium sulphide (WAT (LivSS)).

3.3.3 Results of rate model for *Lucilia* spp.

The best model to describe the rate of catch per week of *Lucilia* spp**.** was a negative binomial model with an R^2 of 0.27. In addition to trap type (Table 3.5 and Figure 3.9), rainfall was found to have a negative relationship with catch ($p < 0.05$) (Table 3.5). The mean, minimum and maximum temperature ($p > 0.05$) was not related to the catch of *Lucilia* spp... A Q-Q plot and simulated residuals plotted against observed data both were found to be normally distributed (plot shown in Appendix section B.1.3).

Table 3.5 Summary of model coefficients, confidence intervals and *p* values for each predictor of the rate of catch per week of *Lucilia* spp. The rate of catch per week model included an offset of the bycatch (i.e., all other flies other than *Lucilia* spp.) in each trap.

Predictors	Co-Efficients	CI	*p* Value
Intercept	−4.73	−5.47 – −3.99	<0.001
WAT (LivSS) [a]	Reference	-	-
LuciTrap [b]	0.49	−0.10 – 1.09	0.105
WAT (Sq) [c]	−2.12	−2.85 – −1.40	<0.001
WAT (Luci) [d]	−0.94	−1.50 – −0.37	0.004
Week [e]	0.53	0.36 – 0.70	<0.001
Rainfall [f]	−0.04	−0.07 – 0.00	0.026

[a] Western Australian Trap with sodium sulphide and sheep liver.
[b] LuciTrap with LuciLure A, B and C.
[c] Western Australian Trap with squid.
[d] Western Australian Trap with LuciLure A, B and C.
[e] Week was defined as the week of catch.
[f] Rainfall was designated at the total rainfall each week.

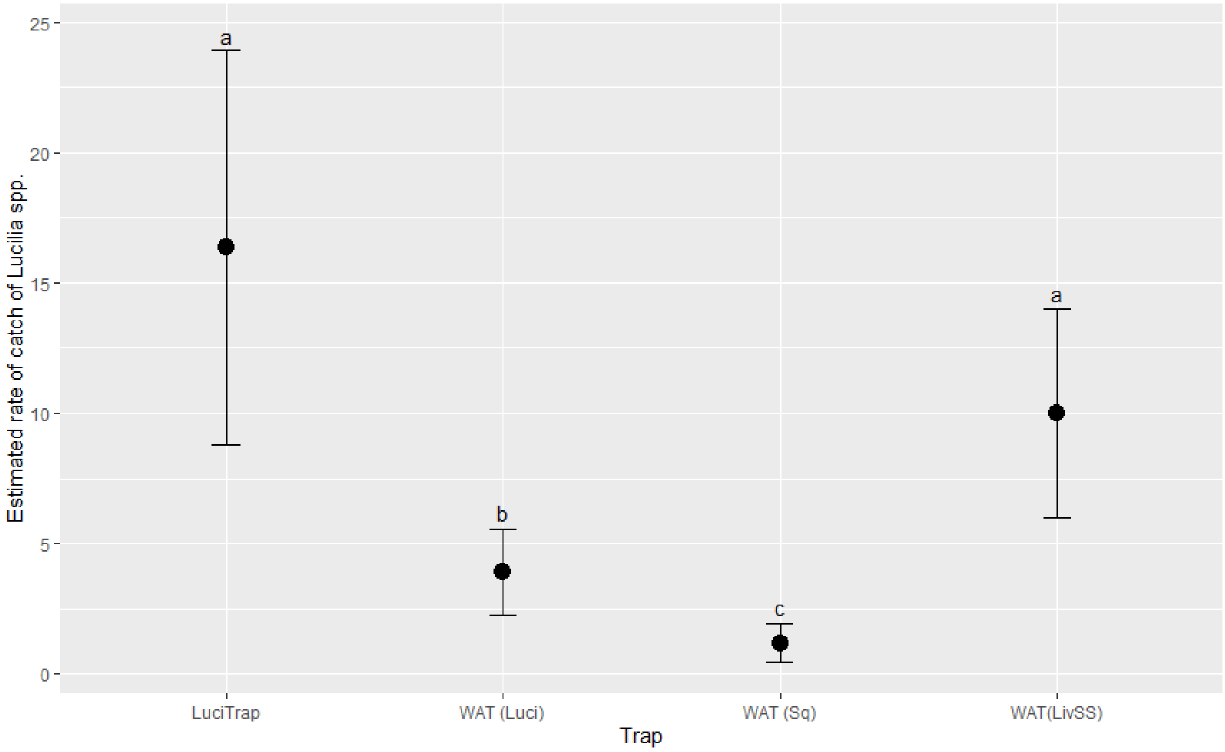


Figure 3.9 The estimated rate per week of the catch of *Lucilia* spp. adjusting for the effect of rainfall. Trap types with differing letters (a, b, and c), within target species, are significantly different based on a Tukey comparison of least square means ± standard error ($p < 0.05$). LuciTrap with LuciLure A, B and C (LuciTrap); Western Australian Trap with squid (WAT (Sq)), Western Australian Trap with LuciLure A, B and C (WAT (Luci)); Western Australian Trap with sheep liver and sodium sulphide (WAT (LivSS)).

3.4 Discussion

The aim of this study was to identify the most efficient trap and bait combination that New Zealand farmers could utilise as part of routine surveillance to monitor the presence of *Lucilia* spp. and management of flystrike prevention on farms. Overall, this study found that both the LuciTrap® with the LuciLures® and the Western Australian Trap with sheep liver and sodium sulphide were the most effective for *Lucilia* spp...

The high catch of Muscidae and Sarcophagidae was notable from each of the trap and bait combinations; however, these were not relevant from a flystrike monitoring or control perspective. This study occurred in the mid-late summer period, which is recognised as the mid-season for flystrike target species in New Zealand (Heath and Bishop, 2006). The location of the treatments within the Latin square array was not found to have any influence on the fly catch rates or species and, therefore, had no impact on the results found.

In this study, two models were presented to contrast the catch of *Lucilia* spp. by each trap bait combination. Due to the morphological misidentifications identified in Chapter Two, the counts of *Lucilia* spp. were combined. The first model estimated the mean catch per week of both target species and the second model estimated the rate of catch per week of the target species by including an offset to adjust for the total catch from each trap each week. Further analysis could not be made regarding *C. stygia* due to the low sample size, and it is unclear why such a result was obtained.

In the mean catch model, the maximum temperature positively affected the catch of *Lucilia* spp... Temperature has previously been found to be an important environmental variable regarding the activity of blowflies (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2019), this result was not unexpected. Dipterans are thermophilic and, therefore, more active in warmer temperatures. Wind was found not to have an effect in either model.

In the rate of catch model, the catch of *Lucilia* spp. was negatively influenced by rainfall but not by temperature. Rainfall has been found to depress the activity of Calliphoridae; thus, it has been linked with lower observed catches in flytraps (Mahat et al., 2009, Mulieri et al., 2008). Interestingly, rainfall had no influence on the mean catch model; the reasoning for this is uncertain. However, it could be hypothesised that, in the rate of catch model, the effect of rain was on the bycatch, and therefore this changed the rate by having a positive effect on the denominator (total catch) rather than a negative effect on the numerator (target species catch). Otherwise, the effect of rainfall would have been found in the mean model. The negative influence of rainfall on the activity of various Calliphoridae including *L. sericata* has been previously reported in Germany (Lutz et al., 2019). Whereas, for Sarcophagidae and Muscidae species, climatic variables aside from temperature have not been identified to significantly alter abundance (Mulieri et al., 2008, Oliveira et al., 2002).

This study indicated that certain trap treatments were better than others for monitoring these target species. Regarding *Lucilia* spp., for the mean model, the WAT (LivSS) caught more than the LuciTrap, while, in the rate model, there was no difference between either treatment. These findings do not agree with previous studies in Australia (Urech et al., 2004, Urech et al., 2009) where it was found that the LuciLures (i.e., the same treatment as the LuciTrap in the present study) were more selective and attractive for *L. cuprina* than sheep liver in 30 % sodium sulphide when using StickyTraps. The differences between the present study and others (Urech et al., 2004, Urech et al., 2009) are due to the experimental set-up as the comparisons made in this study are on the basis of the trap and bait type. That being said, it should be noted that the present study is limited to being conducted in one location over a relatively short period of time and that there was no differentiation between *L. cuprina* and *L. sericata*. Given the results from this study, further variation in different regions with different climates would be expected, whereas the previous Australian studies were conducted across multiple regions over several years.

Furthermore, there are fundamental differences in the statistical measures used in this study compared to previous studies in this area. In previous studies the fly count data was log-transformed by +1 in an effort to normalise the data prior to analysis in these studies (Urech et al., 2004, Urech et al., 2009). In the present study the approach was to analyse the results as count data, which recognises the importance of zero counts and their statistical implications.

In this study, it was proposed that squid could be an effective replacement for either sheep liver and 30 % sodium sulphide or the LuciLure for attracting and monitoring the target species in the WA Traps. This would have the added benefit that it would be an easier bait to source and much safer to use on-farm from a health and safety perspective. Squid would also be advantageous as it retains a state of moist decay, and it would remove the need for using chemicals to attract necrophagous flies.

Squid has been reported to have a relatively high success rate for trapping species in the family Calliphoridae (Baz et al., 2007). However, in the present study, it was found that the WAT (Sq) had the lowest catch of all trap groups for all target species. Therefore, it is not suitable for this purpose in New Zealand. Although the data from this study would suggest it may be more suitable as bait if the aim was to catch Sarcophagidae and Muscidae.

The combined analysis in the present study indicates that either the WAT (LivSS) or the LuciTrap could be used to catch *Lucilia* spp. in New Zealand. Given its low bycatch, the LuciTrap makes it far easier for a farmer to quickly identify the presence of either target species. This is particularly important when a farmer is only using a trap to monitor when the first appearance of these target species in a season, so that control procedures can be initiated. The LuciTrap is advantageous given the length of time its chemical bait lasts (i.e., six months).However, there are legal limitations for the use of its chemicals on farms in New Zealand (Government, 1997) which may restrict their future use. This suggests the WAT (LivSS) may be a more acceptable option; however, its use is limited by the need to change the bait weekly, and there are also the same limitations with the use of sodium sulphide on farms in New Zealand (Government, 1997).

This study appears to be the first report of *C. megacephala* being caught on a farm in New Zealand. It has previously only been recorded at the New Zealand border (Heath, 2021). *Chrysomya megacephala* is noted to be a secondary fly strike species that cannot initiate flystrike in the absence of wounds (Badenhorst and Villet, 2018). Therefore, it may be expected that the potential effect on the rate of flystrike by this new species in New Zealand would be negligible.

3.5 Conclusion

The aim of this study was to determine the better trap and bait combination for use on New Zealand farms. The results of this study indicate that the best trap and bait combinations of those tested were the LuciTrap® with its LuciLures® or the WAT (LivSS) for monitoring *Lucilia* spp... Overall, a clear difference in the catch of flies in the family

Calliphoridae was identified between the three treatments that had chemicals (LuciTrap®, WAT (Luci) and WAT (LivSS) and the squid bait.

Future studies should consider the effectiveness of combinations of sheep liver and/or other types of offal with other fish baits to attract *Lucilia* spp. as alternatives to using chemical baits. In addition, future studies could also consider contrasting these non-chemical baits with aged offal in a similar manner to this study. The adaptation of the most effective treatments from this study on New Zealand farms may also be limited by the current legislation regarding the use of chemicals on farms.

Chapter Four. The seasonal population dynamics of *Lucilia* spp. on the North and South Islands of New Zealand 2018/2019, 2019/2020 and 2020/2021

4.1 Introduction

Cutaneous myiasis commonly referred to as flystrike can be defined as an infestation of the living tissue of live vertebrates by larvae of flies that feed for varying periods on the hosts living tissue or bodily substances (Zumpt, 1965). Flystrike in sheep can occur on the head, body, tail, breech, poll or foot (French et al., 1995). The hindquarters of sheep are the predominant site for flystrike (Heath and Bishop, 1995, French et al., 1995, Greeff et al., 2018) and are generally referred to as breech strike. The body, shoulder, chest and belly of sheep are less prone to strike (Heath and Bishop, 1995). Flystrike can cause severe weight loss (Heath et al., 1987), permanent damage to pelts (Cranston et al., 2017), reduced fertility (Heath et al., 1987) and in more extreme cases can lead to death (Guerrini, 1988). In addition, not treating cases of flystrike can lead to repeated strikes by other flies, known as secondary strike (Heath and Bishop, 2006). In New Zealand, there are three species within the family Calliphoridae associated with primary flystrike: *L. cuprina, L. sericata* and *C. stygia* with *C. rufifacies* associated with cases of secondary strike (Heath and Bishop, 1995, Heath and Bishop, 2006).

Flystrike has been recognised as an animal health and welfare problem in New Zealand since the 1890s (Miller, 1939). It is estimated to affect 3-5 % of the national flock (Heath and Bishop, 1995, Corner-Thomas et al., 2017) and approximately $60.2 million is spent annually reducing the risk of flystrike, with chemicals accounting for half this figure (B+LNZ Genetics, 2014). A 2016 survey by Corner-Thomas et al. (2017) found that 83% of farmers considered flystrike to be an important disease in New Zealand. Fifteen per cent of 1,200 responses reported >2 % of sheep were struck during that year despite the current farm preventative measures (Corner-Thomas et al., 2017). It has been estimated that production losses due to flystrike in New Zealand are $37 million annually (Heath and Bishop, 1995). To prevent and control flystrike New Zealand sheep farmers are encouraged to use Integrated Pest Management (IPM) to keep sheep less susceptible to flystrike during risk periods (Cole and Heath, 1999, Heath and Bishop, 2006). Integrated Pest Management is a combination of chemical and non-chemical strategies that renders sheep less susceptible over a certain time span.

Until the arrival of *L. cuprina* in the late 1970s, the New Zealand flystrike season was quite predictable, lasting from November until late March, with minimal losses due to flystrike (Tenquist and Wright, 1976, Dear, 1986). With a predictable season, farmers were able to shear their sheep at the appropriate time and then apply suitable insecticides to extend the period of protection (Tenquist and Wright, 1976, Dear, 1986). However, with the arrival of *L. cuprina* in the late 1970s, the status quo changed substantially and it rapidly became apparent that the season was longer and less predictable than before (Bishop et al., 1991).

Heath and Bishop (2006) presented mean seasonality data for the three common flystrike species for the North and South Islands, but the differences between sites and regions could not be identified. This was due to the self-referral and self-selection methodology utilised, which did not allow for a statistical interpretation of the data set. *Lucilia cuprina* was found to have a mean season from November until May in the North Island whereas, in the South Island, it was from October until May. *Lucilia sericata* was found to have a shorter but more definite season on both islands being from November until April each year. In contrast, *C. stygia* was potentially present all year round, with a much longer season in both the North and South Islands depending on temperatures (Roberts, 1977, Roberts et al., 1983, Heath and Bishop, 1995). Climate records show that temperatures in New Zealand have increased by one degree in the past 100 years and are predicted to increase by a further 1-4°C this century (Environment and NZ, 2020, IPCC, 2016). This possibly means that the previous data gathered (Heath and Bishop, 1995, Heath and Bishop, 2006) may no longer be valid, with the likely effects of climate change expanding both the season, number and distribution of primary flystrike species within New Zealand.

To allow farmers to use current IPM strategies and licensed chemicals more effectively, there is an urgent need to develop a predictive model to forecast the seasonality and numbers of the main flystrike species in New Zealand. Models to predict the fly season are currently available to sheep farmers in the United Kingdom for *L. sericata* (Wall et al., 2002) and in Australia for *L. cuprina* (Wardhaugh et al., 2007, Horton and Hogan, 2010). These models allow farmers to make strategic management decisions for their sheep considering these predictions. To replicate this in New Zealand, accurate data on the occurrence and numbers of the main fly species which cause flystrike across the country is required. At the same time, weather data needs to be collected to determine which climatic variables are driving fly numbers under New Zealand conditions.

This study reported in this chapter aimed to update and extend the work of Heath and Bishop (1995; 2006) by estimating the start, end, and peak of the season for *L. cuprina*, *L. sericata* and *C. stygia* on eight extensive farmed sheep farms across the North and South Islands of New Zealand over three fly catch seasons (2018/2019, 2019/2020 & 2020/2021) and record climatic associations using on-farm weather station. It also aimed to investigate the usefulness of using a simple degree day model to predict the start and end of the season as well as to compare catch rates with the LuciTrap® and a StickyTrap attachment across the season.

4.2 Materials and Methods

Eight farms were enrolled in the study with the assistance of the study sponsor New Zealand Merino Ltd (five farms) and Massey University (three farms). Farm selection was non-random and based on a willingness by farm owners or managers to be involved in this study. Each enrolled farm had between 1,000 and 20,000 ewes and over all farms there were two breed types (Table 2.1). For the North Island farms, five farmed coarse wooled, dual-purpose Romney breed sheep (“T”, “R”, “BH” and “LD”) and on one they were Perendale breed sheep (“BH”). For the South Island farms, all were dual-purpose Romney breed sheep (“KD”, “ST”, “S”).

4.2.1 Trapping Methods

Figure 4.1 Photo illustrating the standardized trap layout used on each of the study farms, a LuciTrap with a StickyTrap adhered to the lid. The LuciTrap was positioned 1.5m in height with a rain deflecting lid above the trap.

The LuciTrap® (Bugs for Bugs, 3 Rocla Court, Toowoomba Queensland, 4350, Australia) was employed as the baited fly trap for this study given its practicality and design, which includes a bait that lasts for up to six months. It had previously been used in Australia to monitor *L. cuprina* (Urech et al., 1998, Urech et al., 2001b, Horton et al., 2001a, Urech et al., 2009) and in Hungary to survey *L. sericata* (Hall et al., 2003). On each farm, three LuciTraps were placed at similar sites on all farms, one next to the woolshed, one beside the weather station and another on pasture frequently grazed by sheep. Each LuciTrap® was bolted to a sturdy post, at a height of 1.5 m and a strip of self-adhesive StickyTrap (EasyTrap Stickies for Flying Insect, Gubba, Greenlane, PO Box 74435, Auckland 1546, New Zealand), see Figure 4.1, measuring 11 cm x 3.3 cm, was placed between the two circular plastic inserts on tops of the lid. The combination of the StickyTrap with the LuciTrap® has not been used before to monitor *L. cuprina* and *L. sericata*. In addition, a solid orange lid was placed above the trap to reduce the amount of rainfall falling on the trap as previously suggested by (Urech et al., 2014); based on a study conducted in Indonesia during the rainy season. The lures in the LuciTrap® were all changed before the start of each sample collection season.

For the 2020/2021 fly collection study, replacement chemicals were purchased instead of purchasing additional LuciLures® from Bugs for Bugs Ltd. This was due to the realistic delays and substantial costs of importation from Australia. Therefore, using the chemicals listed on the Bugs for Bugs safety data sheet for the chemical composition of the LuciLures® (https://bugsforbugs.com.au/), an in-house version was made to match the commercial chemical. This included: Sodium Sulphide at 12 % as for the LuciTrap® Lure A and Butanoic Acid at 98 % as for the LuciTrap Lure C.

4.2.2 Health and safety

A booklet was distributed to each farm outlining the hazards posed by the chemicals in the LuciTrap. The booklet included health and safety sheets for each of the chemicals from the LuciTrap. In the unlikely event of ingestion of the products from the traps by animals, the farmers were instructed to give the folder with the health and safety forms to their veterinarian. Also, the farm managers were given suitable equipment in the event they had to handle the traps.

4.2.3 Climatic data

4.2.3.1 Soil Temperature Probes

Three soil temperature probes (Hobo MX2201 Temperature probes, Onset Headquarters, 470 MacArthur Boulevard, Bourne, Massachusetts, 02532, United States of America) were installed on each farm using the same three locations as the LuciTraps. The Hobo MX2201 Temperature probes are accurate to ±0.5°C and can be accessed using Bluetooth via the Hobo mobile app (https://www.onsetcomp.com/products/software/hobomobile/). This allowed for the data to be downloaded to a mobile phone and then exported via email as a CSV file. Each soil probe was set to record the hourly mean, maximum, minimum, and standard deviation of 10 cm soil temperature.

4.2.3.2 Weather stations

One weather station Vantage Pro2 6322C (Davis Instruments, Hayward, California, United States of America) was installed on each of the farms in the North Island in June of 2018 (Figure 4.2). These weather stations in the South Island had previously been installed in 2017. On "S", a Campbell Scientific CR200 weather station, (Campbell Scientific, Logan, Utah, 84321, United States of America) was already installed and was therefore utilised. The data from the Davis weather stations was accessed at www.weatherlink.com and from the Campbell Scientific weather stations were accessed at www.envirodata.co.nz. All the weather data from the Davis Instruments Vantage Pro2 6322C (Table 4.1), were available on an hourly basis whereas only some weather variables from the Campbell Scientific weather station were recorded as an hourly summary (Table 4.2).

Table 4.1 The variables, measurement and scale of measurements recorded hourly by the Davis Instruments Vantage Pro2 6322C on “T”, “R”, “BH”, “M”, “LD”, “ST”, “KD.

Variable	Units	Accuracy
Mean Temperature	°C	±0.3°C
Minimum Temperature	°C	±0.3°C
Maximum Temperature	°C	±0.3°C
Relative Humidity	%	±2%
Dew Point	°C	±1°C
Wet Bulb	°C	±1°C
Wind Speed	Km/hr	±2 mph (= 2 kts, 3.2 km/h, 0.9 m/s) or ±5%, whichever is greater
Wind Direction	Km	±3°
Wind Run	Km	±5%
High Wind Speed	Km/hr	±2 mph (= 2 kts, 3.2 km/h, 0.9 m/s) or ±5%, whichever is greater
High Wind Direction	Km	±3°
Wind Chill	°C	±1°C
Heat Index	°C	±1°C
THW Index	°C	±2°C
Rainfall	mm	For rain rates up to 10"/hr (250 mm/hr): ±3% of total or ± one tip of the spoon (0.01"/0.2mm)
Evapotranspiration	mm	±5%
Heating Degree Days	Degree Day	±0.3 Degree-Day
Cooling Degree Days	Degree Day	±0.3 Degree-Day

Table 4.2 The variables, measures, scale of measurements and frequency when measured recorded by the Campbell Scientific CR200 weather station on “S”.

Variable	Units	Error of measurement	When recorded
Mean Temperature	°C	±0.6°C	Hourly
Minimum Temperature	°C	±0.6°C	Daily
Maximum Temperature	°C	±0.6°C	Daily
Relative Humidity	%	±3%	Hourly
Rainfall	mm	0.2mm	Hourly/Daily
Soil Moisture	%	±0.5%	Hourly
10cm Soil Temperature	°C	±0.2°C	Hourly
Wind Speed	m/s	< 0.1 m/s for the range 5 to 25 m/s	Hourly

The following are definitions of climate variables that were available from the Vantage Pro2 6322C (Davis Instruments, Hayward, California, United States of America) installed in “T”, “R”, “BH”, “M”, “LD”, “KD” and “ST”. The equations used for these variables are as indicated in the Davis Instruments instructions manual (https://www.davisinstruments.com/).

Average Temperature is the mean of the highest and lowest temperature of that day.

Maximum Temperature is the highest temperature that occurs over a continuous time interval.

Minimum Temperature is the lowest temperature that occurs over a continuous time interval.

Relative Humidity describes the concentration of water vapour in air at a specific temperature as a percentage. It is calculated as follows:

$$Relative\ humidity = \frac{actual\ vapour\ density}{saturation\ vapour\ density} \times 100\%$$

Dew Point is the temperature at which a volume of air cools to immediately before it becomes saturated with water vapour. It is calculated as follows:

$$Dew\ Point = \frac{(237.3\ \times [ln\left(\frac{RH}{100}\right) + \left(\frac{(17.27\ \times T)}{(237.3 + T)}\right)]}{(17.27 - [ln\ \left(\frac{RH}{100}\right) + \left(\frac{17.27\ \times T}{237.3 + T}\right)]})$$

Wind Chill is the measure of heat loss due to the effect of wind. It is calculated using the following formula taken from (Siple and Passel, 1945, Steadman, 1979).

$$Wind\ Chill\ ℃ = 13.12 + 0.6215\ (T) - 11.37\ (V^{0.16}) + (\ 0.3965(T)(V^{0.16}))$$

T - Temperature in degrees Celsius ℃
V – Wind Velocity in kilometres per hour

THSW Index uses a combination of humidity, temperature, and wind to calculate an apparent temperature. The index is calculated by a series of formulas found in (Steadman, 1979).

Heat Index uses a combination of temperature and relative humidity to determine how the equivalent temperature feels to the human body. This is calculated using the best curve fit from values found in (Steadman, 1979, Quayle and Steadman, 1998).

Heating Degree Days is a measure of how many degrees average daily temperature is below 18.33℃. This value is the standard used in New Zealand (Anon, NIWA per comm) and is the value typically chosen and is used in this study. Heating degree days can be calculated using the equation formula from (Schoenau and Kehrig, 1990):

$$DD_{bm} = NS_d[Z_b F(Z_b) + f(Z_b)]$$

Cooling Degree Days is a measure of how many degrees average daily temperature is below 18.33℃. This value is the standard used in New Zealand (Anon, NIWA per comm). Cooling degree days can be calculated using the formula from (Schoenau and Kehrig, 1990):

$$DD_{bm} = NS_d(Z_b(F(Z_b) - 1) + f(Z_b)$$

4.2.3.3 Daylength

Daylength for each farm was calculated using the *insol* package on Rstudio (Corropio, 2003). To calculate the daylength, the latitude and longitude of each farm were defined (see Table 2.1). The daylength per week was then calculated by inputting the desired time period with the location of each farm; the code for doing so can be found in Appendix C.1.1.

Figure 4.2 The Davis Vantage Pro2 6322C in position on "M" Station, North Island.

4.2.4 Monitoring of Population Dynamics for the 2018/2019, 2019/2020 and 2020/2021 Seasons

Sampling began in mid to late September 2018 on all farms in the North Island and the first week of October in the South Island (Table 4.3). Each week, the traps were emptied of any specimens and the StickyTraps were replaced (Table 4.3). Samples from the flytrap and the StickyTrap were placed in separate containers, containing 70 % alcohol for preservation. On each of the sampling sites for 2018/2019, samples were collected on a weekly basis (Table 4.3). Each site was collected on the same day each week (± one day) (Table 4.3). The start and end of the season for each target species was determined after two successive weeks of zero catches, in a similar manner to that previously described (Lacour et al., 2015).

Table 4.3 The day of collection and start date for each population dynamic study on the farms for the 2018/2019 season.

Day of collection	Farm	Start date of studies
Thursday	"T"	20th September 2018
Thursday	"R"	20th September 2018
Thursday	"BH"	20th September 2018
Tuesday	"LD"	25th September 2018
Tuesday	"M"	25th September 2018
Wednesday	"KD"	9th October 2018
Wednesday	"S"	9th October 2018
Wednesday	"ST"	9th October 2018

Flytraps were placed before the start of the fly season on all North and South Island farms in late September until the second week of December 2019 and from mid-March until the last week of June 2020 on all North Island farms (Table 4.4). The traps were removed between these two periods (Table 4.4). On the South Island farms, the collection of samples in 2019 began a week later in October (Table 4.4). The study to monitor the end of the fly season was carried out only on one farm, “LD”, due to travel restrictions during the Covid 19 pandemic in New Zealand (Table 4.4). Due to considerable travel restrictions implemented in New Zealand during this time, it was not possible to collect samples via on-farm visits. Samples were only collected at “LD” as the person collecting samples each week was living on the farm.

A further study to monitor the start of the flystrike season for 2020 was conducted from the third week of September 2020 until the second week of December 2020 on two farms only, “R” and “T” (Table 4.4). It was initially planned to conduct this study on all farms on the North Island, but due to difficulty in securing replacement chemicals from Australia (as outlined previously), and the potential for further covid travel restrictions, the study was restricted to two farms only.

Table 4.4 The start and end dates of fly collection for each population dynamic study for each farm for the 2019/2020 and 2020/2021 collection season.

			Start of season		End of season	
Season	Day of collection	Farm	Start date of study	End date of study	Start date of study	End date of study
2019/2020	Wednesday	“T”	25th September 2019	4th December 2019	4th March 2020	Cancelled
		“R”	25th September 2019	4th December 2019	4th March 2020	Cancelled
		“BH”	25th September 2019	4th December 2019	4th March 2020	Cancelled
		“M”	25th September 2019	4th December 2019	4th March 2020	Cancelled
		“LD”	25th September 2019	4th December 2019	4th March 2020	24th June 2020
	Thursday	“KD”	2nd October 2019	5th December 2019	Cancelled	Cancelled
		“S”	2nd October 2019	5th December 2019	Cancelled	Cancelled
		“ST”	2nd October 2019	5th December 2019	Cancelled	Cancelled
2020/2021	Wednesday	“T” “R”	28th September 2020	8th December 2020	Cancelled	Cancelled

4.2.5 Monitoring of flystrike on sheep

All farmers or farm managers were asked to record the date of the first case of flystrike.

4.2.6 Identification Keys

The following identification keys were used to identify the flies in the family Calliphoridae (Dear, 1986, Wallman, 2001a, Szpila, 2012), including *Chrysomya* spp. (Dear, 1986, Wallman, 2001a, Akbarzadeh et al., 2015, Lutz et al., 2018) and *Lucilia* spp. (Dear, 1986, Holloway, 1991, Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018). The following identification keys were used to identify the following dipterans to family level: Sarcophagidae (Szpila, 2012, Meiklejohn et al., 2013a, Meiklejohn et al., 2013c), Muscidae (Grzywacz et al., 2017), Faniidae (Domínguez and Pont, 2001) and the following morphological keys were used to identify other families of Diptera (Zumpt, 1965, Oosterbroek, 2006, Szpila, 2012). All specimens were taken from 70% alcohol and identified as wet samples using a Leika EZ4 Stereo Microscope (Leica Microsystems GmbHErnst-Leitz-Straße 17-37, 35578 Wetzlar, Germany).

4.2.7 Statistics

Augmented Dickey-Fuller test was applied using the *adf.test* from the *tseries* package (Trapletti and Hornik, 2022). The Augmented Dickey fuller test is used to define the stationarity of the *Lucilia* spp. data sets collected on each farm (Dickey and Fuller, 1979, Cheung and Lai, 1995). If the data was found to be stationary, then there would be no dependence on the time of year at which the population of *Lucilia* spp. were observed. While if the data was found to be non-stationary, then we could conclude that there is a time-dependent trend and seasonality which would affect the population of *Lucilia* spp. at different times of the year.

A cross-correlation function (CCF) was used to explore the relationship between various climatic variables and the occurrence of *Lucilia* spp. for the 2018/2019 season using the *ccf* function from the *forecast* package (Hyndman and Khandakar, 2008, Hyndman et al., 2023). CCF is a useful function to use as it will identify the time lags at which a variable may be useful to predict the future population. CCF are defined based on the autocorrelation coefficients (ACF) between x_{t+h} and y_t for w = 0, ±1, ±2, ±3, ±4 etc. The lags were investigated up to a maximum of eight weeks. The crucial relationship is to discern if the correlation relationship is either at a negative or positive lag. If a climatic variable at a certain time was found to be negative, then one may say that this change would be observed in the distribution of *Lucilia* spp. at a later stage. Conversely, if the relationship is found to be at a positive stage, then the observed change in the distribution of *Lucilia* spp. would be observed after the change in the climatic variable.

Weather data for each farm was summarised over a week with the day of the fly collection being the last day of the previous week. The mean of the following variables for each farm over the seven days: temperature, relative humidity, dew point, wet bulb, wind speed, wind run, high wind speed, wind chill, heat index, THW index. Rainfall was summarised as the total rainfall during that week. Cooling and heating degree days were summarized as the sum of degree days over the entire week. The 10 cm soil temperature probes were averaged from all three probes on each farm.

4.2.8 Degree Days

Degree day models are used to predict insect activity by estimating temperature thresholds when activity will begin. Degree days are then accumulated each day when this threshold is exceeded which allows for the prediction of whether a stage of development has been met. These temperature thresholds are typically based on laboratory experiments with a species at differing stages of developmental life stages at constant temperatures (Grassberger and Reiter, 2001, Cervantes et al., 2018, Pruna et al., 2019). The majority of experimental studies have shown that the development of calliphorid flies exhibit a sigmoid curve with the growth rate slowing at the upper and lower registers (Vogt and Woodburn, 1980, Harvey et al., 2016, Shin et al., 2021). An arbitrary date is typically chosen for when Degree Days (DD) models are utilised. For example, in studies conducted in the temperate Northern Hemisphere, the first of January is typically chosen (Wall et al., 2002). The 1st of August was chosen in the present study as it is the equivalent month to January in the Southern Hemisphere in terms of environmental conditions in a New Zealand context using the following formula:

$$DD = mean\ 10cm\ Soil\ temperature\ per\ day\ - T_{base}$$

There are currently no published developmental data at constant temperatures available from New Zealand for *L. cuprina* or *L. sericata*. Therefore, this method aimed to assess whether the lower developmental threshold calculated from the developmental data from other countries could fit the observed seasonality of *L. cuprina* and *L. sericata* under New Zealand conditions (Table 4.6). The lower developmental threshold is estimated by taking the developmental time of each temperature measured in the study as 1/developmental time in hours (y-axis) and linearly extrapolating a line through until it exceeds the lower limit of temperature (℃) on the x-axis.

However, the Hobo soil temperature probes were not installed on any farms at 10 cm soil depth by the 1st of July. Therefore, daily 10 cm soil temperature from the nearest Virtual Climate Station was used in this calculation. The Virtual Climate Station Network (VCS) is operated by the National Institute of Water and Atmospheric Research (NIWA), which provides a spatial interpolation of several climatic variables from the national weather stations across New Zealand at a ~5 km grid resolution (Tait and Turner, 2005, Tait et al., 2006a, Tait and Woods, 2007, Cichota et al., 2010, Tait et al., 2012, Mason et al., 2017). The VCS agents used for each farm are listed in Table 4.5.

Table 4.5 outlines the name of the farm, its location, and the closest Virtual Climate Station (VCS) agent number.

Farm Name	Latitude	Longitude	VCS Agent number
“KD”	-42.####	172.####	20098
“ST”	-42.####	173.####	20496
“S”	-42.####	173.####	20500
“LD”	-37.####	174.####	26837
“BH”	-40.####	176.####	27906
“R”	-40.####	175.####	28791
“M”	-38.####	175.####	29239
“T”	-40.####	175.####	30371

Table 4.6 The estimated base temperature for *Lucilia cuprina* and *Lucilia sericata* from publications worldwide and the required number of degree days required for emergence of adult from the base temperature.

Species	Publication	Country	Base Temperature (℃)	Accumulated degree day estimation from Base Temperature (℃)	Acronym
L. cuprina	(Dallawitz, 1984)	Australia	11.03	None	Cuprina_1
L. cuprina	(Kotze et al., 2015)	South Africa	12.02	None	Cuprina_2
L. cuprina	(Bambaradeniya et al., 2018)	Sri Lanka	14	None	Cuprina_3
L. cuprina	(Sa et al., 2016)	India	13.4	None	Cuprina_4
L. sericata	(Wall et al., 1992a)	England	9.2	153.7	Sericata_1
L. sericata	(Cervantes et al., 2018)	France	8.3	223-243	Sericata_2
L. sericata	(Grassberger and Reiter, 2001)	Austria	9.14	214	Sericata_3
L. sericata	(Gosselin et al., 2010)	Belgium	9.55	317.97	Sericata_4
L. sericata	(Greenberg, 1991)	USA	11.3	485	Sericata_5

4.3 Results

4.3.1 Seasonal dynamics of species caught by LuciTrap and Stickytrap

4.3.1.1 Fly Collection 2018/2019

A total of 60,428 flies were caught and identified for the 2018/2019 season over the 24 traps across the eight farms (Table 4.7). The following species from the family Calliphoridae were caught *Lucilia* spp. (*L. cuprina and L. sericata*), *C. stygia*, *C. quadrimaculata*, *Calliphora vicina* (Diptera: Calliphoridae; Robineau-Desvoidy, 1830), *Calliphora hilli* (Diptera: Calliphoridae; Patton, 1925) and *C. rufifacies*. The most common calliphorid species were 8,553 *Lucilia* spp. (as combined *L. cuprina* and *L. sericata* species) (14.15 % of the total catch) (Table 4.7). The highest weekly and total catches of *Lucilia* spp. were found on the South Island farms (Table 4.7). The most numerous families were Sarcophagidae with 27,159 specimens (44.94 %) and Muscidae with 12,373 (20.48 %) (Table 4.7). There was a low catch rate of *C. stygia* throughout this study on all sites throughout each fly collection season (Table 4.7, 4.9, 4.10). Due to the low sample size for this species, no further inferences and analyses could be made regarding this species.

Table 4.7 Total catch of flies in the family Calliphoridae (by separate species) and other dipteran families across eight farms on the North and South Islands 2018/2019.

Family		Farms								Total	Total (%)
	Species	North Island					South Island				
		“T”	“R”	“LD”	“BH”	“M”	“KD”	“S”	“ST”		
Calliphoridae	*Lucilia* spp.	479	599	670	347	1,116	1.575	1,542	2,225	8,553	14.15
	Calliphora stygia	7	20	23	36	14	25	75	27	227	0.38
	Calliphora quadrimaculata	1	3	4	1	0	5	1	4	19	0.03
	Calliphora vicina	7	26	2	17	20	37	0	40	149	0.25
	Calliphora hilli	0	0	0	1	4	0	0	0	5	0.01
	Chrysomya rufifacies	4	5	10	2	26	1	1	1	50	0.08
Sarcophagidae		4,946	4,243	3,725	1,113	2,090	4,365	2,500	4,177	27,159	44.94
Muscidae		991	865	891	761	692	1,570	2,945	3,658	12,373	20.48
Tachinidae		0	3	12	0	0	17	19	8	59	0.10
Psycodidae		183	4	28	2	20	252	11	21	521	0.86
Polleniidae		6	1	39	2	3	0	9	34	94	0.16
Xenocalliphora		7	1	14	0	6	11	20	9	68	0.11
Sphaeorceridae		0	0	0	0	0	70	63	89	222	0.37
Heleomyzidae		3	9	0	9	3	8	58	14	104	0.17
Piophilidae		34	3	0	1	3	1	29	7	78	0.13
Phoridae		350	109	839	108	407	633	159	361	2,966	4.91
Unknown		691	315	1208	330	2574	708	630	670	7,126	11.79
Other families		50	40	140	62	80	90	73	120	655	1.08
	Total	7,759	6,246	7,605	2,792	7,058	9,368	8,135	11,465	60,428	100%

4.3.1.1.1 Interpolation of missing data

In the South Island sites of “S” and “ST”, samples were not collected on the following dates: 24th December and 31st of December 2018 with the count on the 7th of January being the cumulative catch from the previous three weeks as no collection of samples occurred during the previous fortnight (Table 4.8). It was therefore necessary to remove the catch of *Lucilia* spp. (*L. cuprina* and *L. sericata*) from the data set for the 7th of January 2019 (Table 4.8). Any missing data, as a result of failure to empty traps over a fortnight (Table 4.8), were imputed using a Steinman linear interpolation method using the *imputeTS* package (Moritz et al., 2017), code for the method is included Appendix C.1.2.

Table 4.8 Recorded and interpolated values of *Lucilia* spp. (*Lucilia cuprina* and *Lucilia sericata*) from “S” and “ST”.

Date	Farm	Recorded values	Interpolated values
26/12/2018	“S”	No collection	1
02/01/2019	“S”	No collection	1
09/01/2019	“S”	16	0
26/12/2018	“ST”	No collection	19
02/01/2019	“ST”	No collection	35
09/01/2019	“ST”	66	59

4.3.1.2 Fly Collection 2019/2020

Flies were successfully collected on all eight farms for the start of the 2019/2020 season but at the end of the 2019/2020 season only for “LD” (Table 4.9). A total of 6,291 specimens were collected with most samples being collected at “LD” (due to it being the only farm where samples were collected at the end of the 2019/2020 season). The following calliphorid species were caught: *Lucilia* spp. (*L. cuprina* and *L. sericata*), *C. stygia*, *C. quadrimaculata*, *C. vicina*, *C. hilli* and *C. rufifacies*. The most common calliphorid species were *Lucilia* spp. (3.8 %) followed by *C. stygia* (0.7 %; Table 4.9). The most numerous families were Psycodidae, Sarcophagidae and Phoridae (Table 4.9).

Table 4.9 Total catch of flies trapped on eight farms across the North and South Islands of New Zealand for the 2019/2020 fly collection season.

		Farms									
Family	Species	North Island					South Island			Total	Total (%)
		“T”	“R”	“LD” (*)	“BH”	“M”	“KD”	“S”	“ST”		
Calliphoridae	*Lucilia* spp.	15	2	84	12	100	7	12	6	238	3.8
	Calliphora stygia	2	0	18	16	0	5	0	3	44	0.7
	Calliphora quadrimaculata	0	0	0	17	0	0	0	0	17	0.3
	Calliphora vicina	0	4	7	0	12	0	0	0	23	0.4
	Calliphora hilli	0	0	0	0	0	0	0	0	0	0.0
	Chrysomya rufifacies	0	0	2	0	0	0	0	0	2	0.0
Sarcophagidae		26	17	520	42	118	38	9	68	838	13.3
Muscidae		69	5	210	22	13	44	78	84	525	8.3
Tachinidae		0	0	0	0	0	0	0	0	0	0.0
Psycodidae		631	27	88	6	0	5	0	2	759	12.1
Polleniidae		0	0	0	0	0	0	0	0	0	0.0
Xenocalliphora		0	0	0	0	0	0	0	0	0	0.0
Spaeroceridae		32	3	0	5	1	0	1	0	42	0.7
Heleomyzidae		0	0	0	0	0	0	0	0	0	0.0
Piophilidae		0	0	0	0	0	0	0	0	0	0.0
Phoridae		204	21	700	60	96	63	16	125	1,285	20.4
Unknown		127	143	770	162	312	398	292	34	2,518	40.1
Total		1106	222	2,399	342	652	560	408	602	6,291	100%

*”LD” was the only farm that was collected for the start and end of the season due to Covid 19 restrictions

4.3.1.3 Fly Collection 2020/2021

A total of 1,786 samples were collected on these two farms (Table 4.10). The following species from the family Calliphoridae were caught: *Lucilia* spp. (*L. cuprina* and *L. sericata*), *C. stygia* and *C. vicina*. The most common calliphorid species were *Lucilia* spp. (Table 4.10). The most common families caught were Phoridae and Psycodidae (Table 4.10).

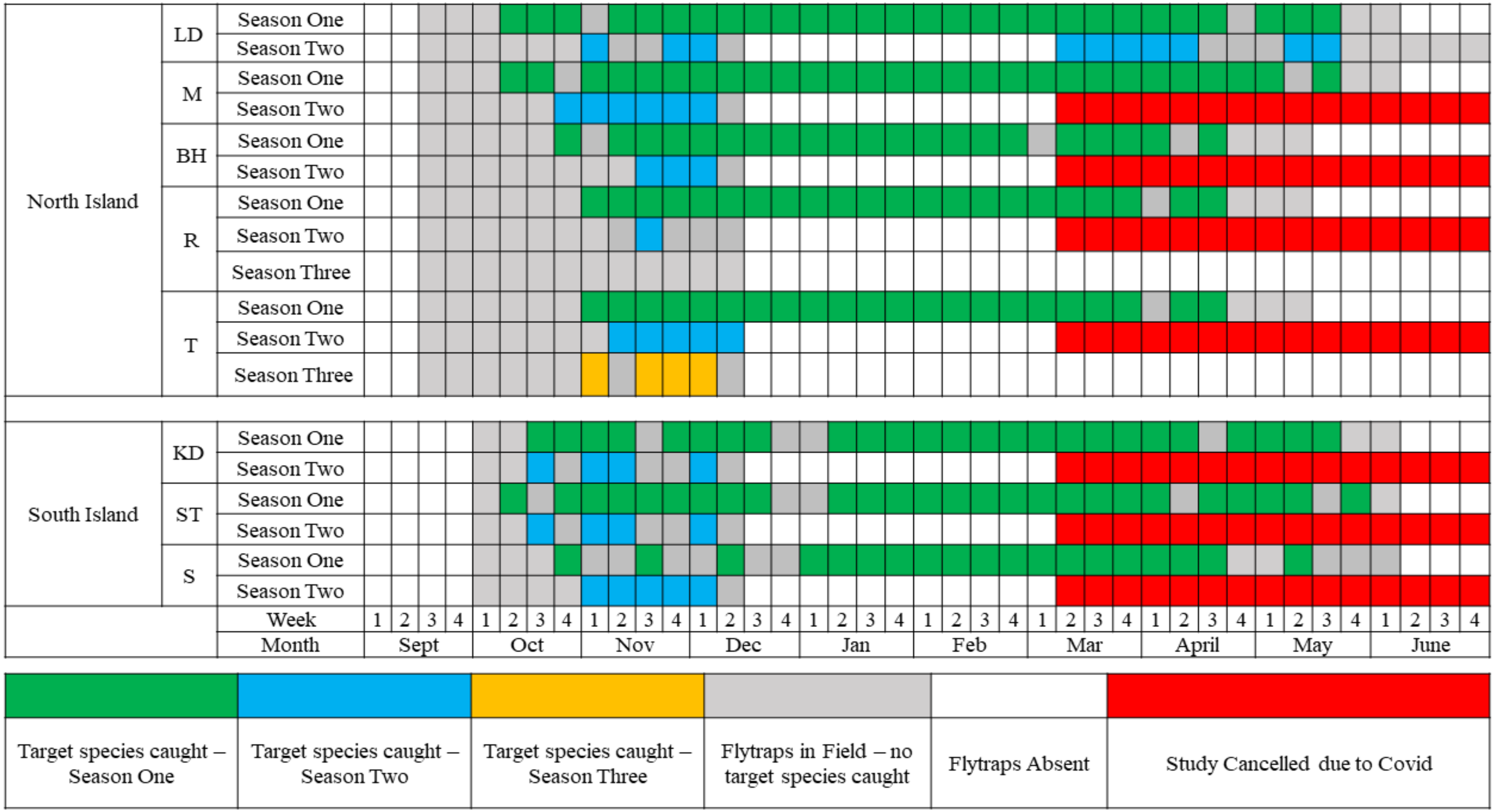
Table 4.10 Total catch of flies trapped on "T" and "R" in the North Island of New Zealand for the 2020/2021 fly collection season. Flies in the family Calliphoridae were differentiated into species.

		Farms			
Family	Species	"T"	"R"	Total	Total (%)
Calliphoridae	*Lucilia* spp.	12	0	12	0.7
	Calliphora stygia	1	3	4	0.2
	Calliphora quadrimaculata	0	0	0	0.0
	Calliphora vicina	2	6	8	0.4
	Calliphora hilli	0	0	0	0.0
	Chrysomya rufifacies	0	0	0	0.0
Sarcophagidae		30	42	72	4.0
Muscidae		15	9	24	1.3
Tachinidae		0	0	0	0.0
Psycodidae		579	15	594	33.3
Polleniidae		0	0	0	0.0
Xenocalliphora		0	0	0	0.0
Spaeroceridae		0	0	0	0.0
Heleomyzidae		0	0	0	0.0
Piophilidae		19	1	20	1.1
Phoridae		648	67	715	40.0
Unknown		223	114	337	18.9
Total		1,529	257	1,786	100%

4.3.2 Contrasting the seasonality of *Lucilia* spp. across three fly collection seasons (2018/2019, 2019/2020, 2020/2021)

Overall, the results showed that *Lucilia* spp. have a season beginning in mid-October and lasting until mid to late May (Figure 4.3). The length of the season for *Lucilia* spp. varied by up to a month when comparing sites on the upper North Island (“LD” and “M”) and the South Island (“KD”, “ST”, “S”) with those on the lower North Island (“T”, “R” and “BH”) (Figure 4.3).

On “KD”, *Lucilia* spp. were observed in the same week at the start of the fly season across both the 2018/2019 and 2019/2020 seasons (Figure 4.3). On “T”, “S” and “ST”, a difference of one week was observed across seasons (Figure 4.3). However, larger differences in the start of the season for *Lucilia* spp. were observed between the 2018/2019 and 2019/2020 seasons of a fortnight on “M” and “R” and “BH”; while a three-week difference was found on “LD” and “BH” (Figure 4.3). No difference was observed at the end of the 2019/2020 season for *L. cuprina* on “LD” compared to the 2018/2019 season (Figure 4.3). This data could not be collected for all the other farms across both seasons due to the Covid restrictions.



North Island	LD	Season One			▒	▒	▒	🟩	🟩	🟩	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	🟩	▒	▒			
		Season Two			▒	▒	▒	▒	▒	▒	🟦	▒	▒	🟦	🟦	▒											🟦	🟦	🟦	🟦	🟦	▒	▒	▒	🟦	🟦	▒	▒	▒	▒	▒
	M	Season One			▒	▒	▒	🟩	🟩	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	▒	▒			
		Season Two			▒	▒	▒	▒	▒	🟦	🟦	🟦	🟦	🟦	🟦	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	BH	Season One			▒	▒	▒	▒	▒	🟩	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	🟩	🟩	▒	🟩	▒	▒	▒						
		Season Two			▒	▒	▒	▒	▒	▒	▒	▒	🟦	🟦	🟦	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	R	Season One			▒	▒	▒	▒	▒	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	▒	▒	▒						
		Season Two			▒	▒	▒	▒	▒	▒	▒	▒	🟦	▒	▒	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Season Three			▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒																									
	T	Season One			▒	▒	▒	▒	▒	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	▒	▒	▒						
		Season Two			▒	▒	▒	▒	▒	▒	▒	🟦	🟦	🟦	🟦	🟦											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Season Three			▒	▒	▒	▒	▒	▒	🟨	▒	🟨	🟨	🟨	▒																									
South Island	KD	Season One					▒	▒	🟩	🟩	🟩	🟩	▒	🟩	🟩	🟩	🟩	▒	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	🟩	🟩	▒	▒			
		Season Two					▒	▒	🟦	▒	🟦	🟦	▒	▒	🟦	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	ST	Season One					▒	🟩	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	🟩	🟩	🟩	🟩	▒	🟩	▒			
		Season Two					▒	▒	🟦	▒	🟦	🟦	▒	▒	🟦	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	S	Season One					▒	▒	▒	🟩	▒	▒	🟩	▒	▒	🟩	▒	▒	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▒	▒	🟩	▒	▒	▒			
		Season Two					▒	▒	▒	▒	🟦	🟦	🟦	🟦	🟦	▒											🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		Month	Sept				Oct				Nov				Dec				Jan				Feb				Mar				April				May				June			

🟩	🟦	🟨	▒		🟥
Target species caught – Season One	Target species caught – Season Two	Target species caught – Season Three	Flytraps in Field – no target species caught	Flytraps Absent	Study Cancelled due to Covid

Figure 4.3 Contrasting the seasonality of *Lucilia* spp. across eight farms in the North Island of New Zealand for the first (2018/2019 season), second (2019/2020 season) and third year (2020/2021 season).

4.3.3 The seasonal dynamics of *Lucilia* spp. across three fly collection seasons (2018/2019, 2019/2020, 2020/2021)

The total weekly catches of *Lucilia* spp. peaked from January until March on all farms with the month of February having the overall peak weekly catch on all farms in the 2018/2019 season (Figures 4.4). The highest catch of 561 *Lucilia* spp. per week was recorded in the fourth week of February at “S” (Figure 4.4). Singular peaks of *Lucilia* spp. were found in “T”, “M” and “S” (Figures 4.4). Whereas in “R”, “KD” and “ST” a bimodal peak *Lucilia* spp. was observed. In “BH” and “LD” an accumulative seasonality with multiple peaks for *Lucilia* spp. was found (Figures 4.4). Overall, there were higher catches of both species on the South Island sites compared to the North Island sites in 2018/2019 season.

The first case of flystrike was recorded on all eight farms in 2018/2019 (Figures 4.6 and 4.7). The first case of flystrike was recorded on five out of the eight farms in the 2019/2020 season and on one farm in the 2020/2021 season (Figures 4.8, 4.9, 4.10). On each farm, where the occurrence of flystrike was recorded, there were relatively low numbers of *Lucilia* spp. (i.e., less than 5 per week) caught during the week of the first observed flystrike case (Figures 4.6, 4.7, 4.8, 4.9 and 4.10). However, on “BH” in 2019/2020, the first *Lucilia* spp. was caught a week after the first case of flystrike was detected.

The population dynamics of *Lucilia* spp. appeared to differ across farms. For example, during the 2018/2019 season, “BH” (Figure 4.6A) displayed a pattern with each subsequent generation or peak was larger than the last with a sharp decline at the end of the season. Whereas on each of the other seven farms, there is a low population of both species of less than 30 flies caught per week until late December or early January, when the population suddenly spikes on each of the farms (Figure 4.6 and 4.7). It is unknown if these population spikes are biological in nature with reaction to climatic changes or if they are due to on-farm actions by the farmers.

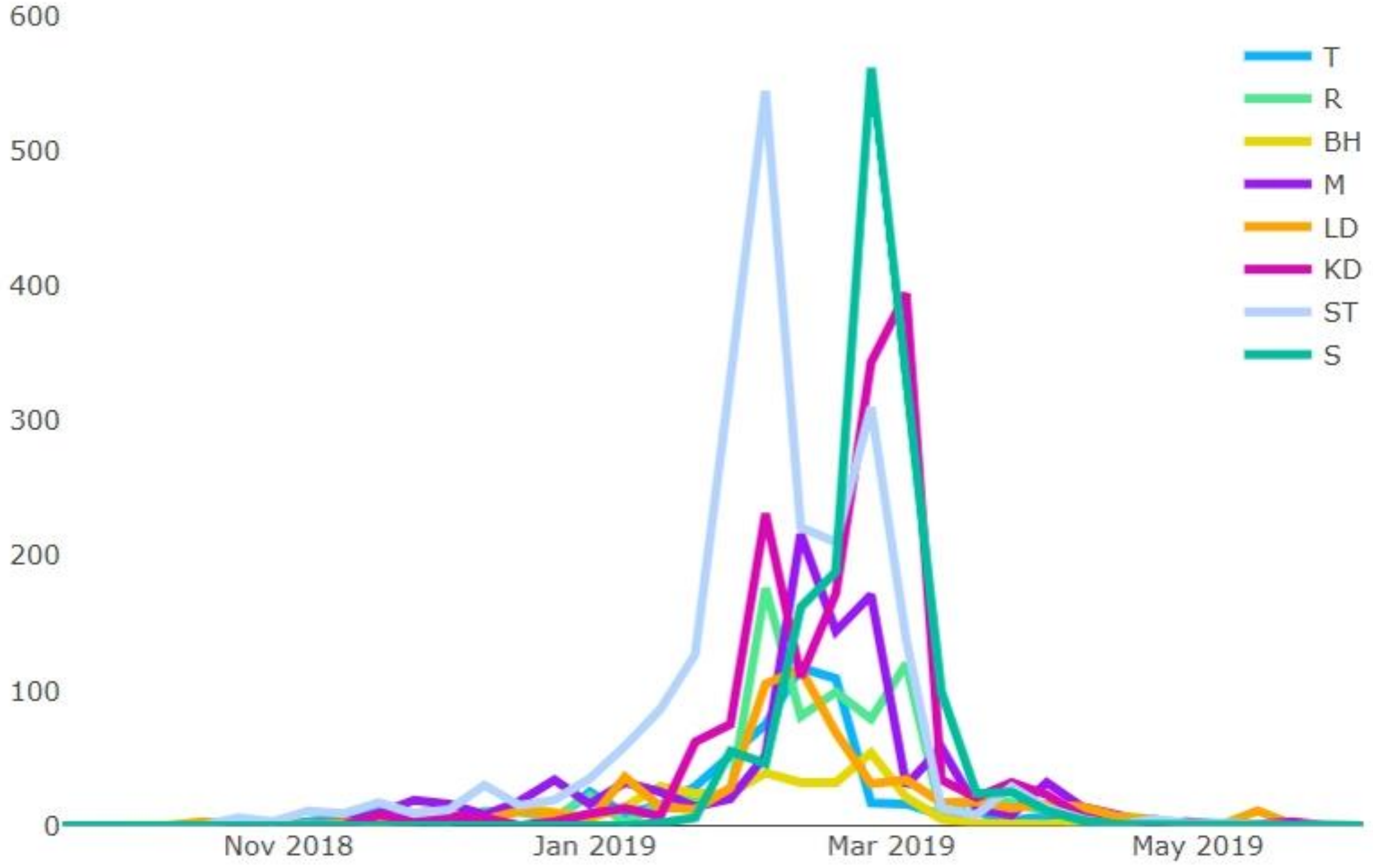


Figure 4.4 Weekly catch of *Lucilia* spp. in the 2018/2019 fly collection season from each farm. Individual farms were collected on different days during each week; therefore, the data were standardised to the first day of each week to allow for the comparison of the catch between farms.

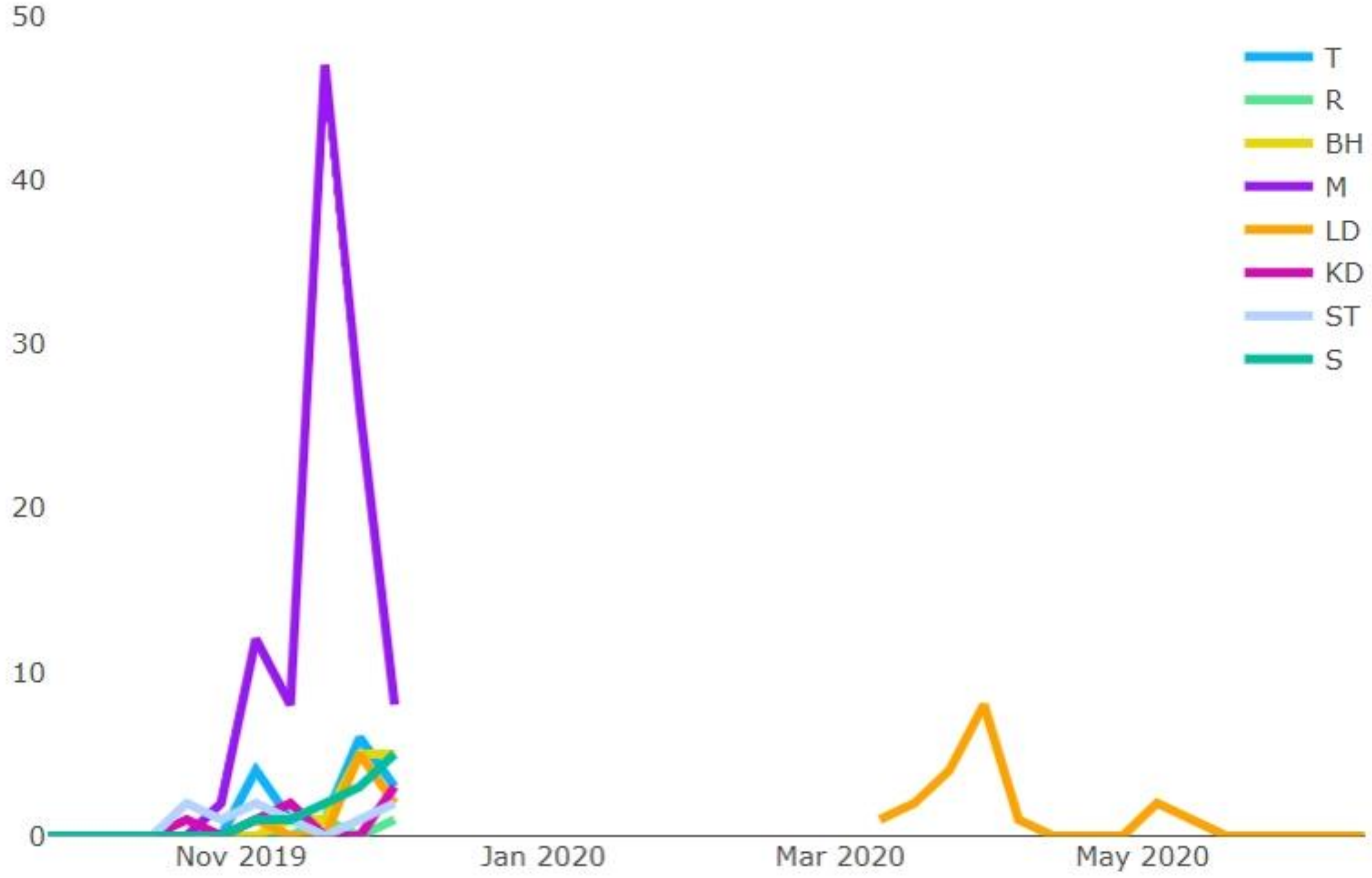


Figure 4.5 Weekly catch of *Lucilia* spp. in the 2019/2020 fly collection season from each farm. Individual farms were collected on different days during each week; therefore, the data were standardised to the first day of each week to allow for the comparison of the catch between farms.

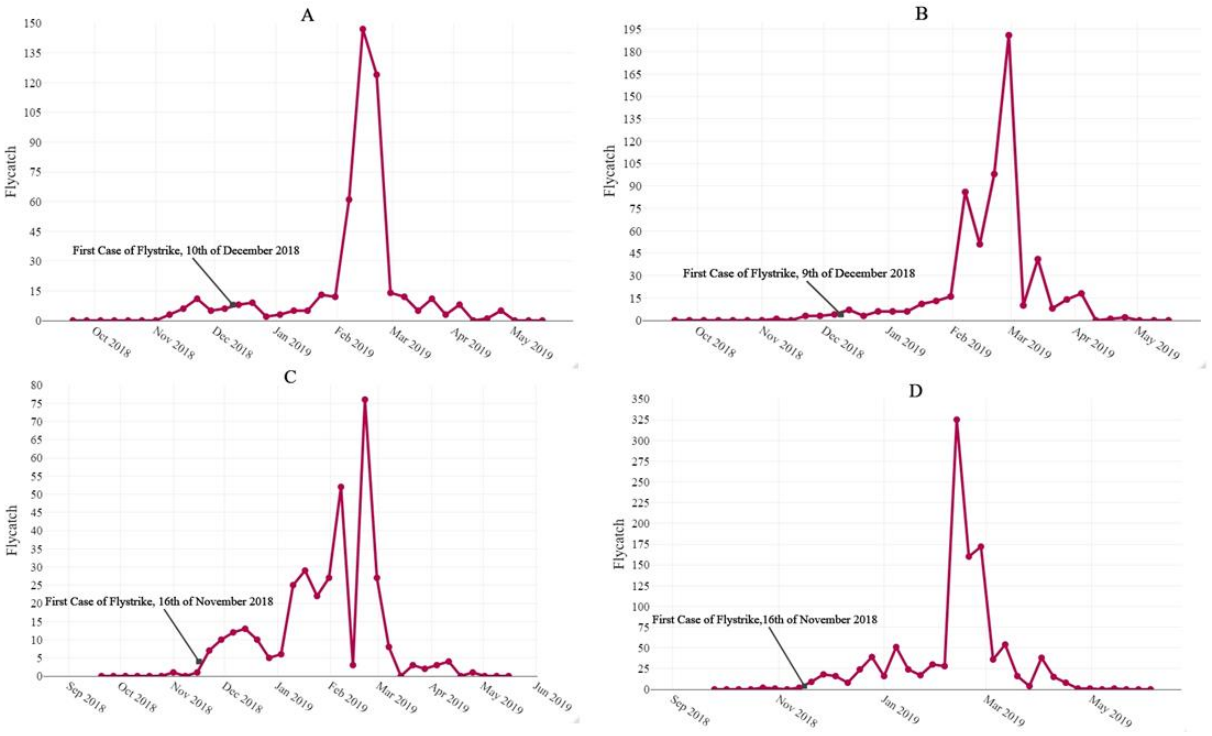


Figure 4.6 Weekly catch of *Lucilia* spp. for the 2018/2019 fly collection season on the following farms: “T” (Figure 4.6A), “R” (Figure 4.6B), “BH” (Figure 4.6C) and “M” (Figure 4.6D). The first case of flystrike is denoted for each farm.

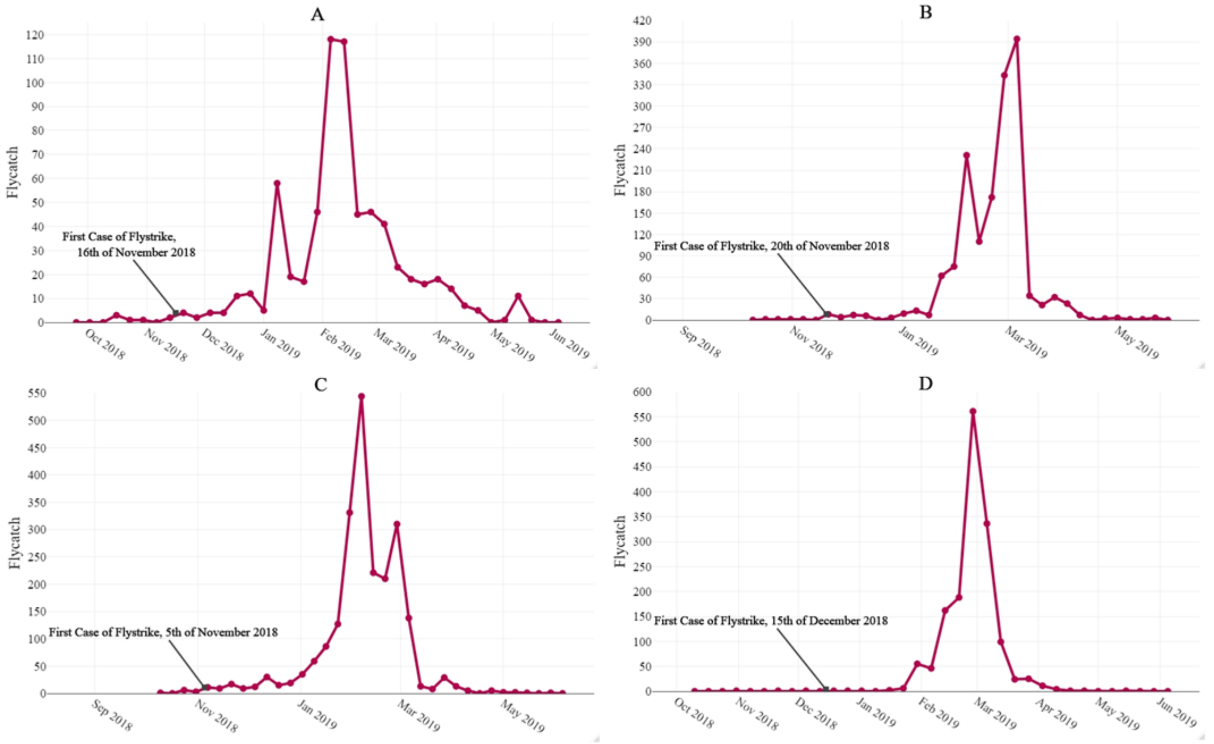


Figure 4.7 Weekly catch of *Lucilia* spp. for the 2018/2019 fly collection season on the following farms: “LD” (Figure 4.7A), “KD” (Figure 4.7B), “ST” (Figure 4.7C) and “S” (Figure 4.7D) The first case of flystrike is denoted for each farm.

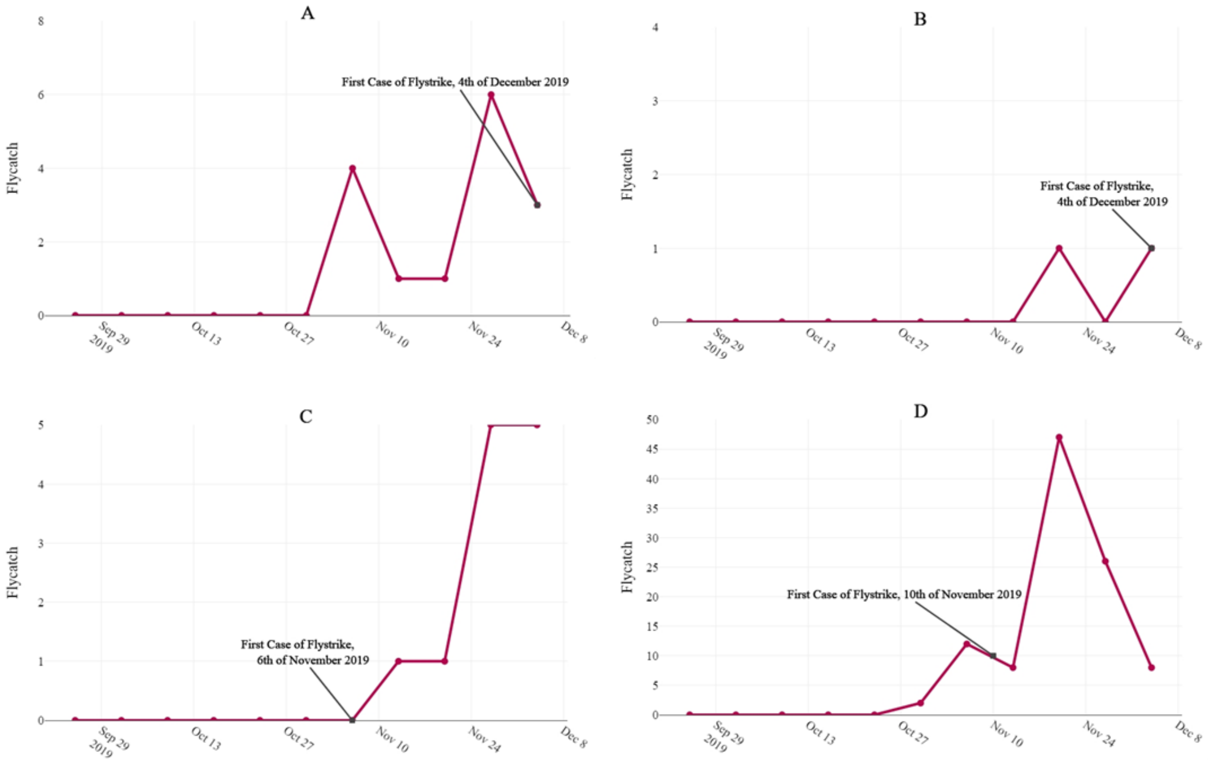


Figure 4.8 Weekly catch of *Lucilia* spp. for the 2018/2019 fly collection season on the following farms: “T” (Figure 4.8A), “R” (Figure 4.8B), “BH” (Figure 4.8C) and “M” (Figure 4.8D). The first case of flystrike is denoted for each farm.

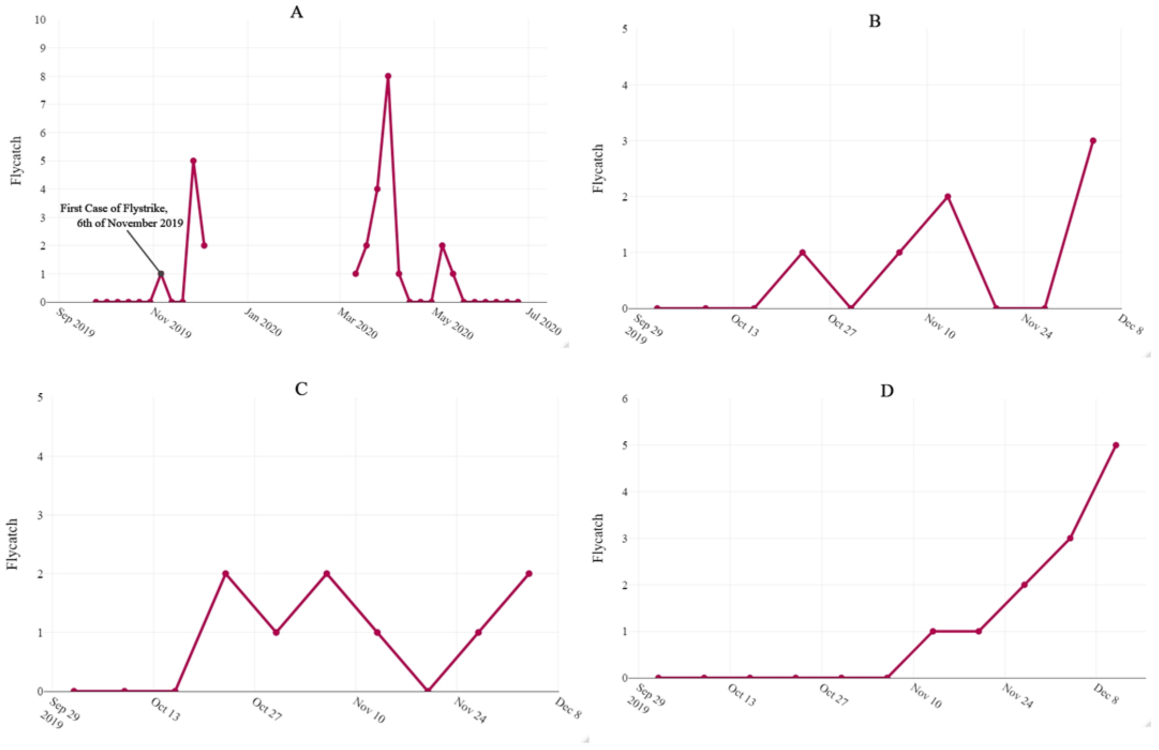


Figure 4.9 Weekly catch of *Lucilia* spp. for the 2018/2019 fly collection season on the following farms: “LD” (Figure 4.9A), “KD” (Figure 4.9B), “ST” (Figure 4.9C) and “S” (Figure 4.9D). The first case of flystrike is denoted in “LD” only (Figure 4.9 A).

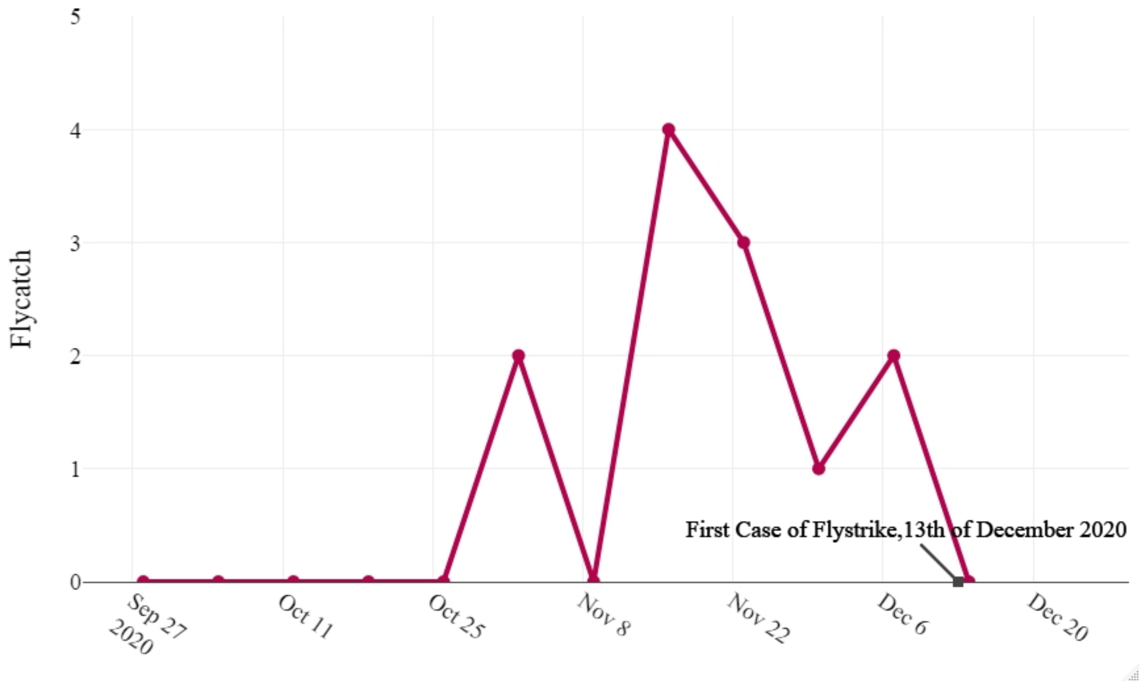


Figure 4.10 Weekly catch of *Lucilia* spp. for the start of the 2020/2021 fly collection season on the following farm: "T" (Figure 4.10). The first case of flystrike is noted at "T". The first case of flystrike was noted on "R" on the 15th of December. No *Lucilia* spp. were caught at "R" during the period traps were monitored at the start of the 2020/2021 season.

4.3.4 Results of the Augmented Dickey Fuller test

The Augmented Dickey Fuller Test found the population data of *Lucilia* spp. on all farms to be non-stationary ($p<0.05$). Therefore, there is a time-dependent structure to the population data which is seasonal. In addition, the non-stationary structure to the data was also evident by the high proportion of low counts of *Lucilia* spp. on each farm during the months of October until December as well as from April until May. There were high counts of these species in the months of December until March, although this varied from farm to farm.

4.3.5 Autocorrelation plots of the climatic variables

Initial exploratory analysis was conducted using cross-correlation of possible explanatory variables between the seasonality of *Lucilia* spp. with time lags of up to eight weeks. The results of these plots indicate that a change in distribution occurred for *Lucilia* spp. after a lag of five weeks in photoperiod on Farm “R” (Figure 4.12A) and at a lag of seven weeks in photoperiod on each of the other farms (Figure 4.11A, Figure 4.13A – 4.18A). Rainfall exhibited a cyclical pattern on all farms and on “LD” there was a negative correlation association at a lag of two weeks (Figure 4.15B). There was no significant association on the other farms.

The temperature variables of 10cm soil temperature, mean temperature, minimum temperature and maximum temperature exhibited a seasonal trend. Lower temperatures would lead to lower populations of *Lucilia* spp. at varying lags of up to seven weeks for each variable (Figures 4.11 C, E, F, G – 4.18 C, E, F, G). Similar trends were observed whereby higher temperatures at positive lags of up to four weeks were found to be significant (Figures 4.11 C, E, F, G – 4.18 C, E, F, G). This suggests that these temperature variables may influence each farm’s population size of *Lucilia* spp..

Mean Relative Humidity was found to be significant at a lag of one week for “BH” (Figure 4.13D), “M” (Figure 4.14D) and “ST” (Figure 4.17D). While for “T” (Figure 4.11D) and “LD” (Figure 4.15D), it was found to be significant with a two weeks lag. No significant lag was found on “R” (Figure 4.12D), “KD” (Figure 4.16D) or “S” (Figure 4.18D). No significant trend was identified with Mean Wind Speed on “ST” (Figure 4.17H), “KD” (Figure 4.16H), “LD” (Figure 4.15H), “M” (Figure 4.14H), “BH” (Figure 4.13H). Graphical associations of the trends between these climatic variables and the weekly catch of *Lucilia* spp. for each season may be found in Appendix C.

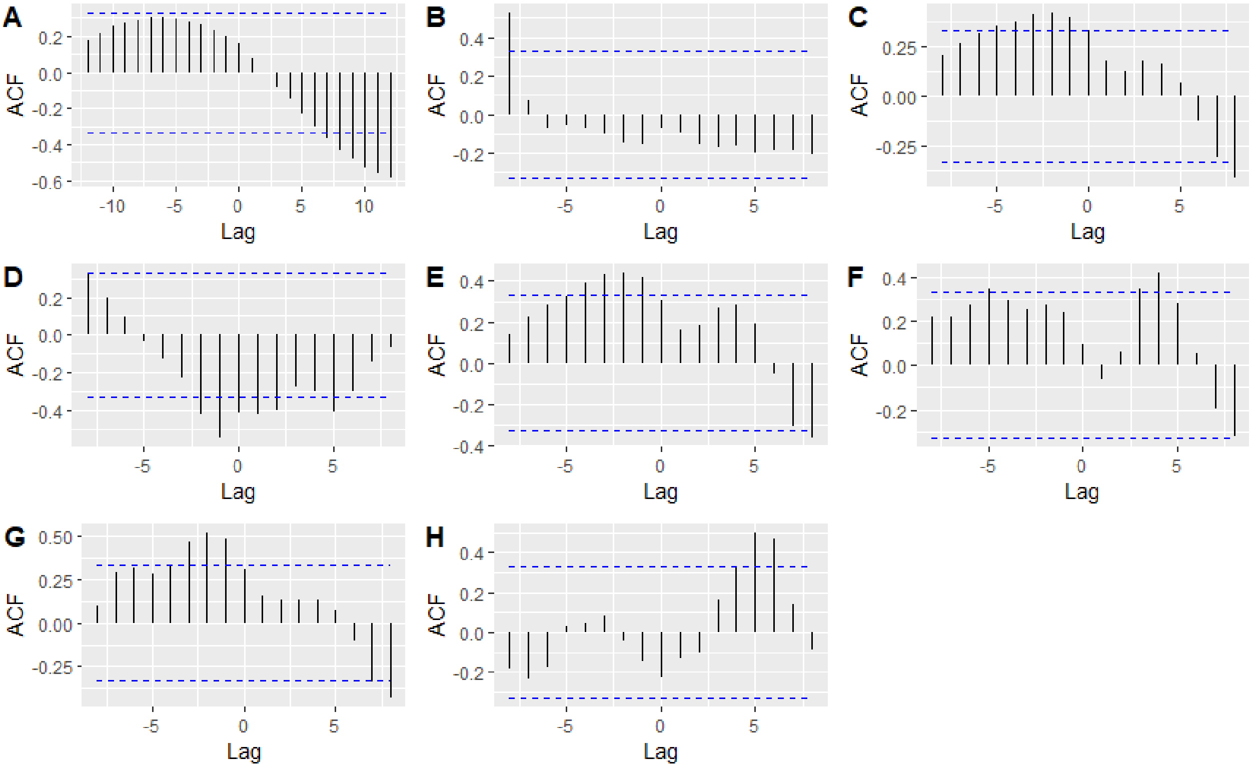


Figure 4.11 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm “T” in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature €, Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

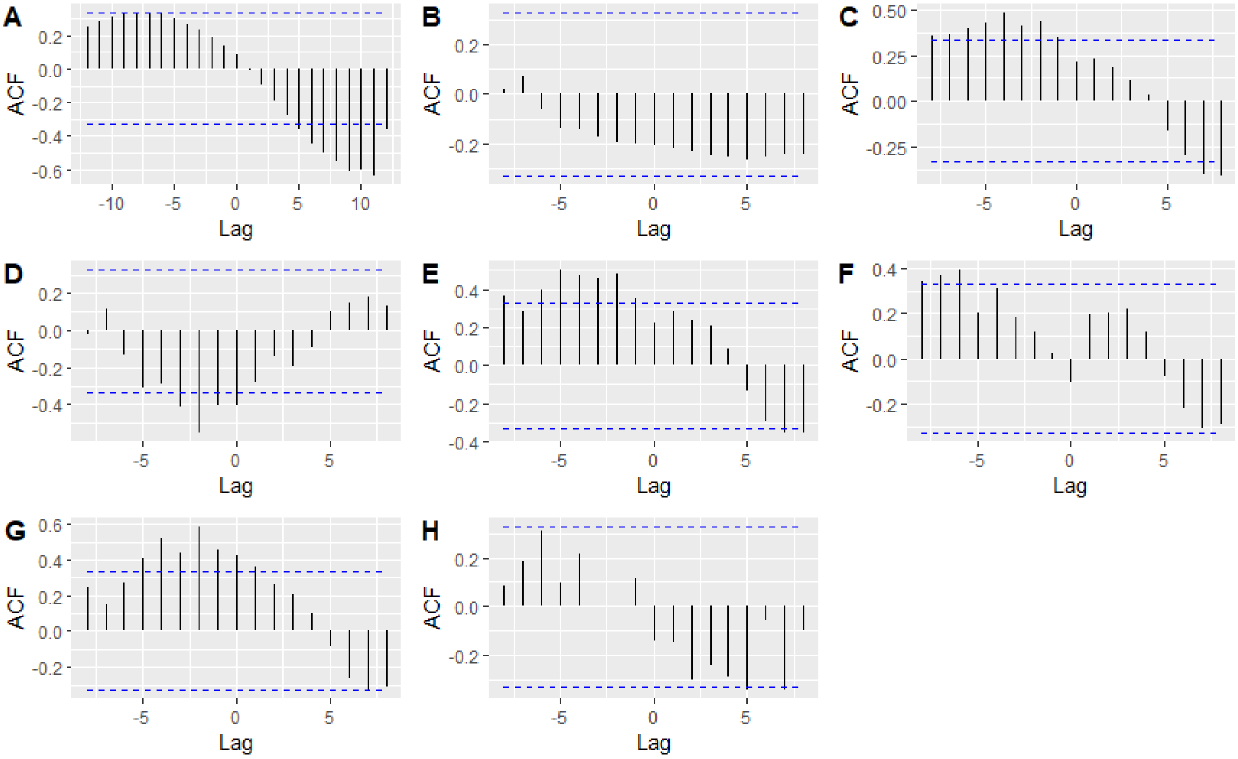


Figure 4.12 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "R" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

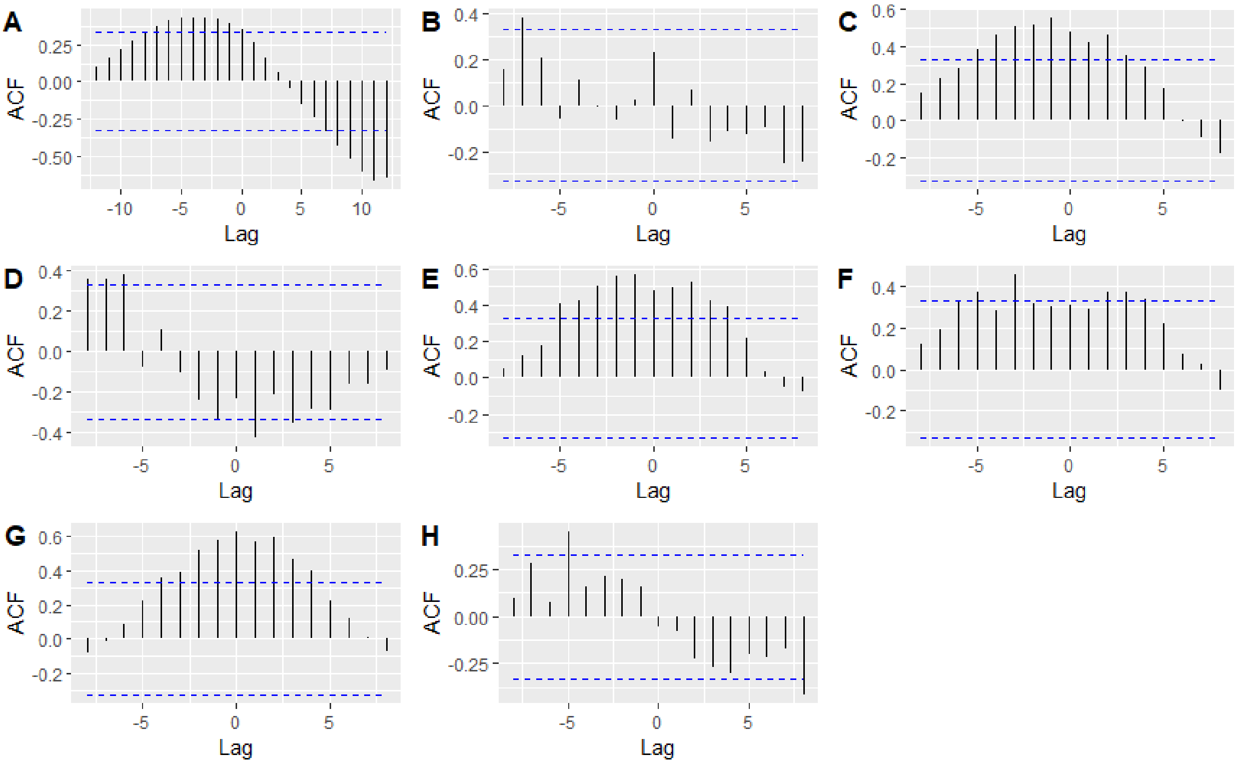


Figure 4.13 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "BH" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

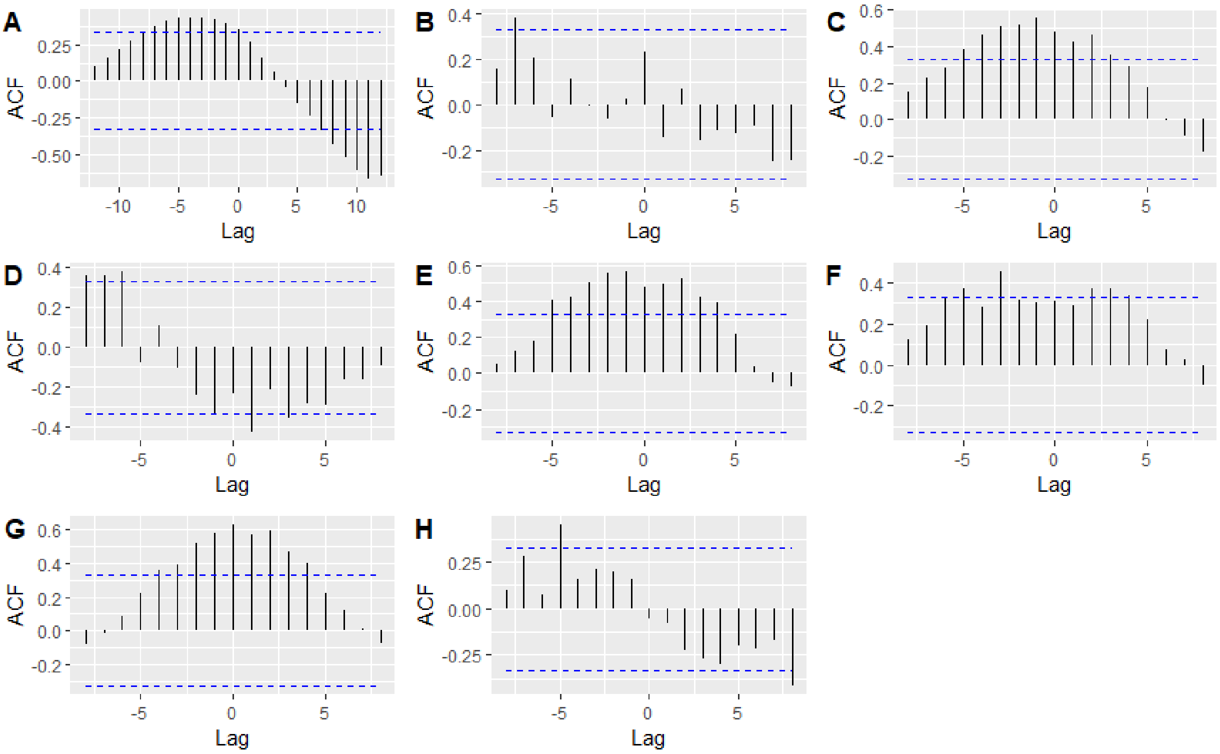


Figure 4.14 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "M" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

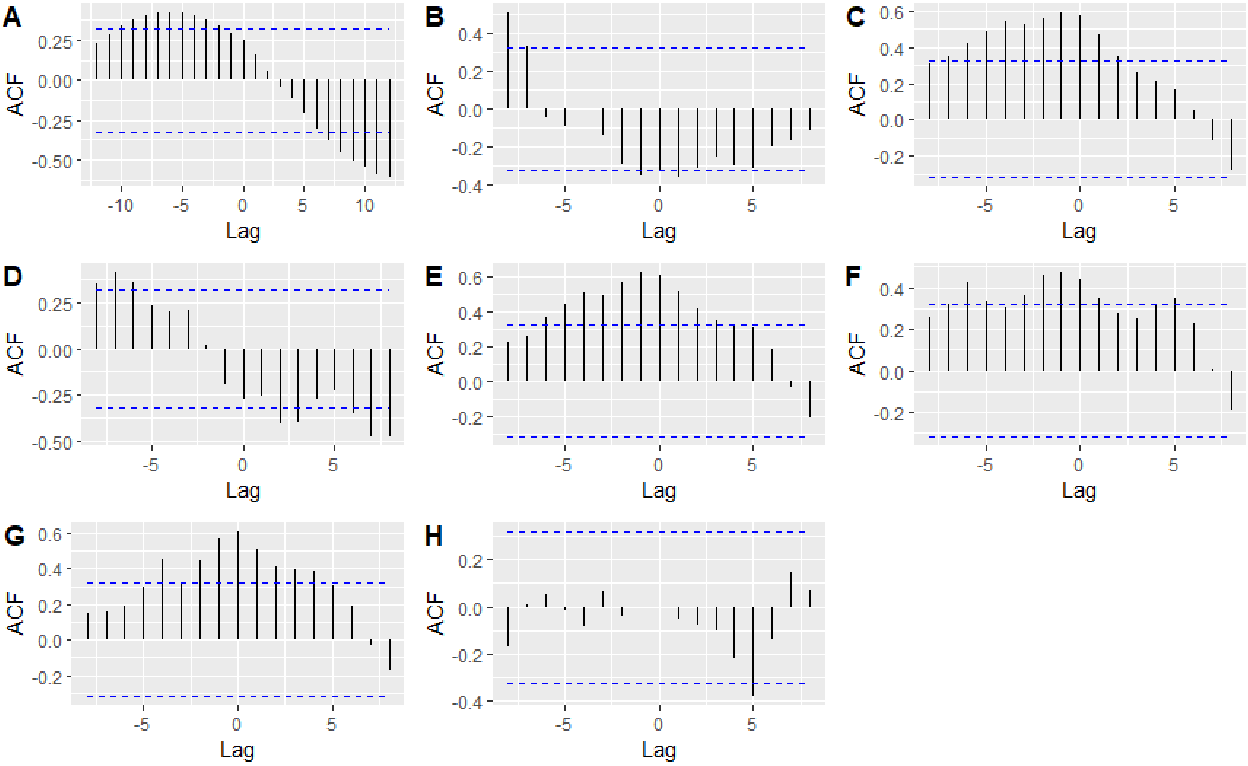


Figure 4.15 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "LD" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

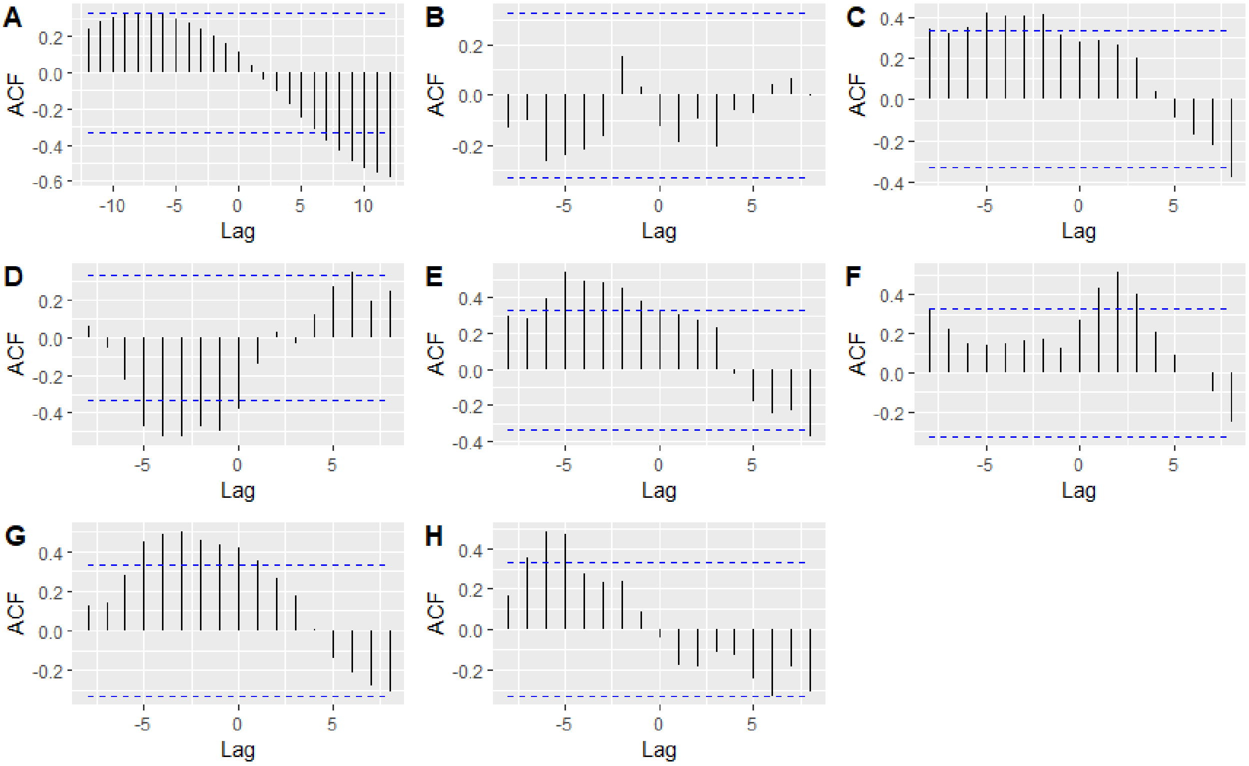


Figure 4.16 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "KD" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

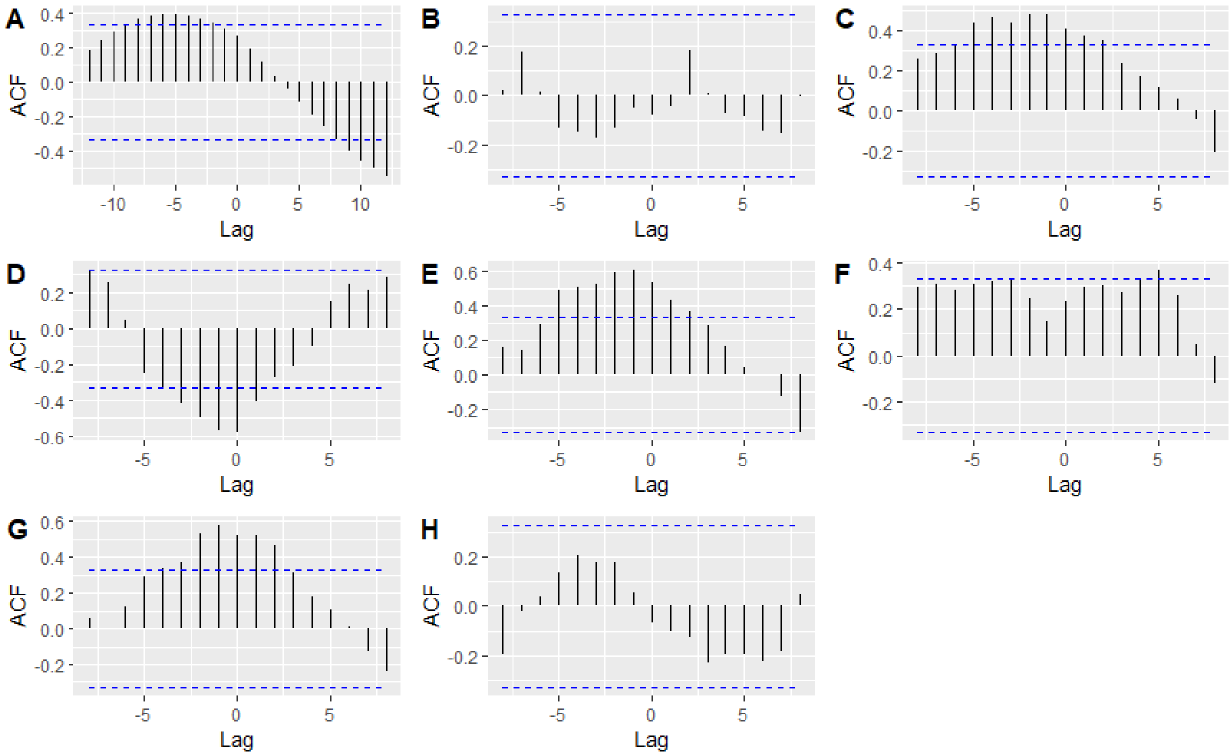


Figure 4.17 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm "ST" in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

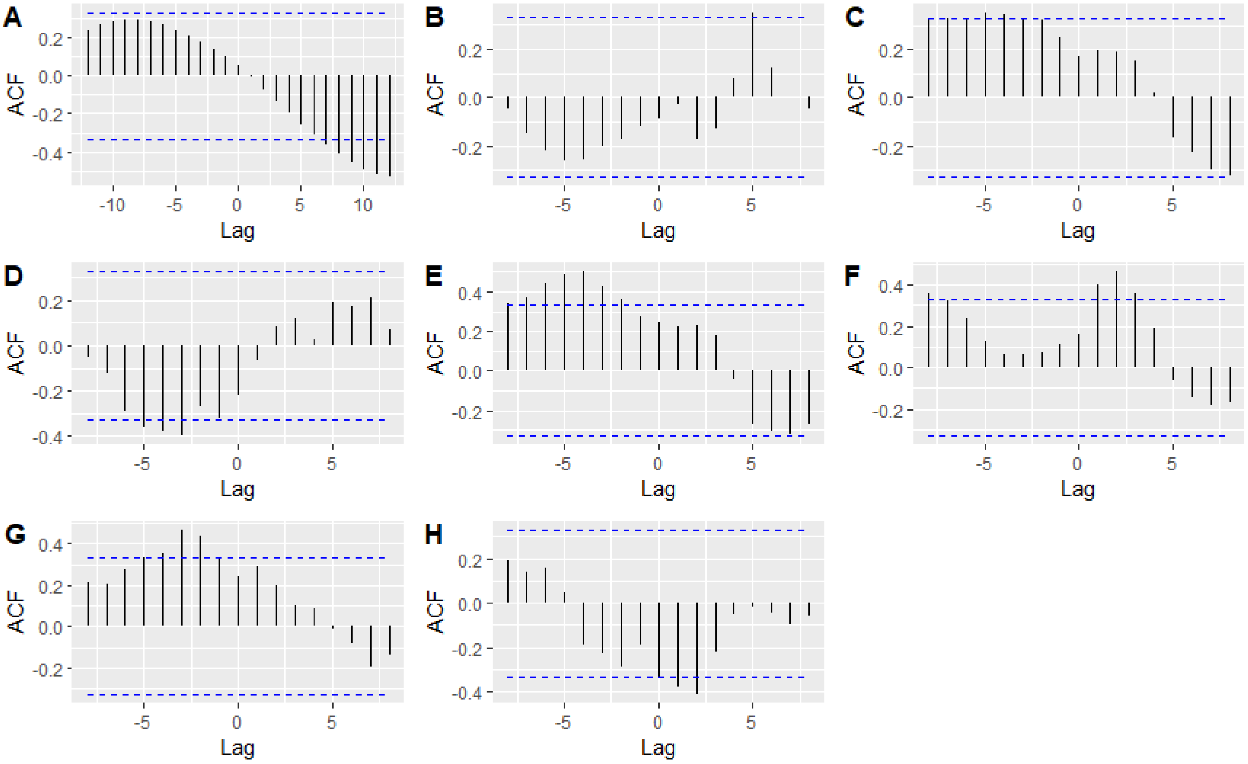


Figure 4.18 A cross-correlation plot examining the correlation for the following climatic variables lagged over a maximum of eight weeks (+/-) for Farm “S” in response to *Lucilia* spp... The following climatic variables were assessed: Mean Photoperiod (A), Total Rainfall (B), Mean 10 cm Soil Temperature (C), Mean Relative Humidity (D), Mean Temperature (E), Minimum Temperature (F), Maximum Temperature (G) and Mean Wind Speed (H). The autocorrelation function (ACF) is used to assess the correlation (positive, negative, or independent) between a time series and a lagged version of itself over successive time intervals. The blue lines indicate an approximate 95 % confidence interval for the corresponding time intervals.

4.3.6 Degree Days

The number of degree days from the lower developmental threshold was not specified by the publications of *L. cuprina* outlined in Table 4.6. Nevertheless, the number of degree days (DD) accumulated from the 1st of August until the emergence of the first *Lucilia* spp. (presumed to be *L. cuprina*) were estimated for each farm by applying the lower developmental thresholds reported from four different countries (Table 4.11). For each of these thresholds a wide disparity was found between each farm and between each threshold which suggested lower developmental thresholds in New Zealand (Table 4.11). This is especially notable in the South Island farms, where no DD were accumulated before the emergence of the first *Lucilia* spp. for any of the thresholds investigated (Table 4.11). This suggests that the lower developmental thresholds outlined previously for *L. cuprina* may be too high for New Zealand's climatic conditions and that there is pronounced variation between estimated threshold temperatures.

The number of degree days required for the emergence of *Lucilia* spp. was also estimated by utilizing the minimum DD required for *L. sericata* from five different countries: England (Wall et al., 1992a), France (Cervantes et al., 2018), Austria (Grassberger and Reiter, 2001), Belgium (Gosselin et al., 2010), USA (Greenberg, 1991). On each farm, the week of the observed occurrence of *Lucilia* spp. were compared to the predicted emergence for each of the five *L. sericata* thresholds (Table 4.12). No single threshold was found to be accurate for all farms (Table 4.12). For example, the 'Sericata_2' threshold from France was able to correctly predict the emergence of *Lucila* spp. on Farm "R" to the correct week (Table 4.12). However, *Lucilia* spp. were predicted to emerge two weeks later on Farms "T" and "M" but one week earlier on Farms "BH" and "LD" (Table 4.12). While on the South Island farms ("KD", "ST", "S") each of these were predicted to emerge one month later (Table 4.12).

Table 4.11 The number of degree days that were accumulated until the emergence of the first *Lucilia* spp. beginning from the 1st of August on each farm, based on the lower developmental thresholds from five different countries for *Lucilia cuprina*.

Farm	Observed Occurrence	Cuprina_1[a]	Cuprina_2[b]	Cuprina_3[c]	Cuprina_4[d]
“T”	07/11/2018 - 13/11/2018	36.74	17.3	1.8	3.8
“R”	07/11/2018 - 13/11/2018	78.23	45.54	8.5	15.9
“BH”	31/10/2018 - 06/11/2018	91.75	51.34	9.6	17.9
“M”	17/10/2018 - 23/10/2018	40.03	16.12	1.1	2.7
“LD”	17/10/2018 - 23/10/2018	114.49	66.96	10.1	22.7
“KD”	17/10/2018 - 23/10/2018	0	0	0	0
“ST”	10/10/2018 - 16/10/2018	0	0	0	0
“S”	31/10/2018 - 06/11/2018	0	0	0	0

a Cuprina_1, an *L. cuprina* degree day model with a developmental threshold of 11.03℃ (Dallawitz et al. 1984), Australia
b Cuprina_2, an *L. cuprina* degree day model with a developmental threshold of 12.02℃ (Kotze et al. 2015), South Africa
c Cuprina_3, an *L. cuprina* degree day model with a developmental threshold of 14℃ (Bambaradeynia et al. 2017), Sri Lanka
d Cuprina_4, an *L. cuprina* degree day model with a developmental threshold of 13.4℃ (Sa Bansode et al. 2016), India

Table 4.12 The observed occurrence of an adult *Lucilia* spp. is contrasted with the estimated date of emergence based on accumulated degree day thresholds for *Lucilia sericata* observed from five different countries.

Farm	Observed Occurrence	Sericata_1a	Sericata_2b	Sericata_3c	Sericata_4d	Sericata_5e
"T"	07/11/2018 - 13/11/2018	21/11/2018 - 27/11/2018	28/11/2018 - 04/12/2018	28/11/2018 - 04/12/2018	19/12/2018 - 25/12/2018	06/02/2019 - 12/02/2019
"R"	07/11/2018 - 13/11/2018	07/11/2018 - 13/11/2018	07/11/2018 - 13/11/2018	21/11/2018 - 27/11/2018	05/12/2018 - 11/12/2018	23/01/2019 - 29/01/2019
"BH"	31/10/2018 - 06/11/2018	24/10/2018 - 30/10/2018	24/10/2018 - 30/10/2018	31/10/2018 - 06/11/2018	21/11/2018 - 27/11/2018	02/01/2019 - 08/01/2019
"M"	17/10/2018 - 23/10/2018	24/10/2018 - 30/10/2018	31/10/2018 - 06/11/2018	14/11/2018 - 20/11/2018	21/11/2018 - 27/11/2018	09/01/2019 - 15/01/2019
"LD"	17/10/2018 - 23/10/2018	10/10/2018 - 16/10/2018	03/10/2018 - 09/10/2018	31/10/2018 - 06/11/2018	07/11/2018 - 13/11/2018	26/12/2018 - 01/01/2019
"KD"	17/10/2018 - 23/10/2018	28/11/2018 - 04/12/2018	28/11/2018 - 04/12/2018	19/12/2018 - 25/12/2018	02/01/2019 - 08/01/2019	13/02/2019 - 19/02/2019
"ST"	10/10/2018 - 16/10/2018	28/11/2018 - 04/12/2018	05/12/2018 - 11/12/2018	02/01/2019 - 08/01/2019	26/12/2018 - 01/01/2019	20/02/2019 - 26/02/2019
"S"	31/10/2018 - 06/11/2018	21/11/2018 - 27/11/2018	28/11/2018 - 04/12/2018	19/12/2018 - 25/12/2018	02/01/2019 - 08/01/2019	20/02/2019 - 26/02/2019

a Sericata_1, an *L. sericata* GDD model with a developmental threshold of 9.2℃ and an accumulated degree days of 153.7 until the emergence of an adult fly (Wall et al. 1992), England

b Sericata_2, an *L. sericata* GDD model with a developmental threshold of 8.3℃ and anaccumulated degree day of 223-243 until the emergence of an adult fly (Cervantes et al. 2018), France

c Sericata_3, an *L. sericata* GDD model with a developmental threshold of 9.14℃ and an accumulated degree day of 214 until the emergence of an adult fly (Grassberger and Reiter 2001), Austria

d Sericata_4, an *L. sericata* GDD model with a developmental threshold of 9.55℃ and an accumulated degree days of 317.97 until the emergence of an adult fly (Gosselin et al. 2010), Belgium

e Sericata_5, an *L. sericata* GDD model with a developmental threshold of 11.3℃ and an accumulated degree days of 485 until the emergence of an adult fly (Greenburg et al. 1991), the United States of America

4.3.7 Contrasting the catch of all flies from the Lucitrap® and the StickyTrap attachment

Across all eight farms, a trend was observed where the LuciTrap® itself had low catch rates for all flies in the early (September – December) and late season (March – June) period; with a peak from January until March (Figure 4.19). In comparison, the StickyTrap had a relatively constant catch rate over the course of the year (Figure 4.19). It could be suggested that there is a limitation with the StickyTrap due to its effective size which may restrict the number of flies that could be caught.

The same trend was observed for *Lucilia* spp. on each farm with a high catch rate from the LuciTrap® between January until March, but for the StickyTrap it tended to extend into March (Table 4.13). The additional sensitivity of this combination was observed in both the early and late season where the addition of the StickyTrap to the LuciTrap® allowed for a more accurate determination of the start and end of the season of *Lucilia* spp. (Table 4.13).

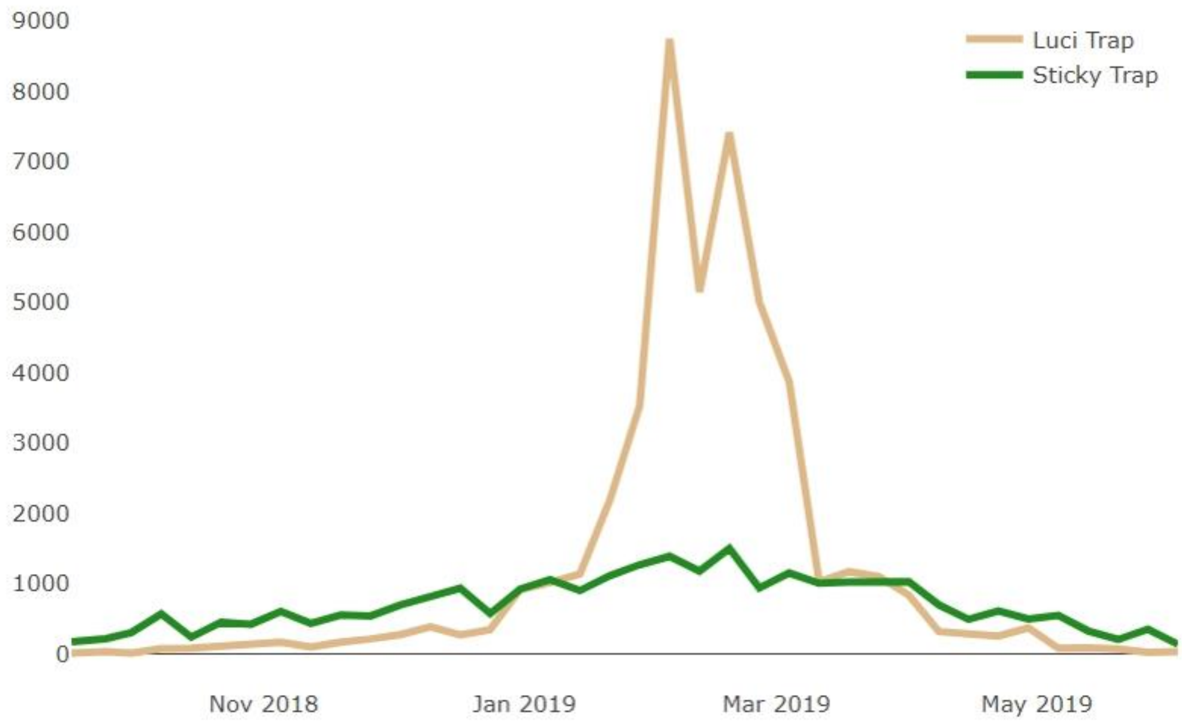


Figure 4.19 The total weekly catch of all fly species from the LuciTrap®and the StickyTrap on all eight farms from September 2018 until June 2019.

Table 4.13 Weekly catch of *Lucilia spp.* per week by LuciTrap® and StickyTrap in "T" 2018/2019

Date	*Lucilia spp.*	
	LuciTrap®	StickyTrap
20/09/2018 - 26/09/2018	0	0
27/09/2018 - 03/10/2018	0	0
04/10/2018 - 10/10/2018	0	0
11/10/2018 - 17/10/2018	0	0
18/10/2018 - 24/10/2018	0	0
25/10/2018 - 31/10/2018	0	0
01/11/2018 - 07/11/2018	0	0
08/11/2018 - 14/11/2018	0	3
15/11/2018 - 21/11/2018	1	5
22/11/2018 - 28/11/2018	0	11
29/11/2018 - 05/12/2018	2	3
06/12/2018 - 12/12/2018	1	5
13/12/2018 - 19/12/2018	1	7
20/12/2018 - 26/12/2018	0	9
27/12/2018 - 02/01/2019	0	2
03/01/2019 - 09/01/2019	0	3
10/01/2019 - 16/01/2019	0	5
17/01/2019 - 23/01/2019	2	3
24/01/2019 - 30/01/2019	6	7
31/01/2019 - 06/02/2019	9	3
07/02/2019 - 13/02/2019	53	8
14/02/2019 - 20/02/2019	141	6
21/02/2019 - 27/02/2019	118	6
28/02/2019 - 06/03/2019	9	5
07/03/2019 - 13/03/2019	6	6
14/03/2019 - 20/03/2019	1	4
21/03/2019 - 27/03/2019	5	6
28/03/2019 - 03/04/2019	2	1
04/04/2019 - 10/04/2019	6	2
11/04/2019 - 17/04/2019	0	0
18/04/2019 - 24/04/2019	1	0
25/04/2019 - 01/05/2019	4	1
02/05/2019 - 08/05/2019	0	0
09/05/2019 - 15/05/2019	0	0
16/05/2019 - 22/05/2019	0	0

4.4 Discussion

This study aimed to determine the start, the peak, and the end of the season for *Lucilia* spp. for eight farms across the North and South Islands of New Zealand over three years (2018/2019,2019/2020 & 2020/2021). The cancellation of studies in 2020 due to the restriction of movement during the COVID 19 epidemic by the New Zealand government restrictions and further restrictions on conducting fieldwork by Massey University policies have greatly impacted the potential results of this study. This cancellation also limited the extent of data analysis and the types of modelling which could have been performed with these data sets.

In addition, difficulties in sourcing chemicals to replace those in the LuciTrap® possibly hindered results although the chemical composition of the three lures used on "R" in 2020/2021 was as published by the company and as had previously been described (Urech et al. 1996, Urech et al. 1998, Urech et al. 2004). However, the variation in the fly catch numbers between "T" and "R" across the 2020/2021 season could have been influenced by this. It is not possible to critically determine the impact of this and therefore this limits the ability of this study to compare directly with the two previous seasons (2018/2019 & 2019/2020).

4.4.1 Seasonality of *Lucilia* spp. In New Zealand

The results of this study showed that the seasonality of *Lucilia* spp. in New Zealand begins in October until late May. It was also found that there was a variation of up to a month between the farms across the lower half of the North Island ("T", "R" and "BH") and those in the upper North Island ("LD" and "M") and/or the South Island ("KD", "ST" and "S"). This amount of variation in seasonality across New Zealand has not been described previously as previous studies have presented an averaged season for the North and South Islands (Heath and Bishop, 1995, Heath and Bishop, 2006). Between the South Island farms, there was little variation in the season found. This might be expected due to the clustering of the South Island farms around the North Canterbury region.

Upon comparison of each season of *Lucilia* spp., it was found that there was generally a high degree of conformity in the seasonality. *Lucilia* spp. appeared up to three weeks earlier in the 2018/2019 season on some farms compared to the 2019/2020 season. However, a variation between each season is to be expected due to the low numbers of *Lucilia* spp. caught at the start of the season when only single flies of this genus were caught. In general, there is a regional difference between farms, where those found in the lower North Island, Upper North Island and the South Island all have differing lengths of seasonality's.

4.4.2 *Calliphora stygia*

There was a consistently low catch of *C. stygia* on all farms and as a result, no further inferences could be made in terms of its seasonality. Previous studies have described the importance of this species and it is unclear if this fly has become less prevalent (MacFarlane, 1938, MacFarlane, 1941, Tenquist and Wright, 1976, Heath and Bishop, 2006). An alternative hypothesis could be suggested in that the holes in the lid of the LuciTrap® were too small for the fly to enter which may account for the low sample size. However, this species was also not caught to any extent on the StickyTrap in the current chapter. Earlier in the thesis, Chapter Three, where the Western Australian Traps were used which have no limitation on access size, *C. stygia* were only occasionally caught.

4.4.3 The occurrence of Flystrike

Of interest was the timing of the first report of flystrike (when recorded), which suggests that flystrike can occur when only low numbers of primary strike flies are trapped (i.e., less than 5 per week). This confirms that the occurrence of flystrike can occur with low fly populations (French et al., 1995). The occurrence of flystrike also coincided with periods of high rainfall on five of the farms ("T", "R", "BH", "LD" and "KD"). The identification of flystrike is subjective in this study as preventative measures on sheep to reduce cases of flystrike were not recorded and it was not part of a case-controlled aspect of this study. Furthermore, identifying cases of flystrike on large farms with high populations of sheep can be difficult. Future studies should consider intensely monitoring instances of flystrike on a limited number of sheep to increase the accuracy of this measure.

On each farm, there was a clear peak occurrence and sample size of the main target species. Peak occurrence of *Lucilia* spp. on all farms occurred across the period from late

January until late March. For instance, on Farm “ST”, the highest population of flies was over eight weeks from mid-January until the first week of March whereas at Farm “T” this peak was for just three weeks in February. The South Island farms, all had higher catches than each of the North Island farms and displayed longer periods of a sustained high catch rate, except for Farm “S”.

The on-farm measures to treat sheep were not measured on each of the farms during these studies. Thus, it is unclear whether the local variation of the population dynamics of *Lucilia* spp. was influenced by the environmental conditions or by the individual farm management programmes. It should be noted that the intention of this study was just to monitor the weekly population of the primary strike flies on each farm. It was not within the realms of this study to ask these commercial farms to follow the exact same management plans over the course of these studies.

4.4.4 Degree days

This study explored whether a degree day model utilising developmental data from other countries for *L. cuprina* and *L. sericata* could be utilised to predict the start of the season from each of the farms. The results showed poor alignment and would suggest that using lower developmental thresholds from other countries does not accurately predict the start of the season for *Lucilia* spp. in this study. August 1st was arbitrarily chosen as the start date to begin accumulating degree days. The 1st of August was chosen based on the methodology used in the United Kingdom (Wall et al., 2002); where the model began a month before the start of spring in England. This study used this approach to replicate that methodology in New Zealand's seasonal conditions. It has previously been identified that there is an inherent error in using developmental data from other countries as there are differences in the lower developmental thresholds which can account for the differences observed (Amendt et al., 2007). Furthermore, the use of a single developmental threshold in New Zealand may not be suitable, given the variation in environmental conditions across the country. Alternative means (or models) of estimating the start of the season for *Lucilia* spp. may have to be investigated if a predictive model for fly emergence and fly numbers is to be progressed.

4.4.5 Climatic Variables

A change in photoperiod has been suggested to be associated with the seasonality of *Lucilia* spp… The influence of a change in photoperiod inducing a developmental arrest has been shown for *L. sericata*; whereby Tachibana and Numata (2004), showed that the frequency of instances of diapause by *L. sericata* increased with a decrease of photoperiod below 13 hours of light. While for *L. cuprina*, it has been suggested, based on observations from field studies in Australia (Dallawitz and Wardhaugh, 1984, McLeod, 1997, McLeod, 2001), that it is subject to facultative diapause. As evidenced in Chapter Two, *L. cuprina* was the sole species present at both the beginning and the end of the season on each farm. It is unclear why there is a is a difference of up to a month for the end of the season for *Lucilia* spp. between farms. To date, there have not been any controlled studies that have examined the effects of photoperiod on *L. cuprina*, and it is unclear if any other variables may be of some influence such as temperature for instance.

The 10 cm soil temperature was found to be a variable of considerable significance on each of these farms. Third instar larvae of *Lucilia* spp. would drop off sheep as third instar larvae and pupate in soil and eventually emerge depending on temperatures (Heath and Bishop, 1995, Heath and Bishop, 2006) and thus, soil temperatures are clearly important for pupal development. In addition, prepupal larvae of *Lucilia* spp. typically overwinter in the top 10 cm of soil in a diapausal state (Foster et al., 1975, Wardhaugh, 2001, Pitts and Wall, 2005). This overwintering diapause stage would be broken once soil temperatures exceed a given threshold. This would trigger development of the prepupae that leads to the eventual emergence of the adult *Lucilia* fly.

Humidity and ambient temperature have all been found to be significant variables that positively affect *Lucilia* species population dynamics (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2019). Rainfall has been found to have differing effects, depending on the life stage of the fly. In adults, it will delay instances of oviposition for several days, as calliphorid flies are known to have a reduced distribution due to rainfall (Mahat et al., 2009, Lutz et al., 2019) and in the pupal stage, rainfall may cause prolongation of pupation (McLeod, 2012). It could be suggested that after periods of heavy rain there could be a sharp increase in the populations of *Lucilia* spp. It was hypothesized that this relationship was more associated with the occurrence of cases of flystrike and opportunistic occurrences of oviposition both of which are commonly linked with warm humid weather rather than with the population dynamics of these species *per* *se* (Wardhaugh, 2001, Wall et al., 2002, Wardhaugh et al., 2007). It can also be hypothesised that at the start of the season (i.e., from September until mid-December), heavy rainfall may have caused a delay in pupation in the soil, and which could have affected the success rate of emergence of adult flies, given the seemingly false start found on each of the farms. For example, on Farm "ST", in both the 2018/2019 (Figure 4.7 C) and 2019/2020 (Figure 4.9 C) seasons, the first *Lucilia* spp. appears but another is not observed for another week.

Regarding Mean, Maximum and Minimum temperature in the present study, it was found that the peak catch coincided with peak temperatures. This is to be expected as dipterans are thermophilic, and one would naturally expect to catch the most flies at the highest temperatures. Temperature is noted to be an important climatic variable in promoting the activity of numerous necrophagous flies whereby with higher temperatures more flies were caught in the flytraps (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2018).

Wind speed and wind run have previously been found to have a negative effect on flycatch numbers of various dipteran species (Vogt et al., 1985a, Vogt, 1988, Vogt et al., 1995, Vogt and Bedo, 2001). These assertions were based on daily catches and comparing the catch of flies from month to month. However, in the present study, the weather was averaged on a weekly basis and no discernible trend was observed on any farm. Further studies may wish to utilise daily wind and fly catches data to allow for accurate associations to be determined.

4.4.6 Climate in New Zealand during these studies

The mean temperature in New Zealand in 2018 was the equal 2nd highest on record; 2019 was the 4th warmest on record and 2020 was the 7th warmest on record (NIWA, 2019, NIWA, 2020, NIWA, 2021). Each of these years had temperatures that were 0.5°C higher than historical mean temperatures (NIWA, 2019, NIWA, 2020, NIWA, 2021). The year 2018 was classed as being a neutral year for both La Niña and El Niño (NIWA, 2019), while 2019 was noted to be an El Niño event bringing dry weather until July with lower rainfall and higher temperatures than average persisting until winter (NIWA, 2020). August until October of 2020 was marked by a negative Southern Annular Mode where temperatures were near average (NIWA, 2020). The year 2020 was noted to have a neutral

El Niño phase until May (NIWA, 2021). A transition to La Niña conditions was noted in June with the changes in the atmosphere being noted in October (NIWA, 2021). This meant that climate conditions from January until late April were marked by high pressure and dry conditions (NIWA, 2021). The winter of 2020 was noted to be the warmest on record with a persistent dryness which persisted until Spring with sporadic rainfall events noted in November (NIWA, 2021). Thus, overall, there were variations, but temperatures in general were higher than recorded mean temperatures.

Warmer temperatures in New Zealand would affect the seasonality of both species by increasing the population sizes and frequency of new generations of *Lucilia* spp… It could potentially lead to higher populations of prepupae overwintering thus leading to higher populations of *Lucilia* spp. appearing at the start of the season. During phases of La Niña the higher amounts of rainfall may depress population sizes by causing a delay in the emergence of adults from pupae in the soil.

4.4.7 StickyTrap/LuciTrap®

The addition of the StickyTrap to the LuciTrap® resulted in earlier identification of the seasonality of *Lucilia* spp. Further, the StickyTrap consistently caught flies throughout the season. The LuciTrap® appeared to be more effective during the warmest part of the year on all farms i.e., from mid-December until late March. However, there is a limitation with the StickyTrap due to its effective size which restricted the number of flies that could be caught. The additional sensitivity of this combination was observed in both the early and late seasons where the addition of the StickyTrap to the LuciTrap ®allowed for a more accurate determination of the start and end of the season for *Lucilia* spp... It would be recommended that this combination of StickyTrap and LuciTrap® be used in similar future studies. There is a potential issue with regards to the safe and legal use of the LuciTraps on farms given the chemicals used in the traps (Government, 1997). It could be argued that farmers wanting to utilise a cheap cost-effective method of signalling to signal the start and end of the season for *Lucilia* spp. could just use the StickyTraps. However, it is unclear how well the StickyTraps would perform without the chemical lures from the LuciTrap®; in addition, there may be an additional issue of collecting non-target species. This warrants further investigation.

4.5 Conclusion

In conclusion, the results of this study illustrate that there is a relatively small difference of up to three weeks between farm sites across seasons for *Lucilia* spp… This uniformity indicates farmers could start to plan their flystrike preventative strategies around these timings although biology and climate-driven model supplemented with on-farm fly monitoring would assure that the season was following these dates. However, there was some variation on the farms and knowledge of the cause of this variation would be highly beneficial. Therefore, the next step would be to determine if climatic variables are responsible for this variation and if it can be predicted. The results of these models would allow farmers to plan for preventative measures as part of an integrated pest management plan.

Chapter Five. A seasonal description of *Lucilia* spp. in New Zealand using a hurdle model with on-farm weather stations

5.1 Introduction

Flystrike or cutaneous myiasis is of considerable concern for the health and animal welfare of sheep on pastoral farms in New Zealand. It has been estimated that approximately 3-5 % of New Zealand's sheep population are affected by flystrike each year (Heath and Bishop, 1995, Corner-Thomas et al., 2017), which likely costs the New Zealand economy $60.3 million annually, with half of this being spent on chemicals (B+LNZ Genetics, 2014). Integrated pest management (IPM) is the combination of using biological knowledge of a pest species while using appropriate practices to implement control measures. Farmers may implement certain management measures at specific time points during the year to reduce losses due to flystrike by shearing, crutching, tail docking, removal of carcasses from pasture, the use of fly traps and the timely application of chemicals. To improve the success of the IPM and reduce the incidence of flystrike in New Zealand, there is an urgent requirement to develop mathematical models that could predict the start, end, and high-risk periods of flystrike throughout the year. This requirement is made even more pressing by the knowledge that such models have been available to sheep farmers in the United Kingdom for *L. sericata* (Wall et al., 2002) and in Australia for *L. cuprina* (Wardhaugh et al., 2007) for several years. These models have facilitated and improved the active management of flystrike in these countries.

The models currently available in Australia and the United Kingdom share a similar construct in which they utilise multiple temperature thresholds to predict the start of the season for each country's respective species. The model in the United Kingdom uses differing developmental thresholds for larval – pupal and adult using multiple temperature thresholds in a degree day model with further criteria used for the prediction of flystrike based on a combination of climatic variables and husbandry practices (Wall et al., 1992a, Wall et al., 1993a, Fenton et al., 1997, Wall et al., 2002). By comparison, the model from Australia differs as it uses a single temperature threshold of 5 ℃ and utilises a pasture growth index as a proxy to generate fly numbers (Wardhaugh et al., 2007).

The lagged effect of several climatic variables from the on-site weather stations and photoperiod were assessed for their predictability using Cross-correlation function (CCF) analysis for the seasonality of *Lucilia* spp... The results suggested that the seasonality of *Lucilia* spp. was significantly influenced by a change in weather over the season. Temperature has been widely used as a variable to predict the population dynamics of Diptera (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2019). Photoperiod has been used to predict the onset of diapause for *L. sericata* (Fenton, 1998, Wall et al., 2000, Wall et al., 2002). Rainfall and humidity have been included in previous models which have focused on predicting flystrike (Fenton et al., 1998, Wall et al., 2000, Wardhaugh et al., 2007), but as far as this author can ascertain; it has not been included in models that predict fly numbers. Rainfall was not found to be significant in the CCF analysis from Chapter Four; qualitative analysis would suggest that there may be a lagged negative association between heavy rainfall and depression in the fly numbers over the course of the season (Appendix C.3).

For the successful adoption of a predictive model in New Zealand, it needs to be based on weather variables that are measured at a farm level, for example by on-farm weather stations or via data available through the National Institute of Water and Atmospheric Research (NIWA). A model based on data collected at the farm level would have considerable advantages over the model currently used in the United Kingdom. This United Kingdom model is based on national weather stations which has been noted as a reason for inaccuracies in the predictions provided (Wall et al., 2002).

For a model to provide sheep farmers with sufficient time to implement preventative treatments, a lagged prediction of at least one to two weeks would be advantageous. It is anticipated that farmers would be able to use such a model to identify periods where fly numbers are predicted to increase and decrease due to climatic variables. This knowledge would then allow for farmers to implement preventative measures such as shearing, crutching, and correctly timing the application of chemicals to sheep. If successful, a New Zealand farm-based model predicting fly numbers could be incorporated into each farm’s IPM program leading to improved animal welfare outcomes, reduced reliance on chemicals as a management tool and decreased instances of flystrike.

This chapter aimed to construct a model to describe in advance the seasonal occurrence of *Lucilia* spp. on each farm, using climatic variables collected at the farm level. The mode output of the fly numbers and their occurrence would then be compared to the actual observed field data on each farm site.

5.2 Methods

5.2.1 Data Collection methods

This is as described in Section 4.2.1. In brief, dipteran specimens were collected using the LuciTrap® (Bugs for Bugs, 3 Rocla Court, Toowoomba Queensland, 4350, Australia) with an attachment StickyTrap (EasyTrap Stickies for Flying Insect, Gubba, Greenlane, PO Box 74435, Auckland 1546, New Zealand) and a lid added above the trap to reduce the amount of rainfall entering the trap. Samples were collected every week on the same day (plus or minus one day) from each farm and the flies were preserved in 70 % alcohol. The fly collection for each farm was summarised over a week with the day of the sampling being the last day of the previous week. The collection dates for each farm site are described in Section 4.4.4.

Two consecutive weeks where no target species were caught were the criteria used to determine the end of the season for *L. cuprina* and *L. sericata* (Lacour et al., 2015). Any missing data, such as a result of failure to empty traps in a given week, were imputed using a Steinman linear interpolation method using the *imputeTS* package (Moritz et al., 2017); the code for this can be found in Appendix A.1.1.. This was required on two farms, “S” and “ST”, over a fortnight in December. This was outlined previously in Table 4.8 in Chapter Three.

5.2.2 Fly Count Data

Initially, the flies were morphologically identified as *L. cuprina* and *L. sericata* as outlined in Chapter Four. However, the molecular identification results from Chapter Two showed considerable issues with the accuracy of morphological identification. Therefore, the counts of both species were combined for each week and shall be referred to henceforth as *Lucilia* spp...

5.2.3 Weather variables

As outlined in Chapter Four, seven of the farms (“T”, “R”, “BH”, “M”, “LD”, “KD”, “ST”) had a Vantage Pro2 6322C (Davis Instruments, Hayward, California, United States of America) weather station installed, whereas on “S” a Campbell Scientific CR200 weather station, (Campbell Scientific, Logan, Utah, 84321, United States of America) was utilised. Unfortunately, there were several variables recorded by the Vantage Pro2 Weather Station that were not recorded by the Campbell Weather Station; and vice versa. Rather than removing Farm “S” from the data set, only the weather variables common to both weather stations were used to build the model. These variables were: Mean Temperature, Maximum Temperature, Minimum Temperature, Relative Humidity, Wind Speed, and Rainfall. The following variables were only available on either the Vantage Pro2 Weather Station or the Campbell Weather Station and thus were not included in the model: Wind Direction, Dew Point, Wet Bulb, High Wind Speed, High Wind Direction, Wind Chill, Heat Index, THW Index, Heating Degree Days, Cooling Degree Days, Evapotranspiration and Soil Moisture.

Three soil temperature probes (Hobo MX2201 Temperature probes, Onset Headquarters, 470 MacArthur Boulevard, Bourne, Massachusetts, 02532, United States of America) were installed on each farm in the same locations as the flytraps. These probes were installed a week before the start of the fly trapping study on each of the farms. Soil temperature at 10 cm was also available from the closest Virtual Climate Station (VCS) on each farm. The VCS is a series of weather stations spread on a ~5km grid across New Zealand which used a spatial interpolation of the climatic data from the nearest national weather station (Tait and Turner, 2005, Tait et al., 2006a, Tait and Woods, 2007, Tait et al., 2012, Mason et al., 2017).

Data from the three Hobo MX2201 soil temperature probes placed on each farm were not used due to the notable issues outlined in Chapter Four. Instead, the 10 cm soil temperature data from the closest Virtual Climate Station Network (VCS) station was used in model construction.

Each of the weather variables was summarized for the week of the catch. Mean Temperature, mean 10 cm Soil Temperature, and mean Relative Humidity was calculated over seven days. Rainfall was calculated as the Total Rainfall per week. Maximum and Minimum Temperatures were taken as the Maximum and Minimum Temperatures recorded at any time over the course of the week. Photoperiod was calculated for each farm using the same method as outlined previously in Chapter Four and averaged over the week.

5.2.4 Data set up and time of collection.

The collection of fly samples on the farm sites for the 2018/2019 season began on different dates as outlined in Table 4.3 in Chapter Four. Fly collection on the South Island farms did not start as early as on the North Island farms, nor continue for as long. This meant that a null period of two consecutive weeks was not always available to define the end of the season for the South Island farms. A *Lucilia* spp. was caught on the first week of the fly collection study (10th October 2018) at "ST" and the second week of the fly collection study (17th October 2018) at "KD". In addition, there was not a null catch of two weeks at the end of the season in "KD" and "ST". To standardise the collection data, all farms were designated to begin on the first week of September 2018 and to end on the last week of June 2019, with null catches of *Lucilia* spp. added for these additional weeks.

5.2.5 Statistical Methods

The distribution of *Lucilia* spp. count data was initially assumed to come from a Poisson distribution. Exploratory plots for *Lucilia* spp. showed that the data sets on all farms were zero-inflated and thus over dispersed. As it was important to fit a model that would account for both the zero counts in the early season and the high *Lucilia* spp. counts at the peak it was decided to fit a hurdle model using the *glmmTMB* package (Brooks et al., 2017). A hurdle model is an alternative model for count data that has excessive zeros and can be interpreted as a two-part model. The first part of the model, the hurdle portion, estimates and sets the threshold, over which the model has to "jump" to begin generating positive counts. The first part is a binary logistic regression model and models the likelihood that the number of flies will be greater than zero. The second part of the model is a count model which models the number of flies caught and is truncated at zero. Using a hurdle model allows for the separate investigation of the climatic variables which influence both the occurrence and abundance of *Lucilia* spp. and likely mimics what happens on individual farms more accurately. The following distributions were tested to describe the count part of the model with the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) selected: a truncated generalized Poisson, a truncated Poisson, and a truncated negative binomial.

For each climatic variable, a lag version from one to twelve weeks was created, grouped by each individual farm using the *dplyr* package in R studio (Wickham et al., 2021); the code for doing so may be found in Appendix D.3. Farm was included in the model as a random effect. As there could be collinearity between the temperature variables; only one temperature variable was tested in the model at a time. All lagged variables were added to the initial model. Interactions between variables that were biologically plausible were assessed; an interaction between 10 cm soil temperature and rainfall was tested in the model. Variables were removed using a backward selection method and retained if significant at $p < 0.05$, with all deleted variables set aside and retested in the final model. The model was checked for multicollinearity and if present then the variable with the highest variance inflation factor (VIF) value was removed and the model was rebuilt. The mean squared error (MSE) was used to assess how accurately the predictions of the model fitted the observed data on each farm. The final model was selected on the basis of the lowest AIC and BIC value. The simulated residuals of the resulting model were then compared to the observed data using the *DHARMa* package (Hartig, 2020) to assess the fit of the models. Simulations of the response of the variables from the fitted model with the other variables held at their mean value were then generated based on a 95 % confidence interval where a fixed number of iterations were run using the *ggeffects* package (Ludecke, 2018). These iterations were run until adding additional simulations was not observed to change the estimated mean of the simulated variable, the code for doing so may be found in Appendix D.3.

5.3 Results

A total of 60,428 dipterans were collected from 2018/2019 which included 8,553 *Lucilia* spp. (*L. cuprina* and *L. sericata*) (14.15 % of the total catch). The highest weekly and total catches of *Lucilia* spp. were found on the South Island farms. Further details regarding the catch from each farm may be found in Table 4.7 and the corresponding graphs for the population dynamics may be found in Figures 4.6 and 4.7.

The final model identified six lagged climatic variables that were significantly associated with the seasonal population dynamics of *Lucilia* spp.: mean 10 cm soil temperature at a lag of three weeks, total rainfall per week at a lag of five weeks, the maximum temperature per week at lags of one, three and four weeks and mean photoperiod per week at a lag of six weeks (Table 5.1). Interactions between mean 10 cm soil temperature and total rainfall were also assessed; however, these interactions were not found to be significant ($p > 0.05$). Mean wind speed and mean relative humidity were not found to have a significant effect ($p > 0.05$) in the model.

The final model was fitted using a truncated negative binomial distribution and had an R^2 of 0.57 and an MSE of 46; this MSE value illustrates the lack of precision of the model output to the observed data. A Q-Q plot and simulated residuals plotted against observed data both were found to be normally distributed (plot shown in Appendix section D.1).

Table 5.1 Summary of the model coefficients, confidence intervals, and p values for the Count and Logistic portions of the hurdle model to describe the seasonality of *Lucilia* spp. using the following climatic variables at appropriate lags: mean 10 cm soil temperature per week, total rainfall per week, the maximum temperature per week and mean photoperiod per week.

Predictors	Coefficients	95 % Confidence interval low and high	*p*-Value
Count Model			
Intercept	-9.42	-10.80 – -8.04	<0.001
Mean Ten cm soil temperature at a lag of three weeks	0.27	0.18 – 0.36	<0.001
Total Rainfall at a lag of five weeks	-0.01	-0.02 – -0.01	<0.001
Maximum Temperature at a lag of one week	0.12	0.07 – 0.17	<0.001
Maximum Temperature at a lag of three weeks	0.06	0.01 – 0.12	0.015
Maximum Temperature at a lag of four weeks	0.10	0.06 – 0.15	<0.001
Logistic Model			
Intercept	18.84	15.10 – 22.58	<0.001
Mean Photoperiod at a lag of seven weeks	-1.58	-1.89 – -1.27	<0.001

5.3.1 Count Model

The exponentiated coefficients for the final model are shown in Table 5.2. As the model is on a log scale the effect of each variable on the count part of the model is multiplicative. This means that for each 1°C increase in 10 cm soil temperature in that week there was an increase in the fly count by 1.2-fold after a lag of 3 weeks. For example, if 30 flies were caught in a week with a mean 10 cm soil temperature of 11°C then the model indicates that you would catch 1.2*30 = 36 flies in a further three weeks (Table 5.2). There was a similar positive effect for the maximum temperature where for each 1°C increase, together at a lag of one, three and four weeks, fly numbers caught per week increased by 1.07, 1.01 and 1.05-fold respectively after those time-periods (Table 5.2). In contrast, the model indicates that for each mm of rainfall the fly count decreased by 0.99 after a lag of five weeks. For example, if 100 flies were caught in a week with a rainfall of 10 mm, then five weeks later it would be expected that 98 flies (0.98*100) would be caught (Table 5.2).

Table 5.2 The exponentiated coefficients and 95% confidence intervals from Table 5.1 give the estimated effect on the population size of *Lucilia* spp. due to the fixed effects variables in the count aspect of the hurdle model.

Variable	Estimate	95 % Confidence interval low and high
Mean Ten cm soil temperature at a lag of three weeks	1.20	1.20 – 1.43
Total Rainfall at a lag of five weeks	0.98	0.98 – 0.99
Maximum Temperature at a lag of one week	1.07	1.07 – 1.19
Maximum Temperature at a lag of three weeks	1.01	1.01 – 1.12
Maximum Temperature at a lag of four weeks	1.05	1.06 – 1.16

The marginal effects of each of the variables from the count aspect of the hurdle model based on the mean value were computed. This showed that higher populations of *Lucilia* spp. would be observed with an increased 10cm soil temperature (Figure 5.1 A). The model indicates lower developmental threshold on the eight farms for the occurrence of ≥ 1 *Lucilia* spp. was estimated to be 10°C with a lower confidence interval of 7°C and a higher confidence interval of 12.5°C (See Appendix D.2). This is representative of the variability of the climatic conditions on each of the farms. Higher abundances of *Lucilia* spp. were observed once maximum temperatures for the week increased above 25°C per week, albeit with a relatively wider confidence interval, which may be farm-specific (Figure 5.1 B, C, D). In contrast, lower populations of *Lucilia* spp. would be expected with higher amounts of rainfall per week (Figure 5.1 E).

The effects on the occurrence of these species were also illustrated further upon a simulation of these climatic variables. For each of the temperature variables, it was observed that higher populations of *Lucilia* spp. will be observed with increased temperatures (Figures 5.2, 5.3). In addition, by observing the values in Figure 5.2A it can be expected that these higher populations will be observed once mean 10cm soil temperatures are above about 15°C per week and from Figure 5.2B and Fig 5.3, when maximum temperatures are above about 25°C per week. While for rainfall, one could expect higher populations of *Lucilia* spp. on all farms once rainfall is below ~50-60 mm of rainfall per week (Figure 5.4), although the data for this is less clear.

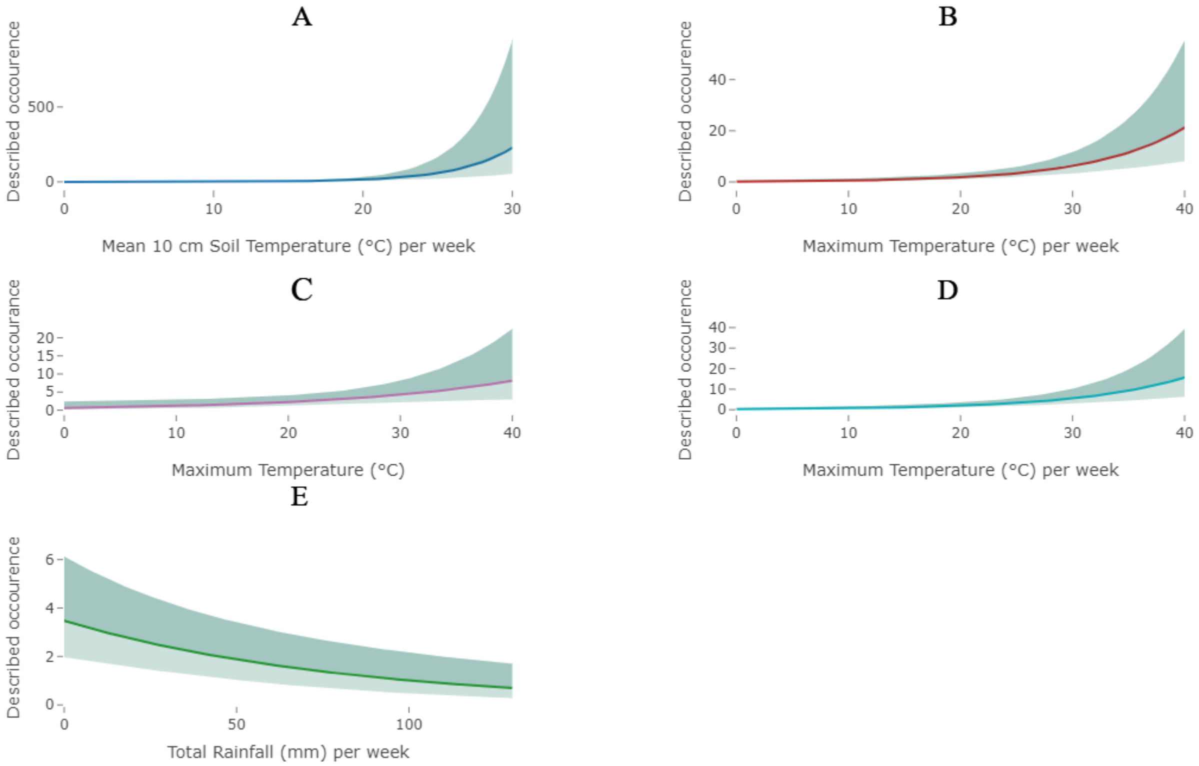


Figure 5.1 An exponentiated transformation to describe the population occurrence of *Lucilia* spp. due to the following fixed lagged climatic variables: (A) Mean 10 cm soil temperature at a lag of three weeks, (B) Maximum Temperature at a lag of one week (B), (C) Maximum Temperature at a lag of three weeks (C), (D) Maximum Temperature at a lag of four weeks (D) and (E)Total Rainfall at a lag of five weeks (E). The mean is shown in bold with the 95% upper and lower confidence intervals shaded.

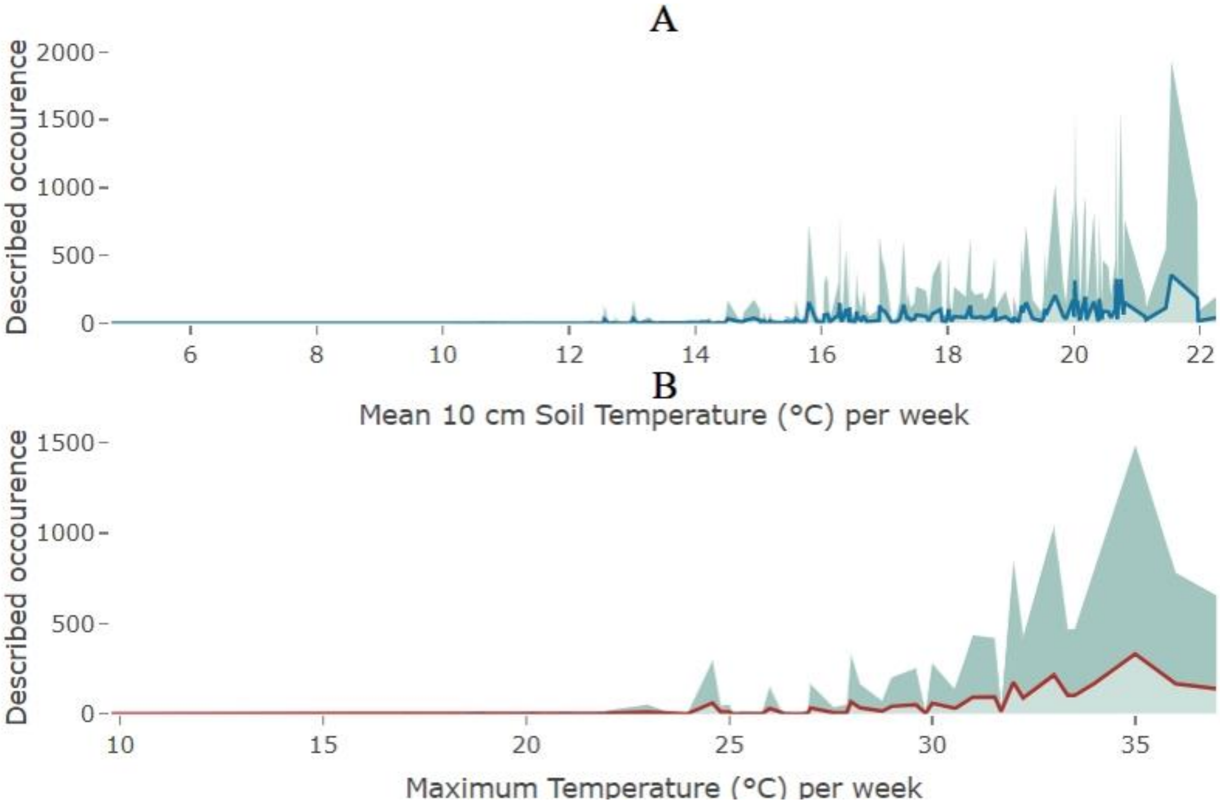


Figure 5.2 A simulation to describe the population occurrence of *Lucilia* spp. over all farms because of (A) Mean ten 10 cm soil temperature at a lag of three weeks and (B) Maximum Temperature at a lag of one week. The mean is shown in a bold line with the 95 % upper and lower confidence intervals shaded.

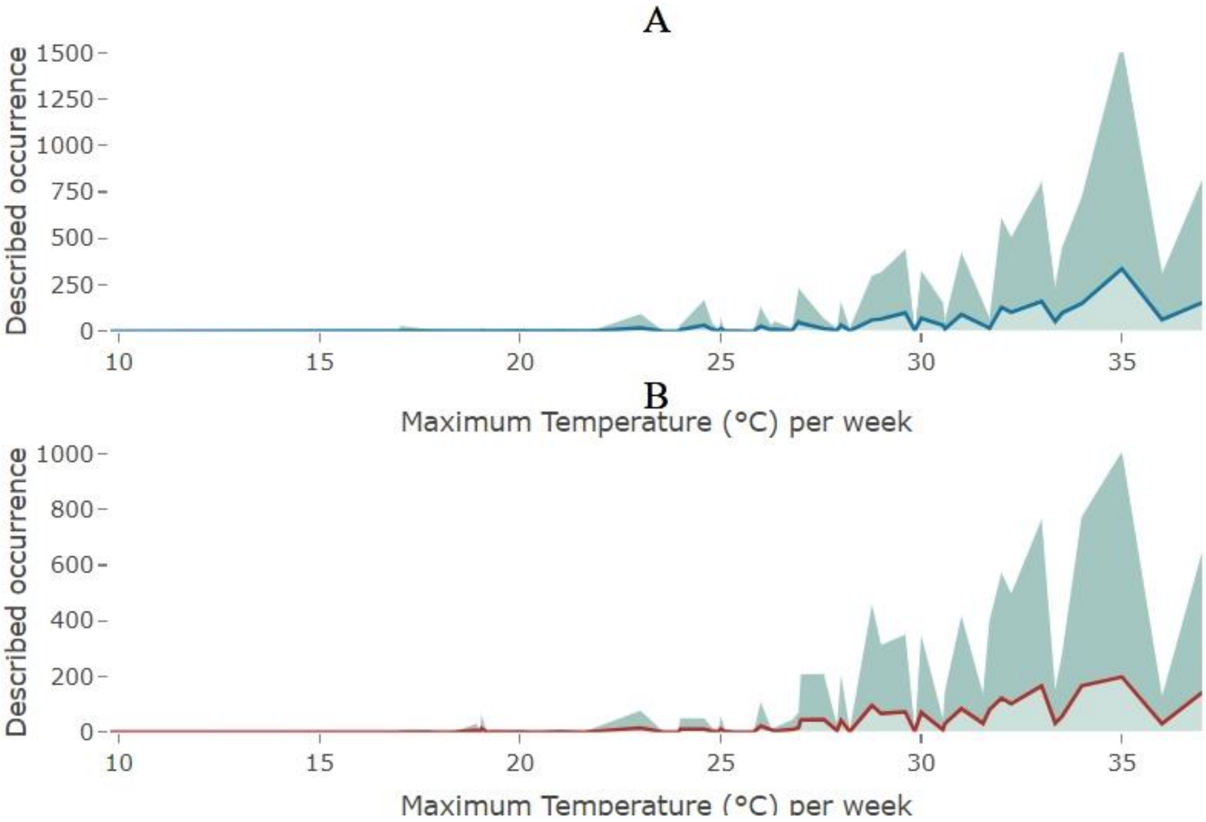


Figure 5.3 A simulation to describe the population occurrence of *Lucilia* spp. over all farms because of (A) Maximum Temperature at a lag of two weeks and (B) Maximum Temperature at a lag of four weeks. The mean is shown in a bold line with the 95 % upper and lower confidence intervals shaded.

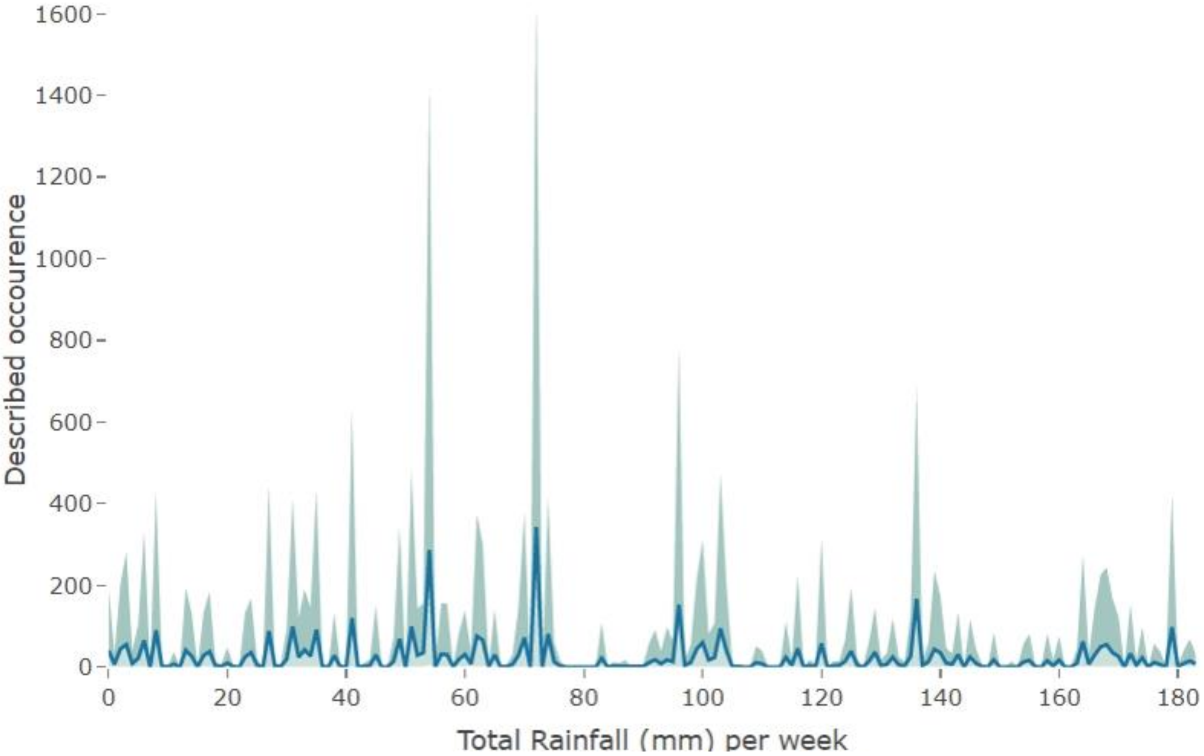


Figure 5.4 A simulation to describe the population occurrence of *Lucilia* spp. over all farms because of total rainfall per week at a lag of five weeks. The mean is shown in a bold line with the 95 % upper and lower confidence intervals shaded.

5.3.2 Logistic Model

The probability of the occurrence of a *Lucilia* spp. being observed was found to have a negative effect ($p < 0.001$) due to a decline in mean photoperiod at a lag of six weeks (Table 5.1).

The probability of a zero count of a single *Lucilia* spp. being observed due to a change in the value of each of these variables was then assessed by the following equation:

Equation 1:

$$Odds = exponential\ (intercept + \beta_1 X_1)$$

Intercept = the intercept from the logistic regression portion of the model, 18.84 (Table 5.1)

β_1 = intercept of Mean Photoperiod at a lag of seven weeks, -1.58 (Table 5.1)

X_1 = the number of hours of Mean Photoperiod from 8 – 16 hours

To estimate the probability of zero occurrences using Equation 1. the intercept from the logistic model and the coefficients for each variable were transformed to describe the probability of occurrence of *Lucilia* spp… The probability of zero-occurrence was investigated independently for each variable. This was estimated using the mean photoperiod of daylight per week at 0.5-hourly increments from 8 – 16 hours.

As observed in (Figure 5.5 A), the probability of zero occurrences of *Lucilia* spp. increases with decreasing photoperiod over the course of the season. This means that the likelihood of the occurrence of zero *Lucilia* spp. will increase once the seasonal mean photoperiod begins to decrease below 12 hours of light per week (Figure 5.5).

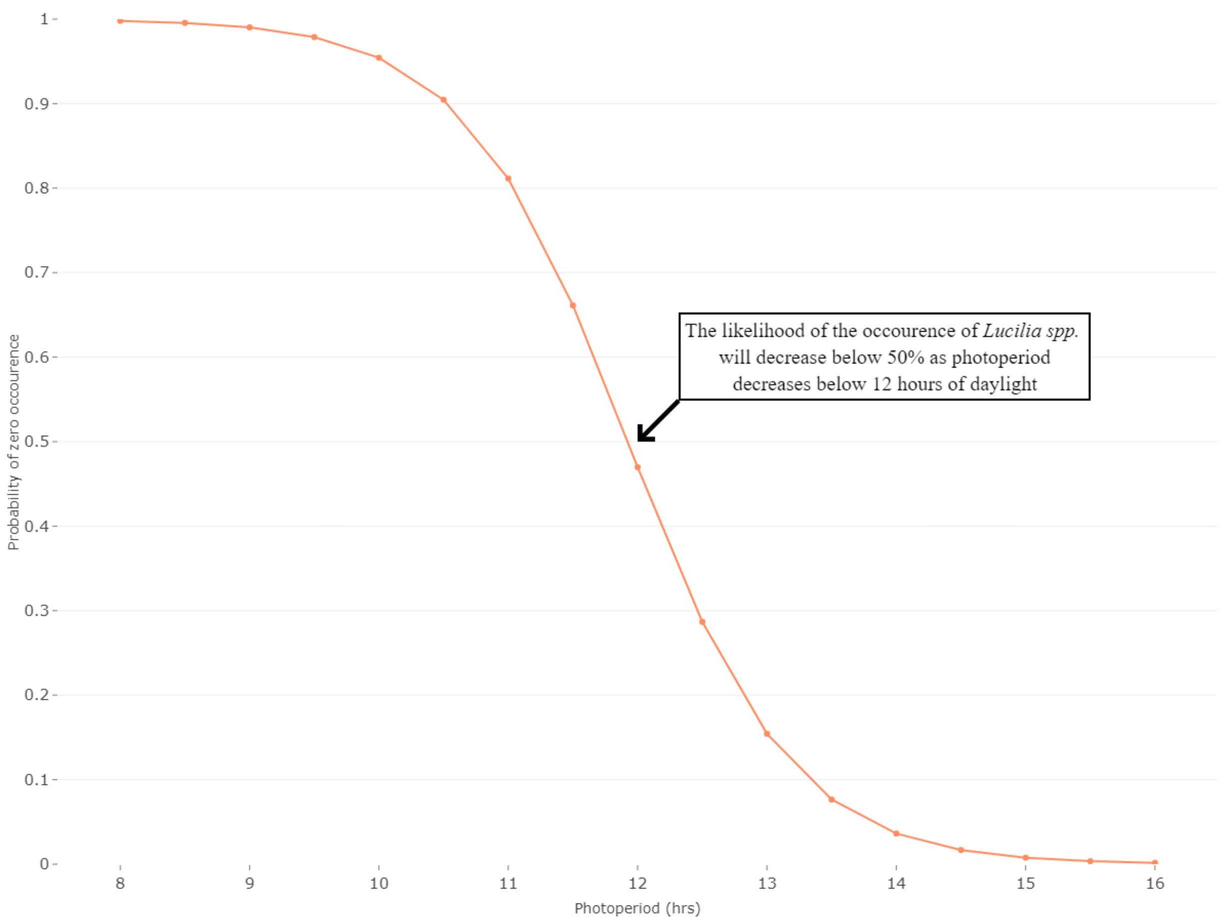


Figure 5.5 The probability of zero occurrence of *Lucilia* spp. due to the effect of the mean photoperiod per week at a lag of six weeks.

5.3.3 A description of the Population Dynamics of *Lucilia* spp. on farms

There is a general pattern on all farms that the described start and end of the season varies by being one to two weeks earlier or later than the observed data (Figure 5.6). This can be likely attributed to the capture of single specimens of *Lucilia* spp. which was used to define the start and end of the season. It is possible that there was a small population of these flies present at this time, as the model describes, but they were simply not captured.

North Island	LD	Described									▨	▨	▨	▨	▨	🟪	🟪	▨	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	▨	▨	▨
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		Observed									▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨
South Island	KD	Described									▨	▨	▨	▨	▨	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	▨	▨	▨	▨	▨
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		Observed									▨	▨	▨	▨	▨	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	▨	🟩	▨	▨	▨	▨
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		Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		Month	July				Aug				Sept				Oct				Nov				Dec				Jan				Feb				March				April				May				June			

🟪	🟩	▨
Described	Observed	No Target Species

Figure 5.6 The observed and described occurrences of *Lucilia* spp. on all farms for the 2018/2019 season.

The described population dynamics versus the observed fly population of *Lucilia* spp. on each farm are illustrated in Figures 5.7–5.10. Consistently there are low numbers of *Lucilia* spp. caught during the early season, from late October until the end of 2018 (Figures 5.7–5.10). However, by mid-January 2019 there is a sudden and dramatic increase in the number of trapped flies which was sustained for six weeks until the start of March (Figures 5.7–5.10). From March onwards there is a sharp decline in the number of trapped flies which occurs over nine weeks until the end of the season for *Lucilia* spp. (Figures 5.7–5.10). It is unclear if the high peaks of *Lucilia* spp. caught are due to the influence of the climatic variables or if they are due to the preventative IPM measure implemented on the farms to combat instances of flystrike on each farm.

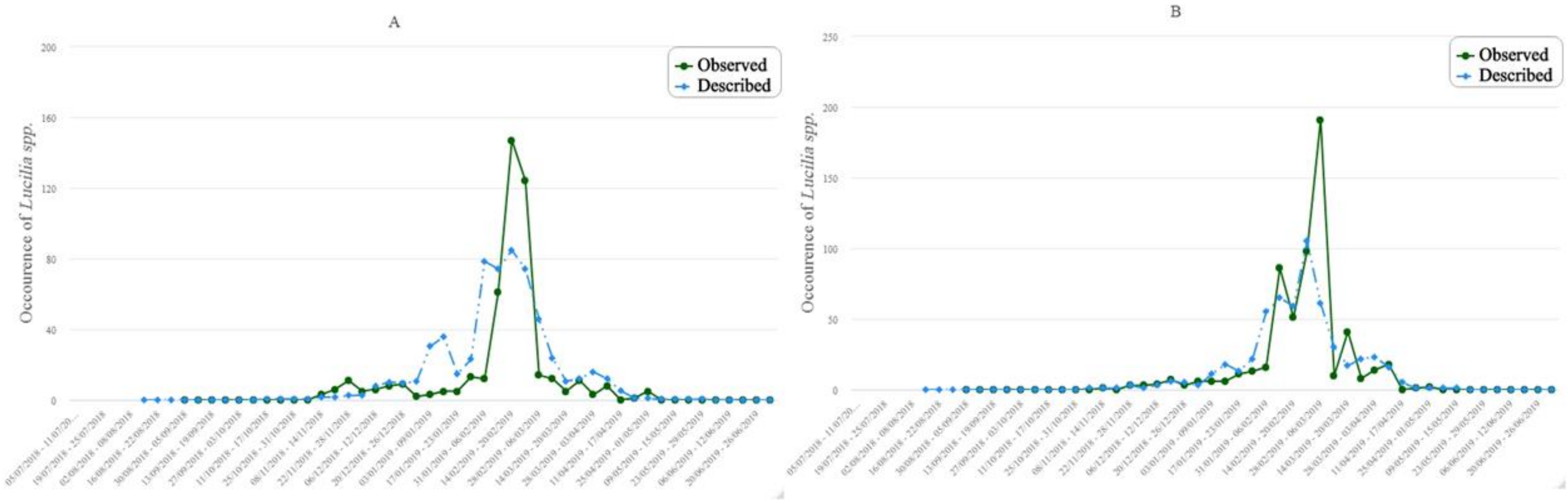


Figure 5.7 The observed and described occurrences of *Lucilia* spp. on Farms "T" (A) and "R" (B).

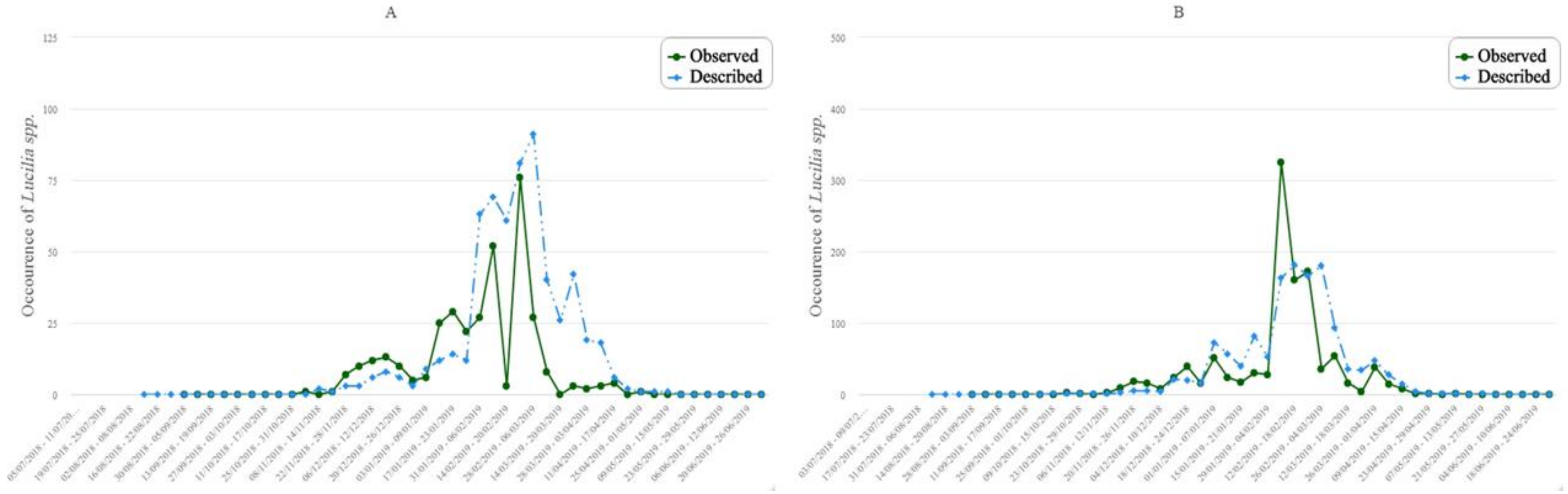


Figure 5.8 The observed and described occurrences of *Lucilia* spp. on Farms “BH” (A) and “M” (B).

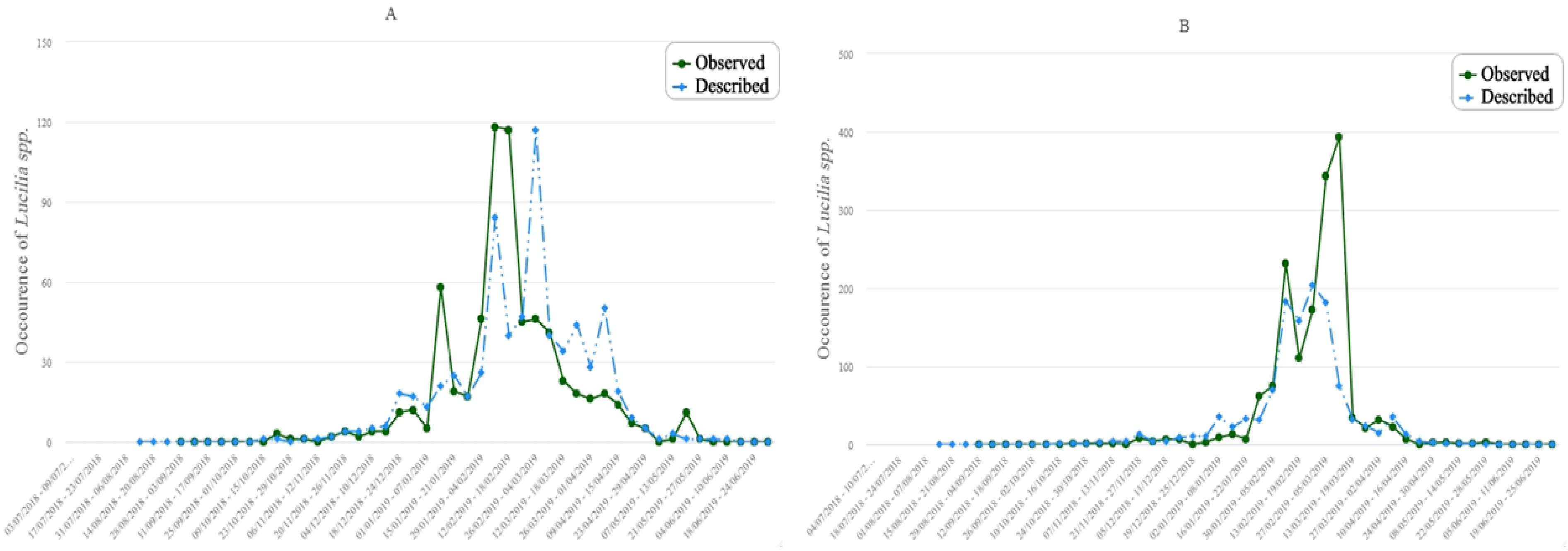


Figure 5.9 The observed and described occurrences of *Lucilia* spp. on Farms “LD” (A) and “KD” (B).

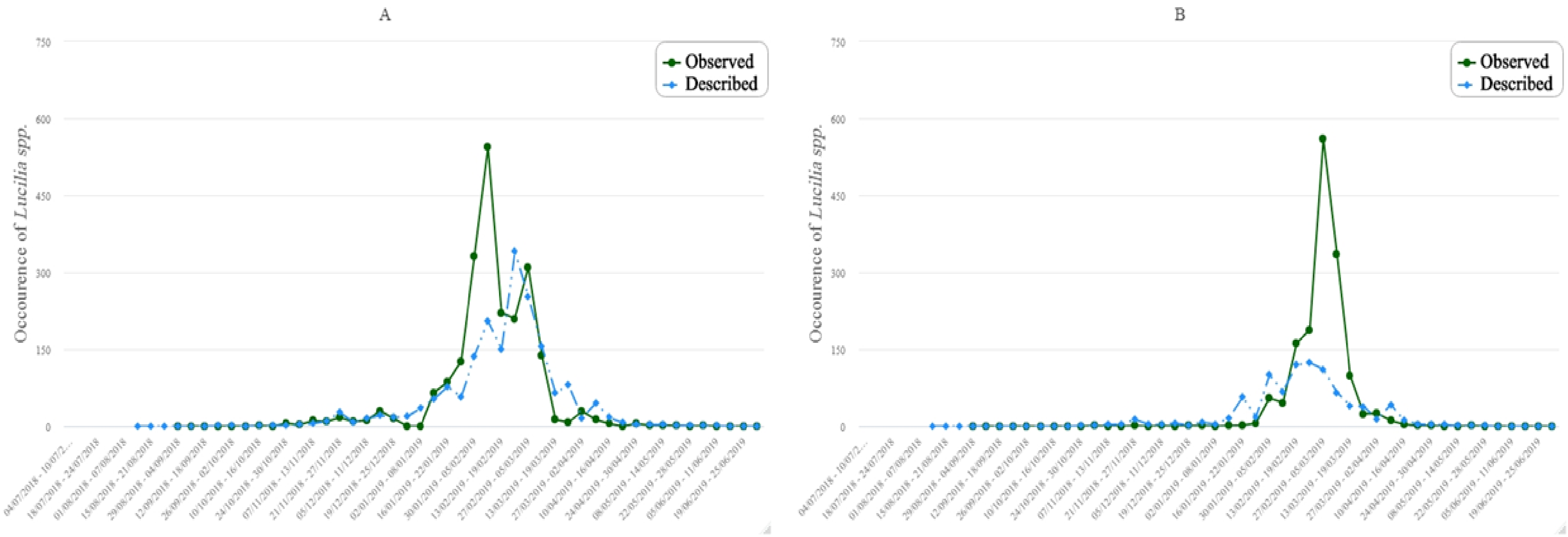


Figure 5.10 The observed and described occurrences of *Lucilia* spp. on Farms “ST” (A) and “S” (B).

5.4 Discussion

5.4.1 Overview

The study aimed to develop a hurdle model using climatic variables that could be used to explain the observed biological season for *Lucilia* spp. on eight farms across New Zealand. Overall, this study found that the counts of the *Lucilia* spp. could be explained using a combination of 10 cm soil temperature at a lag of two weeks, rainfall at a lag of five weeks, and the maximum temperature at a lag of one, three and four weeks respectively. Whereas the probability of observing a zero count of *Lucilia* spp. was attributed to photoperiod at a lag of six weeks.

There is an agreement in the literature on the use of 10 cm soil temperature to account for the start of the season for *L. cuprina* (Dallawitz and Wardhaugh, 1984, McLeod, 2001, De Cat et al., 2012) and for *L. sericata* (Wall et al., 1992, Wall et al., 1993b, Wall et al., 1993a, Fenton et al., 1997, Wall et al., 2002). This is likely explained by the prepupal larvae of both *Lucilia* spp. typically overwintering in the top 10 cm of soil in a diapausal state (Foster et al., 1975, Wardhaugh, 2001, Pitts and Wall, 2005). This overwintering stage is broken once temperatures exceed a given temperature which would break the diapausal state of the prepupae. This will trigger development that leads to the eventual emergence of the adult *Lucilia* fly.

The model output illustrated that the lower developmental threshold for *Lucilia* spp. is approximately 10℃ across all farms. Due to the morphological misidentifications identified in Chapter Two, the counts of *Lucilia* spp. were combined. However, there was evidence (Chapter Two) to suggest that *L. cuprina* was the sole *Lucilia* spp. present during October, with *L. sericata*, first appearing in November in the 2018/2019 season. Nevertheless, both species were considered together as *Lucilia* spp., but the start of the season is likely primarily influenced by the emergence of *L. cuprina*. The modelling based on the marginal effects in the present study suggested a developmental threshold of 10 ℃ at 10 cm soil temperature which is lower than that previously reported for *L. cuprina* (Dallawitz, 1984, Kotze et al., 2015, Sa et al., 2016, Bambaradeniya et al., 2018). These previous studies suggest a minimum temperature in the range of 11.03 ℃ to 14 ℃ for *L. cuprina*. The model output suggests a temperature range for *L. cuprina* in New Zealand conditions are more akin to those found for *L. sericata* which ranged from 8.3 ℃ to 11.3

°C respectively (Greenberg, 1991, Wall et al., 1992a, Grassberger and Reiter, 2001, Gosselin et al., 2010, Cervantes et al., 2018). Further work should aim to confirm the models' output for the lower developmental threshold for *L. cuprina* in New Zealand under controlled laboratory settings.

As dipterans are ectothermic, the effect of temperature has been found to be an important climatic variable to explain the activity of blowflies in flytraps (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2019, Brett et al., 2021). With higher temperatures, one would expect to observe higher populations at shorter generational intervals (Grassberger and Reiter, 2001, Kotze et al., 2015, Sa et al., 2016, Cervantes et al., 2018). These changes in temperature over the course of the season may account for the three differing maximum temperature lags which were included in the final model. Therefore, one could expect to find the highest populations of *Lucilia* spp. when the temperatures are at the highest during the season. This matches the present findings.

The present model for *Lucilia* spp. identified that rainfall at a lag of five weeks was negatively associated with the number of flies caught per week. Where it was found with each 1 mm increase in rainfall caused the fly catch to decrease by a proportion of 0.99 flies per week. However, a simulation from the model, as shown in Figure 5.4, indicated that below ~50-60 mm of total rainfall per week minimal effects on *Lucilia* spp. are to be expected. In published studies, the effects of rainfall have been found to differ depending on the life stage of the fly. Studies on the effects of rainfall on adult calliphorid flies have concluded that fewer flies were abundant during periods of heavy rain which occurred on a daily basis (Mahat et al., 2009, Lutz et al., 2019). As heavy rain reduces fly activity, this could result in less oviposition occurring and thus reducing the size of the fly population later up to five weeks later as this model may suggest.

It would also be expected that with heavier rainfall, waterlogged soils may occur which could affect the survivorship and emergence of further generations of these dipterans. From an agronomical point of view, soils that are waterlogged typically experience anoxic saline conditions which result in reduced pasture growth rates due to a plant's ability to respire and acquire nutrients (Barrett-Lennard, 2003, Colmer and Greenway, 2011, Shabala et al., 2014, Najeeb et al., 2015). It has been observed that fewer adult calliphorid flies emerged during periods of heavy rainfall in previous field studies (McLeod, 2012). It has also been shown experimentally with *L. sericata* pupae, that high rates of soil moisture cause a delay in the emergence and slow the rate of development (Kokdener and Sahin Yurtgan, 2022). Furthermore, the type of soil, its ability to retain heat, and its porosity, may also be variables that should be considered in future models to predict the emergence rate of flies in the family Calliphoridae.

The present results suggest that the probability of zero occurrence of *Lucilia* spp. may be induced by a decrease in photoperiod with a six-week lag. This finding supports other work which found that the initiation of diapause in female *Lucilia* spp. is governed by a response to decreasing photoperiod (Vinogradova and Zinovjeva, 1972, Danks, 1987, Saunders, 1987, Danks, 2007). This change induces the female *Lucilia* spp. to begin to lay eggs which develop to a prepupal stage and then overwinter at this stage (Tauber and Tauber, 1970, Danks, 1987). The influence of photoperiod to induce obligatory diapause has been shown experimentally for *L. sericata* at light durations below 13 hours of daylight (Tachibana and Numata, 2004). The photoperiod has also been used successfully to predict the end of the season for *L. sericata* in the United Kingdom (Fenton et al., 1998, Wall et al., 2000, Wall et al., 2002). However, for *L. cuprina* there is no published experimental work to confirm the results of the present model and earlier debate as to the actual type of diapause which is used by this species. Nevertheless, based on observations from field studies in Australia, it has been suggested it undergoes facultative diapause (Dallawitz and Wardhaugh, 1984, McLeod, 1997, McLeod, 2001).

As outlined previously in Chapter Two, the counts of *Lucilia* spp. were combined due to the morphological misidentifications that occurred. However, the results indicated that *L. cuprina* was probably the sole *Lucilia* spp. present at the end of the season in May of the 2018/2019 season (Chapter Two). Therefore, it is likely that the photoperiod effect described by the model is predominantly based on the numbers of *L. cuprina* present at the end of the season. Thus, it can be suggested that *L. cuprina* undergoes a form of diapause which is induced by a decrease in photoperiod with a 50 % threshold occurring below 12 hours of daylight, which is lower than the experimental threshold for *L. sericata* (Tachibana and Numata, 2004). Future studies in New Zealand should aim to confirm experimentally whether this is apparent and whether *L. cuprina* undergoes either obligatory or facultative diapause.

Mean wind speed and mean relative humidity were variables that were not found to have an effect in this model. Mean wind speed has previously been described to have a negative effect on the catch of various dipteran species in fly traps (Vogt et al., 1985a, Vogt, 1988, Vogt et al., 1995, Vogt and Bedo, 2001). However, this negative effect was found with daily catches not with weekly. In this study, the weekly averaged population counts of *Lucilia* spp. did not appear to be affected by wind speed. The reason for this variation is unclear.

Mean relative humidity have been associated with the occurrences of flystrike in the United Kingdom (Fenton et al., 1998, Wall et al., 2000, Wall et al., 2002) and in Australia (Wardhaugh, 2001, Wardhaugh et al., 2007). The present study did not find that mean relative humidity had any effect on population dynamics. However, the occurrence of flystrike requires appropriate conditions on the sheep which includes damp fleece as a recognised risk factor. Hence, changes in relative humidity are likely to dictate conditions that allow flies to strike sheep and this may then also affect the population size of these species as *L. cuprina* in particular is dependent on striking sheep as a means to continue its life cycle (Fenton et al., 1998, Wall et al., 2000, Wardhaugh, 2001, Wall et al., 2002, Wardhaugh et al., 2007). Further work could explore the association between the population size of *Lucilia* spp., mean relative humidity and instances of flystrike with the other variables in the model outlined in this chapter.

5.4.2 Comparison to prediction models currently used in the United Kingdom and Australia

The model developed from this study can describe the start and end of the season for *Lucilia* spp. with reasonable accuracy under New Zealand conditions when compared to the data from which it was developed on the eight farms. This model differs from the models currently used in the United Kingdom (Wall et al., 1993a, Fenton et al., 1998, Wall et al., 2000, Wall et al., 2002) and Australia (Wardhaugh et al., 2007, Horton and Hogan, 2010) as it utilises a multivariable approach averaged across eight farms to explain the seasonality of *Lucilia* spp. In the United Kingdom, for *L. sericata*, there is an assumption that the threshold temperature of prepupae was the same throughout the entire country (Wall et al., 2002). From the initial exploratory analysis of the data presented in this thesis, a single threshold temperature for 10 cm soil temperature could not be identified. Therefore, a multivariable approach was used instead, in which a combination of different climatic variables was identified that were associated with *Lucilia* spp. population dynamics. This approach should improve the external validity of the present model when applied across New Zealand. It should also have the ability to adjust for differences in climate and the multiple photoperiods found across New Zealand.

Furthermore, including the effect of photoperiod, soil temperature and maximum temperature on *Lucilia* spp. in the present model allowed for an effective explanation of the zero occurrences of these species. However, the drawback of this model is that it is a generalisation of the biology of both species, when in fact they may undergo differing types of diapause (Cragg and Cole, 1952, Fraiser and Smith, 1963b, Dallawitz and Wardhaugh, 1984). In comparison with the model currently used in Australia, the model developed here does not predict the end of the season in advance (Wardhaugh et al., 2007, Horton and Hogan, 2010). The model developed in the United Kingdom to predict diapause indicates there is an explicit day number each year which is effectively a substitute for photoperiod (Wall et al., 1993b, Fenton et al., 1998, Wall et al., 2002) and thus incorporates one of the factors from the present study but not the others. The methods used to account for the end of the season in this chapter could readily be adapted for the United Kingdom and Australia.

5.4.3 Limitations of the models

When examining the data for the combined species, there were a considerable number of null catches on all farms through November and December. The model presented in this chapter was only able to explain null catches at the start and end of the season for both species and not during the midseason. In addition, the model was not able to explain the sudden spikes in population that were observed in January, February, and March of the 2018/2019 season (Chapter Four). This was particularly evident with the data set from “Farm S” on the South Island. It is unknown if there were on-farm activities that may have affected the distribution of *Lucilia* spp. such as the timing of shearing or the application of various chemicals on sheep as this data was not recorded. In addition, there was no way to include any consideration for the life expectancy of adult flies.

5.4.4 How this model may be used by farmers in New Zealand

The results of this model could allow farmers in New Zealand to plan in advance for the seasonal occurrence of these species due to the inclusion of lagged variables. Notably, there was little variation in the seasonality of *Lucilia* spp. from farm to farm across the country. This would suggest that farmers could proactively plan preventative strategies. It would be expected that there will be some local variations from farm to farm due to climate and this present model allows for that.

Further work should consider conducting studies in different regions across the North and South Islands over several years. Studies in the North Island could examine regions such as Northland, the wider Waikato region, Gisborne, northern Hawkes Bay, Bay of Plenty, Northland, and Auckland. Whereas in the South Island, regions such as Tasman, Marlborough, Otago, West Coast and Southland should be considered given their potential considerable differences in climatic conditions to those areas studied. Conducting these studies would overcome the key issue for this study being the lack of independent data to verify the observed population dynamics from this study.

5.5 Conclusion

The results of this study show that with suitable lagged climatic variables, the biological season for *Lucilia* spp. can be explained under New Zealand conditions. Seasonality of *Lucilia* spp. may be described using a combination of the following lagged climatic variables: 10 cm soil temperature, rainfall, maximum temperature, and photoperiod. The results of this model could allow farmers in New Zealand to make informed proactive decisions for the management of flystrike in their sheep with independent data.

Chapter Six. A mixed-effects logistic regression model to predict the occurrence of *Lucilia* spp. in New Zealand at the start of the 2019/2020 season using the Virtual Climate Station Network

6.1 Introduction

Lucilia spp., in particular, *L. cuprina* and *L. sericata* are the main dipterans responsible for causing flystrike in New Zealand (Bishop, 1995, Heath and Bishop, 2006). A recent survey of sheep farmers in New Zealand suggests that the threat of flystrike is now throughout all the sheep-rearing areas in New Zealand (Corner-Thomas et al., 2017). The change in the spatial and temporal distribution of flystrike in New Zealand changed in the late 1970s due to the arrival of *L. cuprina* (Heath and Bishop, 1995, Heath and Bishop, 2006). The cost of flystrike to the New Zealand economy was estimated to be in excess of $60 million each year, of which half was spent on chemicals (B+LNZ Genetics, 2014). Several interventions can be used to prevent outbreaks of flystrike on sheep farms, such as shearing, dagging and the application of chemicals (Levot, 1995, Savage, 1998, Cole and Heath, 1999, Cranston et al., 2017). The appropriate timing of using these measures would benefit greatly from the use of a prediction model that would identify the seasonality of these species.

In Chapter Five, a model was built using data largely from on-farm weather stations. However, more realistically, not all sheep farmers in New Zealand will have their own weather stations. Therefore, another option is to explore whether using climate data from the National Institute of Water and Atmospheric Research (NIWA) could be utilised to build an alternative model. NIWA currently operates two national weather station networks, the first is known as CliFlo (http://cliflo.niwa.co.nz/), which is a series of 600 weather stations that are found throughout New Zealand and the second is the Virtual Climate Station Network (VCS). The VCS provides a spatial interpolation of the climatic data from the CliFlo weather stations at a ~5 km grid across New Zealand (Tait and Turner, 2005, Tait et al., 2006b, Tait and Woods, 2007, Tait et al., 2012, Mason et al., 2017). Using the VCS data, it should theoretically be possible to predict the seasonality of *Lucilia* spp. at a local level with greater precision in comparison to using data from CliFlo where the nearest weather station may still be some distance from a given farm.

There is a difference in variables used in VCS compared to the on-farm weather stations so it will require a different model to be created.

The overarching aim of this thesis was to build a prediction model that would be widely available to sheep farmers, to estimate the start and the end of the season for *Lucilia* spp. and to forecast risk periods within the season. The use of VCS weather data in such a model would then make it possible to make predictions for individual farms and to make nationwide prediction maps for New Zealand. These results could be made available to all farmers, either through rural newsletters or using an application such as a mobile phone and updated in real-time. The present chapter aimed to assess whether the climatic variables from the nearest VCS could be used to predict the first appearance of adult flies in the 2019/2020 season for *Lucilia* spp. using a model trained on data from the 2018/2019 season, for each of the farms in the North and South Islands.

6.2 Methods

6.2.1 Data Collection Methods

The methods for the collection of samples and the fly count data have been outlined previously, in Chapter Four. As in Chapter Five, the counts of *L. cuprina* and *L. sericata* were combined to give the total count of *Lucilia* spp. caught each week given the issues regarding the accuracy of the morphological identification described in Chapter Two. Also, as explained in Chapter Four, due to the government-mandated Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) lockdown response within New Zealand (Ministry of Health, New Zealand, https://www.health.govt.nz/), the collection of samples was only possible at “LD” on the North Island during the second half of the 2019/2020 season. Therefore, the predictive model was only fitted to the first half of the 2019/2020 season: i.e., from September until December 2019.

6.2.2 Weather Variables

Climate data from the closest Virtual Climate Station (VCS) to each farm was accessed from the National Institute of Water and Atmospheric Research (NIWA). The VCS agent numbers and the farm to which they relate are identified in Table 6.1. Photoperiod was calculated for each farm using the same method as described in Chapter Four. Each station presents interpolated data on the following variables: Potential Evapotranspiration, Rainfall, Relative Humidity, Soil Moisture Deficit Index, 10 cm Soil Temperature, Soil Wetness Index, Temperature (Maximum, Minimum and Mean) and Wind Speed. The majority of these climatic variables have been defined previously in Chapter Four, as they have the same interpretation as those recorded by the on-farm weather stations. The exceptions being for Soil Moisture Deficit Index, Soil Wetness Index and Potential Evapotranspiration. These variables are defined as follows:

Potential Evapotranspiration (PET) is defined as the expected amount of water that could be evaporated due to the average daily temperature. This variable is calculated based on a combination of the Penman method and Priestly-Taylor method for calculating the value of this variable and the equations are outlined in (Tait and Woods, 2007).

Soil Moisture Deficit Index (SMDI) is calculated using a single-layer water balance model based on the fixed water capacity that soil can hold based on the incoming daily rainfall and the outgoing daily evapotranspiration (Porteous et al., 1994). A single soil moisture-holding capacity of 150 mm for loam soil is used for all of New Zealand, this has been accepted as being sufficient to account for the variation in the soil moisture capacity across the country (Mol et al., 2017). Soil moisture deficit index is expressed as a negative number meaning that the soil is drier with a more negative number and wetter with the contrary.

Soil Water Index (SWI) is defined as the amount of water currently in the soil in mm and is calculated as follows:

$$SWI = (Soil\ water\ index\ yesterday + Daily\ Rainfall) - (PET \times Moisture\ index)$$

In this equation moisture index is a value of between 0 and 1; where at a value of 0, the soil has no moisture whereas at a value of 1, the soil is totally saturated. This considers the soil wetness relative to the maximum soil water holding capacity and the soil drying coefficient (i.e., how efficient the soil is at releasing moisture).

Each of the weather variables was summarized for the week of the catch, with the day of trapping being taken as the last day of the week. Mean 10 cm Soil Temperature, mean PET, mean Photoperiod, mean SMDI, mean SWI, and mean Relative Humidity were calculated over seven days for each farm. Rainfall was calculated as the Total Rainfall per week. Maximum and Minimum Temperatures were taken as the Maximum and Minimum Temperatures recorded at any time over the course of the week.

The annual variation in the climate on each farm was described for each variable on each farm for the 2018/2019 and 2019/2020 seasons beginning on the 1st of July until the following 30th of June for each season. The average annual climate from each of the VCS which were closest to each farm during this period was calculate for each variable. This is the methodology used to summarize annual climate data by several national agencies' organizations such as NIWA in New Zealand (https://niwa.co.nz/) and the NOAA (National Oceanic and Atmospheric Administration) in the United States of America (https://www.ncei.noaa.gov/). The mean, standard deviation and range of values were defined for each farm.

Table 6.1 The name of the farm, its location, and the closest Virtual Climate Station (VCS) Agent number.

Farm Name	VCS Agent number
"KD"	20098
"ST"	20496
"S"	20500
"LD"	26837
"BH"	27906
"R"	28791
"M"	29239
"T"	30371

6.2.3 Statistical Methods

A generalized linear mixed-effects model (GLMM) was fitted to the first year's data (2018/2019) and then used to predict the start of the second year (2019/2020 season) for *Lucilia* spp. on each farm. The 2018/2019 trapping season for each farm site began on the 3rd week of September 2018 and ended on the last week of June 2019. As the collection date of samples varied on each farm, the climatic variables were summarized as outlined previously in the Method section of Chapters Four. The period the traps were out in the 2019/2020 season was from the 3rd week of September 2019 to the last week of December 2019. The data for *Lucilia* spp. for each week of trapping was transformed into a binary notation (i.e., 1 = *Lucilia* spp. caught, 0 = no *Lucilia* spp. caught). The *glmer* function in R was used to fit a GLMM (Brooks et al., 2017) to predict the probability of the *Lucilia* spp. caught on each farm for each week. Farm was entered into the model as a random effect. The 2018/2019 season was used to train the model and the 2019/2020 season was used to test the model. For each climatic variable, a lag of one to twelve weeks were also investigated, grouped by each farm using the *dplyr* package in R studio (Wickham et al., 2021); the code for doing so may be found in Appendix D.3.

Variables were removed using a backward selection method and variables were retained if they were significant to a $p < 0.05$. A partial F test was used to compare nested models. Individual weather variables were tested and retained if at $p < 0.05$, with all deleted variables set aside and retested in the final model. However, due to the potential for temperature variables to be co-linear to each other, only one temperature variable was tested in the model at each time. Interactions between variables were also tested in the model and retained when they were $p < 0.05$.

The final model built on the first year data was then used to predict the occurrence of *Lucilia* spp. for the start of the second year and the sensitivity, specificity, positive likelihood ratio and accuracy of the model were calculated using the packages *caret* and *performance* (Ludecke et al., 2021, Kuhn et al., 2022). Receiver operating curves (ROC) were used to plot the true positive rate (Sensitivity) versus the false positive rate (Specificity) at a number of specified cut off points using the *ROCR* package (Sing et al., 2005). This optimal cut-off point was then used to make the predictions and to evaluate the performance of the model. The Hosmer and Lemeshow test was used to calibrate the model's goodness of fit (Hosmer and Lemeshow, 2013). The simulated residuals of the resulting models were then compared to the observed data using the *DHARMa* package (Hartig, 2020), to assess the fit of the models. As both Soil Moisture Deficit Index and Soil Water Index used potential evapotranspiration in their calculation; it was considered that the inclusion of both variables in the same model could be problematic and could result in introducing multicollinearity. To circumvent this problem two models were built: the first with SMDI and the second with SWI. Each of these two models was compared using the Akaike information criterion (AIC) and Bayesian information criterion (BIC) and the model with the lowest AIC or BIC was selected as the final model.

6.3 Results

The following tables summarise the climate variables for the 2018/2019 and 2019/2020 seasons for the VCS closest to each of the farms (Tables 6.2, 6.3). It should be noted that the South Island farms are all found within the same region. This somewhat limits the comparison as they all have a similar climate.

The soil temperatures were broadly similar each season between each farm by 0.5℃ except for “KD”, where the second season was 2.3 ℃ warmer (Table 6.2). Rainfall was similar from one season to the next, except for “BH”, where the amount decreased from 86.2 mm in the 2018/2019 to 61.1 mm 2019/2020 season. While in “KD”, there was more rainfall in 2019/2020 (10.7 mm) than in the 2018/2019 season. Mean SMDI was found to differ between farms, with “M” and “R” being the lowest overall for both seasons (Table 6.3).

The mean PET was similar between seasons and between farms (Table 6.2). While for wind speed, this was also similar between seasons; in “BH”, the highest annual mean wind speeds were recorded each season compared to the other farms (Table 6.3). Furthermore, the South Island farms had higher maximum and minimum temperatures than the North Island farms (Table 6.3). It is also notable that annual Maximum temperatures were higher on each farm in 2019/2020 compared to the previous season (Table 6.3). The mean SWI differed considerably between each farm, except for “KD” and “ST” (Table 6.2). A further comparison between seasons could not be made as data for Soil Water Index was only available for the 2019/2020 season until December 2019.

Table 6.2 The annual Mean 10 cm Soil Temperature, Mean Soil Moisture Deficit Index, Mean Soil Water Index, Mean Potential Evapotranspiration and Mean Relative Humidity for the 2018/2019 and 2019/2020 seasons. The annual variation in the climate for each variable on each farm for the 2018/2019 and 2019/2020 seasons from the closest Virtual Climate Station, beginning on the 1st of July until the following 30th of June for each season. The mean value, and standard deviation (sd) with the maximum and minimum range (range) are stated for each variable on each farm.

		Climatic variables									
	Farm	Mean 10 cm soil temperature (°C) (sd; range)		Mean Soil Moisture Deficit Index (mm) (sd; range)		Mean Soil Water Index (mm) (sd; range)		Mean Potential Evapotranspiration (PET) (sd; range)		Mean Relative Humidity (°C) (sd; range)	
		2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020a	2018/2019	2019/2020	2018/2019	2019/2020
North Island	“T”	14.1 (4.5; 8.0–20.8)	13.3 (4.2; 8.4–20.2)	-61.3 (47.2; 2.2– -130.2)	-51.4 (51.0; 2.1– -135.8)	21.6 (16.7; 2.7–45.6)	32.9 (14.0; 9.5–45.0)	2.6 (1.5; 0.6–5.1)	2.6 (1.5; 0.6–4.9)	82.1 (5.3; 74.2–90.1)	80.7 (6.3; 70.4–90.1)
	“R”	12.8 (4.3; 6.8–19.6)	12.6 (4.0; 7.2–19.2)	-39.4 (40.8; 5.9– -116.9)	-38.3 (47.8; 8.2– -126.0)	20.6 (12.9; 1.6–35.5)	25.5 (10.1; 9.4–35.2)	2.4 (1.4; 0.5–5.0)	2.5 (1.5; 0.5–4.5)	81.3 (4.7; 72.5–88.1)	79.3 (6.3; 69.9–89.1)
	“BH”	13.7 (4.4; 7.6–20.3)	13.5 (4.2; 7.8–20.5)	-55.8 (39.2; -1.7– -118.4)	-69.1 (53.5; -2.1– -143.2)	17.1 (11.3; 3.1–34.7)	18.4 (13.0; 1.1–34.8)	2.7 (1.5; 0.8–5.5)	3.0 (1.7; 0.7–5.3)	79.2 (4.1; 72.4–86.1)	75.8 (7.1; 64.5–87.4)
	“M”	13.8 (4.5; 7.7–20.4)	13.4 (3.9; 8.4–19.3)	-31.3 (34.4; 3.7– -104.0)	-37.4 (48.8; 5.3– -132.5)	53.1 (24.0; 8.1–80.8)	69.8 (12.3; 50.4–77.5)	2.2 (1.3; 0.5–4.5)	2.4 (1.5; 0.5–4.6)	86.9 (5.7; 79.2–94.1)	85.3 (6.8; 74.7–93.4)
	“LD”	15.6 (4.0; 9.8–21.2)	15.6 (3.6; 10.9–21.2)	-50.5 (44.7; 4.5– -132.3)	-52.5 (52.7; 4.2– -141.8)	49.2 (26.8; 2.9–83.4)	62.0 (26.1; 17.9–83.2)	2.6 (1.4; 0.8–4.9)	2.8 (1.6; 0.8–5.1)	84.6 (5.6; 76.8–92.4)	83.5 (6.4; 74.7–93.6)
South Island	“KD”	10.7 (4.5; 4.3–17.4)	13.0 (5.4; 4.5–21.2)	-63.9 (36.3; -7.3– -126.5)	-63.8 (45.2; 1.4– -141.8)	25.7 (16.2; 2.0–50.2)	28.8 (17.1; 5.5–47.7)	2.5 (1.5; 0.6–5.4)	2.8 (1.6; 0.8–5.1)	77.4 (6.0; 65.8–84.0)	78.0 (9.0; 62.8–92.4)
	“ST”	10.7 (4.4; 4.4–17.3)	11.2 (4.9; 4.8 –18.4)	-61.0 (37.8; -10.1– -127.9)	-71.4 (48.6; -8.4– -141.0)	29.5 (18.; 2.6–61.8)	31.6 (25.0; 2.4–59.7)	2.6 (1.5; 0.7–5.5)	2.9 (1.7; 0.6–5.3)	76.8 (5.6; 66.0–83.8)	73.5 (7.4; 61.2–86.2)
	“S”	11.1 (4.4; 4.9–17.7)	11.8 (4.8; 5.4–18.9)	-64.0 (32.7; -13.0– -131.6)	-76.3 (46.0; -9.3– -141.0)	32.2 (22.7; 4.8–62.1)	39.9 (30.0; 2.1–77.3)	2.7 (1.5; 0.7–5.5)	2.9 (1.7; 0.6–5.3)	76.9 (5.3; 66.2–83.8)	73.4 (7.3; 61.2–86.2)

[a] measured from September 2019 until December 2019/2020

Table 6.3 The annual Total Rainfall, Maximum Temperature, Minimum Temperature, Mean Wind Speed, Mean Vapour for the 2018/2019 and 2019/2020 seasons. The annual variation in the climate for each variable on each farm for the 2018/2019 and 2019/2020 seasons from the closest Virtual Climate Station, beginning on the 1st of July until the following 30th of June for each season. The mean value, standard deviation (sd) with the maximum and minimum range (range) are stated for each variable on each farm.

	Farm	Climatic variables									
		Total Rainfall (mm) (sd; range)		Maximum Temperature (℃) (sd; range)		Minimum Temperature (℃) (sd; range)		Mean Wind Speed (mph) (sd; range)		Vapour Pressure (Pa) (sd ; range)	
		2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
North Island	“T”	75.0 (34.3; 24.1–126.5)	78.1 (33.9; 18.9–126.5)	24.3 (4.3; 15.7–30.1)	22.5 (4.9; 15.7–31.9)	1.5 (2.8; -2.7–6.5)	2.0 (3.7; -2.9–8.4)	3.4 (0.5; 2.7–4.3)	3.5 (0.5; 2.7–4.2)	12.1 (2.6; 9.0–16.8)	11.5 (1.9; 9.1–15.2)
	“R”	99.6 (44.7; 27.9–176.0)	98.3 (51.9; 16.0–162.7)	22.3 (4.3; 15.5–30.7)	21.8 (4.6; 15.2–29.1)	0.7 (3.0; -3.0–6.5)	0.6 (3.1; -2.9–6.3)	2.8 (0.3; 2.3–3.4)	2.9 (0.4; 2.1–3.4)	12.1 (2.5; 9.0–16.7)	11.6 (1.9; 8.9–15.2)
	“BH”	86.2 (54.6; 20.2–168.2)	61.1 (36.6; 6.5–113.1)	22.4 (4.3; 15.7–29.6)	23.4 (5.1; 15.6–33.6)	3.9 (3.1; 0.2–9.5)	3.7 (2.8; 0.4–8.5)	5.4 (0.8; 3.9–6.9)	5.6 (0.8; 4.0–6.7)	11.8 (2.7; 8.3–16.4)	11.3 (1.8; 8.5–14.7)
	“M”	93.9 (35.8; 45.9–149.5)	105.7 (53.8; 7.0–201.6)	22.7 (5.3; 14.0–31.8)	23.3 (5.6; 15.3–31.3)	0.8 (3.0; -3.1–7.6)	1.0 (2.6; -3.2–4.6)	1.1 (0.2; 0.7–1.3)	1.1 (0.2; 0.7–1.5)	12.0 (2.8; 8.4–16.7)	11.6 (2.2; 9.1–15.4)
	“LD”	89.7 (52.3; 16.5–191.4)	95.6 (58.7; 4.4–184.1)	22.9 (4.4; 16.5–29.7)	23.2 (4.3; 17.3–29.4)	4.7 (3.3; -0.4–10.6)	5.1 (3.1; 0.0–9.2)	3.1 (0.4; 2.4–3.8)	3.5 (0.7; 2.4–4.6)	13.8 (2.8; 10.3–18.4)	13.7 (2.0; 11.1–17.1)
South Island	“KD”	59.3 (27.7; 17.1–109.1)	70.0 (41.5; 4.4–161.8)	23.1 (5.3; 15.2–31.6)	23.7 (4.1; 17.5–29.4)	0.5 (3.0; -3.8–5.8)	2.7 (4.2; -3.8–9.2)	1.9 (0.4; 1.3–2.6)	2.2 (0.3; 1.6–2.5)	10.3 (2.5; 6.9–14.0)	9.0 (1.6; 6.8–11.2)
	“ST”	53.4 (33.3; 9.7–120.6)	51.3 (37.4; 0.0–101.5)	23.4 (4.8; 16.2–30.2)	24.4 (4.7; 16.9–32.8)	2.1 (3.0; -2.1–7.1)	1.8 (2.8; -2.2–6.6)	2.1 (0.3; 1.6–2.7)	2.3 (0.3; 1.7–2.8)	10.6 (2.5; 7.2–14.3)	10.1 (2.0; 7.0–13.7)
	“S”	53.1 (36.1; 12.5–133.3)	49.1 (35.6; 0.0–101.0)	24.5 (4.8; 1.7–31.3)	25.6 (4.8; 17.8–34.2)	2.9 (3.0; -1.2–8.0)	2.7 (2.7; -1.1–7.0)	2.9 (0.3; 2.4–3.4)	3.2 (0.4; 2.5–3.9)	10.6 (2.5; 7.2–14.4)	10.1 (2.0; 6.9–13.6)

6.3.1 Model Overview

The model built with daily mean SMDI and mean 10 cm soil temperature had the lowest AIC/BIC and was adopted as the final model to predict the occurrence of adult flies at the beginning of the season. The model coefficients are shown in Table 6.4. As a logistical function, it does not attempt to predict fly numbers. All other variables, interactions and lagged variables tested in the model were not significant ($p>0.05$). The model had an R^2 value of 0.65, and the Hosmer-Lemeshow Test showed a good fit; (chi-squared 4.549, degrees of freedom of 8, $p<0.05$). The coefficients for the final model are found in Table 6.4. Mean 10 cm soil temperature had a positive effect indicating that an increase in 10 cm soil temperature would increase the probability that *Lucilia* spp. will be caught. An increase of one degree in 10 cm soil temperature will increase the probability of catching flies by 1.6 (Table 6.4). Whilst mean SMDI was found to have a negative coefficient (Table 6.4). This means that as the mean SMDI becomes less, i.e., it becomes wetter, the probability of catching *Lucilia* spp. decreased by 0.97 for each unit change (Table 6.4).

The relationship between the mean 10 cm soil temperature, Mean SMDI and the probability of catching *Lucilia* spp. is illustrated with a 3D contour plot in Figure 6.1. This plot shows that there are lower probabilities for the occurrence of *Lucilia* spp. with saturated soils i.e., where the mean SMDI is closer to zero, and with lower mean 10 cm soil temperatures of less than 12 °C (Figure 6.1). Conversely, there is a higher probability of the occurrence of *Lucilia* spp. with higher mean 10 cm soil temperatures and lower mean SMDI i.e., drier soils which are more negative (Figure 6.1). It should also be noted that to have a higher degree of the probability of occurrence of *Lucilia* spp. of greater than 0.8, mean 10 cm soil temperatures must be above 16 °C, where mean SMDI is fully saturated (Figure 6.1).

A Q-Q plot and simulated residuals plotted against observed data both were found to be normally distributed (plot shown in Appendix E.1). The ROC curve showed that the optimum cut point was 0.73 (Figure 6.2). Using a cut-point of 0.73 the model was found to have a sensitivity of 0.80, a specificity of 0.85, an accuracy of 0.82, a kappa of 0.65 and a no information rate of 0.51 (Table 6.5).

Table 6.4 Summary of the model coefficients, 95 % confidence intervals, an exponentiated transformation of the coefficients, and p values for the logistic regression model for *Lucilia* spp. with the variables Mean 10 cm Soil Temperature per week and Mean Soil Moisture Index.

Predictors	Coefficients	95 % Confidence interval low and high	Exponentiated Transformation of Coefficients	*p*-Value
Intercept	-7.30	-9.12 – -5.47		<0.001
Mean 10 cm Soil Temperature	0.45	0.33 – 0.57	1.6	<0.001
Mean Soil Moisture Deficit Index	-0.03	-0.05 – -0.02	0.97	<0.001

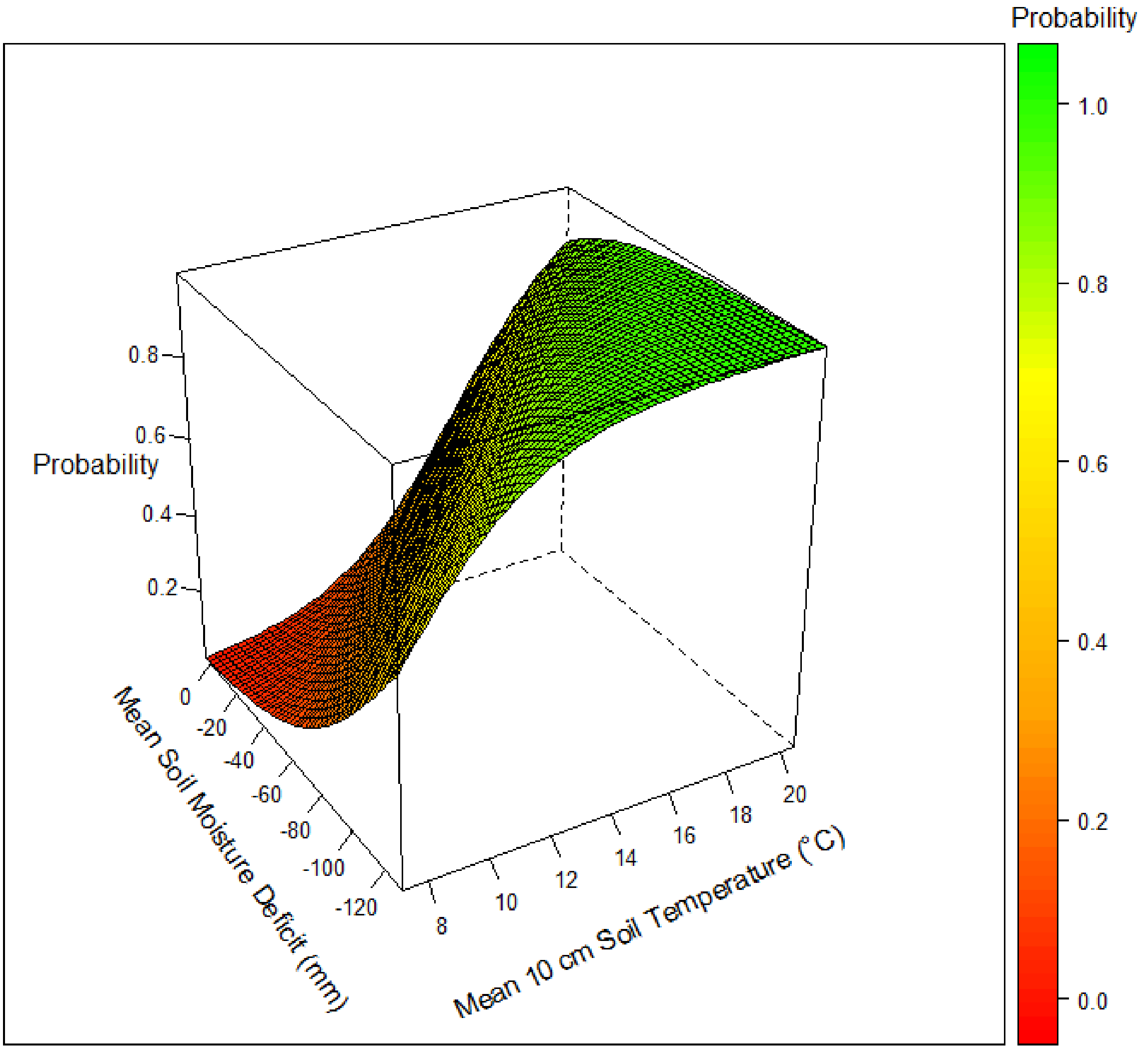


Figure 6.1 A 3D contour plot showing the change in probability of occurrence of *Lucilia* spp. with changes in Mean Soil Moisture Deficit per week and mean 10 cm soil temperature per week. For example, at a Mean Soil Moisture Deficit Index of -60 mm and mean 10 cm soil temperature of 14 °C with a probability of occurrence of 0.6.

Table 6.5 A Confusion Matrix showing the actual and predicted values for the 2019/2020 season for *Lucilia* spp. at a cut point of greater than 0.73.

		Actual values	
		0	1
Predicted values	0	49	9
	1	12	50

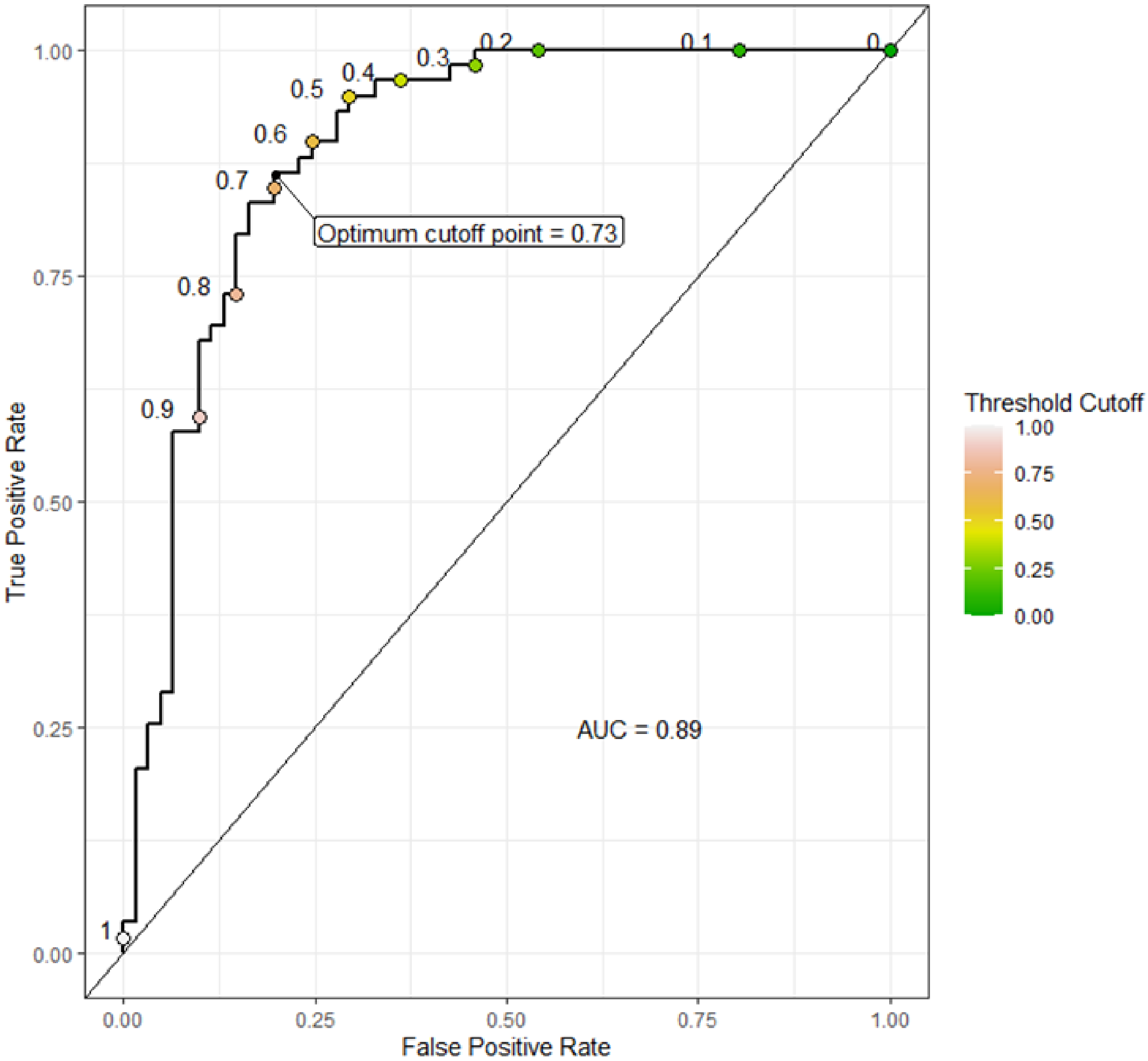
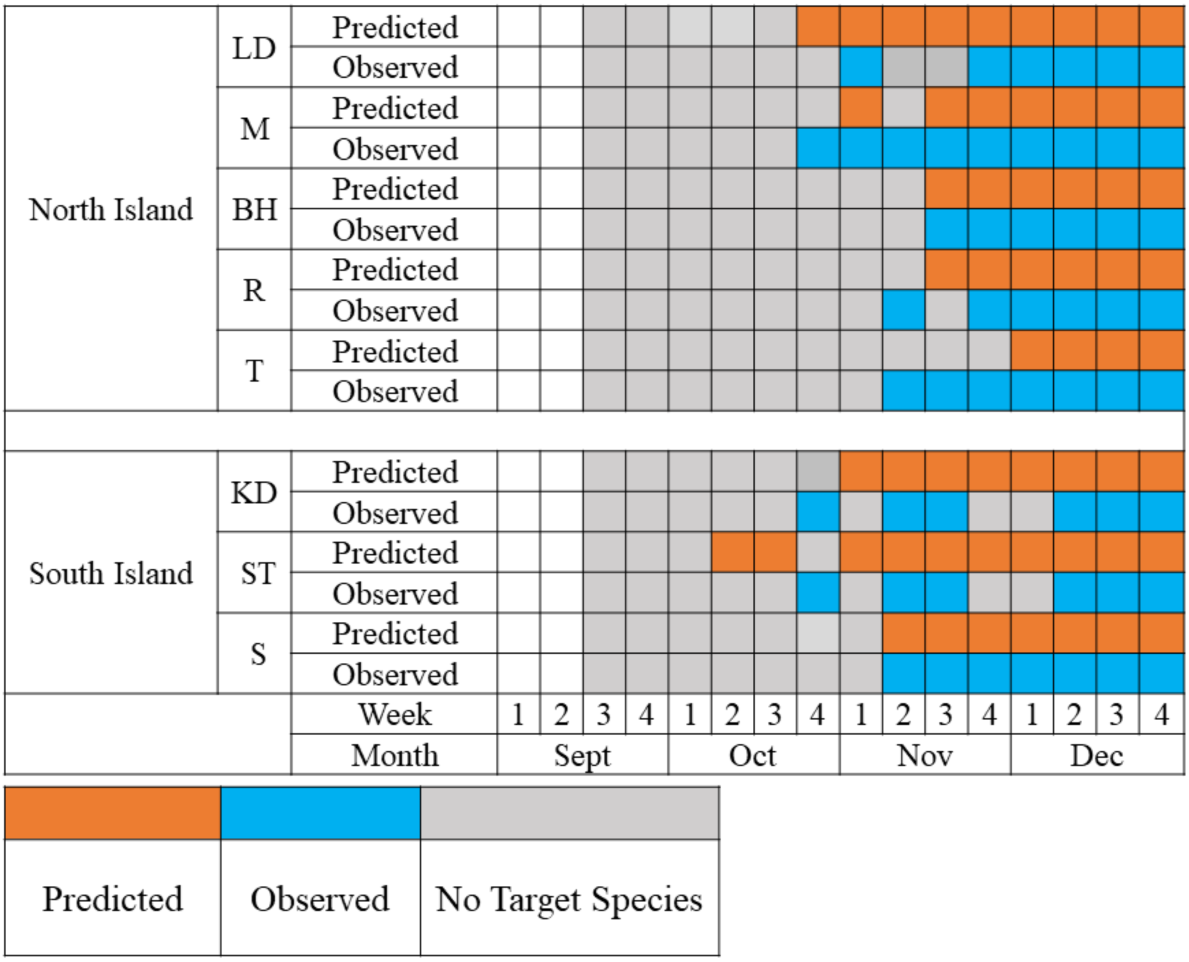


Figure 6.2 Receiver operating characteristic curve (ROC) for the prediction of *Lucilia* spp. based on the effects of the Mean 10 cm Soil Temperature per week and Mean Soil Moisture Deficit Index with an area under the curve (AUC) value of 0.89.

6.3.2 The occurrence of *Lucilia* spp. on each farm

The observed and predicted occurrence of *Lucilia* spp. on the North and South Island farms in 2019 is illustrated in Figure 6.3. For every farm, the prediction is either in agreement with the observed data (“S” and “BH”), anticipates the start of the season by one to three weeks earlier than observed (“LD” and “ST”) or lags the observed data by one to three weeks (“M”, “R”, “T” and “KD”). It should be observed that for farms “M” and “ST”, there were null catches predicted to occur after the start of the season.



North Island	LD	Predicted			⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	⬜	🟦	⬜	⬜	🟦	🟦	🟦	🟦	🟦
	M	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	🟧	⬜	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
	BH	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦
	R	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟦	⬜	🟦	🟦	🟦	🟦	🟦
	T	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦	🟦
South Island	KD	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	🟦	⬜	🟦	🟦	⬜	⬜	🟦	🟦	🟦
	ST	Predicted			⬜	⬜	⬜	🟧	🟧	⬜	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	🟦	⬜	🟦	🟦	⬜	⬜	🟦	🟦	🟦
	S	Predicted			⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		Observed			⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦	🟦
		Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		Month	Sept				Oct				Nov				Dec			

🟧	🟦	⬜
Predicted	Observed	No Target Species

Figure 6.3 illustrates the observed (blue blocks) versus predicted (orange blocks) occurrences of *Lucilia* spp. on the North and South Island farms for the first half of the 2019/2020 season from September until December 2019.

6.4 Discussion

This study aimed to predict the start of the *Lucilia* spp. 2019/2020 season using a model fitted to the 2018/2019 seasonal data utilising weather data from the closest virtual climate (VCS) station for each farm. The final model had only two significant weather variables: mean 10 cm soil temperature and Mean Soil Moisture Deficit Index. This is the first time the combination of mean 10 cm soil temperature and Mean Soil Moisture Deficit Index has been used to predict the occurrence of *Lucilia* spp. according to this author's knowledge. The model suggests that *Lucilia* spp. are more likely to survive in warmer drier soils, with colder wetter soils thus decreasing the probability of catching these species. When these variables were considered with a lag to allow for subsequent effects on any developing flies due to a change in the values of these variables, neither variable was found to be significant. Thus, this suggests that there would likely be no advanced warning for farmers when the season is likely to begin. However, as long-range weather forecasts are becoming more available, it could be possible to base this model on these long-range weather forecasts and thus achieve the same effect with a warning available several weeks in advance although this will have limitations based on the accuracy of these long-term predictions.

Temperature is widely used to predict the times of the year when calliphorid flies will be active and thus the start of their season, due to them being ectothermic (Vogt et al., 1983, Vogt et al., 2001, George et al., 2013, Lutz et al., 2019). Predicting the seasonality of *L. sericata* in the United Kingdom has been made by the accumulation of degree days until the emergence of the adult fly (Wall et al., 1992a, Fenton et al., 1997). However, the use of just temperature as a measure to predict the start of the season may not reflect the overall environmental conditions experienced by these species.

Including both the Soil Moisture Deficit Index and 10 cm soil temperature in a model to predict the start of the season is likely more realistic, as it reflects the soil conditions that the pupae experience. It has been shown experimentally for both *L. sericata* pupae and larvae, that high levels of soil moisture can delay their emergence although it does not impact their survivorship (Kokdener and Sahin Yurtgan, 2022). This lag might be reflected both in the results of the present model and that observed in the data by the gaps in the occurrence of *Lucilia* spp. each week. It could be hypothesized that waterlogged soils have lower oxygen levels thus becoming anoxic which would impact the development of both larvae and pupae. A similar finding has been reported in several plants (Barrett-Lennard, 2003, Najeeb et al., 2015). This may also account for these gaps in both the observed data and the predictions from the model.

In published reports to date, the effect of soil moisture on pupal development was not found to differ significantly due to the three different soil types investigated: loam, clay and sand (Kokdener and Sahin Yurtgan, 2022). In New Zealand, the Soil Moisture Deficit Index variable currently used by NIWA assumes that loam is the sole soil type throughout the country (Mol et al., 2017). However, it is unlikely that all the farms have the same soil type (or even one soil type), although this was not a factor considered in the analysis. Kokdener and Sahin Yurtgan (2022) suggested that soil type was not the most important factor, rather it is just a simple matter of high moisture content in any soil type. Future studies should consider if different soil types may impact the emergence of *Lucilia* spp., other than those previously examined (Kokdener and Sahin Yurtgan, 2022). Establishing this may improve the accuracy of the current predictions.

On the South Island farms, the predicted start of the season was similar at Farms “KD” and “S” with each being within a week of the other. Interestingly, there was an observed difference of up to four weeks for these two farms compared to nearby Farm “ST”. All three of these farms were close to each other and thus question the accuracy of this model. The variation might be explained as a sampling bias as on these farms generally only a single *Lucilia* spp. fly was caught per week at this time of the season. Therefore, it is quite possible that *Lucilia* spp. were present in low numbers but were not caught.

Formulating a prediction for the entire 2019/2020 season was not possible due to travel restrictions from March to July 2020 implemented to the New Zealand government-mandated SARS-CoV-2 lockdown response within New Zealand in 2020. Further work should be undertaken to observe the end of the season for both species at these same farm locations or perhaps in other locations across New Zealand. These further studies should encompass regions which have a variety of climatic conditions across New Zealand. It is unknown if this model will also be suitable to predict the end of the season.

In conclusion, the model outlined in this study predicted the start of the season for *Lucilia* spp. on eight farm sites across New Zealand utilising NIWA VCS data. It was not intended to predict the number of flies occurring throughout the season. This model shows that the VCS data could be used with the same efficacy and accuracy as the model outlined in Chapter Six which used on-farm weather stations. The results of this model will allow farmers to make effective proactive integrated pest management plans to monitor the start of the season for *Lucilia* spp. in New Zealand. Given that instances of flystrike were recorded very soon after the first appearance of flies as indicated in Chapter Four; close monitoring of sheep for instances of flystrike should be undertaken once *Lucilia* spp. are predicted to occur. As this model had no lagged variables, farmers would have a minimum window to react when the season for *Lucilia* spp. is forecast to begin.

Chapter Seven. General Discussion

The overall aim of this thesis was to develop mathematical models to describe the abundance of flies which cause flystrike in New Zealand. In this country, the situation is somewhat different to both Australia and the United Kingdom which each have one dominant species causing flystrike whereas in New Zealand; both *L. sericata* and *L. cuprina* are found. Two types of approaches were taken to describe fly abundance, and both were able to provide a sufficiently accurate description of the fly abundance. The hurdle model described in Chapter 5 gave a more complete description of the life cycle but unfortunately was not able to be tested by fly catches in later seasons whereas the logistic model to describe the start of the season in Chapter 6 was able to be tested. The inclusion of lagged variables in the hurdle model will allow predictions to be made some weeks earlier whereas the logistic model did not find lagged variables to be significant and hence only able to use current weather to predict emergence of flies in spring. The latter is not particularly helpful to farmers who need to plan, but as discussed, the advent of newer techniques to predict the weather some weeks in advance will allow this model to used.

It was found that *Lucilia* spp. have a season beginning in mid-October and lasting until mid to late May. There was a difference of up to a month between farms in the lower half of the North Island (farms “T”, “BH”, “R”) compared to those in the upper North Island (Farms “M”, “LD”) and/or the South Island (Farms “KD”, “ST”, “S”) for *Lucilia* spp... It was also found that there was a seasonal variability on the farms of up to three weeks. Thus overall, there is some variation from farm to farm and year to year. This is in part due to very low fly numbers being caught at the beginning and end of the season, so it is very easy to get null catches despite low numbers of flies being present. The other obvious explanation is the variation of weather from year to year and location to location. The hurdle model used data from on-farm weather stations and no attempt was made to generally apply Virtual Climate Station (VCS) weather data to this model. If the latter is shown to be suitable then all farmers would be able to make local predictions. Alternatively, industry organisations could formulate country wide risk maps in real-time and farmers could orientate themselves and take the available information from this source. Similarly, the logistic model (Chapter 6) could be applied nationally in much the same way. No single model can be applied to New Zealand as a whole and the results do need to be applied farm by farm preferably.

Local confirmation of the presence of *Lucilia* spp. would be useful and perhaps only necessary on a district basis. Whilst identifying between the two *Lucilia* species would not be possible it is still likely that a farmer could maintain one or more traps on a farm to at least gauge the start and end of the fly season on that farm. Identification of metallic green *Lucilia* species should be possible with the main point of confusion being *C. rufifacies*. However, the latter is not that common and unlikely to materially affect the interpretation. Choice of bait for such on-farm traps needs further development. Unfortunately, the use of squid as a bait which would be simple for farmers was shown to be ineffective. Regulations prohibiting chemicals which have been used to date will restrict the use of such as those in LuciLures®. Use of offal as a bait is possible but needs to be refreshed at short intervals so is unlikely to be used long term. Thus, further work is required to refine a suitable trap for farmers to use. It is possible that one local farm could use traps and inform his neighbours and that would be sufficient.

Unfortunately, the morphological identification between the two *Lucilia* species was inadequate in the present studies to allow the identification originally made to be used in the development of separate models for both species. The combination of the two *Lucilia* species together is unfortunate as they do differ in their biological characteristics such as diapause induction, critical temperatures etc. Nevertheless, the data was able to confirm that *L. cuprina* was the first species to appear and the last to disappear each season. Hence, the logistic model for the commencement of the season is effectively for *L. cuprina*. Further work is required to confirm this and investigate how that varies between the two species. Clearly, the subsequent comparison with the morphological keys after the molecular identification was made confirm the actual keys themselves are accurate.

There still is some confusion about speciation within the genus *Lucilia* especially for the species *L. cuprina.* The results of the present study suggest that there are three groupings within the *Lucilia* genus found in New Zealand. The first grouping comprises *L. c. dorsalis, L. sericata* and the first New Zealand variant of *L. cuprina* (New Zealand Variant 1). The second grouping contains the second New Zealand variant of *L. cuprina* (New Zealand Variant 2) with a third comprising *L. c. cuprina*. How the discovery of these two New Zealand variants affects the occurrence of flystrike is uncertain. There may be no effective difference but further work to describe this is now required. As described elsewhere, insecticide resistance is becoming an issue for these flystrike species in general and it is now required to consider these two variants and how they may be involved. They should also be considered when registering new insecticides to ensure both are included. The absence of a hybrid species of *L. cuprina/L. sericata* present in New Zealand at least provides some evidence that it is not necessary to consider such a genotype when investigating insecticide efficacy or any varying epidemiological patterns for the hybrid genotype.

This study also explored whether a degree day model utilising developmental data from other countries for *L. cuprina* and *L. sericata* could be utilised to predict the start of the 2018/2019 season for *Lucilia* spp. on the farms. Such Degree Day models already exist for *L. sericata* and applying those to the present data showed quite a bit of variation with no single threshold being found that could be applied to all farms. Similarly, although no Degree Day models exist for *L. cuprina* threshold temperatures have been published and when these are applied to the present data, they also did not provide a single useful Degree Day model. Thus, the present results suggest that the use of such a model is not currently suitable for New Zealand. Further experimental work is required to define the key factors for any such model for New Zealand.

The focus of this PhD was primarily the seasonal collection of *L. cuprina* and *L. sericata* over three fly seasons (2018/2019, 2019/2020 and 2020/2021) from eight farms across the North and South Islands across New Zealand. However, it could be considered that the eight farms utilized may not be representative of the wide diversity of locations and climates found throughout New Zealand. The farms in this study were chosen based on their willingness to participate rather than solely on their location and/or climate differences. The following regions in the North Island were covered: Manawatu, Wairarapa, Hawks Bay, Ruapehu and the Waikato. Several other regions such as Northland, Taranaki, Gisborne, and Taupo/Rotorua/Tauranga region were not covered. It is unknown if the seasonality of *L. cuprina* or *L. sericata* would be similar in these regions especially given both Northland and Gisborne are regarded as the warmest regions in New Zealand (See Table 1.9).

The South Island farms were all clustered in the North Canterbury region which was not ideal for achieving the aims of this study. Regions in the South Island such as Southland and Otago have considerably different climate conditions from the rest of New Zealand (Table 1.9). The photoperiod for one is quite different from the top of the North Island to the bottom of the South Island. In the original plan, there were farms in these more southern regions but a reliable weekly collection of samples on these other sites could not be organized and hence they were removed before the start of the study. Therefore, the number of sites and locations utilised in this study is a limitation.

Ideally, the timing of the first case of flystrike and further outbreaks should have been collected along with the timing of the chemical treatment of sheep on each farm. This would have helped to answer questions such as: was there a correlation between the spikes in the population of both species with higher instances of flystrike and how this may interact with various climatic variables. Conversely, did the use of control measures lead some of the dips in fly abundance seen? However, each of the farm managers specifically stated from the outset of this project that they did not have enough staff to allocate for this role on a daily basis. Furthermore, having only eight farms may have also limited the value of the data. In addition, it was not possible for each farm to have the same chemical treatment plan as this was outside the remit of this thesis. Future studies could use a similar approach and assess the usefulness of control measures used in altering the abundance of flies.

As noted above the poor accuracy of the original morphological identification was a limitation. The difficulty of reliably differentiating *L. cuprina* and *L. sericata* has been reported in multiple publications (Williams and Villet, 2014, Akbarzadeh et al., 2015, Lutz et al., 2018). It was simply not feasible to molecularly identify the many thousands of flies collected over the course of this thesis, therefore visual identification was the only practical solution. Further, in Chapters Three and Four, these species were identified out of alcohol which has been traditionally viewed as being more difficult than identifying dry, pinned samples. This approach to storing samples in the present studies was necessitated by the location of farms and the number of flies involved. Future studies will likely need to develop a different approach and probably molecularly identify at least a subset and extend that to the general collection. Development of simpler, yet equally accurate molecular methods such as LAMP, Digital droplet PCR, or Maldi-Tof assays will likely assist.

The effect of the Covid 19 pandemic significantly affected the collection of samples in the 2019/2020 season as outlined in Chapter Four. Restrictions were introduced by the New Zealand government and subsequently by Massey University in March 2020. This occurred in the second week of the collection of samples and the study was essentially cancelled. Samples could only be collected on “LD” as the person collecting the samples lived on the farm. The consequences of losing this data are most noticeable in Chapters Five and Six as it was envisioned that the first year’s data could be used to predict the results of the second year's study. Consequently, the model described in Chapter Five was not tested for accuracy with subsequent years’ collection data so it effectively just describes the data from 2018/19 and would benefit from being tested against another independent set of flytrap data.

The impact of Covid 19 fundamentally affected the original design of this PhD. Insect cultures of *L. cuprina* and *L. sericata* could not be accessed over three months, thus the cultures failed due to lack of maintenance. These cultures would have been used for two further experimental studies. Firstly, to examine the effect of temperature on the larval stages at a constant temperature for both *L. cuprina* and *L. sericata*. Secondly, investigate the effect of decreasing photoperiod on adult *L. cuprina* and *L. sericata*. The aim was for the results of these experimental studies to help predict the start, the end, and the weekly population growth of both species of flies over the two seasons to the data collected on the farms. A further comparison of the fit of these predictions could have been made to the seasonal field data.

The LuciTrap® has not previously been trialled in New Zealand and notable issues were seen before the start of the study in Chapter Four regarding the collection of rainfall in the lid of the trap. To reduce this potential source of error, a cover was added to the trap to reduce the amount of rainfall drops caught in the entrances into the trap effectively closing the trap, and a StickyTrap was added to the lid of the trap. The chemical lures in the LuciTraps are thought to mimic the volatile organic compounds (VOC’s) which are released by sheep that render them attractive to primary strike flies (Urech et al., 2004, Urech et al., 2009). As they may imitate a natural phenomenon than the number of *Lucilia* spp. caught by the LuciTrap® should reflect the natural population dynamics on the farm.

The results of this study suggest that the modifications made to the LuciTrap® were appropriate as the highest catches were found when temperatures peaked on each farm and the lowest catches were during high rainfall as evidenced in Appendix Section C.3.2. The addition of the StickyTrap was clearly beneficial, especially at the beginning and end of the seasons. Their use would be recommended for future use of LuciTraps. Overall, these findings agree with our understanding of the biology of the target species and in the descriptive model in Chapter Five. Thus, the performance of the LuciTrap® and StickyTrap allowed for the monitoring of the subsequent trends of the minimum and maximum population of both species.

Considerable issues were encountered with the Hobo MX2201 soil temperature probes. These probes did not perform to expectation, possibly due to problems with the battery life and the Bluetooth functionality. These issues were communicated to the manufacturers and no issues were found with the probes themselves. It would be strongly advocated that a soil temperature probe affixed to a weather station rather than separate units should be used in future studies as there is no guarantee that the probes will continue to function. However, to mitigate potential issues three probes were placed on each farm.

Further studies are required for both *L. cuprina* and *L. sericata* to confirm if the trends observed in Chapter Four are similar throughout New Zealand. For instance, in the North Island, regions such as Northland, and Gisborne were not surveyed, both are known to be the warmest regions in the North Island (See Table 1.9). In contrast on the South Island, further sampling is required to assess the variability of both species. Regions such as Otago, Southland, West Coast, Nelson, and Fiordland were not represented in this study. This is particularly important for Otago and Southland which have large populations of sheep. However, from the present studies, it would be expected that the seasonality of *L. sericata* would be similar across these regions given the agreement of the studies in Chapter Two to earlier studies (MacFarlane, 1938, Miller, 1939, MacFarlane, 1941, Murray, 1956, Dear, 1986). Whereas for *L. cuprina*, there is unknown if the variation observed from Chapters Two and Four will be found as it appears no sampling studies have been conducted south of Christchurch (MacFarlane, 1938, MacFarlane, 1941, Dear, 1986). Given the wide disparity in climate across the South Island, one would expect a substantial difference in the length of the season for *L. cuprina* (See Table 1.9). One aspect that might be crucial to examine is the potential effect of altitude particularly in the South Island regions. Farm "M" in the North Island was the farm site with the highest altitude in this study (Chapter Four). However, no difference in the species distribution was noted compared to the other farm sites. In Scotland, it was found that the distribution of flies in the family Calliphoridae changed with altitude and the species which caused flystrike also changed (Morris, 1997, Morris and Titchener, 1997). A change in the distribution of species of Calliphoridae due to altitude has also been noted in the Alps (Wyss et al., 2003) and the Himalayas (Bharti, 2012). As some farm stations in the South Island, especially in the Otago region, are found at high altitudes, it is recommended that an altitudinal study, with flytraps placed at descending heights, is undertaken to assess if there is a change in the fly population structure.

La Niña and El Niño have contrasting effects on the New Zealand climate. n El Niño phase trends to increase temperatures (Salinger and Mullan, 1999) and reducing rainfall leading to drought. In contrast, La Niña phase commonly results in heavier rainfall, especially along the eastern coast (Hay et al., 1993). It is unknown if the timing and length of the season, for either species would change due to a La Niña or an El Niño phase as data sets for either species over a considerable length of time (i.e., 5 years) are currently unavailable. One would expect that period of higher temperatures could result in an earlier season onset with higher populations of *L. cuprina* and *L. sericata* whereas, with periods of heavy rainfall, a shorter season could be expected with lower populations. The magnitude and onset of La Niña and El Niño can be forecast in advance, therefore a change in the length of the fly season could also be forecast in theory (Meng et al., 2018, Park et al., 2018, Ham et al., 2019, Dijkstra et al., 2019), but ideally would be tested first.

Several potential future measures would allow for the improvement of the models to predict the seasonality of *Lucilia* spp... This should include the availability of developmental data for both species in New Zealand, the effect of photoperiod and the effect of rainfall. Much of this can be obtained from laboratory trials under controlled conditions. Developmental data for both species via a constant temperature and humidity study in an incubator would have been beneficial as a starting point to be followed by more natural temperature variations as seen day to day in the field. Modern incubators will allow these types of experiments which historically were not possible. This data is typically collected to document the development of each developmental stage from egg, 1st instar, 2nd instar, 3rd instar, pupae, and adult. The lower developmental threshold is estimated from a linear regression due to the low survivorship of the egg and instar stages (Cervantes et al., 2018). Currently, models which predict the population growth of insects generally just consider the effect of temperature in a sigmoidal fashion, using a growing degree model to predict the growth from one stage to another as in a Leslie matrix prediction model (Hansen, 1989). There is currently no developmental data for either species available for New Zealand. These models use only a single threshold to estimate the start of the season whereas the models presented in this thesis use a mean threshold across all farms. Studies are required to investigate which method is more accurate for predicting the seasonality of these species and whether a combination of these statistical methods should be adopted.

Rainfall appears to cause a depression in the population growth of *Lucilia* spp. while a decrease in rainfall appears to result in spikes in the population growth. The effect of rainfall on the adult population dynamics is a measure that is purely based on observation in the literature (Mahat et al., 2009). Likewise, the effect of water-laden soil on the emergence of calliphorid flies is purely based on observations (Van Hoven - McLeod, 2012). The effect of high moisture on the pupal development of *L. sericata* has been shown experimentally with three different soil types with contrasting soil moisture (Kokdener and Sahin Yurtgan, 2022). The high soil moisture content of three different soil types has been shown to delay the development and reduce the size of pupae of *L. sericata* in a controlled laboratory setting (Kokdener and Sahin Yurtgan, 2022). Understanding the rainfall thresholds which would induce these spikes in the larval, pupal, and adult populations would greatly benefit an effective population prediction of these species. Furthermore, the effects of rainfall and soil moisture should be included in future models given their demonstrated effects in the current studies. In addition, further studies could also consider if there are wider differences between other dipterans.

Further clarification is required regarding the effect of photoperiod and temperature for both species which would add further clarity regarding a prediction for the end of the season as the model presented in Chapter Five is a fit of the trapping data. There are currently no experimental results to confirm the results from this model and there is no independent data to verify the observed patterns on each farm. Independent data from the same farms or from different farms from across New Zealand over three years would be required to give further clarity to this apparent distribution at the end of the season. Further consideration should be given to examining the impacts of the combination of the effect of photoperiod and temperature on the induction of diapause as this is not fully understood experimentally for *L. cuprina*.

The use of VCS would allow a dynamic 'whole of New Zealand' risk profile to be developed. The VCS is spaced out on a 5 km grid across the entire country. The only drawback of using data from the closest VCS is that it tends to underestimate the local variation in rainfall (Cichota et al., 2010). Although this may not be a major issue considering that rainfall was not a variable included in the final model in Chapter Six and thus this is an issue which may not have any impact. Alternatively, the use of on-farm weather stations could alleviate this issue but could encounter basic issues such as whether all the farms use the same weather station and collect the same weather variables.

7.1 Conclusion

Lucilia cuprina arrived in New Zealand in the late 1970s, where it lengthened the flystrike season and increased the number of cases of this disease. Consequently, sheep farmers are not able to make management decisions which would allow for the appropriate timing of measures to mitigate against this disease as it is unclear when the season starts and ends. The aims of this thesis were to evaluate the seasonality of the main species which cause flystrike on individual farms, create a climatic prediction model, and evaluate a supplementary fly trap for on farm use.

This thesis presented the seasonality of the combined catches of *Lucilia* spp. (*L. cuprina* and *L. sericata*) which were evaluated on a weekly basis over three seasons on eight sites across New Zealand. It was found that these species were present from early October until late May with a variability of up to a month between farms. Upon a comparison between the seasons, there was a difference of up to three weeks. The accuracy of the molecular and morphological identification of *L. cuprina* and *L. sericata* found a lack of consistency between these methods, which was attributed to the identifier. Thus, the catches of these species were combined for the evaluation of the measures to be used on-farm.

The results from this thesis will allow for farmers to proactively manage their sheep in New Zealand. If farmers know when the start, peak and the end of season will occur then they would be able to utilise integrated pest management strategies to reduce losses in productivity due to flystrike. Farmers may have to adopt differing management plans depending on their location in the country and the given the regional difference in the season for these species. Although this may be verified upon wider sampling in regions in New Zealand which were not evaluated in this thesis. Insecticides are typically applied on sheep to reduce instances of flystrike with a variety of length-of-time effectiveness, contrasting withholding periods and with a variety of costs. With this knowledge of the seasonality of *Lucilia* spp., farmers will be able to gauge how long they require chemicals to remain active and gauge other integrated pest management plan measures they may use. This would remove the uncertainty for farmers and veterinarians concerning the application of chemicals, thus reducing the number of times they are needed.

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Appendices

Appendix A.

Table A.1 The following table outlines the morphological and molecular identification based on 28s and ND4 of *Lucilia cuprina* and *Lucilia sericata* from eight farms in 2018/2019 season. A dash indicates that the sequencing was unsuccessful. The code corresponds with the sequences used to create the phylogenetic tree in Figure 2.9.

Date	Farm	Morphological identification	Molecular identification		Code
			28s	ND4	
29/11/2018	"T"	*L. cuprina*	*Pales pavida*		
13/12/2018	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	
13/12/2018	"T"	*L. cuprina*	*L. sericata*		
17/01/2019	"T"	*L. cuprina*	*L. cuprina*	-	
17/01/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	
07/02/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	
07/02/2019	"T"	*L. cuprina*	*L. sericata*		
07/02/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	T_Feb1
21/02/2019	"T"	*L. cuprina*	*L. sericata*		
21/02/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	T_Feb2
07/03/2019	"T"	*L. cuprina*	*Pales pavida*		
14/03/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	T_March1
28/03/2019	"T"	*L. cuprina*	*L. cuprina*	*L. cuprina*	
28/03/2019	"T"	*L. cuprina*	-		
28/03/2019	"T"	*L. cuprina*	*L. cuprina*	-	
18/04/2019	"T"	*L. cuprina*	*Chrysomya rufifacies*		
17/01/2019	"T"	*L. sericata*	*L. cuprina*	-	
17/01/2019	"T"	*L. sericata*	*L. cuprina*	*L. cuprina*	
07/02/2019	"T"	*L. sericata*	*L. cuprina*	*L. cuprina*	
07/02/2019	"T"	*L. sericata*	*L. sericata*		
14/02/2019	"T"	*L. sericata*	*L. sericata*		
21/02/2019	"T"	*L. sericata*	*L. cuprina*	*L. cuprina*	
21/02/2019	"T"	*L. sericata*	-		
07/03/2019	"T"	*L. sericata*	*L. cuprina*	*L. cuprina*	
14/03/2019	"T"	*L. sericata*	-		
22/11/2018	"R"	*L. cuprina*	*L. cuprina*		
29/11/2018	"R"	*L. cuprina*	*L. sericata*		
20/12/2018	"R"	*L. cuprina*	*L. sericata*		
03/01/2019	"R"	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_Jan1
10/01/2019	"R"	*L. cuprina*	*L. cuprina*	-	
10/01/2019	"R"	*L. cuprina*	-		
14/02/2019	"R"	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_Feb1
14/02/2019	"R"	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_Feb2
28/02/2019	"R"	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_Mar1

21/03/2019	“R”	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_Mar2
28/03/2019	“R”	*L. cuprina*	*L. cuprina*	*L. cuprina*	R_April1
28/03/2019	“R”	*L. cuprina*	-		
24/01/2019	“R”	*L. sericata*	*L. cuprina*	*L. cuprina*	R_Jan2
24/01/2019	“R”	*L. sericata*	*L. sericata*		
14/02/2019	“R”	*L. sericata*	-		
14/02/2019	“R”	*L. sericata*	*L. cuprina*	*L. cuprina*	R_Mar3
28/02/2019	“R”	*L. sericata*	*L. sericata*		
21/03/2019	“R”	*L. sericata*	-		
08/11/2018	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Nov1
15/11/2018	“BH”	*L. cuprina*			
22/11/2018	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
29/11/2018	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Dec1
29/11/2018	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Dec2
17/01/2019	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
17/01/2019	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Jan1
14/02/2019	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Feb1
14/02/2019	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Feb2
28/02/2019	“BH”	*L. cuprina*	*L. cuprina*	*L. cuprina*	BH_Mar1
14/03/2019	“BH”	*L. cuprina*	-		
04/04/2019	“BH”	*L. cuprina*	*L. cuprina*	-	
04/04/2019	“BH”	*L. cuprina*	*Pales pavida*		
18/04/2019	“BH”	*L. cuprina*	-	-	
22/11/2018	“BH”	*L. sericata*	*L. cuprina*	*L. cuprina*	BH_ Nov3
22/11/2018	“BH”	*L. sericata*	*L. cuprina*	*L. cuprina*	BH_ Nov2
17/01/2019	“BH”	*L. sericata*	*L. sericata*		
17/01/2019	“BH”	*L. sericata*	*L. sericata*		
14/02/2019	“BH”	*L. sericata*	*L. sericata*		
14/02/2019	“BH”	*L. sericata*	*L. sericata*		
28/02/2019	“BH”	*L. sericata*	*L. cuprina*	*L. cuprina*	BH_Mar2
14/03/2019	“BH”	*L. sericata*	*L. cuprina*	*L. cuprina*	
16/10/2018	“M”	*L. cuprina*	*L. cuprina*	*L. cuprina*	M_Oct1
23/11/2018	“M”	*L. cuprina*	*L. cuprina*		
18/12/2018	“M”	*L. cuprina*	*L. cuprina*	-	
25/12/2018	“M”	*L. cuprina*	*L. cuprina*	-	
22/01/2019	“M”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
22/01/2019	“M”	*L. cuprina*	*L. cuprina*	-	
05/02/2019	“M”	*L. cuprina*	*L. cuprina*	*L. cuprina*	M_Feb1
05/02/2019	“M”	*L. cuprina*	*L. cuprina*	*L. cuprina*	M_Feb2
26/03/2019	“M”	*L. cuprina*	*Chrysomya rufifacies*		
26/03/2019	“M”	*L. cuprina*	*Chrysomya rufifacies*		
26/03/2019	“M”	*L. cuprina*	*L. cuprina*	*L. cuprina*	M_March1
02/04/2019	“M”	*L. cuprina*	*L. cuprina*	-	
02/04/2019	“M”	*L. cuprina*	*Chrysomya rufifacies*		
25/12/2018	“M”	*L. sericata*	*L. sericata*		
25/12/2018	“M”	*L. sericata*	-		

22/01/2019	“M”	*L. sericata*	*L. sericata*		
22/01/2019	“M”	*L. sericata*	*L. sericata*		
05/02/2019	“M”	*L. sericata*	*L. cuprina*	*L. cuprina*	M_Feb3
05/02/2019	“M”	*L. sericata*	*Chrysomya rufifacies*		
26/03/2019	“M”	*L. sericata*	*L. sericata*		
26/03/2019	“M”	*L. sericata*	*L. cuprina*	-	
30/10/2018	“LD”	*L. cuprina*	-	-	
27/11/2018	“LD”	*L. cuprina*	-	-	
11/12/2018	“LD”	*L. cuprina*	*L. sericata*		
22/01/2019	“LD”	*L. cuprina*	*L. cuprina*	-	
22/01/2019	“LD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	LD_Jan1
22/01/2019	“LD”	*L. cuprina*	*L. sericata*		
19/02/2019	“LD”	*L. cuprina*	*L. sericata*		
19/02/2019	“LD”	*L. cuprina*	-		
19/02/2019	“LD”	*L. cuprina*	*L. cuprina*	-	
19/03/2019	“LD”	*L. cuprina*	*L. cuprina*	L. cuprina	
19/03/2019	“LD”	*L. cuprina*	*L. sericata*		
02/04/2019	“LD”	*L. cuprina*	*L. sericata*		
16/04/2019	“LD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	LD_April1
14/05/2019	“LD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	LD_May1
14/05/2019	“LD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	LD_May2
14/05/2019	“LD”	*L. cuprina*	*L. cuprina*	-	
14/05/2019	“LD”	*L. cuprina*	*L. cuprina*	-	
22/01/2019	“LD”	*L. sericata*	-		
22/01/2019	“LD”	*L. sericata*	-		
19/02/2019	“LD”	*L. sericata*	*L. cuprina*	-	
19/02/2019	“LD”	*L. sericata*	*L. sericata*		
19/03/2019	“LD”	*L. sericata*	*L. sericata*		
19/03/2019	“LD”	*L. sericata*	*L. sericata*		
19/03/2019	“LD”	*L. sericata*	*L. sericata*		
24/10/2018	“ST”	*L. cuprina*	*L. cuprina*	-	
21/11/2018	“ST”	*L. cuprina*	*L. sericata*		
21/11/2018	“ST”	*L. cuprina*	*L. cuprina*	-	
28/11/2018	“ST”	*L. cuprina*	*L. cuprina*	-	
28/11/2018	“ST”	*L. cuprina*	*L. sericata*		
05/12/2018	“ST”	*L. cuprina*	-		
05/12/2018	“ST”	*L. cuprina*	-		
05/12/2018	“ST”	*L. cuprina*	-		
16/01/2019	“ST”	*L. cuprina*	*L. cuprina*	*L. cuprina*	ST_Jan1
16/01/2019	“ST”	*L. cuprina*	-		
27/02/2019	“ST”	*L. cuprina*	*L. cuprina*	*L. cuprina*	ST_Jan2
27/02/2019	“ST”	*L. cuprina*	*L. sericata*	*L. cuprina*	
06/03/2019	“ST”	*L. cuprina*	*L. sericata*		
06/03/2019	“ST”	*L. cuprina*	*L. cuprina*	*L. cuprina*	ST_March1
03/04/2019	“ST”	*L. cuprina*	-		
03/04/2019	“ST”	*L. cuprina*	*L. cuprina*	*L. cuprina*	ST_April1

29/05/2019	“ST”	*L. cuprina*	*L. cuprina*	*L. cuprina*	ST_May1
12/12/2018	“ST”	*L. sericata*	*L. sericata*		
12/12/2018	“ST”	*L. sericata*	*L. cuprina*	-	
16/01/2019	“ST”	*L. sericata*	*L. cuprina*	-	
16/01/2019	“ST”	*L. sericata*	*L. cuprina*	-	
27/02/2019	“ST”	*L. sericata*	*L. sericata*		
27/02/2019	“ST”	*L. sericata*	*L. sericata*		
06/03/2019	“ST”	*L. sericata*	*L. sericata*	-	
03/04/2019	“ST”	*L. sericata*	-		
03/04/2019	“ST”	*L. sericata*	*L. cuprina*	*L. cuprina*	ST_April1
03/04/2019	“ST”	*L. sericata*	*L. sericata*		
24/10/2018	“KD”	*L. cuprina*	*L. cuprina*	-	
07/11/2018	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_Nov1
21/11/2018	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_Nov2
28/11/2018	“KD”	*L. cuprina*	*L. cuprina*		
28/11/2018	“KD”	*L. cuprina*	-		
28/11/2018	“KD”	*L. cuprina*	-		
05/12/2018	“KD”	*L. cuprina*	*L. sericata*		
05/12/2018	“KD”	*L. cuprina*	*L. sericata*		
09/01/2019	“KD”	*L. cuprina*	*L. sericata*		
09/01/2019	“KD”	*L. cuprina*	*L. sericata*		
16/01/2019	“KD”	*L. cuprina*	*L. sericata*		
23/01/2019	“KD”	*L. cuprina*	*L. cuprina*		
30/01/2019	“KD”	*L. cuprina*	*L. cuprina*		
06/02/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_Feb1
06/02/2019	“KD”	*L. cuprina*	*L. sericata*		
06/02/2019	“KD”	*L. cuprina*	*L. sericata*		
27/03/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_March1
27/03/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
03/04/2019	“KD”	*L. cuprina*	-		
03/04/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_April1
08/05/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	KD_May1
08/05/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
15/05/2019	“KD”	*L. cuprina*	*L. cuprina*	*L. cuprina*	
28/11/2018	“KD”	*L. sericata*	*L. sericata*		
26/12/2018	“KD”	*L. sericata*	*L. cuprina*	*L. cuprina*	KD_Dec1
02/01/2019	“KD”	*L. sericata*	-		
16/01/2019	“KD”	*L. sericata*	*L. cuprina*	*L. cuprina*	KD_Jan1
16/01/2019	“KD”	*L. sericata*	*L. sericata*		
23/01/2019	“KD”	*L. sericata*	*L. sericata*		
23/01/2019	“KD”	*L. sericata*	*L. sericata*		
30/01/2019	“KD”	*L. sericata*	*L. sericata*		
06/02/2019	“KD”	*L. sericata*	*L. sericata*		
06/02/2019	“KD”	*L. sericata*	*L. sericata*		
06/02/2019	“KD”	*L. sericata*	*L. cuprina*	-	
27/03/2019	“KD”	*L. sericata*	*L. cuprina*	*L. cuprina*	KD_March2

27/03/2019	“KD”	*L. sericata*	*L. sericata*		
03/04/2019	“KD”	*L. sericata*	*L. sericata*		
31/10/2018	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_Oct1
21/11/2018	“S”	*L. cuprina*	*L. sericata*		
30/01/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_Jan1
30/01/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_Jan2
27/02/2019	“S”	*L. cuprina*	*L. sericata*		
27/02/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_Feb1
27/03/2019	“S”	*L. cuprina*	-		
27/03/2019	“S”	*L. cuprina*	-		
03/04/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_April1
03/04/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_April2
10/04/2019	“S”	*L. cuprina*	*L. cuprina*	*L. cuprina*	S_April3
15/05/2019	“S”	*L. cuprina*	*L. cuprina*	-	
30/01/2019	“S”	*L. sericata*	*L. sericata*		
30/01/2019	“S”	*L. sericata*	*L. cuprina*	-	
27/02/2019	“S”	*L. sericata*	-		
27/02/2019	“S”	*L. sericata*	*L. sericata*		
27/02/2019	“S”	*L. sericata*	-		
27/02/2019	“S”	*L. sericata*	*L. cuprina*	*L. cuprina*	S_Feb2
27/03/2019	“S”	*L. sericata*	-		
27/03/2019	“S”	*L. sericata*	*L. sericata*		
27/03/2019	“S”	*L. sericata*	-		
03/04/2019	“S”	*L. sericata*	*L. cuprina*	-	
03/04/2019	“S”	*L. sericata*	*L. cuprina*	-	

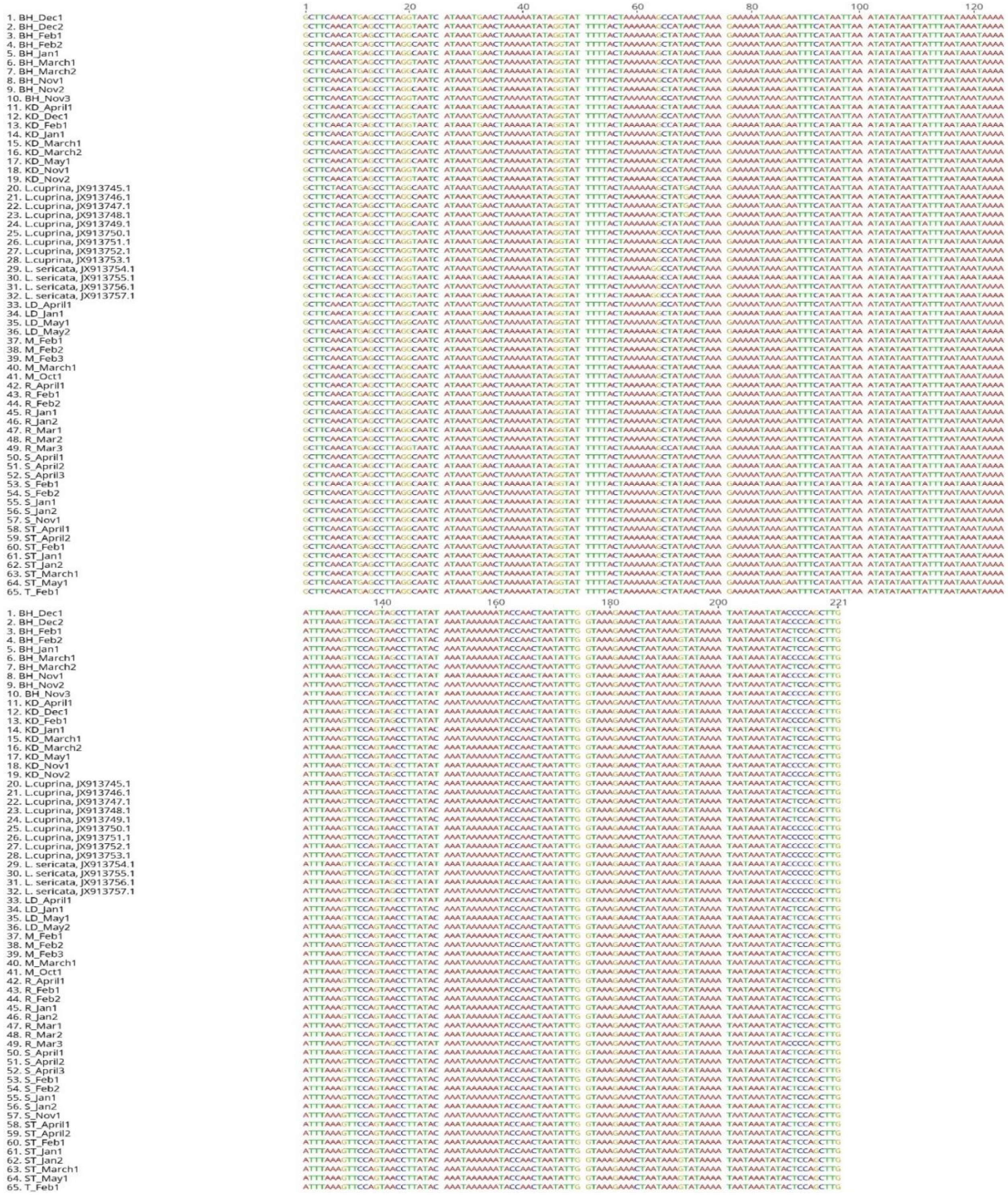


Figure A.2 Illustrates the mutations identified in the mitochondrial dehydrogenase subunit 4 (ND4) gene in nine separate positions for all the New Zealand variant *Lucilia cuprina* sequences compared to previously published sequences of *Lucilia cuprina* (JX913745 - JX913753, NC_019573) and *Lucilia sericata* JX913754 - JX913757

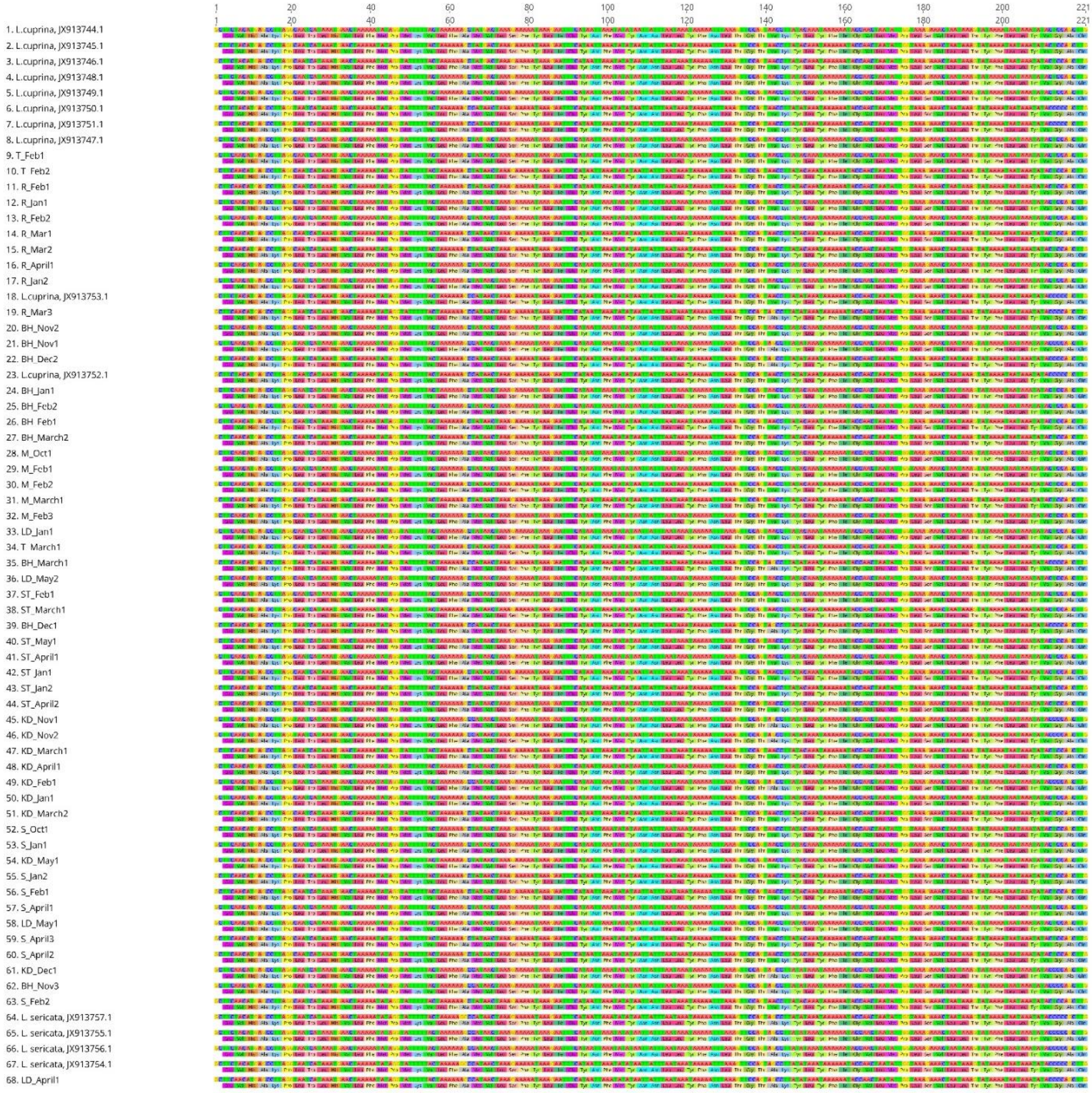


Figure A.3 Translation of proteins in frame 1 in reverse of the mitochondrial dehydrogenase subunit 4 (ND4) sequences for all the New Zealand variants of *Lucilia cuprina* compared to previously published sequences of *Lucilia cuprina* (JX913745 - JX913753, NC_019573) and *Lucilia sericata* JX913754 - JX913757

Appendix B.

Appendix B.1

The following is the output of the Tukey comparisons of each of the trap and bait combinations. In addition, further diagnostic results of each of the models are presented accordingly.

Appendix B.1.1

Rate catch of *Lucilia* spp.

Table B.1 A Tukey contrast of rate catch of *Lucilia* spp. by each trap and bait combinations.

Contrast	Response	SE	95% Lower Confidence interval	95% Upper Confidence interval
Luci [a]	16.35	3.805	8.79	23.91
WAT (Sq) [b]	1.19	0.375	0.45	1.94
WAT (Luci) [c]	3.91	0.816	2.29	5.53
WAT (Liver & SS) [d]	10.00	2.019	5.99	14.01

[a.] LuciTrap® with LuciLure® A, B, C
[b.] Western Australian trap with squid
[c.] Western Australian trap with LuciLure® A, B, C
[d.] Western Australian trap with Sodium Sulphide and sheep liver

Table B.2 Contrasting the difference in the rate catch of *Lucilia* spp. by each trap and bait combinations.

Contrast	Estimate	SE	95% Lower Confidence interval	95% Upper Confidence interval	p-value
Luci [a] – WAT (Sq) [b]	15.15	3.819	5.15	25.153	0.0008
Luci [a] – WAT (Luci) [c]	12.44	3.899	2.26	22.618	0.0102
WAT (Liver & SS) [d] - Luci [a]	6.35	4.254	-4.79	17.489	0.4462
WAT (Sq) [b] – WAT (Luci) [c]	-2.72	0.897	-5.07	-0.369	0.0166
WAT (Liver & SS) [d] – WAT (Sq) [b]	-8.80	2.043	-14.15	-3.453	0.0002
WAT (Liver & SS) [d] - WAT (Luci) [c]	-6.09	2.167	-11.76	-0.412	0.0306

[a.] LuciTrap® with LuciLure® A, B, C
[b.] Western Australian trap with squid
[c.] Western Australian trap with LuciLure® A, B, C
[d.] Western Australian trap with Sodium Sulphide and sheep liver

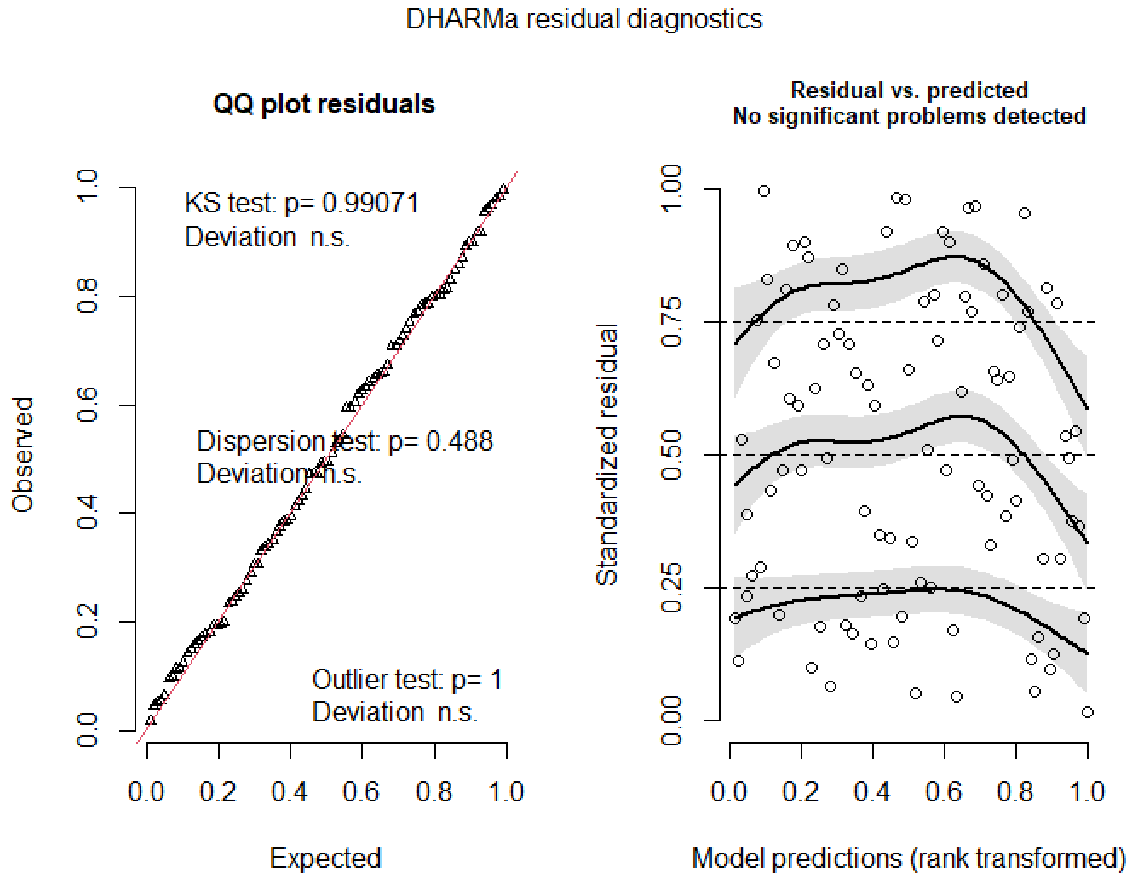


Figure B.1 Q-Q plot and the residuals versus the predicted for the rate catch model of *Lucilia* spp.

Appendix B.1.2

Total of catch of *Lucilia* spp.

Table B.3 A Tukey contrasting the total catch of *Lucilia* spp. by each trap and bait combinations.

Contrast	Response	SE	95% Lower Confidence interval	95% Upper Confidence interval
Luci [a]	3.37	0.789	1.803	4.94
WAT (Sq) [b]	1.27	0.413	0.445	2.09
WAT (Luci) [c]	6.20	1.146	3.922	8.48
WAT (Liver & SS) [d]	8.16	1.322	5.536	10.79

[a.] LuciTrap® with LuciLure® A, B, C

[b.] Western Australian trap with squid

[c.] Western Australian trap with LuciLure A, B, C

[d.] Western Australian trap with Sodium Sulphide and sheep liver

Table B.4 Contrasting the difference in total catch of *Lucilia* spp. by each trap and bait combinations.

Contrast	Estimate	SE	95% Lower Confidence interval	95% Upper Confidence interval	p-value
Luci [a] – WAT (Sq) [b]	2.10	0.834	-0.0803	4.29	0.0633
Luci [a] – WAT (Luci) [c]	-2.83	1.298	-6.2288	0.57	0.1371
WAT (Sq) [b] – WAT (Luci) [c]	-4.79	1.437	-8.5554	-1.03	0.0067
WAT (Liver & SS) [d] - Luci [a]	-4.93	1.158	-7.9648	-1.90	0.0003
WAT (Liver & SS) [d] – WAT (Sq) [b]	-6.90	1.319	-10.3503	-3.44	<.0001
WAT (Liver & SS) [d] - WAT (Luci) [c]	-1.96	1.609	-6.1750	2.25	0.6158

[a.] LuciTrap® with LuciLure® A, B, C

[b.] Western Australian trap with squid

[c.] Western Australian trap with LuciLure A, B, C

[d.] Western Australian trap with Sodium Sulphide and sheep liver

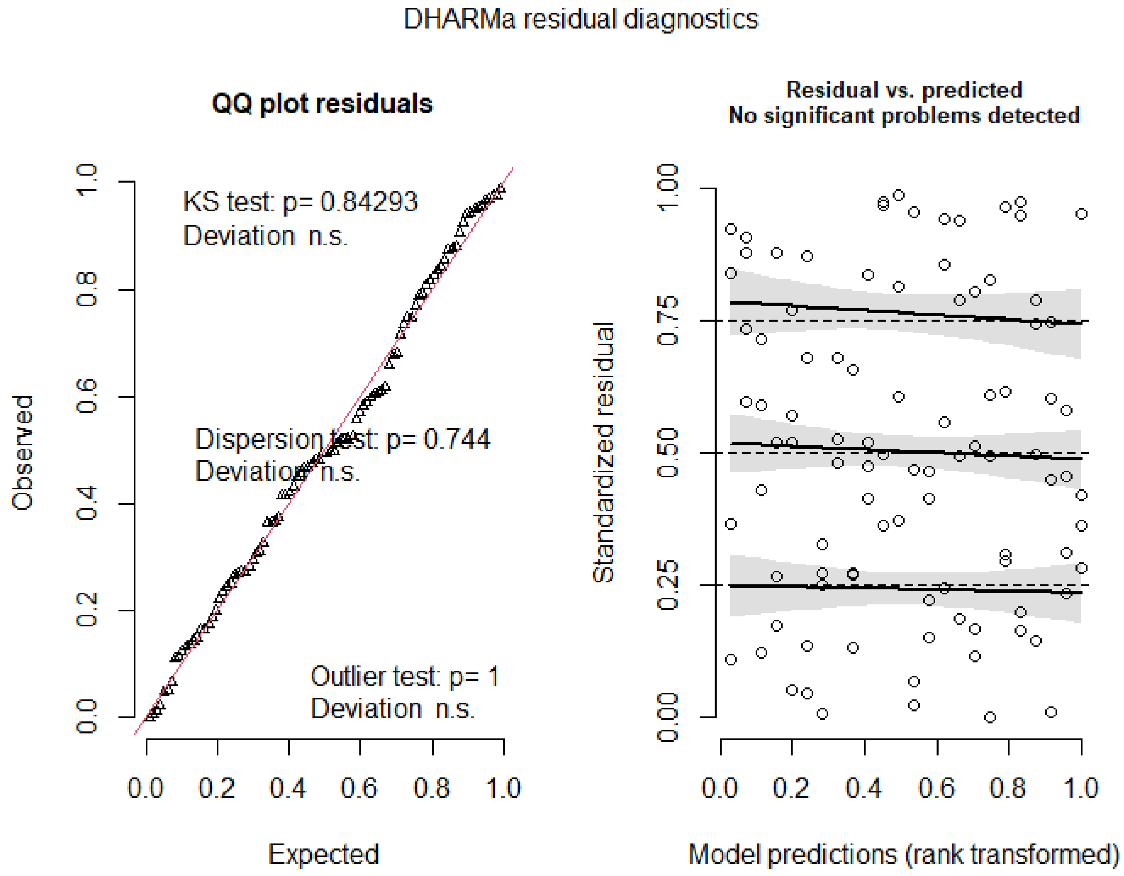


Figure B.2 Q-Q plot of and the residuals versus the predicted the rate of catch model of *Lucilia* spp.

Appendix B.2

The following is the weekly collection data of all specimens caught per week during this study.

A1	B1	C1	D1
B2	C2	D2	A2
C3	D3	A3	B3
D4	A4	B4	C4

Figure B.3 This is the design of the Latin square. Each letter on the Latin square corresponds to the position of the trap and bait combination on the grid. Each number corresponds to the repeat of the trap and bait combination on the grid.

A Luci trap + Luci Lure A, B, C
B Western Australian trap + 50g of Squid
C Western Australian trap + Luci Lure A, B, C
D Western Australian trap + 30% Sodium Sulphide 100ml & 50g of Sheep Liver

Table B.5 The raw catch of dipterans by each trap and bait combination – Week 1

	Week 1										
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora vicina*	*Calliphora quadrameculata*	Sarcophagidae	Faniidae	Muscidae	Tachinidae	Unknown
A1		1					19	3	4		
A2								4	2		
A3							4		4	1	
A4							2		1		
B1	2						325	1	35		
B2									2		
B3							33	1	11		
B4							96		6		
C1							4		9		
C2							11		1		
C3							8		5		
C4				3			124		14		
D1	1						274		16		
D2							354		22		
D3	1						52		5		
D4	4	2					381		28		

Table B.6 The raw catch of dipterans by each trap and bait combination – Week 2

	Week 2												
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora vicina*	*Calliphora quadrameculata*	Sarcophagidae	Faniidae	Muscidae	Tachinidae	Unknown	Staphalinidae	Total
A1	3	2					322		27				354
A2							58		15				73
A3							37		5				42
A4						1	20		8				29
B1	1	1					755		92				849
B2							31		15		2		48
B3							241		59				300
B4	2						688		99				789
C1	5	1					302		35			1	344
C2	1						206		45			1	253
C3	3	1					527		59				590
C4	15	5		2			817		151				990
D1	1	1					974		93				1069
D2	1	2					758		99				860
D3							281		29				310
D4	1	2					690		138				831

Table B.7 The raw catch of dipterans by each trap and bait combination – Week 3

	Week 3											
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora vicina*	Sarcophagidae	Faniidae	Muscidae	Pollenia	Wasp	*Chrysomya megaphala*	Total
A1	1					528		113	0			642
A2	12	5				39		10	0			66
A3	7	9				29		6	0			51
A4	2					6		3	0			11
B1						300		82	0			382
B2						57		22	0			79
B3						184		24	0			208
B4	2	1				17		8	4			32
C1	1	1				250		38	0			290
C2	4	2				373		54	0			433
C3	11	3				484		86	0			584
C4	16	7				744		84	0			851
D1	5	3				94		26	0			128
D2	16	12				430		97	0			555
D3	2	1	1			431		106	0	1	1	543
D4	3	1	1			370		89			1	465

Table B.8 The raw catch of dipterans by each trap and bait combination – Week 4

	Week 4										
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora vicina*	Sarcophagidae	Faniidae	Muscidae	Pollenia	Unknown	Total
A1	6	5				28		12	4		55
A2	2	3				36		22	2		65
A3	1					15		4	3		23
A4						3		1			4
B1						92		68			160
B2	1	1				46		27			75
B3						65		13	1		79
B4						145		41			186
C1	1					149		22			172
C2	3	6				258	2	43	2		314
C3	7	5				190		53			255
C4	9	9				719		119			856
D1	8	4				37		22			71
D2	18	15				86		46			165
D3	9	3				101		28			141
D4	6	3				192		78	2	1	282

Table B.9 The raw catch of dipterans by each trap and bait combination – Week 5

	Week 5											
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora quadrameculata*	*Calliphora vicina*	Sarcophagidae	Faniidae	Muscidae	Pollenia	Unknown	Total
A1	12	5					98		11	1		127
A2	7	3					155		35	2		202
A3	4	1					17		18	4	3	47
A4	1						17		7	4		29
B1	2						78		61			141
B2		1					38		31			70
B3	1						24		27			52
B4	1						112		37			150
C1	3						157		43			203
C2	7	2			1		311		78			399
C3	12	5					448		69			534
C4	12	4					565		184			765
D1	5	2					13		24	1		45
D2	6	4					59		13			82
D3	11	4	2				53		36			106
D4	10	3		1			153		49	1		217

Table B.10 The raw catch of dipterans by each trap and bait combination – Week 6

	Week 6										
	Lucilia cuprina	*Lucilia sericata*	*Chrysomya rufifacies*	*Calliphora stygia*	*Calliphora vicina*	Sarcophagidae	Faniidae	Muscidae	Pollenia	Unknown	Total
A1	10	5			1	42		5	1		64
A2	2					20		6	2		30
A3						5		6		1	12
A4						2		1			3
B1	2					102		30			134
B2						44		14			58
B3						18		28			46
B4	1		1			68		18			88
C1						59		18			77
C2	4	3				218		58			283
C3	7	5				72		54			138
C4	4	2				228		79			313
D1	1	3				23		25			52
D2	14	11				26		30			81
D3	27	6	4			79		68			184
D4	1	2	1			47		3			54

Appendix B.3

The following is the R code used for the analysis conducted in Chapter Three

```
# packages #
library(dplyr)
library(ggplot2)
library(cowplot)
library(glmmTMB)
library(emmeans)
library(plotly)
library(highcharter)
library(performance)
library(MASS)

### Data set up
Trapping_V4<-Trapping_V4_final
Trapping_V4$Trap <- as.factor(Trapping_V4$Trap)
Trapping_V4$Post <- as.factor(Trapping_V4$Post)
Trapping_V4$Bait <- as.factor(Trapping_V4$Bait)
str(Trapping_V4)


# models
# Cuprina
# best model
fit_zipoisson_3 <- glmmTMB(LCLS ~  Trap + Week  + Sum_Rainfall +
offset(log(Total)),ziformula=~1,family=nbinom1,data=Trapping_V4)
summary(fit_zipoisson_3)
r2_zeroinflated(fit_zipoisson_3,method = c("correlation"))

# LCLS models
# poisson
LCLS_1 <- glm(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),family=poisson,data=Trapping_V4)
LCLS_2 <- glm(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),family=quasipoisson,data=Trapping_V4)
LCLS_3 <- glm.nb(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),data=Trapping_V4)
#
LCLS_1 <- glmmTMB(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),family=poisson,data=Trapping_V4)
LCLS_2 <- glmmTMB(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),family=nbinom1,data=Trapping_V4)
LCLS_3 <- glmmTMB(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),family=nbinom2,data=Trapping_V4)
LCLS_4 <- glmmTMB(LCLS ~  Trap + Week  + Sum_Rainfall +
offset(log(Total)),ziformula=~1,family=poisson,data=Trapping_V4)
LCLS_5 <- glmmTMB(LCLS ~  Trap + Week  + Sum_Rainfall +
offset(log(Total)),ziformula=~1,family=nbinom1,data=Trapping_V4)
LCLS_6 <- glmmTMB(LCLS ~  Trap + Week  + Sum_Rainfall +
offset(log(Total)),ziformula=~1,family=nbinom2,data=Trapping_V4)
```

```
# AIC
AIC(LCLS_1,LCLS_2,LCLS_3,LCLS_4,LCLS_5,LCLS_6)
# BIC
BIC(LCLS_1,LCLS_2,LCLS_3,LCLS_4,LCLS_5,LCLS_6)
# R2
r2_zeroinflated(LCLS_3,method = c("correlation"))

# best model
LCLS_3 <- glm.nb(LCLS ~ Trap + Week + Sum_Rainfall +
offset(log(Total)),data=Trapping_V4)
# show non-signficance of average temperature, maximum temperature, and minimum
temperature
LCLS_3a <- glm.nb(LCLS ~ Trap + Week + Tmax  + Sum_Rainfall +
offset(log(Total)),data=Trapping_V4)
summary(LCLSa)
LCLS_3b <- glm.nb(LCLS ~ Trap + Week + Tmin  + Sum_Rainfall +
offset(log(Total)),data=Trapping_V4)
summary(LCLSb)
LCLS_3c <- glm.nb(LCLS ~ Trap + Week + Tavg  + Sum_Rainfall +
offset(log(Total)),data=Trapping_V4)
summary(LCLSc)

# test if there are interactions between the following with cuprina_3 design:
# - average temperature and rainfall
# - maximum temperature and rainfall
# - minimum temperature and rainfall
# - trap and rainfall

LCLS_3a1 <- glm.nb(LCLS ~ Trap + Week + (Sum_Rainfall) *  Tmax   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3a1)
LCLS_3b1 <- glm.nb(LCLS ~ Trap + Week + (Sum_Rainfall) * Tmin   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3b1)
LCLS_3c1 <- glm.nb(LCLS ~ Trap + Week + (Sum_Rainfall) * Tavg   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3c1)

LCLS_3c1 <- glm.nb(LCLS ~ Trap + Week + (Sum_Rainfall) * Tavg   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3c1)

###### three-way interaction
LCLS_3a2 <- glm.nb(LCLS ~ Week + (Trap) * (Sum_Rainfall) *  Tmax   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3a2)
LCLS_3b2 <- glm.nb(LCLS ~ Week + (Trap) * (Sum_Rainfall) * Tmin   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3b2)
LCLS_3c2 <- glm.nb(LCLS ~ Week + (Trap) * (Sum_Rainfall) * Tavg   +
offset(log(Total)),data=Trapping_V4)
summary(LCLS_3c2)

#
AIC(LCLS_3a2, LCLS_3b2,LCLS_3c2,LCLS_3a1, LCLS_3b1,LCLS_3c1)
```

```
BIC(LCLS_3a2, LCLS_3b2,LCLS_3c2,LCLS_3a1, LCLS_3b1,LCLS_3c1)
compare_peformance(LCLS_3a2, LCLS_3b2,LCLS_3c2,LCLS_3a1, LCLS_3b1,LCLS_3c1,
rank = TRUE)

# model output
tab_model(LCLS_3c2,transform = NULL, auto.label = FALSE)
# r2
r2_zeroinflated(LCLS_3c2,method = c("correlation"))

#####
Box plots for results of model

## L cuprina
emmeans(LCLS_3c2,pairwise ~ Trap ,transform = "response",adjust="tukey")
# to add confidence interval to tukey adjustment
a<-emmeans(LCLS,pairwise ~ Trap ,transform = "response",adjust="tukey")
confint(a, adjust.method = "tukey", level = 0.95)

# note i have copied the output, put it in excel and then reimported it into R - hence the different
data point

LCLS_confidence <- ggplot(data = LCLS_1, aes(x= Trap, Y = Rate)) + geom_point(data =
LCLS_1, aes(x= Trap, y = Rate), size=4) + geom_errorbar(aes(x = Trap, ymin = Lower_CL,
ymax = Upper_CL), width =0.1, size=0.5) + labs(x = "Trap",y = "Estimated rate of catch of
Lucilia cuprina")
LCLS_confidence

###
Diagnostic plots
library(DHARMa)
simulationOutput <- simulateResiduals(fittedModel = LCLS_3c2, plot = TRUE)
testResiduals(simulationOutput)
plot(simulationOutput, quantreg = T)

########
Weather plots for trapping study
library(highcharter)
library(dplyr)

airport_weather_graph_2<- highchart() %>% hc_chart(type = "line") %>%
  hc_legend(enabled = TRUE) %>%
  hc_legend(align = "Right", verticalAlign = "top", floating = TRUE,borderRadius = 10 ,
backgroundColor = "white" , borderWidth = 1, layout = "vertical", x=50, y =-5)%>%
  hc_tooltip(shared = TRUE) %>%
  hc_yAxis_multiples(list(title = list(text = "Average Temperature(℃) per
week"),showFirstLabel = FALSE, showLastLabel = FALSE, opposite = FALSE),
                    list(title = list(text = "Average Rainfall (mm) per week"),showFirstLabel = TRUE,
opposite = TRUE)) %>%
  hc_xAxis(categories =
c('1','2','3','4','5','6','7','8','9','10','11','12','13','14','15','16','17','18','19','20','21','22','23','24','25','26','
27','28','29','30','31','32','33','34','35','36','37','38','39','40','41','42','43'), title = list(text =
"Days"),tickmarkPlacement = "on") %>%
  hc_add_series(data = c(Airport_weather_1$Tavg),name = "Average
Temperature",showInLegend = TRUE, title = list(text = "Average Temperature")) %>%
```

```
  hc_add_series(data = c(Airport_weather_1$Tmax),name = "Maximum
Temperature",showInLegend = TRUE, title = list(text = "Maximum Temperature")) %>%
  hc_add_series(data = c(Airport_weather_1$Tmin),name = "Minimum
Temperature",showInLegend = TRUE, title = list(text = "Minimum Temperature")) %>%
  hc_add_series(data = c(Airport_weather_1$Rainfall),yAxis = 1,type = "column", name =
"Rainfall",showInLegend = TRUE, title = list(text = "Rainfall"))
airport_weather_graph_2

############
Fly count plots.

Trapping_allflies2 <-highchart() %>%hc_chart(type ="column") %>%
  +   hc_title(text = "C") %>%
  +   hc_legend(align = "right", layout = "vertical",floating = FALSE, verticalAlign = "middle")
%>%
  +   hc_legend(enabled = TRUE) %>%
  +   hc_xAxis(categories = c('Day 1-7', 'Day 7-14', 'Day 14-21', 'Day 21-28', 'Day 28-35', 'Day
35-42'), tickmarkPlacement = "on") %>%
  +   hc_yAxis(title = list(text = "Total flycatch per week per week")) %>%
  +   hc_plotOptions(line = list (datalabels = list(emabled = TRUE), enableMouseTracking =
FALSE))%>%
  +   hc_series(list (name = "Luci", data = c(Trapping_allflies1$TrapA)),
              +           list (name = "WATrap (sq)", data = c(Trapping_allflies1$TrapB)),
              +           list (name = "WATrap (Luci)", data = c(Trapping_allflies1$TrapC)),
              +           list (name = "WATrap (Liver & SS)", data = c(Trapping_allflies1$TrapD)))
Trapping_allflies2

Trapping_LCLS2 <-highchart() %>%hc_chart(type ="column") %>%
  hc_title(text = "A") %>%
  hc_legend(align = "right", layout = "vertical",floating = FALSE, verticalAlign = "middle")
%>%
  hc_legend(enabled = TRUE) %>%
  hc_xAxis(categories = c('Day 1-7', 'Day 7-14', 'Day 14-21', 'Day 21-28', 'Day 28-35', 'Day 35-
42'), tickmarkPlacement = "on") %>%
  hc_yAxis(title = list(text = "Catch of Lucilia spp. per week")) %>%
  hc_plotOptions(line = list (datalabels = list(emabled = TRUE), enableMouseTracking =
FALSE))%>%
  hc_series(list (name = "Luci", data = c(LCLS_week1$TrapA)),
          list (name = "WATrap (sq)", data = c(LCLS_week1$TrapB)),
          list (name = "WATrap (Luci)", data = c(LCLS_week1$TrapC)),
          list (name = "WATrap (Liver & SS)", data = c(LCLS_week1$TrapD)))
Trapping_LCLS2

####
```

Appendix C.

Appendix C.1

Appendix C.1.1

Imputation for missing data – “ST” and “S”

```
library(forecast)
library(imputeTS)
#### “ST”
STcuprina2 <-
ts(c(1,0,6,3,11,9,17,6,12,22,7,NA,NA,NA,54,85,192,177,158,145,217,99,9,4,15,8,5,0,5,2,2,1,0,
1,0))
STsericata2 <-
ts(c(0,0,0,0,0,0,0,3,0,8,8,NA,NA,NA,32,42,139,167,63,65,93,39,4,4,14,5,0,0,0,0,0,0,0,0,0))
imp<-na_interpolation(STcuprina2, option = "stine")
imp
ggplot_na_imputations(STcuprina2, imp)
imp<-na_interpolation(STsericata2, option = "stine")
imp
ggplot_na_imputations(STsericata2, imp)

#### “S”
Scuprina <-
ts(c(0,0,0,1,0,0,1,0,0,0,1,NA,NA,NA,1,4,37,34,105,124,417,222,59,12,20,5,1,1,1,0,0,1,0,0,0))
SSericata <-
ts(c(0,0,0,0,0,0,0,0,1,0,0,NA,NA,NA,1,2,18,12,57,64,144,114,40,12,5,6,0,0,0,0,0,0,0,0,0))
imp<-na_interpolation(Scuprina, option = "stine")
imp
ggplot_na_imputations(Scuprina, imp)
imp<-na_interpolation(SSericata, option = "stine")
imp
ggplot_na_imputations(SSericata, imp)
```

Appendix C.1.2

The code used to calculate Daylength.

```
###
library(insol)

### “T”
T_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-40.#####,175.#####, T_daylength,1)
data.frame(T_daylength)

### “M”
M_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-38. #####,175. #####, M_daylength,1)
data.frame(M_daylength)

## “LD”
LD_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-37.47651,175.34245, LD_daylength,1)
data.frame(LD_daylength)

## “KD”
KD_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-42.73434,175.34245, KD_daylength,1)
data.frame(KD_daylength)


## “BH”
BH_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-42.73434,175.34245, BH_daylength,1)
data.frame(BH_daylength)

## “R”
R_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-40.83097,175.62093, R_daylength,1)
data.frame(R_daylength)

## “S”
S_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-42.96885873,175.62093, S_daylength,1)
data.frame(S_daylength)

## “ST”
ST_daylength =JD(seq(ISOdate(2018,7,1),ISOdate(2019,7,31),by='week'))
daylength(-42.78993,175.62093, ST_daylength,1)
data.frame(ST_daylength)
```

Appendix C.2

Table C.1 The dates of the first cases of flystrike on each farm as recorded by the farm managers.

Farm	2018/2019	2019/2020	2020/2021
“LD”	16th November 2018	6th November 2019	
“M”	22nd November 2018	10th November 2019	
“BH”	16th November 2018	6th November 2019	
“R”	9th December 2018	4th December 2019	15th December 2020
“T”	10th December 2018	4th December 2019	13th December 2020
“ST”	5th November 2018		
“KD”	20th November 2018		
“S”	15th December 2018		

Appendix C.3

Graphs outlining the association of various climatic variables with the weekly catch of *Lucilia* spp. on each farm during the 2018/2019 season.

Appendix C.3.1

Associations between 10 cm Soil Temperature and Photoperiod with the weekly catch *Lucilia* spp.

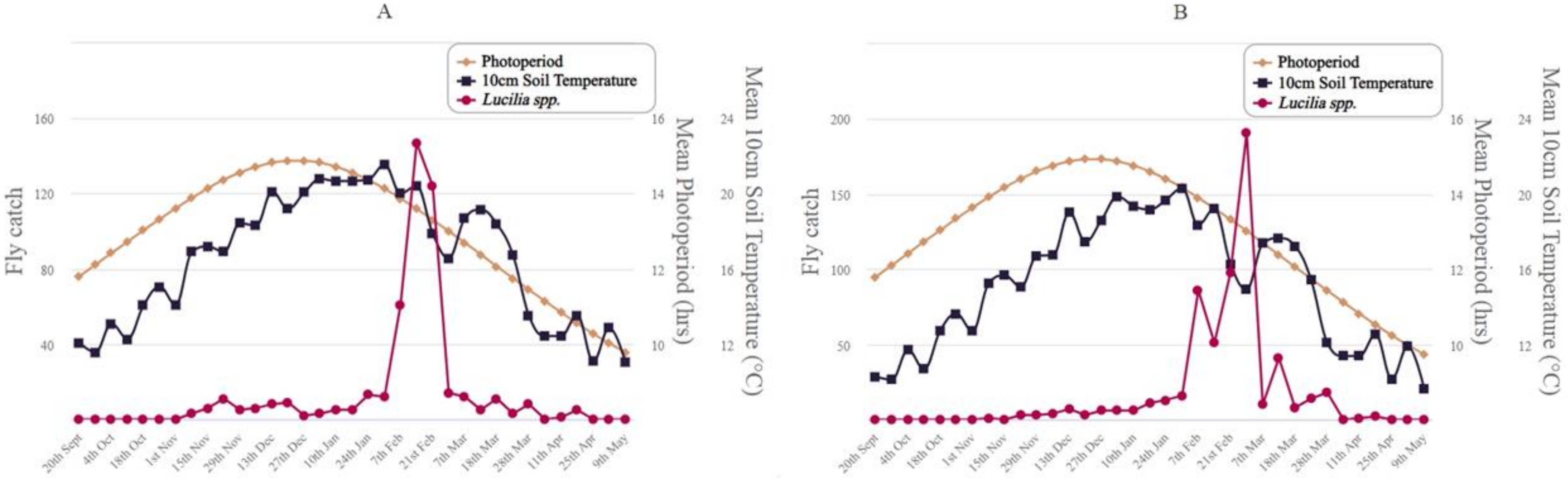


Figure C.1 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on “T” (Figure C.1 A) and “R” (Figure C.1 B).

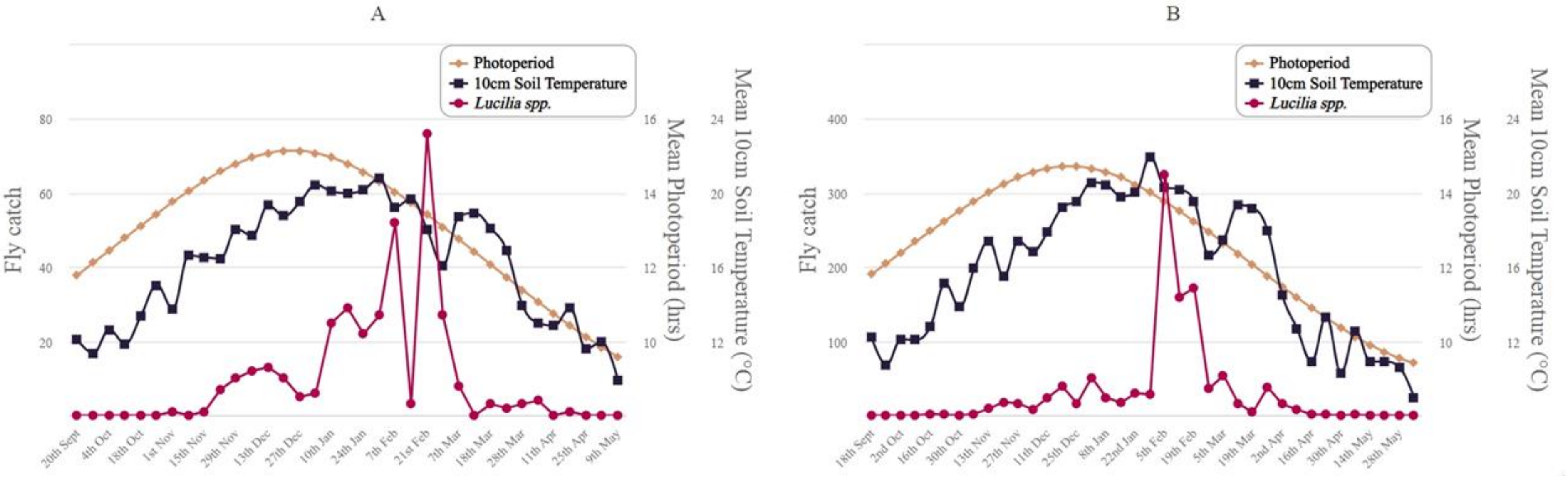


Figure C.2 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on "BH" (Figure C.2 A) and "M" (Figure C.2 B).

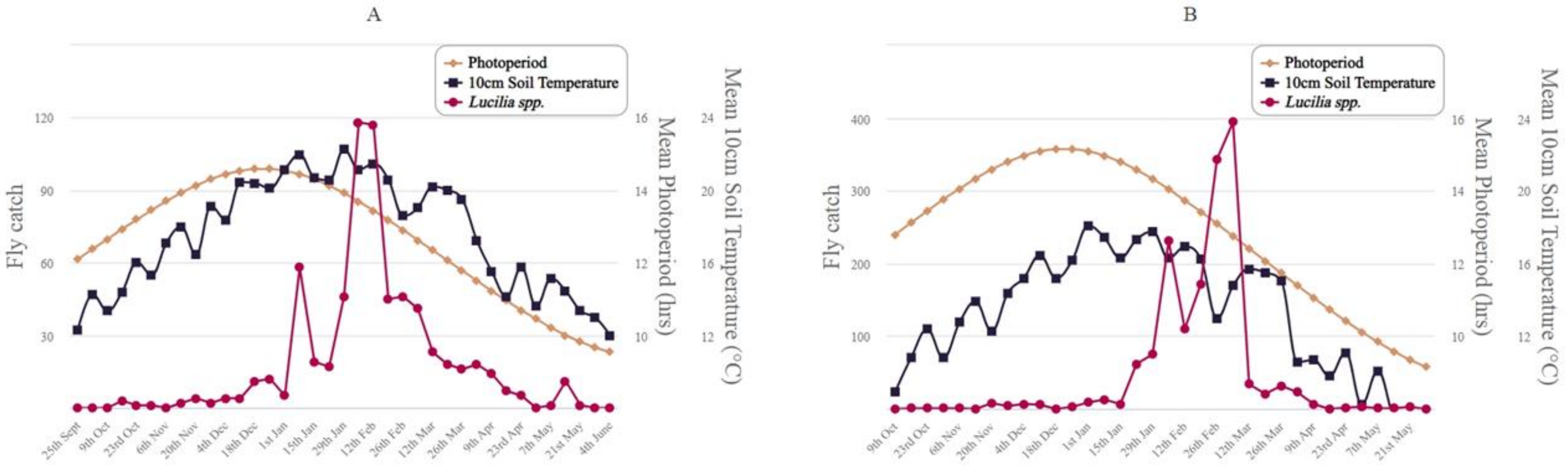


Figure C.3 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on "LD" (Figure C.3 A) and "KD" (Figure C.3 B).

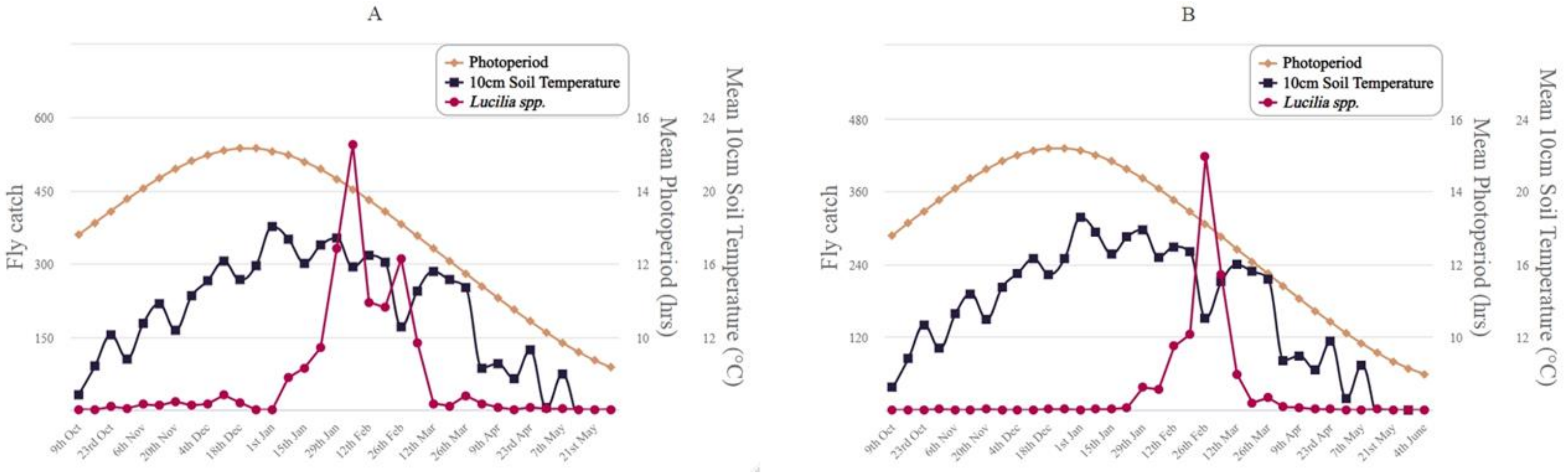


Figure C.4 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of Lucilia spp. for the 2018/2019 season on "ST" (Figure C.4 A) and "S" (Figure C.4 B).

Appendix C.3.2

Association between Relative Humidity and Rainfall with the weekly catch of *Lucilia* spp.

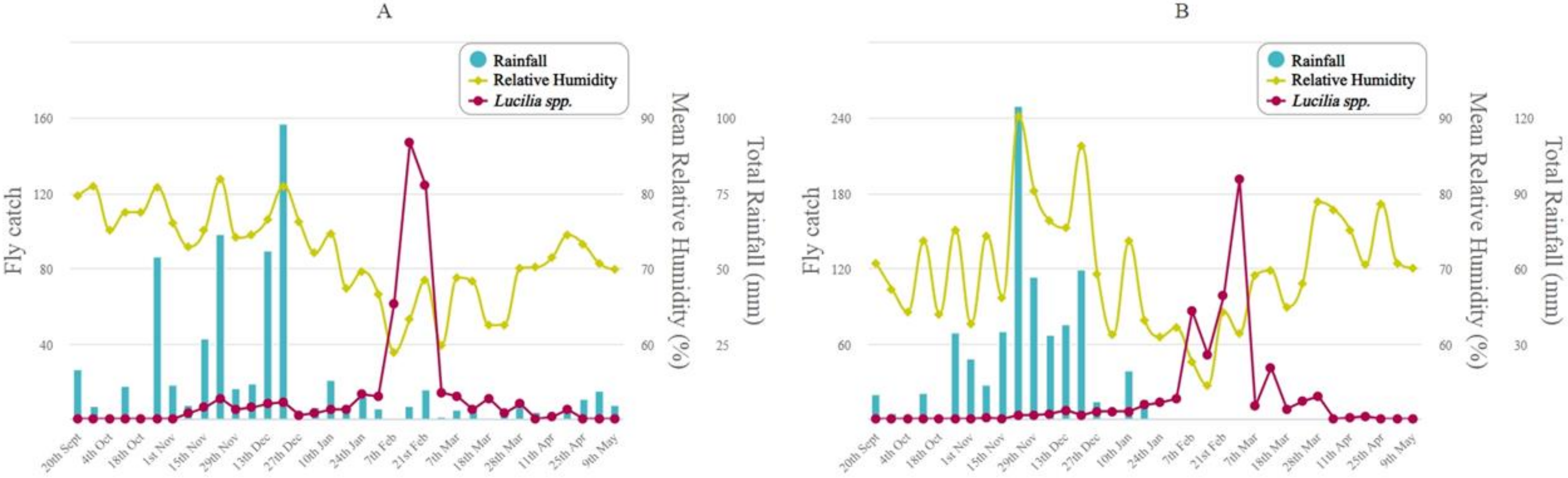


Figure C.5 The association between total rainfall, mean relative humidity, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on “T” (Figure C.5 A) and “R” (Figure C.5 B).

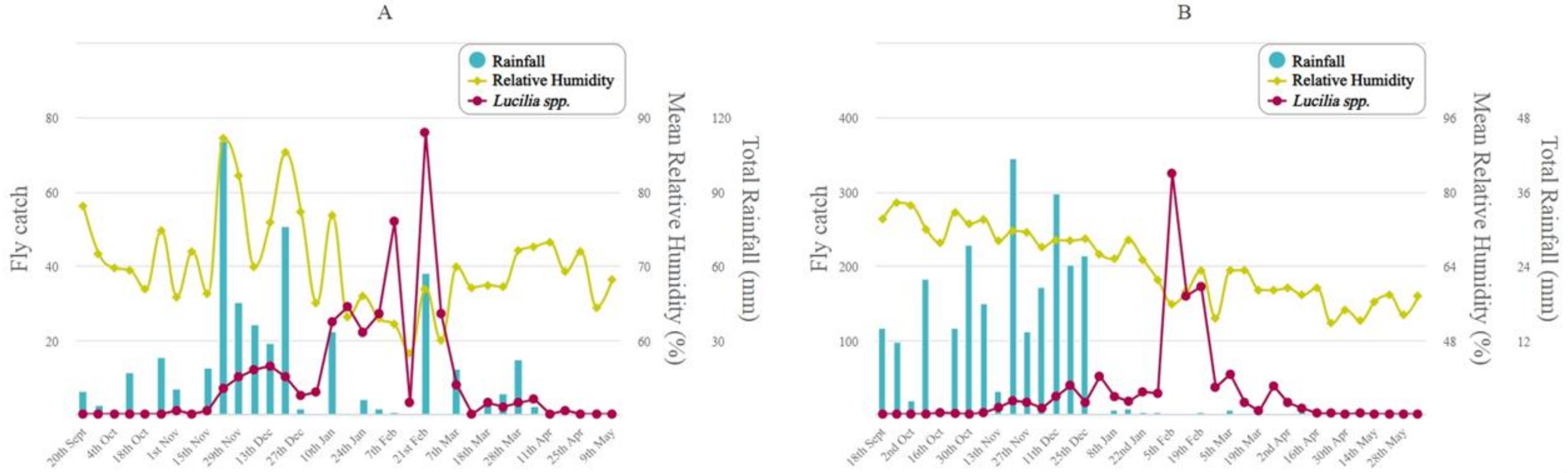


Figure C.6 The association between total rainfall, mean relative humidity, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on “BH” (Figure C.6 A) and “M” (Figure C.6 B).

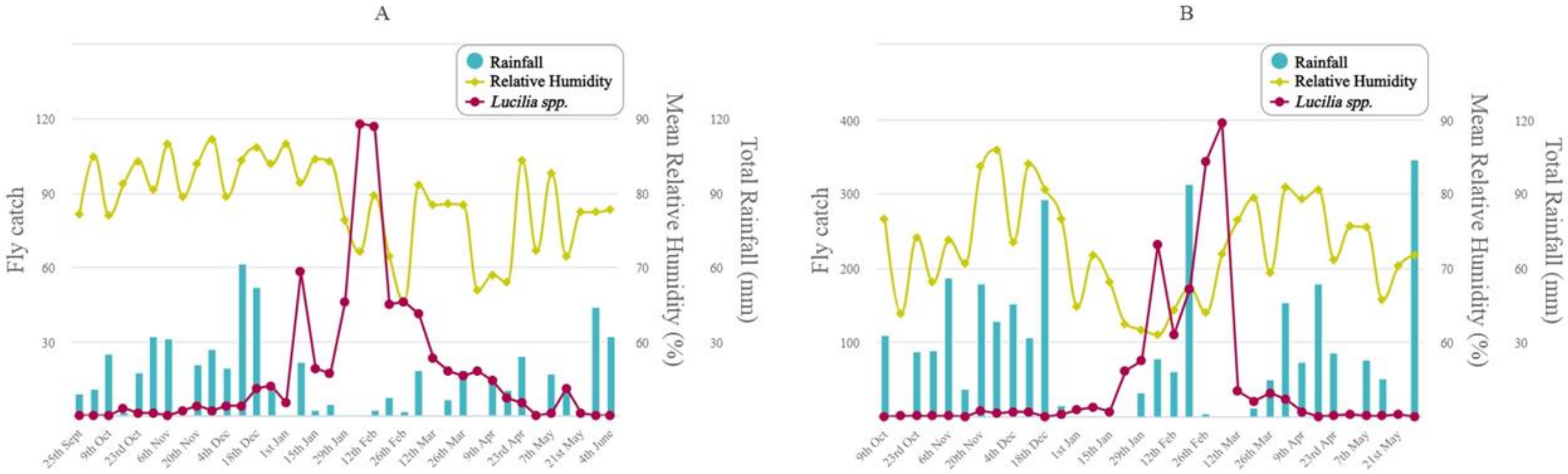


Figure C.7 The association between total rainfall, mean relative humidity, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on "LD" (Figure C.7 A) and "KD" (Figure C.7 B).

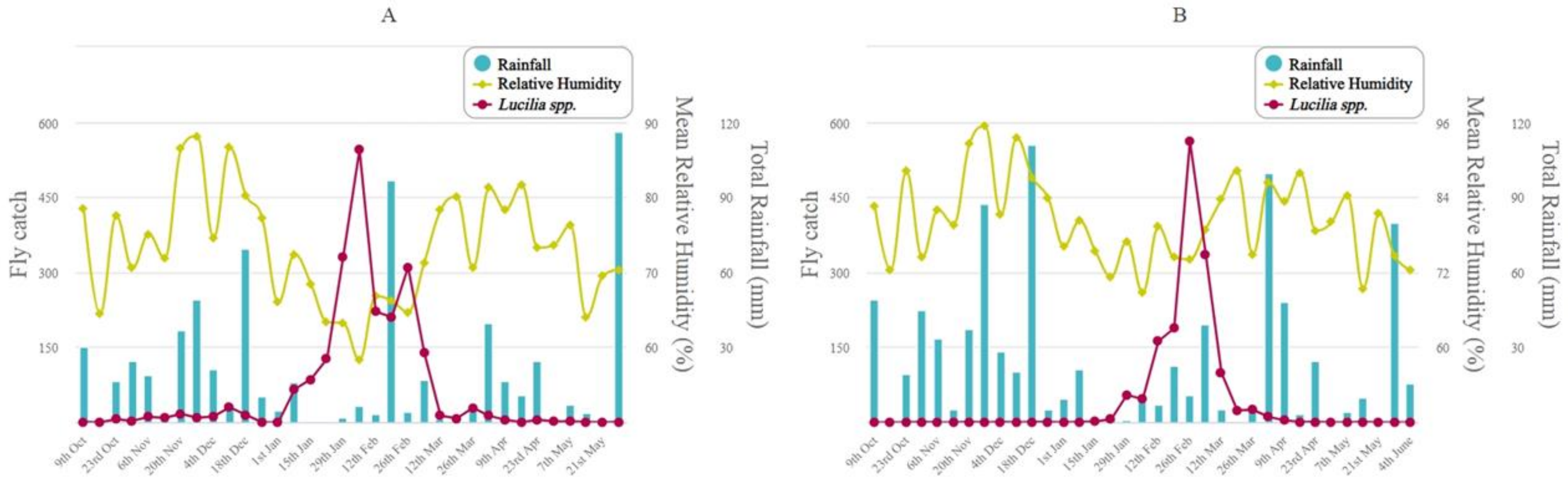


Figure C.8 The association between mean total rainfall, mean relative humidity, and the weekly catch of *Lucilia* spp. for the 2018/2019 season on "ST" (Figure C.8 A) and "S" (Figure C.8 B).

Appendix C.3.3

Association between Mean Wind speed with the weekly catch of *Lucilia* spp.

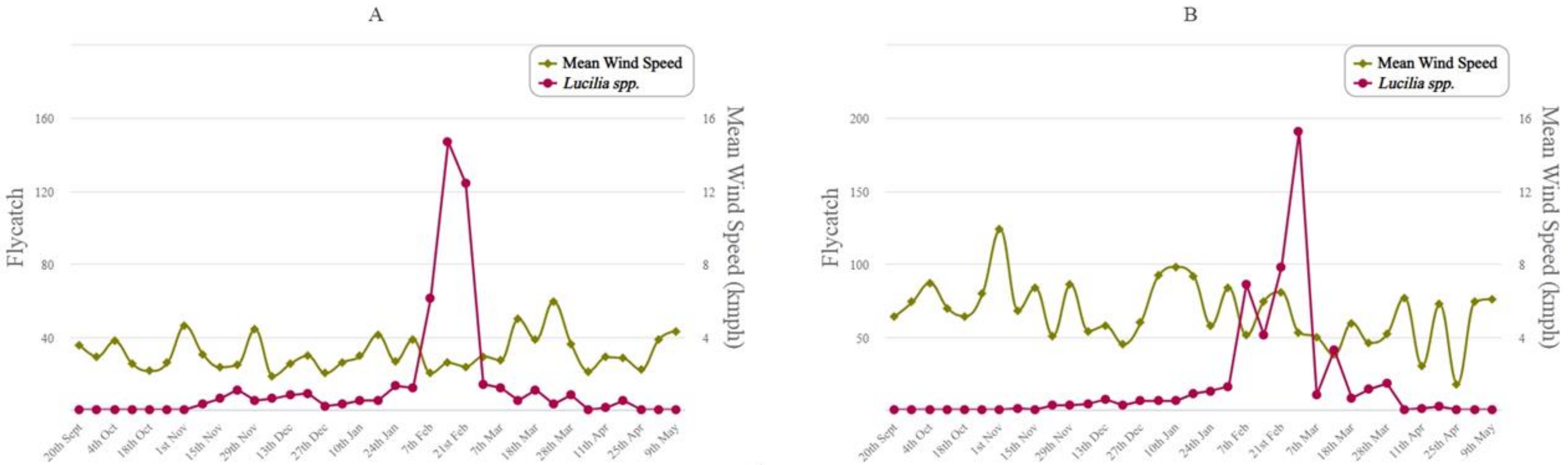


Figure C.9 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2018/2019 season in “T” (Figure C.9 A) and “R” (Figure C.9 B).

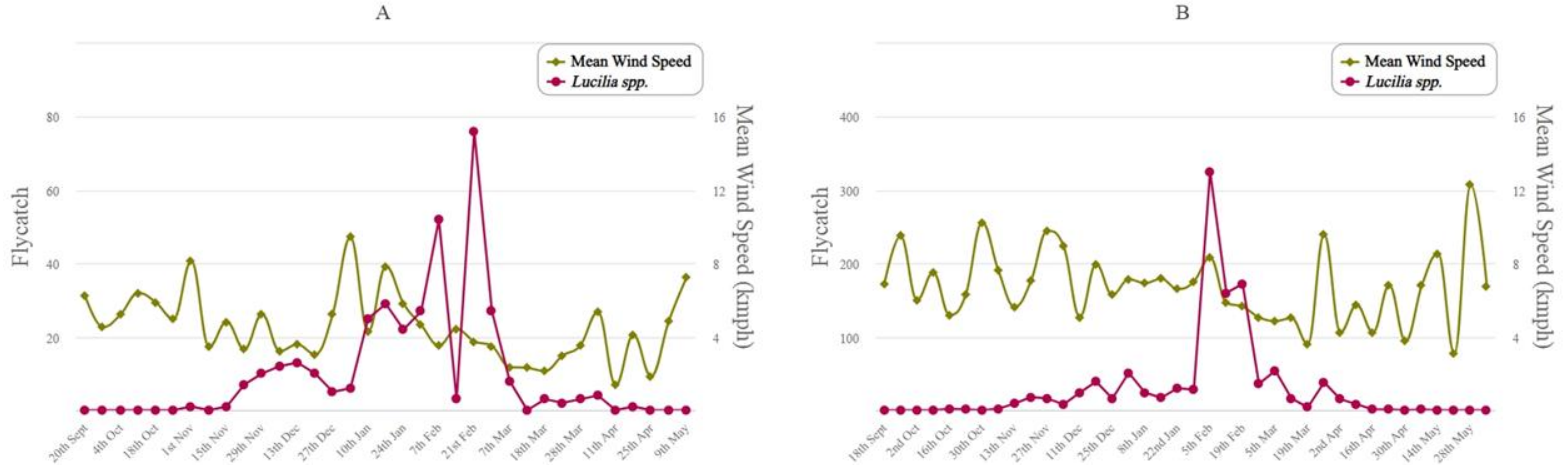


Figure C.10 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2018/2019 season in "BH" (Figure C.10 A) and "M" (Figure C.10 B).

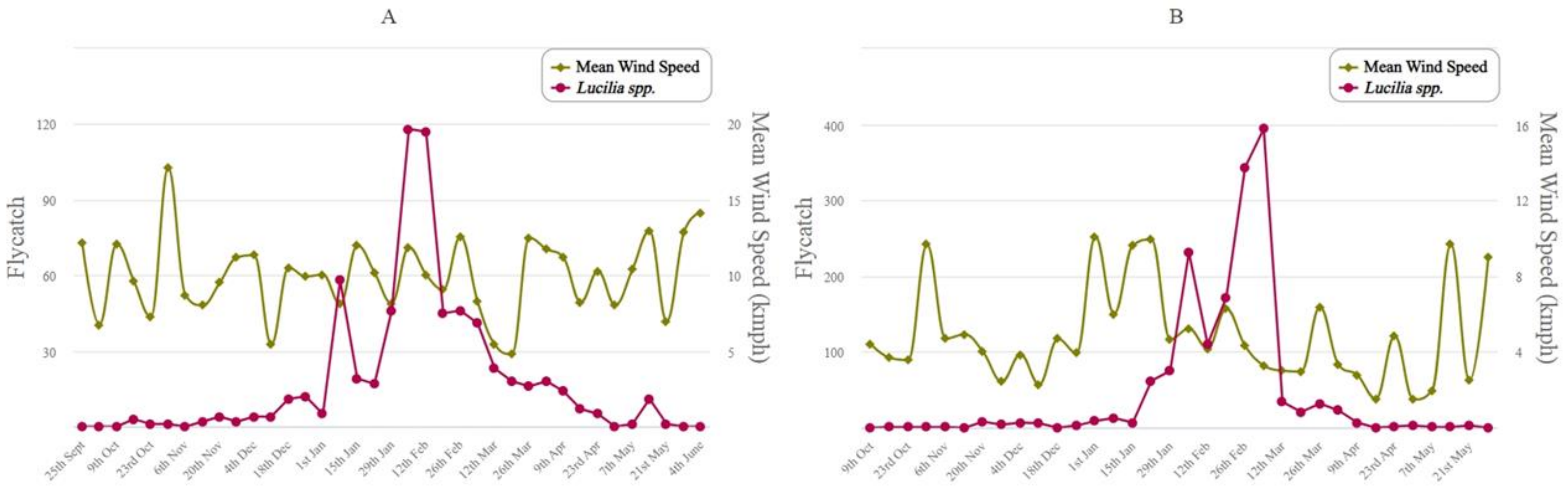


Figure C.11 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2018/2019 season in “LD” (Figure C.11 A) and “KD” (Figure C.11 B).

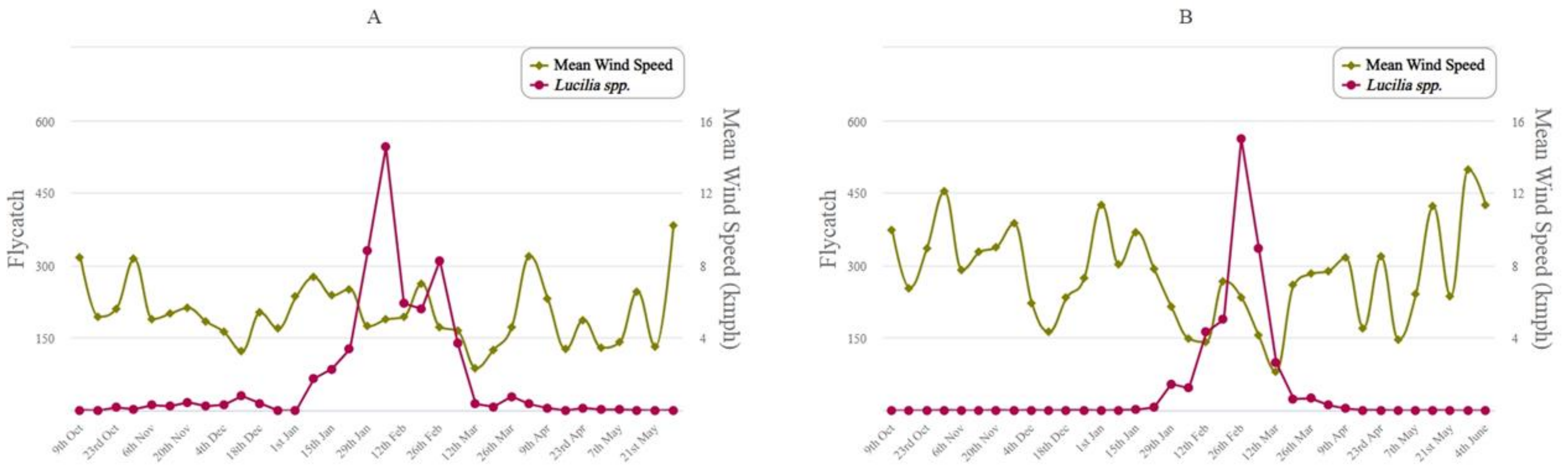


Figure C.12 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2018/2019 season in "ST" (Figure C.12 A) and "S" (Figure C.12 B).

Appendix C.3.4

Association between Mean, Maximum and Minimum Temperature with the weekly catch of *Lucilia* spp.

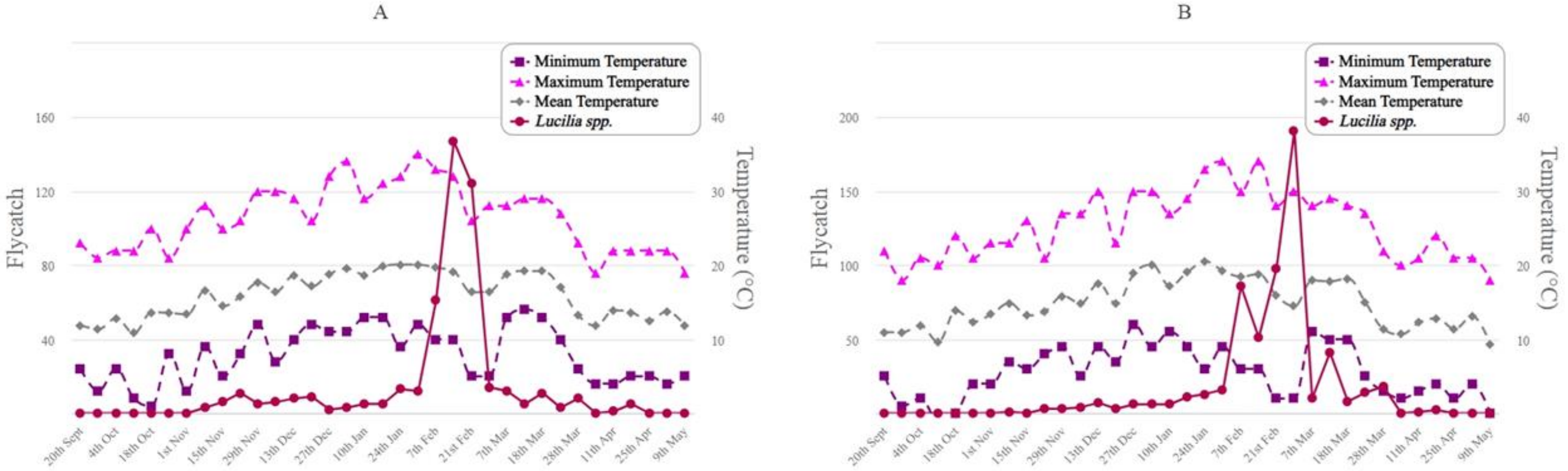


Figure C.13 The association between Mean, Maximum and Minimum Temperature with the weekly catch of *Lucilia* spp. for the 2018/2019 season in "T" (Figure C.13 A) and "R" (Figure C.13B).

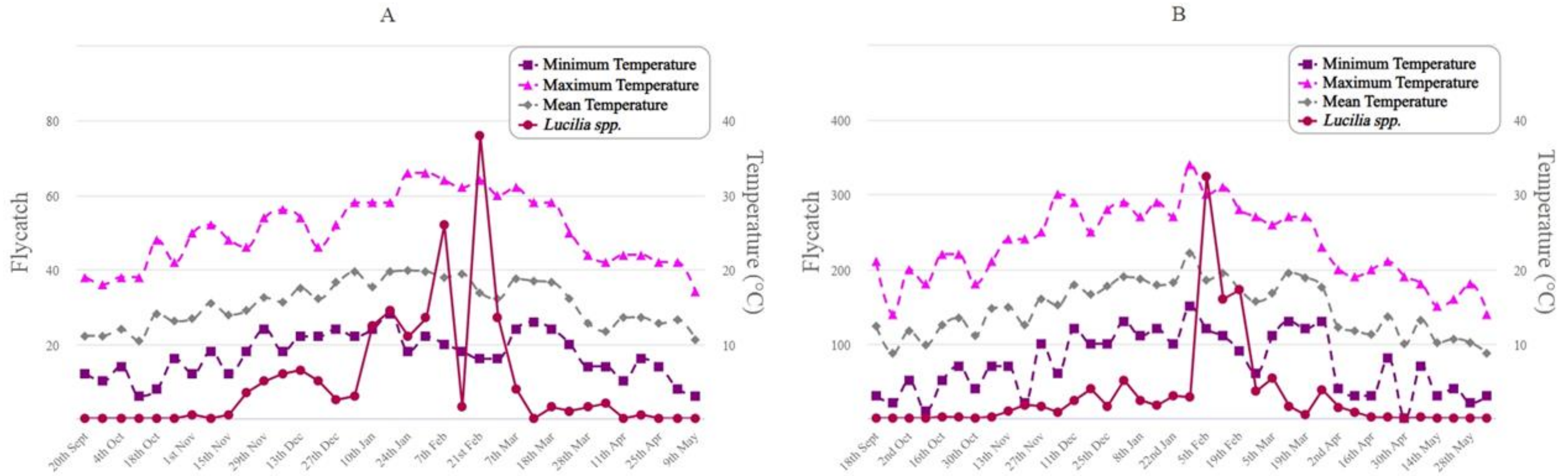


Figure C.14 The association between Mean, Maximum and Minimum Temperature with the weekly catch of *Lucilia* spp. for the 2018/2019 season in “BH” (Figure C.14 A) and “M” (Figure C.14 B).

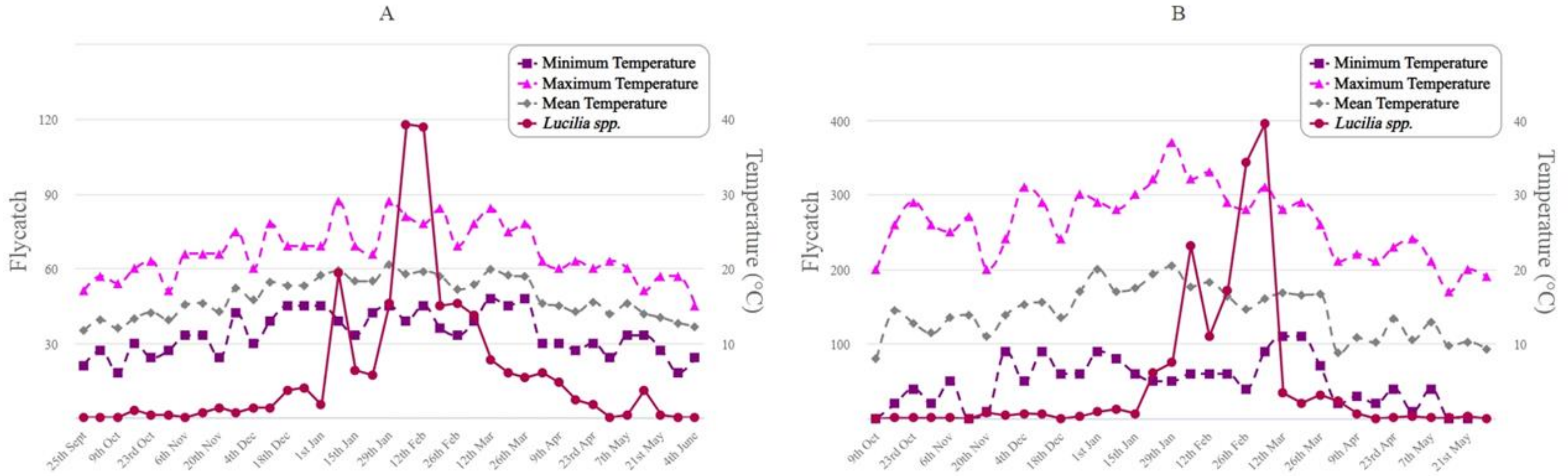


Figure C.15 The association between Mean, Maximum and Minimum Temperature with the weekly catch of *Lucilia* spp. for the 2018/2019 season in "LD" (Figure C.15 A) and "KD" (Figure C.15 B).

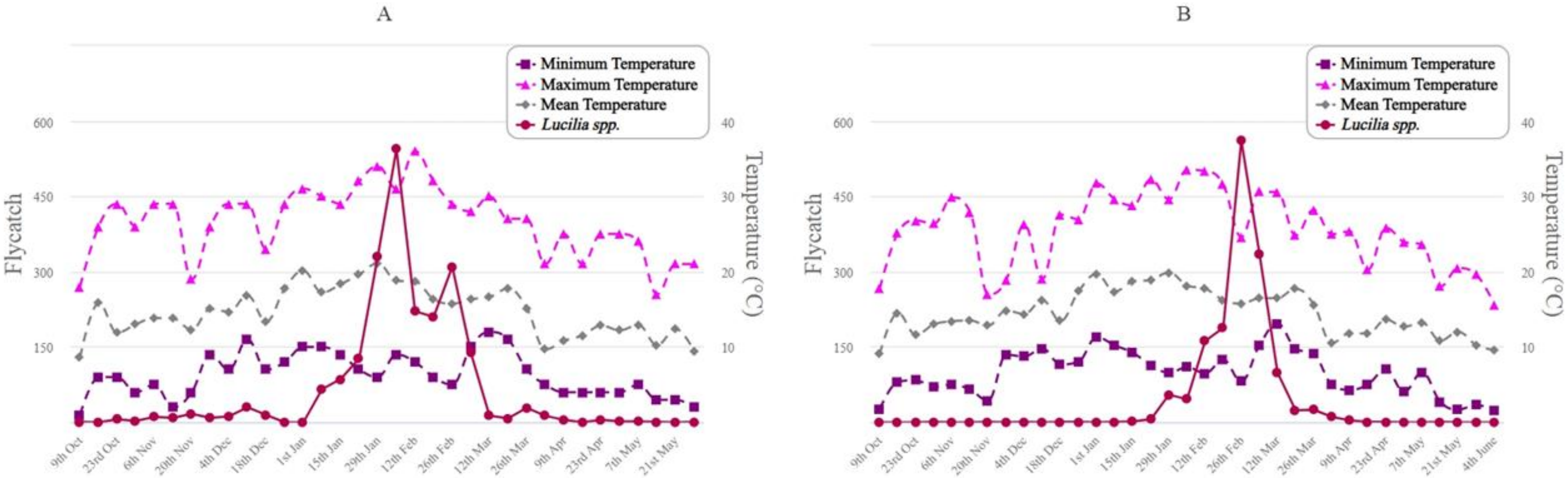


Figure C.16 The association between Mean, Maximum and Minimum Temperature with the weekly catch of *Lucilia* spp. for the 2018/2019 season in “ST” (Figure C.16 A) and “S” (Figure C.16 B)

Appendix C.4

Graphs outlining the association of various climatic variables with the weekly catch of *Lucilia* spp. on each farm during the 2019/2020 season.

Appendix C.4.1

Associations between 10 cm Soil Temperature and Photoperiod with the weekly catch of *Lucilia* spp. on each farm

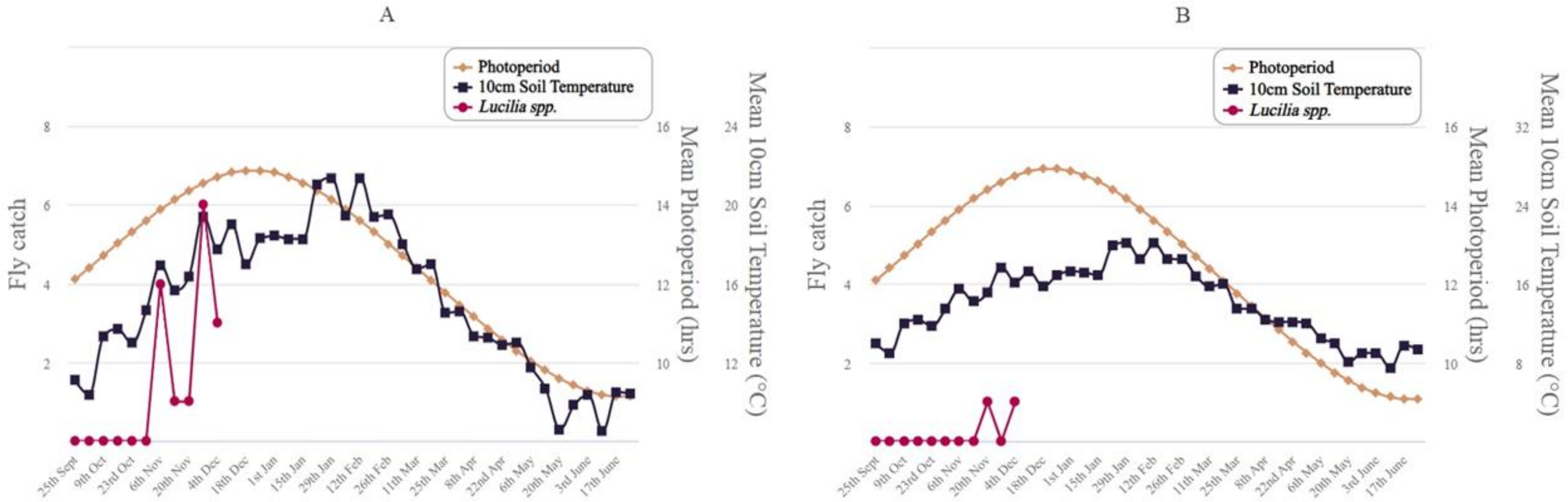


Figure C.17 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2019/2020 season on "T" (Figure C.17 A) and "R" (Figure C.17 B).

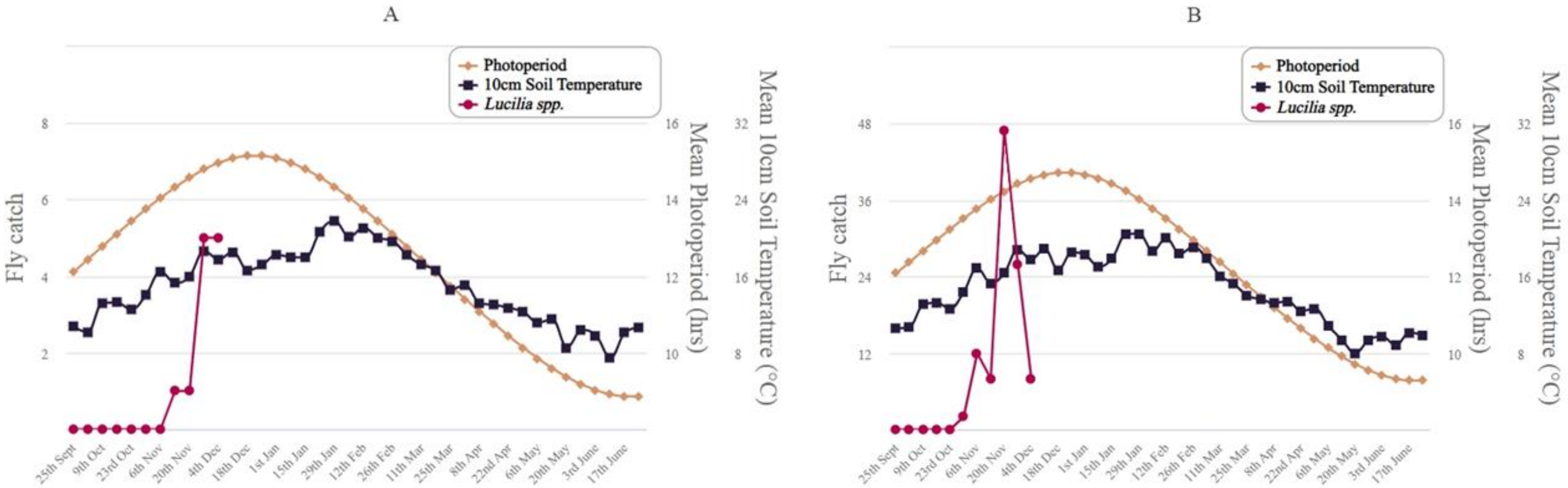


Figure C.18 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2019/2020 season on “BH” (Figure C.18 A) and “M” (Figure C.18 B).

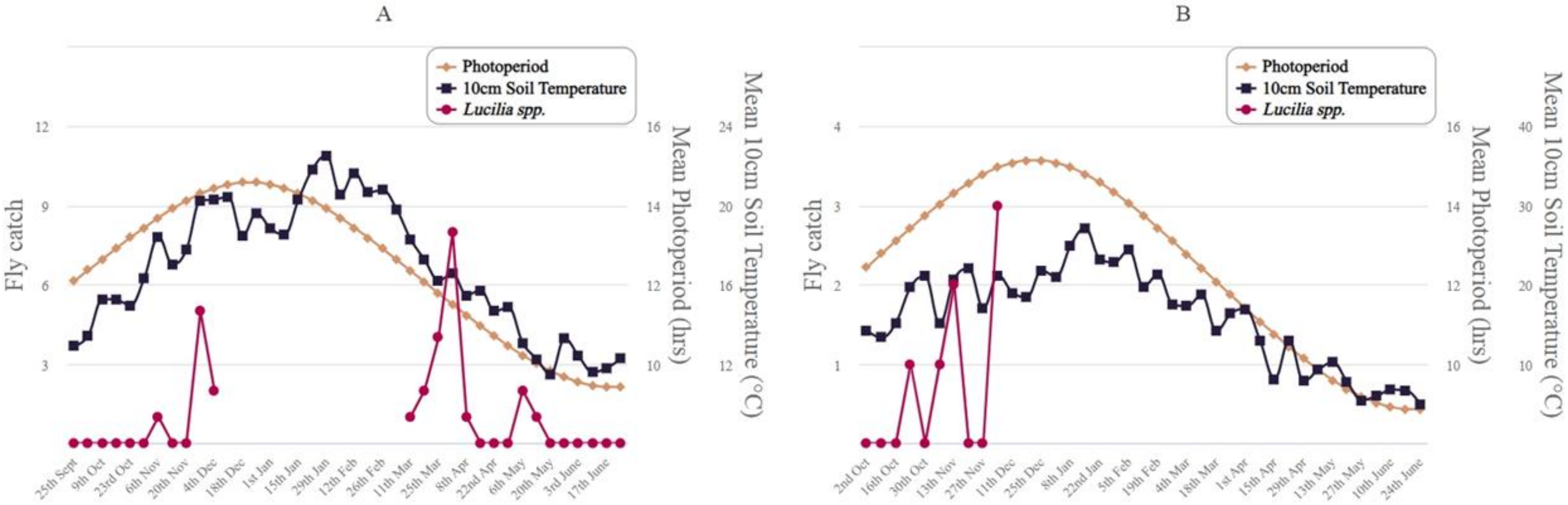


Figure C.19 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2019/2020 season on “LD” (Figure C.19 A) and “KD” (Figure C.19 B).

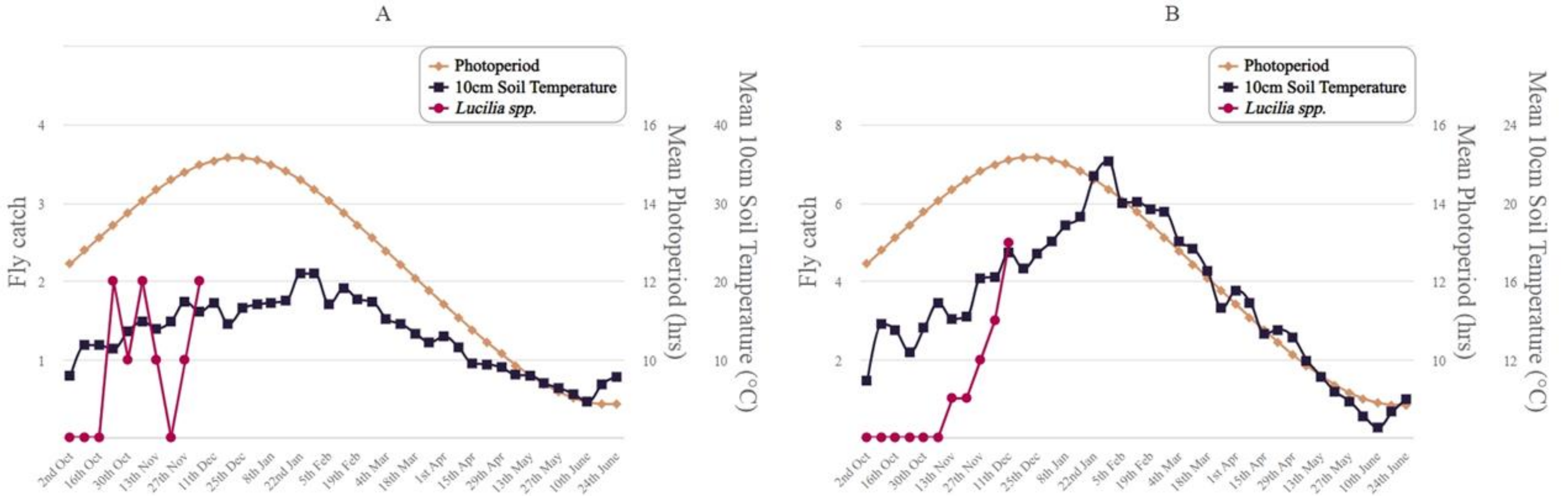


Figure C.20 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2019/2020 season on "ST" (Figure C.20 A) and "S" (Figure C.20 B).

Appendix C.4.2

The association between Relative Humidity and Rainfall with the weekly catch of *Lucilia cuprina* and *Lucilia sericata* on each farm

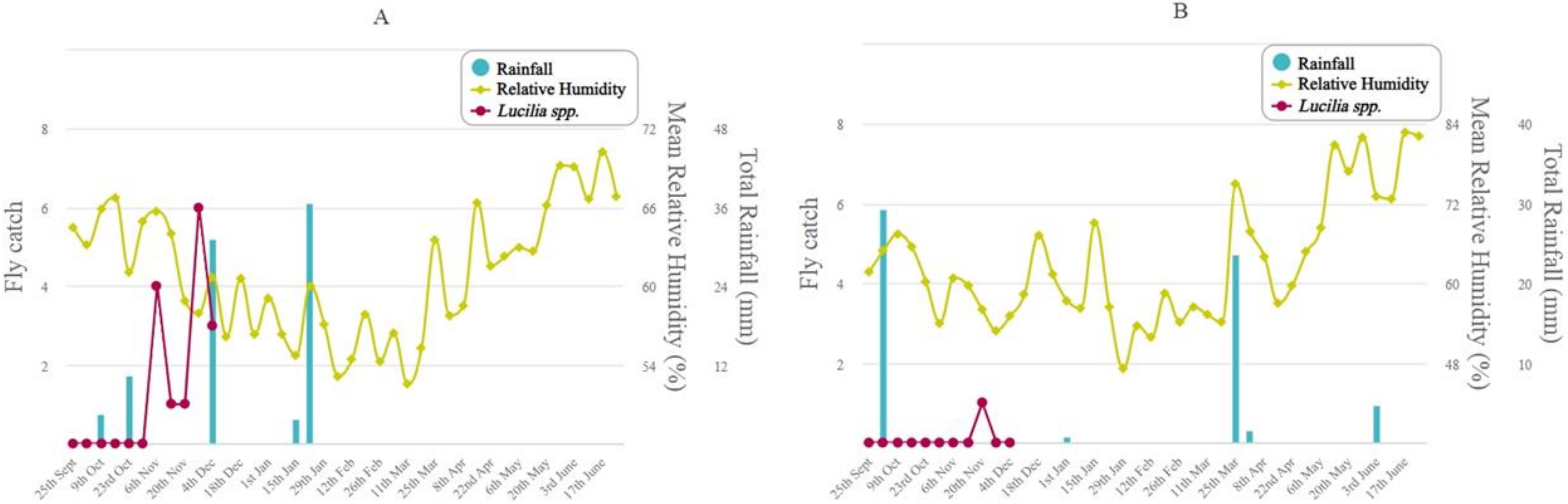


Figure C.21 The association between rainfall and mean relative humidity with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "T" (Figure C.21 A) and "R" (Figure C.21 B).

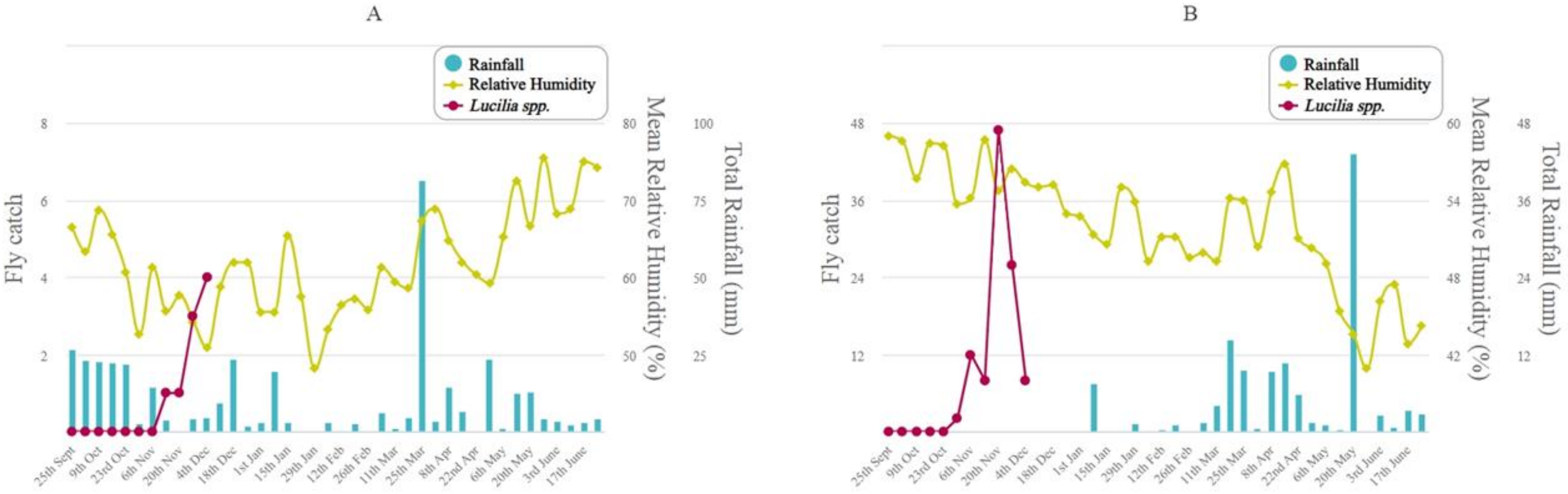


Figure C.22 The association between rainfall and mean relative humidity with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "BH" (Figure C.22 A) and "M" (Figure C.22 B).

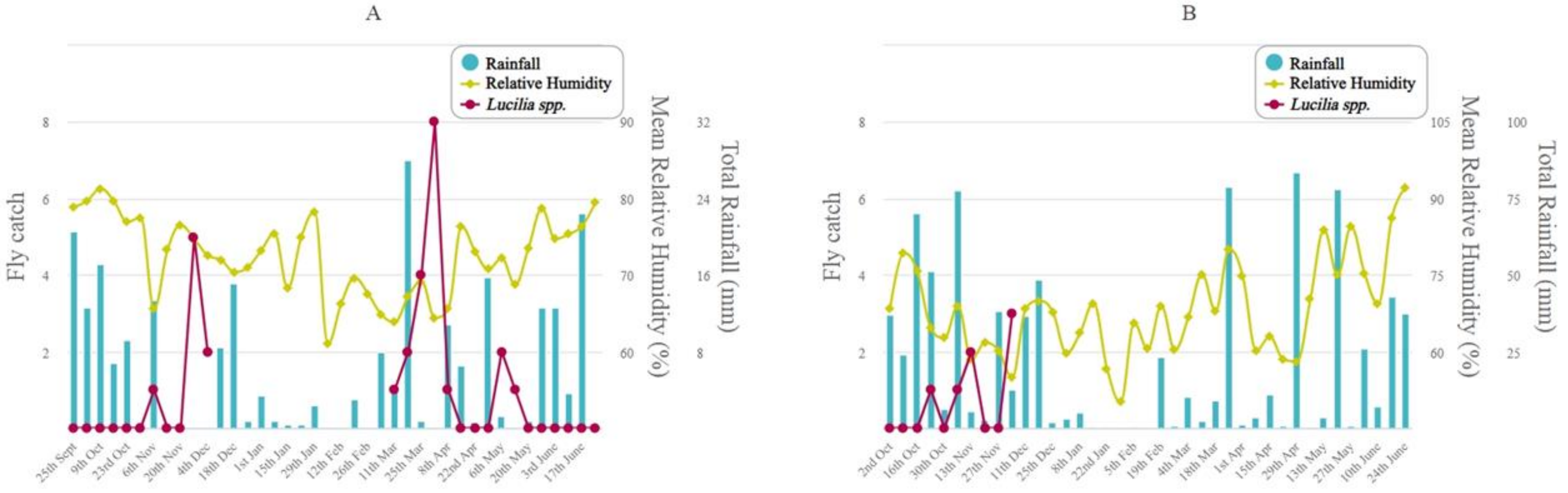


Figure C.23 The association between rainfall and mean relative humidity with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "LD" (Figure C.23 A) and "KD" (Figure C.23 B).

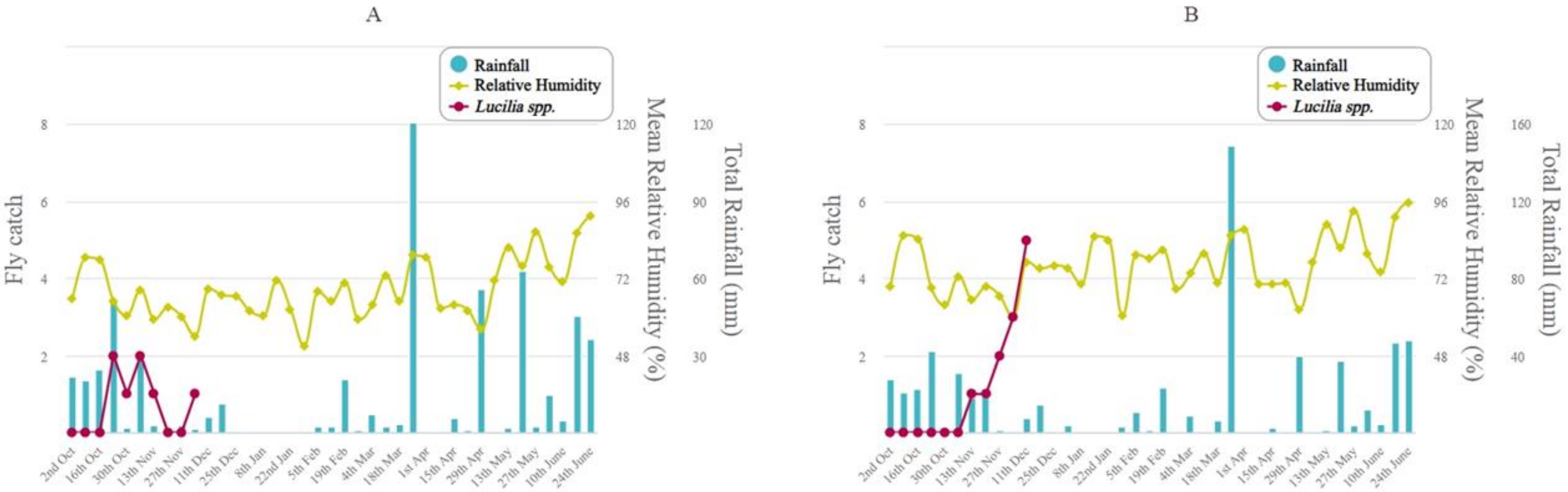


Figure C.24 The association between rainfall and mean relative humidity with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "ST" (Figure C.24 A) and "S" (Figure C.24 B).

Appendix C.4.3

The association between Maximum Wind Speed and Mean Wind Speed with the weekly catch of *Lucilia* spp. on each farm

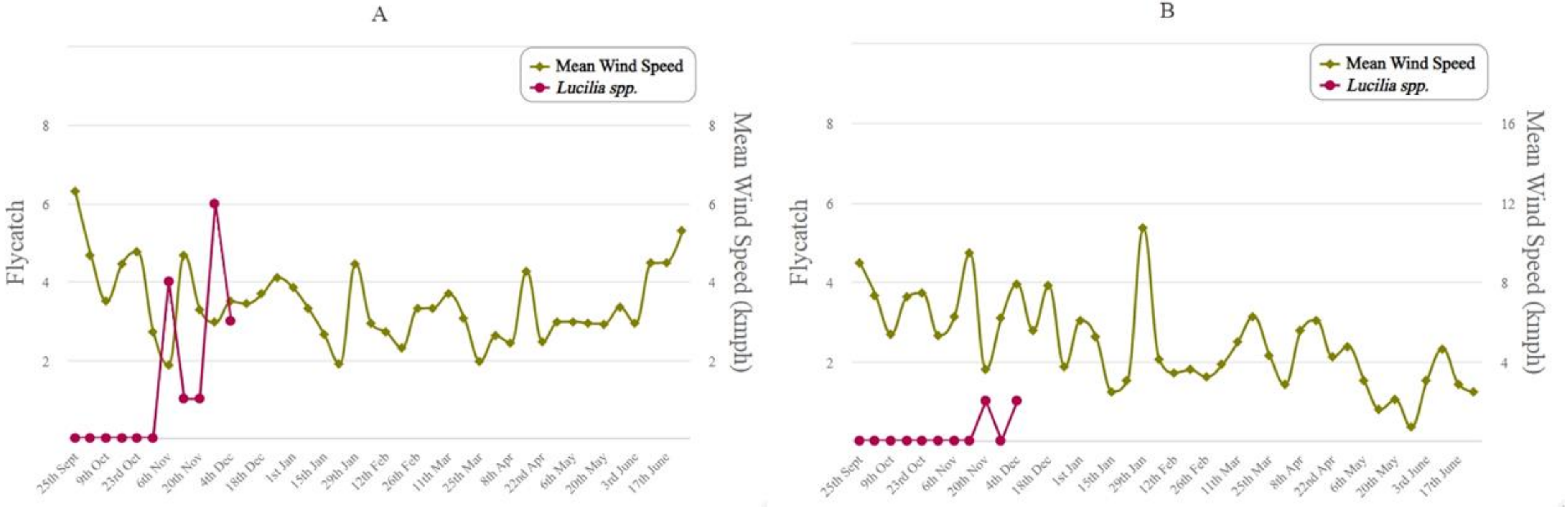


Figure C.25 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "T" (Figure C.25 A) and "R" (Figure C.25 B).

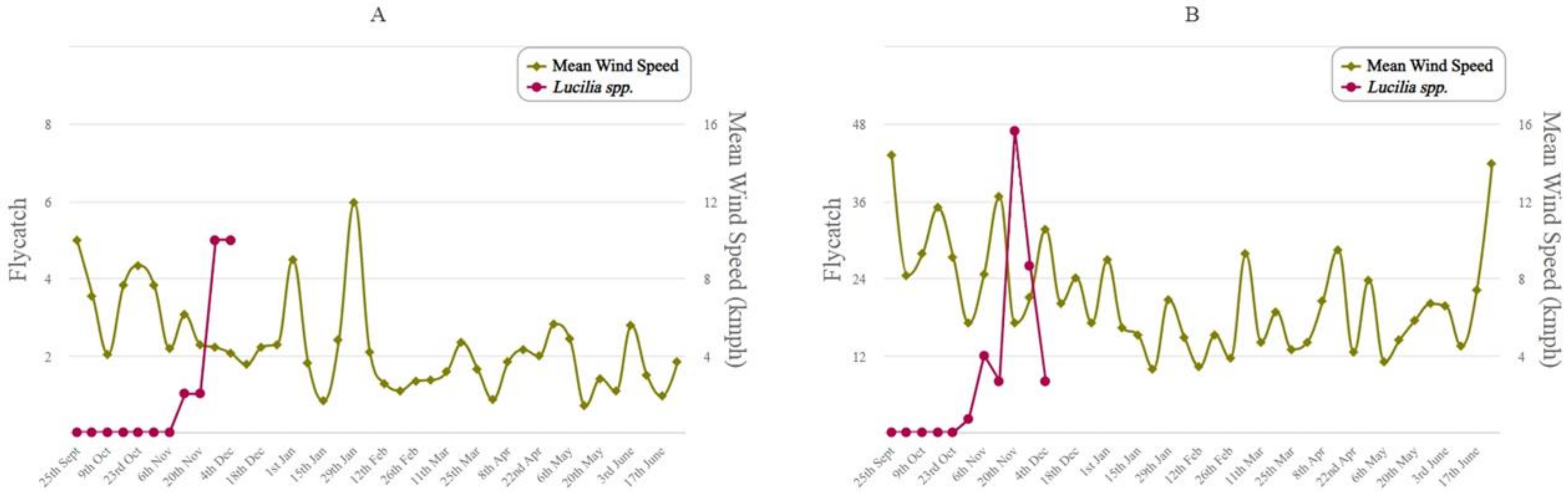


Figure C.26 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “BH” (Figure C.26 A) and “M” (Figure C.26 B).

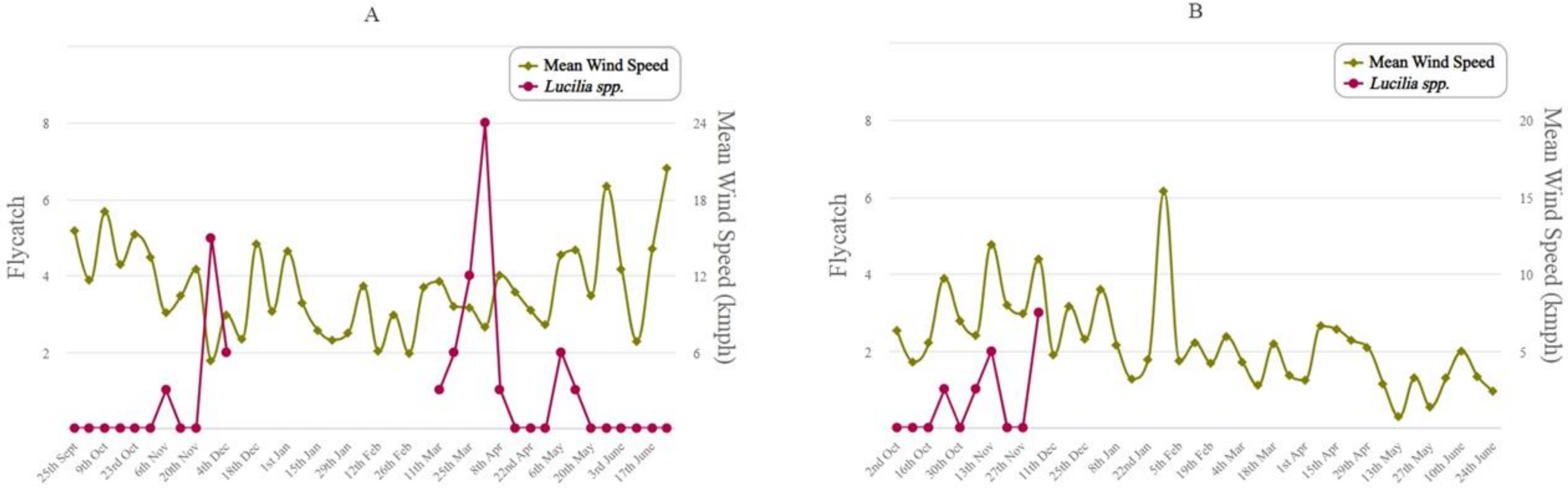


Figure C.27 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “LD” (Figure C.27 A) and “KD” (Figure C.27 B).

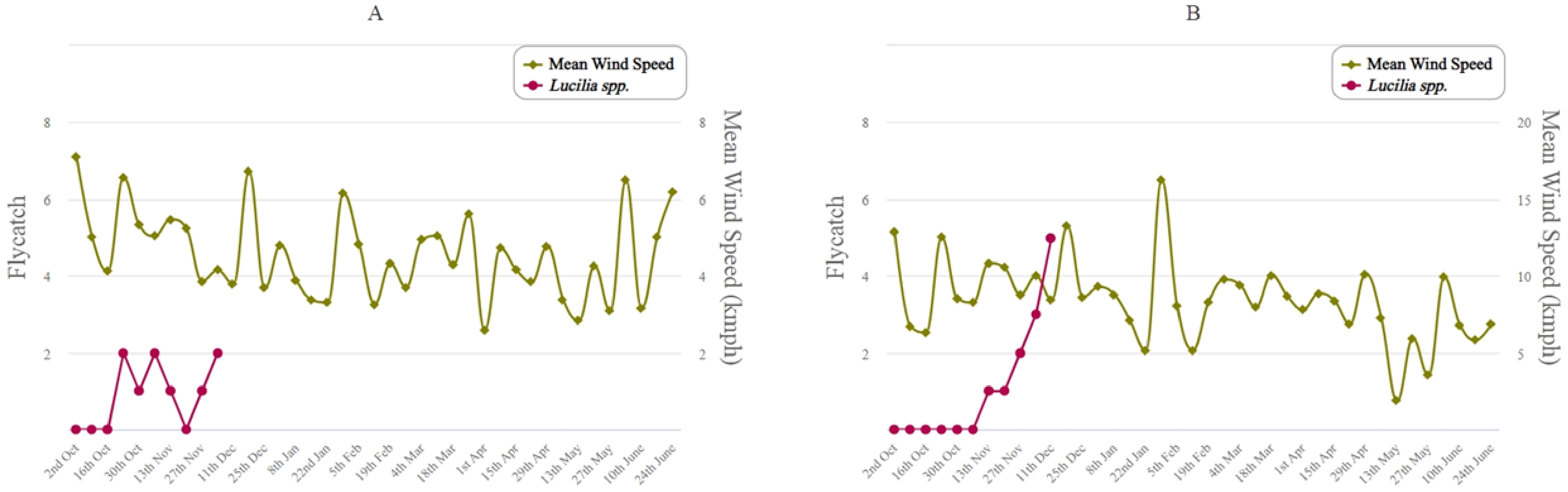


Figure C.28 The association between mean wind speed and maximum wind speed with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “ST” (Figure C.28 A) and “S” (Figure C.28 B).

Appendix C.4.4

The association between Mean, Maximum and Minimum temperature with the weekly catch of *Lucilia* spp. on each farm

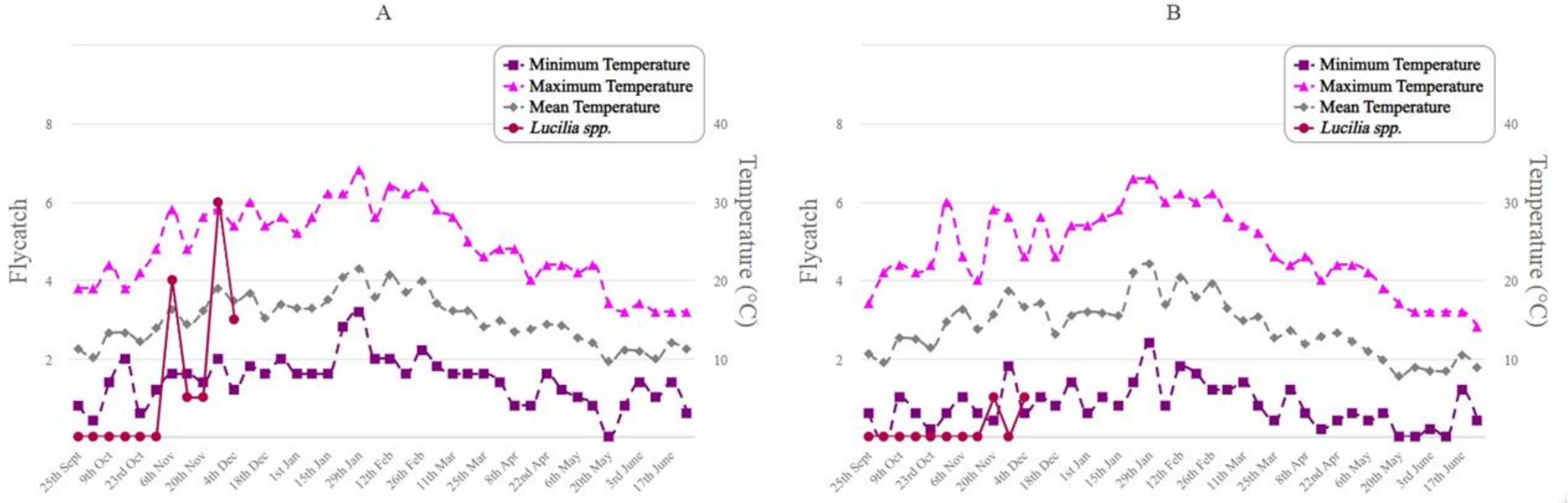


Figure C.29 The association of weekly mean, minimum and maximum temperature with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “T” (Figure C.29 A) and “R” (Figure C.29 B).

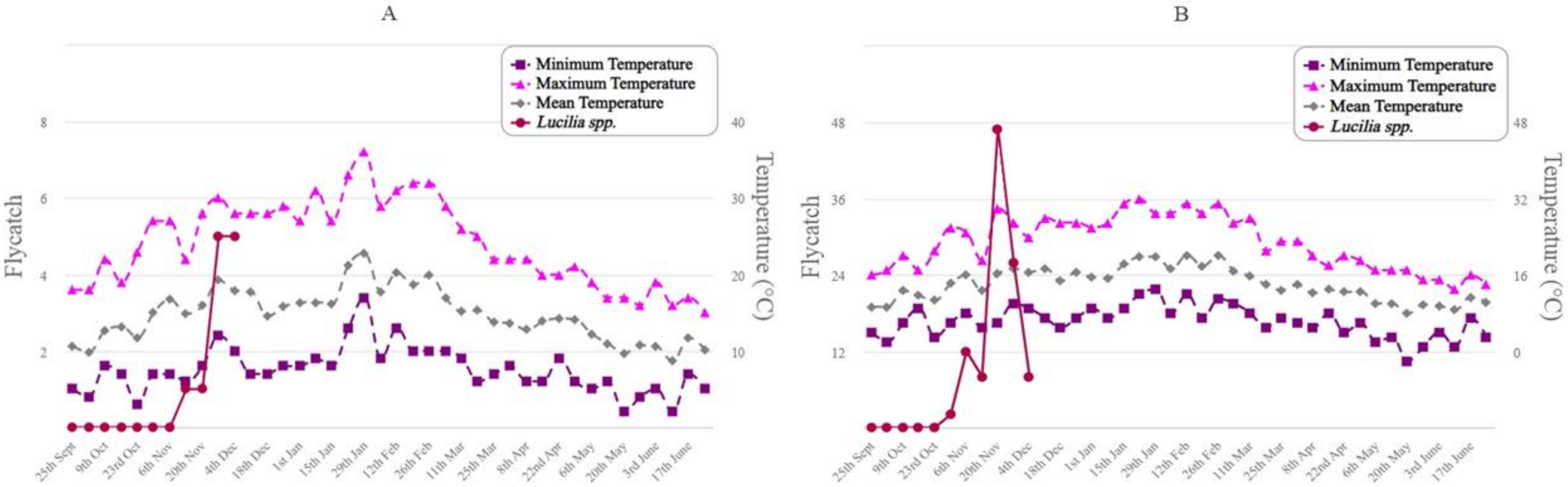


Figure C.30 The association of weekly mean, minimum and maximum temperature with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “BH” (Figure C.30 A) and “M” (Figure C.30 B).

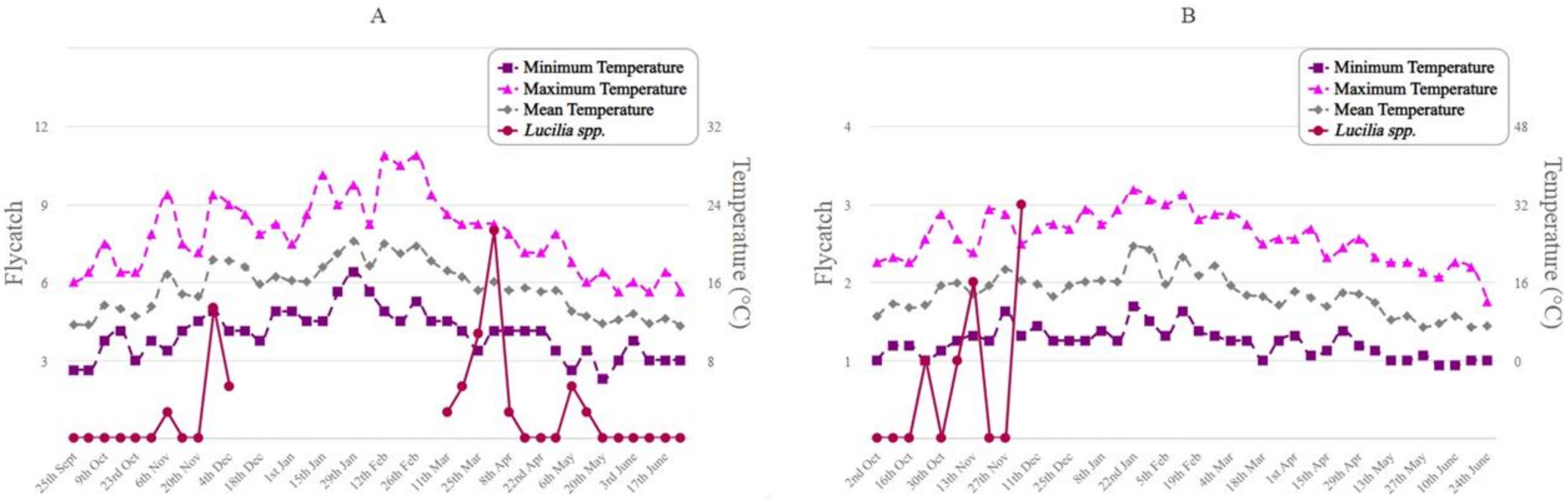


Figure C.31 The association of weekly mean, minimum and maximum temperature with the weekly catch of *Lucilia* spp. for the 2019/2020 season in “LD” (Figure C.31 A) and “KD” (Figure C.31 B).

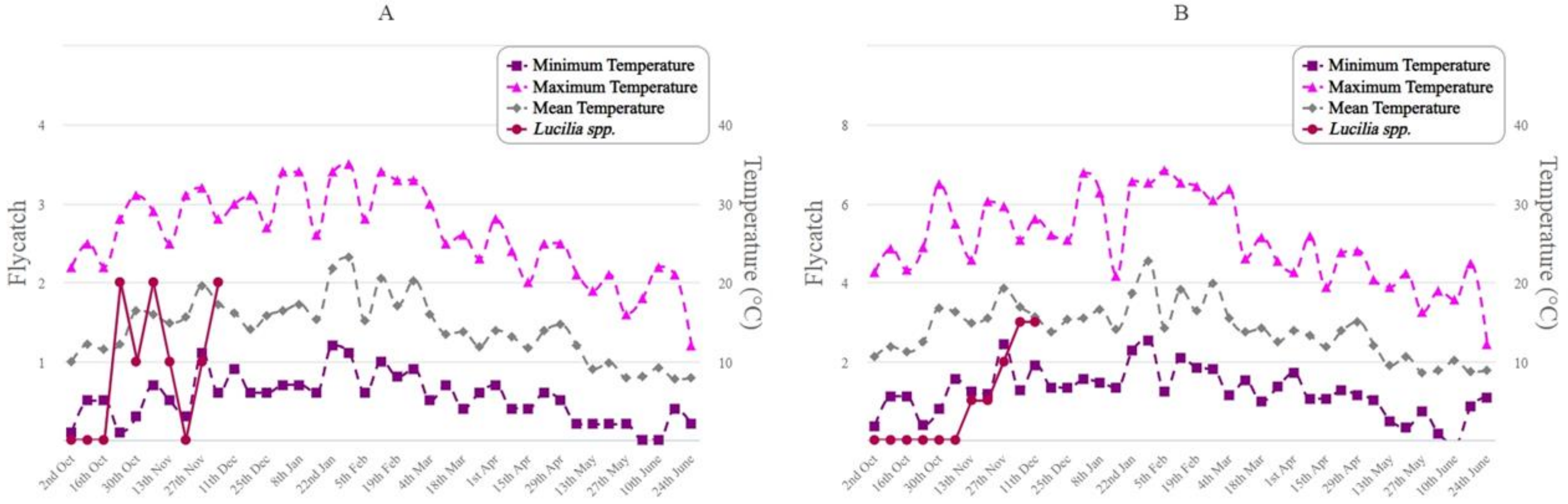


Figure C.32 The association of weekly mean, minimum and maximum temperature with the weekly catch of *Lucilia* spp. for the 2019/2020 season in "ST" (Figure C.32 A) and "S" (Figure C.32 B).

Appendix C.5

Graphs outlining the association of various climatic variables with the weekly catch of *Lucilia* spp. on farm “T” during the 2020/2021 season.

Appendix C.5.1

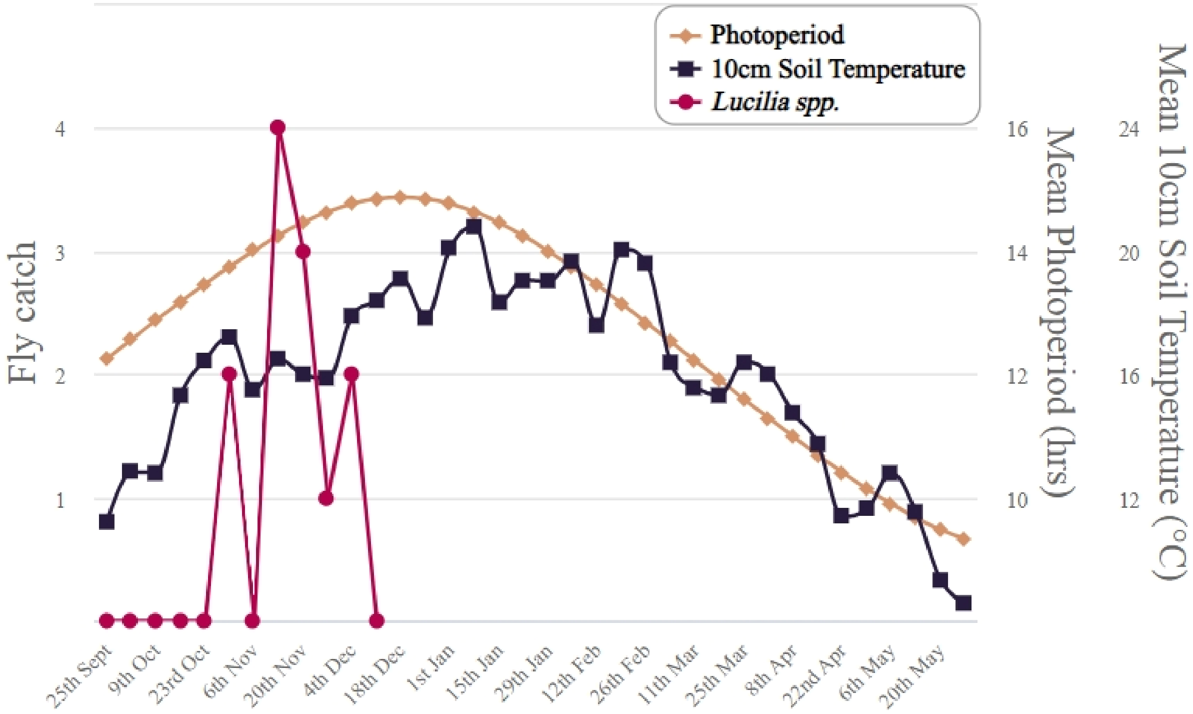


Figure C.33 The association between mean weekly 10 cm soil temperature, mean weekly photoperiod, and the weekly catch of *Lucilia* spp. for the 2020/2021 season on “T”.

Appendix C.5.2

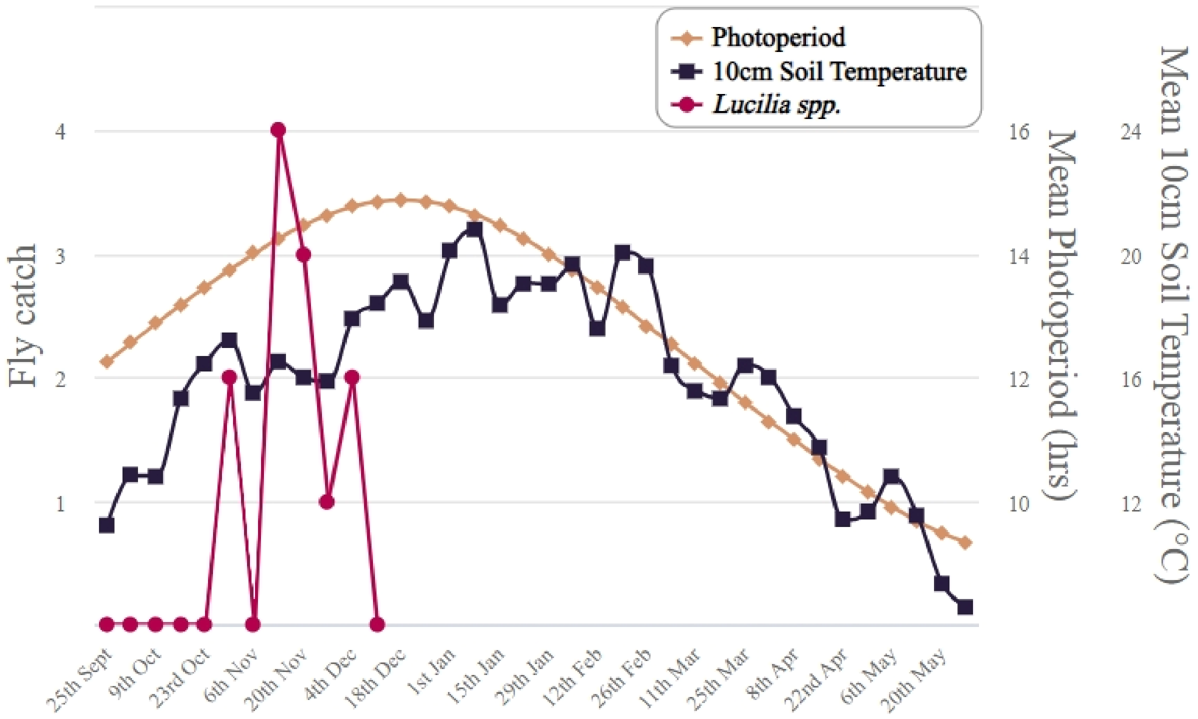


Figure C.34 The association between rainfall and mean relative humidity with the weekly catch of *Lucilia* spp. for the 2020/2021 season in “T”.

Appendix C.5.3

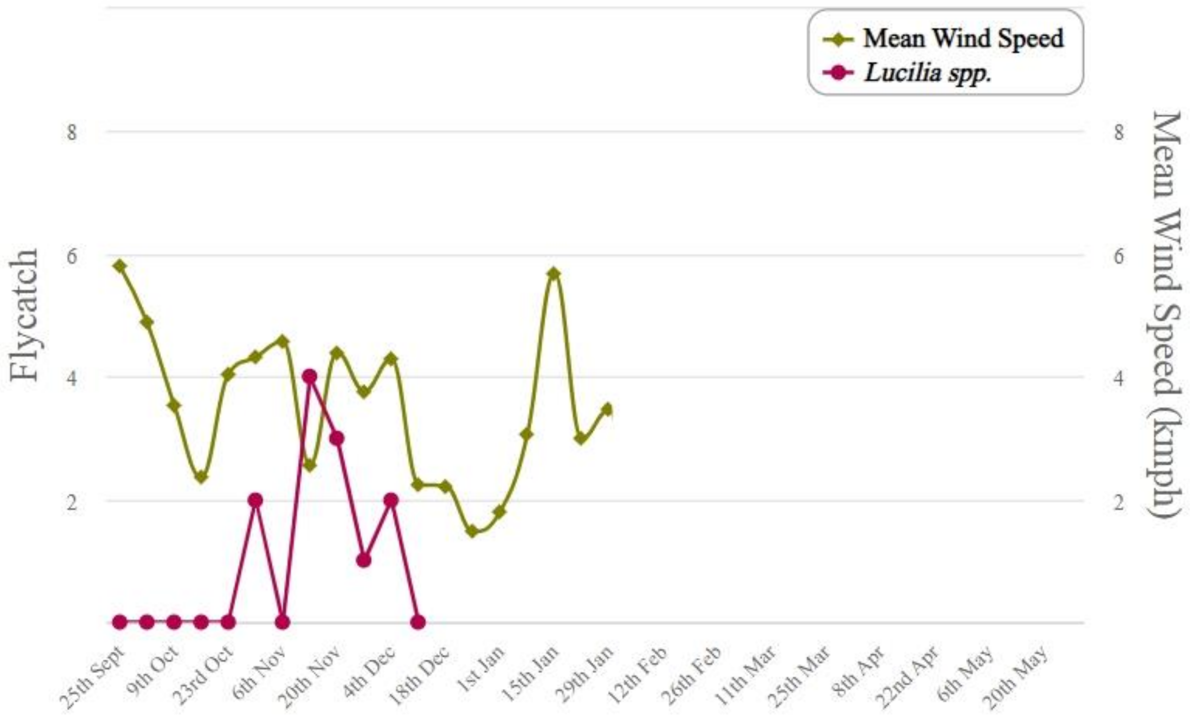


Figure C.35 The association between mean wind speed with the weekly catch of *Lucilia* spp. for the 2020/2021 season in “T”.

Appendix C.5.4

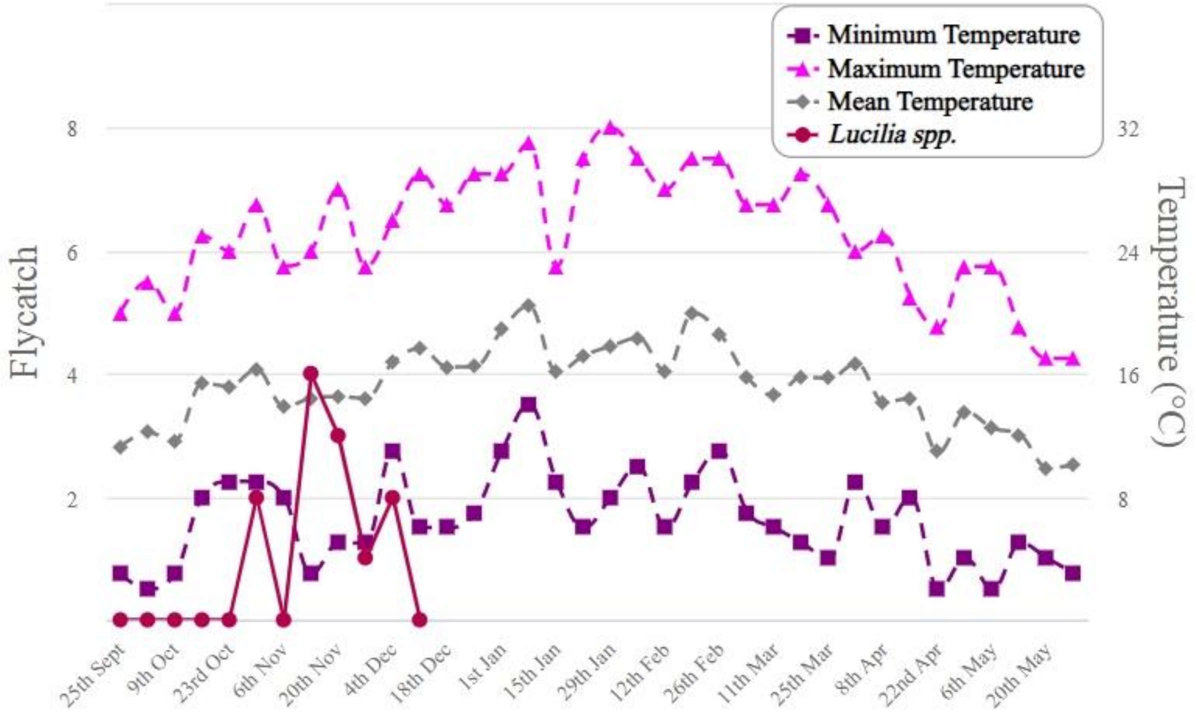


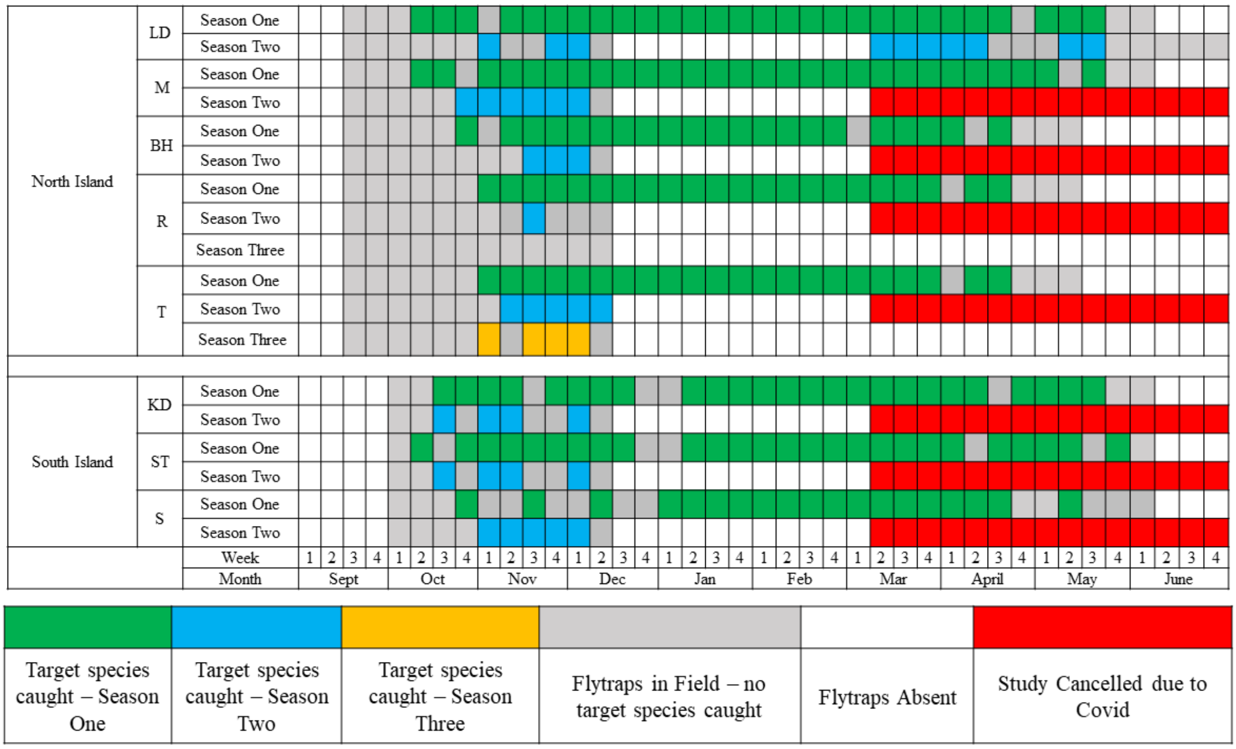
Figure C.36 The association of weekly mean, minimum and maximum temperature with the weekly catch of *Lucilia* spp. for the 2020/2021 season in “T”.

Appendix D

The following appendix section outlines the fly identification according to their original morphological identification on all farms across all seasons. However, due to findings detailed in Chapter 2 indicating the level of inaccuracy in the identification within the genus *Lucilia* all subsequent analyses in the thesis were for the genus *Lucilia* spp. collectively and not for the two separate species as presented in Chapter Four.

Appendix D.1

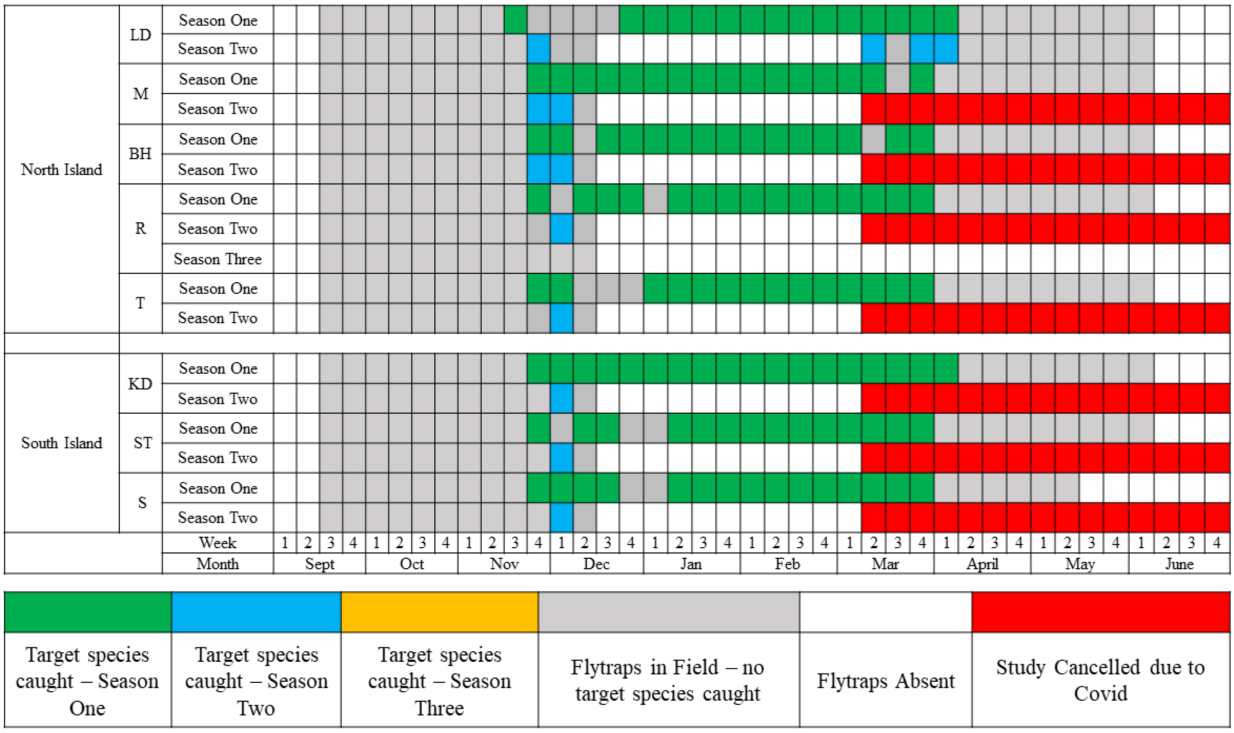
The following outlines weekly seasonal catch of *Lucilia cuprina* and *Lucilia sericata* per week from their original morphological identification on all farms across all seasons.



North Island	LD	Season One	⬜	⬜	▨	▨	▨	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	▨	▨	⬜	⬜	⬜
		Season Two	⬜	⬜	▨	▨	▨	▨	▨	▨	🟦	▨	▨	🟦	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	▨	▨	▨	🟦	🟦	▨	▨	▨	▨	▨
	M	Season One	⬜	⬜	▨	▨	▨	🟩	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	▨	▨	⬜	⬜	⬜
		Season Two	⬜	⬜	▨	▨	▨	▨	▨	🟦	🟦	🟦	🟦	🟦	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	BH	Season One	⬜	⬜	▨	▨	▨	▨	▨	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	▨	🟩	▨	▨	▨	⬜	⬜	⬜	⬜	⬜	⬜
		Season Two	⬜	⬜	▨	▨	▨	▨	▨	▨	▨	▨	🟦	🟦	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	R	Season One	⬜	⬜	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	▨	▨	▨	⬜	⬜	⬜	⬜	⬜	⬜
		Season Two	⬜	⬜	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨	▨	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Season Three	⬜	⬜	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
	T	Season One	⬜	⬜	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	▨	▨	▨	⬜	⬜	⬜	⬜	⬜	⬜
		Season Two	⬜	⬜	▨	▨	▨	▨	▨	▨	▨	🟦	🟦	🟦	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Season Three	⬜	⬜	▨	▨	▨	▨	▨	▨	🟨	▨	🟨	🟨	🟨	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
South Island	KD	Season One	⬜	⬜	⬜	⬜	▨	▨	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	▨	▨	⬜	⬜	⬜
		Season Two	⬜	⬜	⬜	⬜	▨	▨	🟦	▨	🟦	🟦	▨	▨	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	ST	Season One	⬜	⬜	⬜	⬜	▨	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	▨	🟩	▨	⬜	⬜	⬜
		Season Two	⬜	⬜	⬜	⬜	▨	▨	🟦	▨	🟦	🟦	▨	▨	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	S	Season One	⬜	⬜	⬜	⬜	▨	▨	▨	🟩	▨	▨	🟩	▨	▨	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	🟩	▨	▨	▨	⬜	⬜	⬜
		Season Two	⬜	⬜	⬜	⬜	▨	▨	▨	▨	🟦	🟦	🟦	🟦	🟦	▨	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		Month	Sept				Oct				Nov				Dec				Jan				Feb				Mar				April				May				June			

🟩	🟦	🟨	▨	⬜	🟥
Target species caught – Season One	Target species caught – Season Two	Target species caught – Season Three	Flytraps in Field – no target species caught	Flytraps Absent	Study Cancelled due to Covid

Figure D.1 Contrasting the seasonality of *Lucilia cuprina* across eight farms in the North and South Island of New Zealand for the first (2018/2019 season), second (2019/2020 season) and third year (2020/2021 season).



North Island	LD	Season One			▨	▨	▨	▨	▨	▨	▨	▨	🟩	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨	▨												🟦	▨	🟦	🟦	▨	▨	▨	▨	▨	▨	▨	▨			
	M	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	▨	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	BH	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	R	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	▨	🟩	🟩	🟩	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Season Three			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨																										
	T	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
South Island	KD	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	ST	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	▨	🟩	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨	▨	▨	▨			
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	S	Season One			▨	▨	▨	▨	▨	▨	▨	▨	▨	🟩	🟩	🟩	🟩	▨	▨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	▨	▨	▨	▨	▨						
		Season Two			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	🟦	▨												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
		Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		Month	Sept				Oct				Nov				Dec				Jan				Feb				Mar				April				May				June			

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Target species caught – Season One	Target species caught – Season Two	Target species caught – Season Three	Flytraps in Field – no target species caught	Flytraps Absent	Study Cancelled due to Covid

Figure D.2 Contrasting the seasonality of *Lucilia sericata* across eight farms in the North Island and South Island of New Zealand for the first (2018/2019 season) and second year (2019/2020 season).

Appendix D.2

The following outlines weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and StickyTrap from their original morphological identification on all farms.

Table C.2 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and StickyTrap in "T" 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
20/09/2018 - 26/09/2018	0	0	0	0
27/09/2018 - 03/10/2018	0	0	0	0
04/10/2018 - 10/10/2018	0	0	0	0
11/10/2018 - 17/10/2018	0	0	0	0
18/10/2018 - 24/10/2018	0	0	0	0
25/10/2018 - 31/10/2018	0	0	0	0
01/11/2018 - 07/11/2018	0	0	0	0
08/11/2018 - 14/11/2018	0	3	0	0
15/11/2018 - 21/11/2018	1	5	0	0
22/11/2018 - 28/11/2018	0	11	0	0
29/11/2018 - 05/12/2018	2	2	0	1
06/12/2018 - 12/12/2018	1	2	0	3
13/12/2018 - 19/12/2018	1	7	0	0
20/12/2018 - 26/12/2018	0	9	0	0
27/12/2018 - 02/01/2019	0	2	0	0
03/01/2019 - 09/01/2019	0	2	0	1
10/01/2019 - 16/01/2019	0	4	0	1
17/01/2019 - 23/01/2019	2	3	0	0
24/01/2019 - 30/01/2019	2	6	4	1
31/01/2019 - 06/02/2019	7	2	2	1
07/02/2019 - 13/02/2019	27	4	26	4
14/02/2019 - 20/02/2019	100	4	41	2
21/02/2019 - 27/02/2019	81	6	37	0
28/02/2019 - 06/03/2019	6	1	3	4
07/03/2019 - 13/03/2019	5	4	1	2
14/03/2019 - 20/03/2019	1	2	0	2
21/03/2019 - 27/03/2019	4	2	1	4
28/03/2019 - 03/04/2019	2	0	0	1
04/04/2019 - 10/04/2019	6	2	0	0
11/04/2019 - 17/04/2019	0	0	0	0
18/04/2019 - 24/04/2019	1	0	0	0
25/04/2019 - 01/05/2019	4	1	0	0
02/05/2019 - 08/05/2019	0	0	0	0
09/05/2019 - 15/05/2019	0	0	0	0
16/05/2019 - 22/05/2019	0	0	0	0

Table C.3 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in "R" 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
20/09/2018 - 26/09/2018	0	0	0	0
27/09/2018 - 03/10/2018	0	0	0	0
04/10/2018 - 10/10/2018	0	0	0	0
11/10/2018 - 17/10/2018	0	0	0	0
18/10/2018 - 24/10/2018	0	0	0	0
25/10/2018 - 31/10/2018	0	0	0	0
01/11/2018 - 07/11/2018	0	0	0	0
08/11/2018 - 14/11/2018	1	0	0	0
15/11/2018 - 21/11/2018	0	0	0	0
22/11/2018 - 28/11/2018	0	2	0	1
29/11/2018 - 05/12/2018	0	3	0	0
06/12/2018 - 12/12/2018	1	2	0	1
13/12/2018 - 19/12/2018	1	5	0	1
20/12/2018 - 26/12/2018	1	2	0	0
27/12/2018 - 02/01/2019	1	4	0	1
03/01/2019 - 09/01/2019	0	5	1	0
10/01/2019 - 16/01/2019	0	3	1	2
17/01/2019 - 23/01/2019	2	6	0	3
24/01/2019 - 30/01/2019	3	8	0	2
31/01/2019 - 06/02/2019	7	6	2	1
07/02/2019 - 13/02/2019	46	3	37	0
14/02/2019 - 20/02/2019	33	3	10	5
21/02/2019 - 27/02/2019	52	3	43	0
28/02/2019 - 06/03/2019	123	2	60	6
07/03/2019 - 13/03/2019	2	6	2	0
14/03/2019 - 20/03/2019	16	3	15	7
21/03/2019 - 27/03/2019	5	1	1	1
28/03/2019 - 03/04/2019	8	2	1	3
04/04/2019 - 10/04/2019	4	14	0	0
11/04/2019 - 17/04/2019	0	0	0	0
18/04/2019 - 24/04/2019	0	1	0	0
25/04/2019 - 01/05/2019	0	2	0	0
02/05/2019 - 08/05/2019	0	0	0	0
09/05/2019 - 15/05/2019	0	0	0	0
16/05/2019 - 22/05/2019	0	0	0	0

Table C.4 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in "BH" 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
20/09/2018 - 26/09/2018	0	0	0	0
27/09/2018 - 03/10/2018	0	0	0	0
04/10/2018 - 10/10/2018	0	0	0	0
11/10/2018 - 17/10/2018	0	0	0	0
18/10/2018 - 24/10/2018	0	0	0	0
25/10/2018 - 31/10/2018	0	0	0	0
01/11/2018 - 07/11/2018	0	1	0	0
08/11/2018 - 14/11/2018	0	0	0	0
15/11/2018 - 21/11/2018	0	1	0	0
22/11/2018 - 28/11/2018	1	6	0	0
29/11/2018 - 05/12/2018	0	8	0	2
06/12/2018 - 12/12/2018	2	5	0	5
13/12/2018 - 19/12/2018	0	13	0	0
20/12/2018 - 26/12/2018	1	8	0	1
27/12/2018 - 02/01/2019	0	4	0	1
03/01/2019 - 09/01/2019	1	4	0	1
10/01/2019 - 16/01/2019	9	7	2	7
17/01/2019 - 23/01/2019	3	13	3	10
24/01/2019 - 30/01/2019	7	7	7	1
31/01/2019 - 06/02/2019	4	13	5	5
07/02/2019 - 13/02/2019	25	5	18	4
14/02/2019 - 20/02/2019	1	0	1	1
21/02/2019 - 27/02/2019	38	7	19	12
28/02/2019 - 06/03/2019	6	12	3	6
07/03/2019 - 13/03/2019	1	4	0	3
14/03/2019 - 20/03/2019	0	0	0	0
21/03/2019 - 27/03/2019	1	0	1	1
28/03/2019 - 03/04/2019	1	0	1	0
04/04/2019 - 10/04/2019	1	2	0	0
11/04/2019 - 17/04/2019	3	1	0	0
18/04/2019 - 24/04/2019	0	0	0	0
25/04/2019 - 01/05/2019	1	0	0	0
02/05/2019 - 08/05/2019	0	0	0	0
09/05/2019 - 15/05/2019	0	0	0	0
16/05/2019 - 22/05/2019	0	0	0	0

Table C.5 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in "M" 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
18/09/2018 - 24/09/2018	0	0	0	0
25/09/2018 - 01/10/2018	0	0	0	0
02/10/2018 - 08/10/2018	0	0	0	0
09/10/2018 - 15/10/2018	0	0	0	0
16/10/2018 - 22/10/2018	1	1	0	0
23/10/2018 - 29/10/2018	0	1	0	0
30/10/2018 - 05/11/2018	0	0	0	0
06/11/2018 - 12/11/2018	0	2	0	0
13/11/2018 - 19/11/2018	0	9	0	0
20/11/2018 - 26/11/2018	0	18	0	0
27/11/2018 - 03/12/2018	4	11	0	1
04/12/2018 - 10/12/2018	1	6	0	1
11/12/2018 - 17/12/2018	6	12	0	6
18/12/2018 - 24/12/2018	3	30	0	6
25/12/2018 - 31/12/2018	4	11	1	0
01/01/2019 - 07/01/2019	15	14	18	4
08/01/2019 - 14/01/2019	1	21	1	1
15/01/2019 - 21/01/2019	8	4	1	4
22/01/2019 - 28/01/2019	11	6	11	2
29/01/2019 - 04/02/2019	11	4	6	7
05/02/2019 - 11/02/2019	180	21	113	11
12/02/2019 - 18/02/2019	95	6	45	14
19/02/2019 - 25/02/2019	104	1	61	6
26/02/2019 - 04/03/2019	8	20	2	6
05/03/2019 - 11/03/2019	33	3	14	4
12/03/2019 - 18/03/2019	0	11	0	5
19/03/2019 - 25/03/2019	2	2	0	0
26/03/2019 - 01/04/2019	10	21	2	5
02/04/2019 - 08/04/2019	7	8	0	0
09/04/2019 - 15/04/2019	1	7	0	0
16/04/2019 - 22/04/2019	1	0	0	0
23/04/2019 - 29/04/2019	1	0	0	0
30/04/2019 - 06/05/2019	0	0	0	0
07/05/2019 - 13/05/2019	0	1	0	0
14/05/2019 - 20/05/2019	0	0	0	0
21/05/2019 - 27/05/2019	0	0	0	0
28/05/2019 - 03/06/2019	0	0	0	0
04/06/2019 - 10/06/2019	0	0	0	0

Table C.6 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in “LD” 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
18/09/2018 - 24/09/2018	0	0	0	0
25/09/2018 - 01/10/2018	0	0	0	0
02/10/2018 - 08/10/2018	0	0	0	0
09/10/2018 - 15/10/2018	0	0	0	0
16/10/2018 - 22/10/2018	2	1	0	0
23/10/2018 - 29/10/2018	1	0	0	0
30/10/2018 - 05/11/2018	1	0	0	0
06/11/2018 - 12/11/2018	0	0	0	0
13/11/2018 - 19/11/2018	0	2	0	0
20/11/2018 - 26/11/2018	1	2	1	0
27/11/2018 - 03/12/2018	1	1	0	0
04/12/2018 - 10/12/2018	0	4	0	0
11/12/2018 - 17/12/2018	2	2	0	0
18/12/2018 - 24/12/2018	0	11	0	0
25/12/2018 - 31/12/2018	4	5	0	3
01/01/2019 - 07/01/2019	1	3	0	1
08/01/2019 - 14/01/2019	13	22	10	10
15/01/2019 - 21/01/2019	11	3	5	0
22/01/2019 - 28/01/2019	5	2	7	3
29/01/2019 - 04/02/2019	23	2	19	2
05/02/2019 - 11/02/2019	63	12	32	11
12/02/2019 - 18/02/2019	42	31	27	17
19/02/2019 - 25/02/2019	24	8	7	6
26/02/2019 - 04/03/2019	17	7	7	15
05/03/2019 - 11/03/2019	21	10	8	2
12/03/2019 - 18/03/2019	7	8	5	3
19/03/2019 - 25/03/2019	6	7	3	2
26/03/2019 - 01/04/2019	5	7	0	4
02/04/2019 - 08/04/2019	4	12	0	2
09/04/2019 - 15/04/2019	5	8	0	0
16/04/2019 - 22/04/2019	2	5	0	0
23/04/2019 - 29/04/2019	0	5	0	0
30/04/2019 - 06/05/2019	0	0	0	0
07/05/2019 - 13/05/2019	0	1	0	0
14/05/2019 - 20/05/2019	4	7	0	0
21/05/2019 - 27/05/2019	0	1	0	0
28/05/2019 - 03/06/2019	0	0	0	0
04/06/2019 - 10/06/2019	0	0	0	0

Table C.7 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in “KD” 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
09/10/2018 - 15/10/2018	0	0	0	0
16/10/2018 - 22/10/2018	0	1	0	0
23/10/2018 - 29/10/2018	0	1	0	0
30/10/2018 - 05/11/2018	0	1	0	0
06/11/2018 - 12/11/2018	0	1	0	0
13/11/2018 - 19/11/2018	0	0	0	0
20/11/2018 - 26/11/2018	2	6	0	0
27/11/2018 - 03/12/2018	3	0	1	0
04/12/2018 - 10/12/2018	3	4	0	0
11/12/2018 - 17/12/2018	0	5	0	1
18/12/2018 - 24/12/2018	0	0	0	0
25/12/2018 - 31/12/2018	0	0	0	3
01/01/2019 - 07/01/2019	0	2	0	7
08/01/2019 - 14/01/2019	1	7	1	4
15/01/2019 - 21/01/2019	2	0	4	1
22/01/2019 - 28/01/2019	24	12	19	7
29/01/2019 - 04/02/2019	34	8	31	2
05/02/2019 - 11/02/2019	137	10	83	1
12/02/2019 - 18/02/2019	65	14	28	3
19/02/2019 - 25/02/2019	98	10	59	5
26/02/2019 - 04/03/2019	187	34	111	11
05/03/2019 - 11/03/2019	242	19	120	13
12/03/2019 - 18/03/2019	6	13	5	10
19/03/2019 - 25/03/2019	10	5	3	3
26/03/2019 - 01/04/2019	16	4	12	0
02/04/2019 - 08/04/2019	12	7	1	3
09/04/2019 - 15/04/2019	2	5	0	0
16/04/2019 - 22/04/2019	0	0	0	0
23/04/2019 - 29/04/2019	1	1	0	0
30/04/2019 - 06/05/2019	2	1	0	0
07/05/2019 - 13/05/2019	0	1	0	0
14/05/2019 - 20/05/2019	0	1	0	0
21/05/2019 - 27/05/2019	2	1	0	0
28/05/2019 - 03/06/2019	0	0	0	0

Table C.8 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in "ST" 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
09/10/2018 - 15/10/2018	1	0	0	0
16/10/2018 - 22/10/2018	0	0	0	0
23/10/2018 - 29/10/2018	2	4	0	0
30/10/2018 - 05/11/2018	0	3	0	0
06/11/2018 - 12/11/2018	7	4	0	0
13/11/2018 - 19/11/2018	1	8	0	0
20/11/2018 - 26/11/2018	11	6	0	0
27/11/2018 - 03/12/2018	2	4	0	3
04/12/2018 - 10/12/2018	7	5	0	0
11/12/2018 - 17/12/2018	15	7	2	6
18/12/2018 - 24/12/2018	5	2	3	5
25/12/2018 - 31/12/2018				
01/01/2019 - 07/01/2019				
08/01/2019 - 14/01/2019	44	3	17	2
15/01/2019 - 21/01/2019	47	7	30	2
22/01/2019 - 28/01/2019	73	12	38	4
29/01/2019 - 04/02/2019	185	7	136	3
05/02/2019 - 11/02/2019	348	29	154	13
12/02/2019 - 18/02/2019	151	7	62	1
19/02/2019 - 25/02/2019	126	19	58	7
26/02/2019 - 04/03/2019	209	8	78	15
05/03/2019 - 11/03/2019	85	14	34	5
12/03/2019 - 18/03/2019	3	6	4	0
19/03/2019 - 25/03/2019	2	2	0	4
26/03/2019 - 01/04/2019	6	9	4	10
02/04/2019 - 08/04/2019	3	5	3	2
09/04/2019 - 15/04/2019	1	4	0	0
16/04/2019 - 22/04/2019	0	0	0	0
23/04/2019 - 29/04/2019	0	5	0	0
30/04/2019 - 06/05/2019	0	2	0	0
07/05/2019 - 13/05/2019	0	2	0	0
14/05/2019 - 20/05/2019	0	1	0	0
21/05/2019 - 27/05/2019	0	0	0	0
28/05/2019 - 03/06/2019	1	0	0	0
04/06/2019 - 10/06/2019	0	0	0	0

Table C.9 Weekly catch of *Lucilia cuprina* and *Lucilia sericata* per week by LuciTrap and Stickytrap in “S” 2018/2019

Date	*Lucilia cuprina*		*Lucilia sericata*	
	LuciTrap	Stickytrap	LuciTrap	Stickytrap
09/10/2018 - 15/10/2018	0	0	0	0
16/10/2018 - 22/10/2018	0	0	0	0
23/10/2018 - 29/10/2018	0	0	0	0
30/10/2018 - 05/11/2018	0	1	0	0
06/11/2018 - 12/11/2018	0	0	0	0
13/11/2018 - 19/11/2018	0	0	0	0
20/11/2018 - 26/11/2018	0	1	0	0
27/11/2018 - 03/12/2018	0	0	0	0
04/12/2018 - 10/12/2018	0	0	0	1
11/12/2018 - 17/12/2018	0	0	0	0
18/12/2018 - 24/12/2018	0	1	0	0
25/12/2018 - 31/12/2018				
01/01/2019 - 07/01/2019				
08/01/2019 - 14/01/2019	1	15	0	0
15/01/2019 - 21/01/2019	1	0	1	0
22/01/2019 - 28/01/2019	3	1	1	1
29/01/2019 - 04/02/2019	31	6	15	3
05/02/2019 - 11/02/2019	26	8	11	1
12/02/2019 - 18/02/2019	103	2	57	0
19/02/2019 - 25/02/2019	111	13	56	8
26/02/2019 - 04/03/2019	408	9	130	14
05/03/2019 - 11/03/2019	204	18	110	4
12/03/2019 - 18/03/2019	58	1	34	6
19/03/2019 - 25/03/2019	4	8	1	11
26/03/2019 - 01/04/2019	12	8	3	2
02/04/2019 - 08/04/2019	4	1	3	3
09/04/2019 - 15/04/2019	1	3	0	0
16/04/2019 - 22/04/2019	1	0	0	0
23/04/2019 - 29/04/2019	0	1	0	0
30/04/2019 - 06/05/2019	0	0	0	0
07/05/2019 - 13/05/2019	0	0	0	0
14/05/2019 - 20/05/2019	0	1	0	0
21/05/2019 - 27/05/2019	0	0	0	0
28/05/2019 - 03/06/2019	0	0	0	0
04/06/2019 - 10/06/2019	0	0	0	0

Appendix D.3

The following section describes the fly catch data gathered during the 2018/2019 season for the specimens identified to species level only from their original morphological identification. Specimens identified at the family level are outlined in the digital Appendix I.

Table D.1 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in “T” per week during the 2018/2019 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
20/09/2018 - 26/09/2018							
27/09/2018 - 03/10/2018							
04/10/2018 - 10/10/2018							
11/10/2018 - 17/10/2018					1		
18/10/2018 - 24/10/2018							
25/10/2018 - 31/10/2018			1				
01/11/2018 - 07/11/2018							
08/11/2018 - 14/11/2018	3						
15/11/2018 - 21/11/2018	6						
22/11/2018 - 28/11/2018	11						
29/11/2018 - 05/12/2018	4	1					
06/12/2018 - 12/12/2018	3	3			1		
13/12/2018 - 19/12/2018	8		1				
20/12/2018 - 26/12/2018	9						
27/12/2018 - 02/01/2019	2						
03/01/2019 - 09/01/2019	2	1	1				
10/01/2019 - 16/01/2019	4	1					
17/01/2019 - 23/01/2019	5		3		1		
24/01/2019 - 30/01/2019	8	5					

31/01/2019 - 06/02/2019	9	3				
07/02/2019 - 13/02/2019	31	3				
14/02/2019 - 20/02/2019	14	43	1			
21/02/2019 - 27/02/2019	87	37		1		
28/02/2019 - 06/03/2019	7	7		1		1
07/03/2019 - 13/03/2019	9	3				
14/03/2019 - 20/03/2019	3	2		1		
21/03/2019 - 27/03/2019	6	5		1		
28/03/2019 - 03/04/2019	2	1			2	
04/04/2019 - 10/04/2019	8					
11/04/2019 - 17/04/2019						
18/04/2019 - 24/04/2019	1					
25/04/2019 - 01/05/2019	5				1	
02/05/2019 - 08/05/2019					1	
09/05/2019 - 15/05/2019						
16/05/2019 - 22/05/2019						
Total	337	142	7	4	7	1

Table D.2 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in "R" per week during the 2018/2019 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora hili*	*Calliphora quadramaculata*
20/09/2018 - 26/09/2018							
27/09/2018 - 03/10/2018							
04/10/2018 - 10/10/2018							
11/10/2018 - 17/10/2018							
18/10/2018 - 24/10/2018							
25/10/2018 - 31/10/2018							
01/11/2018 - 07/11/2018							
08/11/2018 - 14/11/2018	1				1		
15/11/2018 - 21/11/2018							
22/11/2018 - 28/11/2018	2	1			2		
29/11/2018 - 05/12/2018	3				4		
06/12/2018 - 12/12/2018	3	1	4		2		
13/12/2018 - 19/12/2018	6	1	2		2		
20/12/2018 - 26/12/2018	3		1		1		
27/12/2018 - 02/01/2019	5	1			4		2
03/01/2019 - 09/01/2019	5	1	2		3		
10/01/2019 - 16/01/2019	3	3	1		1		
17/01/2019 - 23/01/2019	8	3	1		2		
24/01/2019 - 30/01/2019	11	2	1				
31/01/2019 - 06/02/2019	13	3			1		
07/02/2019 - 13/02/2019	49	37					
14/02/2019 - 20/02/2019	36	15	1	2			
21/02/2019 - 27/02/2019	55	43					
28/02/2019 - 06/03/2019	125	66		1			
07/03/2019 - 13/03/2019	8	2		1			
14/03/2019 - 20/03/2019	19	22					1

21/03/2019 - 27/03/2019	6	2	1	1			
28/03/2019 - 03/04/2019	1	4			1		
04/04/2019 - 10/04/2019	18		5		1		
11/04/2019 - 17/04/2019							
18/04/2019 - 24/04/2019	1						
25/04/2019 - 01/05/2019	2						
02/05/2019 - 08/05/2019							
09/05/2019 - 15/05/2019					1		
16/05/2019 - 22/05/2019			1				
Total	392	27	2	5	26		3

Table D.3 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in “BH” per week during the 2018/2019 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
20/09/2018 - 26/09/2018							
27/09/2018 - 03/10/2018							
04/10/2018 - 10/10/2018							
11/10/2018 - 17/10/2018							
18/10/2018 - 24/10/2018							
25/10/2018 - 31/10/2018					1		
01/11/2018 - 07/11/2018	1						
08/11/2018 - 14/11/2018			1				
15/11/2018 - 21/11/2018	1						
22/11/2018 - 28/11/2018	7		4		5		
29/11/2018 - 05/12/2018	8	2			3		
06/12/2018 - 12/12/2018	7	5					
13/12/2018 - 19/12/2018	13		1				
20/12/2018 - 26/12/2018	9	1	2		4		
27/12/2018 - 02/01/2019	4	1	6				
03/01/2019 - 09/01/2019	5	1					
10/01/2019 - 16/01/2019	16	9	4		1		
17/01/2019 - 23/01/2019	16	13			1		
24/01/2019 - 30/01/2019	14	8	6				
31/01/2019 - 06/02/2019	17	1	8				
07/02/2019 - 13/02/2019	3	22			1		
14/02/2019 - 20/02/2019	1	2					
21/02/2019 - 27/02/2019	45	31	2	2			
28/02/2019 - 06/03/2019	18	9					
07/03/2019 - 13/03/2019	5	3					
14/03/2019 - 20/03/2019							
21/03/2019 - 27/03/2019	1	2					

28/03/2019 - 03/04/2019	1	1					
04/04/2019 - 10/04/2019	3						
11/04/2019 - 17/04/2019	4						1
18/04/2019 - 24/04/2019			1		1		
25/04/2019 - 01/05/2019	1						
02/05/2019 - 08/05/2019							
09/05/2019 - 15/05/2019			1				
16/05/2019 - 22/05/2019						1	
Overall Total	227	12	36	2	17	1	1

Table D.4 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in “M” per week during the 2018/2019 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
18/09/2018 - 24/09/2018							
25/09/2018 - 01/10/2018							
02/10/2018 - 08/10/2018							
09/10/2018 - 15/10/2018							
16/10/2018 - 22/10/2018	2						
23/10/2018 - 29/10/2018	1						
30/10/2018 - 05/11/2018							
06/11/2018 - 12/11/2018	2				1		
13/11/2018 - 19/11/2018	9				3		
20/11/2018 - 26/11/2018	18				2		
27/11/2018 - 03/12/2018	15	1			2		
04/12/2018 - 10/12/2018	7	1					
11/12/2018 - 17/12/2018	18	6					
18/12/2018 - 24/12/2018	33	6			1		
25/12/2018 - 31/12/2018	15	1			2		
01/01/2019 - 07/01/2019	29	22	2		2		4
08/01/2019 - 14/01/2019	22	2	1		1		
15/01/2019 - 21/01/2019	12	5					
22/01/2019 - 28/01/2019	17	13					
29/01/2019 - 04/02/2019	15	13		1			
05/02/2019 - 11/02/2019	21	124		11	1		
12/02/2019 - 18/02/2019	11	59	8				
19/02/2019 - 25/02/2019	15	67		1			
26/02/2019 - 04/03/2019	28	8					
05/03/2019 - 11/03/2019	36	18		4			
12/03/2019 - 18/03/2019	11	5					
19/03/2019 - 25/03/2019	4		2				

26/03/2019 - 01/04/2019	31	7		6	2		
02/04/2019 - 08/04/2019	15			2			
09/04/2019 - 15/04/2019	8				1		
16/04/2019 - 22/04/2019	1						
23/04/2019 - 29/04/2019	1						
30/04/2019 - 06/05/2019							
07/05/2019 - 13/05/2019	1						
14/05/2019 - 20/05/2019					1		
21/05/2019 - 27/05/2019				1	1		
28/05/2019 - 03/06/2019			1				
04/06/2019 - 10/06/2019							
Total	758	358	14	26	2	0	4

Table D.5 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in "LD" per week during the 2018/2019 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
25/09/2018 - 01/10/2018							
02/10/2018 - 08/10/2018						1	
09/10/2018 - 15/10/2018						1	
16/10/2018 - 22/10/2018	3		1		4		
23/10/2018 - 29/10/2018	1		4		2		
30/10/2018 - 05/11/2018	1				1		
06/11/2018 - 12/11/2018			2		1		
13/11/2018 - 19/11/2018	2						
20/11/2018 - 26/11/2018	3	1	5		3		
27/11/2018 - 03/12/2018	2						
04/12/2018 - 10/12/2018	4						
11/12/2018 - 17/12/2018	4		1				
18/12/2018 - 24/12/2018	11						
25/12/2018 - 31/12/2018	9	3					
01/01/2019 - 07/01/2019	4	1					
08/01/2019 - 14/01/2019	26	23		1	1		
15/01/2019 - 21/01/2019	14	5	1				
22/01/2019 - 28/01/2019	7	1	1				
29/01/2019 - 04/02/2019	25	21					
05/02/2019 - 11/02/2019	75	43		2			
12/02/2019 - 18/02/2019	73	44		1			
19/02/2019 - 25/02/2019	23	13		1			
26/02/2019 - 04/03/2019	24	13		3			
05/03/2019 - 11/03/2019	31	1					
12/03/2019 - 18/03/2019	15	8					
19/03/2019 - 25/03/2019	13	5	1		1		
26/03/2019 - 01/04/2019	12	4	5				

02/04/2019 - 08/04/2019	16	2	1				
09/04/2019 - 15/04/2019	14						
16/04/2019 - 22/04/2019	7		1		1		
23/04/2019 - 29/04/2019	5				1		
30/04/2019 - 06/05/2019							
07/05/2019 - 13/05/2019	1						
14/05/2019 - 20/05/2019	11			1			
21/05/2019 - 27/05/2019	1				1		
28/05/2019 - 03/06/2019				1			
04/06/2019 - 10/06/2019					2		
Total	437	188	23	10	18	2	0

Table D.6 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “KD” per week during the 2018/2019 season.

Date	Species					
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*
09/10/2018 - 15/10/2018			3		1	
16/10/2018 - 22/10/2018	1					
23/10/2018 - 29/10/2018	1					
30/10/2018 - 05/11/2018	1		2			
06/11/2018 - 12/11/2018	1		2		11	
13/11/2018 - 19/11/2018						
20/11/2018 - 26/11/2018	8		1		7	
27/11/2018 - 03/12/2018	3	1			5	
04/12/2018 - 10/12/2018	7				3	
11/12/2018 - 17/12/2018	5	1	1			
18/12/2018 - 24/12/2018			1			
25/12/2018 - 31/12/2018		3				
01/01/2019 - 07/01/2019	2	7			2	
08/01/2019 - 14/01/2019	8	5			5	
15/01/2019 - 21/01/2019	2	5	2			
22/01/2019 - 28/01/2019	36	26				
29/01/2019 - 04/02/2019	42	33				
05/02/2019 - 11/02/2019	147	84	7		1	
12/02/2019 - 18/02/2019	79	31	2			1
19/02/2019 - 25/02/2019	18	64				
26/02/2019 - 04/03/2019	221	122			1	
05/03/2019 - 11/03/2019	261	133		1		1
12/03/2019 - 18/03/2019	19	15	1			
19/03/2019 - 25/03/2019	15	6				1
26/03/2019 - 01/04/2019	2	12				

02/04/2019 - 08/04/2019	19	4	2			
09/04/2019 - 15/04/2019	7				1	
16/04/2019 - 22/04/2019						
23/04/2019 - 29/04/2019	2					
30/04/2019 - 06/05/2019	3					
07/05/2019 - 13/05/2019	1		1			
14/05/2019 - 20/05/2019	1					
21/05/2019 - 27/05/2019	3					1
28/05/2019 - 03/06/2019						1
Overall Total	1023	552	25	1	37	5

Table D.7 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “ST” per week during the 2018/2019 season.

Date	Species					
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*
09/10/2018 - 15/10/2018	1					
16/10/2018 - 22/10/2018					1	
23/10/2018 - 29/10/2018	6		2			
30/10/2018 - 05/11/2018	3		3		3	
06/11/2018 - 12/11/2018	11		5		1	
13/11/2018 - 19/11/2018	9		2		6	
20/11/2018 - 26/11/2018	17				1	
27/11/2018 - 03/12/2018	6	3	2		2	
04/12/2018 - 10/12/2018	12		4		2	1
11/12/2018 - 17/12/2018	22	8	1		6	
18/12/2018 - 24/12/2018	7	8			2	
25/12/2018 - 31/12/2018						
01/01/2019 - 07/01/2019						
08/01/2019 - 14/01/2019	47	19	1		2	
15/01/2019 - 21/01/2019	54	32				
22/01/2019 - 28/01/2019	85	42			2	
29/01/2019 - 04/02/2019	192	139				
05/02/2019 - 11/02/2019	377	167				
12/02/2019 - 18/02/2019	158	63				
19/02/2019 - 25/02/2019	145	65			1	
26/02/2019 - 04/03/2019	217	93				
05/03/2019 - 11/03/2019	99	39			1	
12/03/2019 - 18/03/2019	9	4	1			
19/03/2019 - 25/03/2019	4	4				
26/03/2019 - 01/04/2019	15	14	2			
02/04/2019 - 08/04/2019	8	5	1	1		
09/04/2019 - 15/04/2019	5					

16/04/2019 - 22/04/2019						1
23/04/2019 - 29/04/2019	5					1
30/04/2019 - 06/05/2019	2		1			
07/05/2019 - 13/05/2019	2					
14/05/2019 - 20/05/2019	1					1
21/05/2019 - 27/05/2019						
28/05/2019 - 03/06/2019	1		1		1	
04/06/2019 - 10/06/2019			1			
Overall Total	1520	705	27	1	40	4

Table D.8 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “S” per week during the 2018/2019 season.

Date	Species					
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*
09/10/2018 - 15/10/2018			1			
16/10/2018 - 22/10/2018						
23/10/2018 - 29/10/2018						
30/10/2018 - 05/11/2018	1					
06/11/2018 - 12/11/2018						
13/11/2018 - 19/11/2018						
20/11/2018 - 26/11/2018	1		3			
27/11/2018 - 03/12/2018						
04/12/2018 - 10/12/2018		1	2			
11/12/2018 - 17/12/2018			1			
18/12/2018 - 24/12/2018	1		1			
25/12/2018 - 31/12/2018						
01/01/2019 - 07/01/2019						
08/01/2019 - 14/01/2019	16		2			
15/01/2019 - 21/01/2019	1	1	3			
22/01/2019 - 28/01/2019	4	2	6			
29/01/2019 - 04/02/2019	37	18	3			
05/02/2019 - 11/02/2019	34	12	27			
12/02/2019 - 18/02/2019	15	57	11			2
19/02/2019 - 25/02/2019	124	64	3			
26/02/2019 - 04/03/2019	417	144	5			
05/03/2019 - 11/03/2019	222	114	3			
12/03/2019 - 18/03/2019	59	4	1			
19/03/2019 - 25/03/2019	12	12				
26/03/2019 - 01/04/2019	2	5	1			
02/04/2019 - 08/04/2019	5	6	1	1		
09/04/2019 - 15/04/2019	4					

16/04/2019 - 22/04/2019	1					
23/04/2019 - 29/04/2019	1					
30/04/2019 - 06/05/2019						
07/05/2019 - 13/05/2019						
14/05/2019 - 20/05/2019	1					
21/05/2019 - 27/05/2019			1			
28/05/2019 - 03/06/2019						1
04/06/2019 - 10/06/2019						
Total	958	440	75	1	0	3

Appendix D.4

The following section describes the total catch of calliphorid species from the 2019/2020 season on each farm from their original morphological identification.

Table D.9 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “T” per week during the 2019/2020 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
19/09/2019 - 25/09/2019							
26/09/2019 - 02/10/2019							
03/10/2019 - 09/10/2019			1				
10/10/2019 - 16/10/2019							
17/10/2019 - 23/10/2019							
24/10/2019 - 30/10/2019							
31/10/2019 - 06/11/2019							
07/11/2019 - 13/11/2019	4						
14/11/2019 - 20/11/2019	1						
21/11/2019 - 27/11/2019	1						
28/11/2019 - 04/12/2019	6		1				
05/12/2019 - 11/12/2019	1	2					
12/12/2019 - 18/12/2019							
19/12/2019 - 25/12/2019							
26/12/2019 - 01/01/2020							
Total	13	2	2	0	0	0	0

Table D.10 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “R” per week during the 2019/2020 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hilli*
19/09/2019 - 25/09/2019							
26/09/2019 - 02/10/2019							
03/10/2019 - 09/10/2019							
10/10/2019 - 16/10/2019							
17/10/2019 - 23/10/2019							
24/10/2019 - 30/10/2019							
31/10/2019 - 06/11/2019					2		
07/11/2019 - 13/11/2019							
14/11/2019 - 20/11/2019							
21/11/2019 - 27/11/2019	1						
28/11/2019 - 04/12/2019					2		
05/12/2019 - 11/12/2019		1					
12/12/2019 - 18/12/2019							
19/12/2019 - 25/12/2019							
26/12/2019 - 01/01/2020							
Total	1	1	0	0	4	0	0

Table D.11 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “BH” per week during the 2019/2020 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
19/09/2019 - 25/09/2019							
26/09/2019 - 02/10/2019							
03/10/2019 - 09/10/2019			*1*			1	
10/10/2019 - 16/10/2019							
17/10/2019 - 23/10/2019							
24/10/2019 - 30/10/2019			2				
31/10/2019 - 06/11/2019			5			1	
07/11/2019 - 13/11/2019			4			7	
14/11/2019 - 20/11/2019	1		1				
21/11/2019 - 27/11/2019	1		2			3	
28/11/2019 - 04/12/2019	3	2				3	
05/12/2019 - 11/12/2019	4	1	1			2	
12/12/2019 - 18/12/2019							
19/12/2019 - 25/12/2019							
26/12/2019 - 01/01/2020							
Total	9	3	16	0	0	17	0

Table D.12 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “M” per week during the 2019/2020 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
17/09/2019 - 23/09/2019							
24/09/2019 - 30/09/2019							
01/10/2019 - 07/10/2019							
08/10/2019 - 14/10/2019							
15/10/2019 - 21/10/2019							
22/10/2019 - 28/10/2019					1		
29/10/2019 - 04/11/2019	2		1		3		
05/11/2019 - 11/11/2019	12				2		
12/11/2019 - 18/11/2019	8				2		
19/11/2019 - 25/11/2019	47						
26/11/2019 - 02/12/2019	23	3			4		
03/12/2019 - 09/12/2019	6	2					
10/12/2019 - 16/12/2019							
17/12/2019 - 23/12/2019							
24/12/2019 - 30/12/2019							
Total	98	5	1	0	12	0	0

Table D.13 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “LD” per week during the 2019/2020 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
17/09/2019 - 23/09/2019							
24/09/2019 - 30/09/2019							
01/10/2019 - 07/10/2019					1		
08/10/2019 - 14/10/2019							
15/10/2019 - 21/10/2019					1		
22/10/2019 - 28/10/2019			1				
29/10/2019 - 04/11/2019			1				
05/11/2019 - 11/11/2019	1						
12/11/2019 - 18/11/2019					1		
19/11/2019 - 25/11/2019							
26/11/2019 - 02/12/2019	5		1		2		
03/12/2019 - 09/12/2019	1	1					
10/12/2019 - 16/12/2019							
17/12/2019 - 23/12/2019							
24/12/2019 - 30/12/2019							
31/12/2019 - 06/01/2020							
07/01/2020 - 13/01/2020							
14/01/2020 - 20/01/2020							
21/01/2020 - 27/01/2020							
28/01/2020 - 03/02/2020							
04/02/2020 - 10/02/2020							
11/02/2020 - 17/02/2020							
18/02/2020 - 24/02/2020							
25/02/2020 - 02/03/2020							
03/03/2020 - 09/03/2020							
10/03/2020 - 16/03/2020	7	9	1				
17/03/2020 - 23/03/2020	3						

24/03/2020 - 30/03/2020	26	16	13	1			
31/03/2020 - 06/04/2020	1	1		1			
07/04/2020 - 13/04/2020	1						
14/04/2020 - 20/04/2020							
21/04/2020 - 27/04/2020							
28/04/2020 - 04/05/2020			1		1		
05/05/2020 - 11/05/2020	2						
12/05/2020 - 18/05/2020	1						
19/05/2020 - 25/05/2020							
26/05/2020 - 01/06/2020					1		
02/06/2020 - 08/06/2020							
09/06/2020 - 15/06/2020							
16/06/2020 - 22/06/2020							
23/06/2020 - 29/06/2020							
Total	48	27	18	2	7	0	0

Table D.14 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in "KD" per week during the 2019/2020 season.

Date	*Lucilia cuprina*	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
18/09/2019 - 24/09/2019							
25/09/2019 - 01/10/2019							
02/10/2019 - 08/10/2019							
09/10/2019 - 15/10/2019							
16/10/2019 - 22/10/2019							
23/10/2019 - 29/10/2019	1						
30/10/2019 - 05/11/2019							
06/11/2019 - 12/11/2019	1						
13/11/2019 - 19/11/2019	2		3				
20/11/2019 - 26/11/2019			1				
27/11/2019 - 03/12/2019			1				
04/12/2019 - 10/12/2019	2	1					
11/12/2019 - 17/12/2019							
18/12/2019 - 24/12/2019							
25/12/2019 - 31/12/2019							
Total	6	1	5	0	0	0	0

Table D.15 The catch of Calliphoridae from the LuciTrap® and StickyTrap combination in “ST” per week during the 2019/2020 season.

Date	*Lucilia cuprina*	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
18/09/2019 - 24/09/2019							
25/09/2019 - 01/10/2019							
02/10/2019 - 08/10/2019							
09/10/2019 - 15/10/2019							
16/10/2019 - 22/10/2019							
23/10/2019 - 29/10/2019	2						
30/10/2019 - 05/11/2019			1				
06/11/2019 - 12/11/2019	2						
13/11/2019 - 19/11/2019	1						
20/11/2019 - 26/11/2019			1				
27/11/2019 - 03/12/2019			1				
04/12/2019 - 10/12/2019	1	1					
11/12/2019 - 17/12/2019							
18/12/2019 - 24/12/2019							
25/12/2019 - 31/12/2019							
Total	6	1	3	0	0	0	0

Table D.16 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in "S" per week during the 2019/2020 season.

Date	*Lucilia cuprina*	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
18/09/2019 - 24/09/2019							
25/09/2019 - 01/10/2019							
02/10/2019 - 08/10/2019							
09/10/2019 - 15/10/2019							
16/10/2019 - 22/10/2019							
23/10/2019 - 29/10/2019							
30/10/2019 - 05/11/2019							
06/11/2019 - 12/11/2019	1						
13/11/2019 - 19/11/2019	1						
20/11/2019 - 26/11/2019	2						
27/11/2019 - 03/12/2019	3						
04/12/2019 - 10/12/2019	3	2					
11/12/2019 - 17/12/2019							
18/12/2019 - 24/12/2019							
25/12/2019 - 31/12/2019							
Total	10	2	0	0	0	0	0

Appendix D.5

The following section describes the total catch of calliphorid species gathered during the 2020/2021 season only from their original morphological identification. Specimens identified at the family level are outlined in the digital Appendix I.

Table D.17 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in "T" per week during the 2020/2021 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
14/09/2020 - 20/09/2020	0	0	0	0	0	0	0
21/09/2020 - 27/09/2020							
28/09/2020 - 04/10/2020							
05/10/2020 - 11/10/2020							
12/10/2020 - 18/10/2020							
19/10/2020 - 25/10/2020							
26/10/2020 - 01/11/2020							
02/11/2020 - 08/11/2020	2						
09/11/2020 - 15/11/2020							
16/11/2020 - 22/11/2020	4						
23/11/2020 - 29/11/2020	3						
30/11/2020 - 06/12/2020	1				1		
07/12/2020 - 13/12/2020	2						
14/12/2020 - 20/12/2020			1		1		
Total	12	0	1	0	2	0	0

Table D.18 The catch of Calliphoridae from the LuciTrap® and Stickytrap combination in “R” per week during the 2020/2021 season.

Date	Species						
	Lucilia cuprina	*Lucilia sericata*	*Calliphora stygia*	*Chrysomya rufifacies*	*Calliphora vicina*	*Calliphora quadramaculata*	*Calliphora hili*
14/09/2020 - 20/09/2020							
21/09/2020 - 27/09/2020							
28/09/2020 - 04/10/2020							
05/10/2020 - 11/10/2020							
12/10/2020 - 18/10/2020							
19/10/2020 - 25/10/2020			2				
26/10/2020 - 01/11/2020			1		2		
02/11/2020 - 08/11/2020					1		
09/11/2020 - 15/11/2020					1		
16/11/2020 - 22/11/2020						1	
23/11/2020 - 29/11/2020					1		
30/11/2020 - 06/12/2020							
07/12/2020 - 13/12/2020							
14/12/2020 - 20/12/2020					1		
Total	12	0	1	0	2	0	0

Appendix E.

Appendix E.1

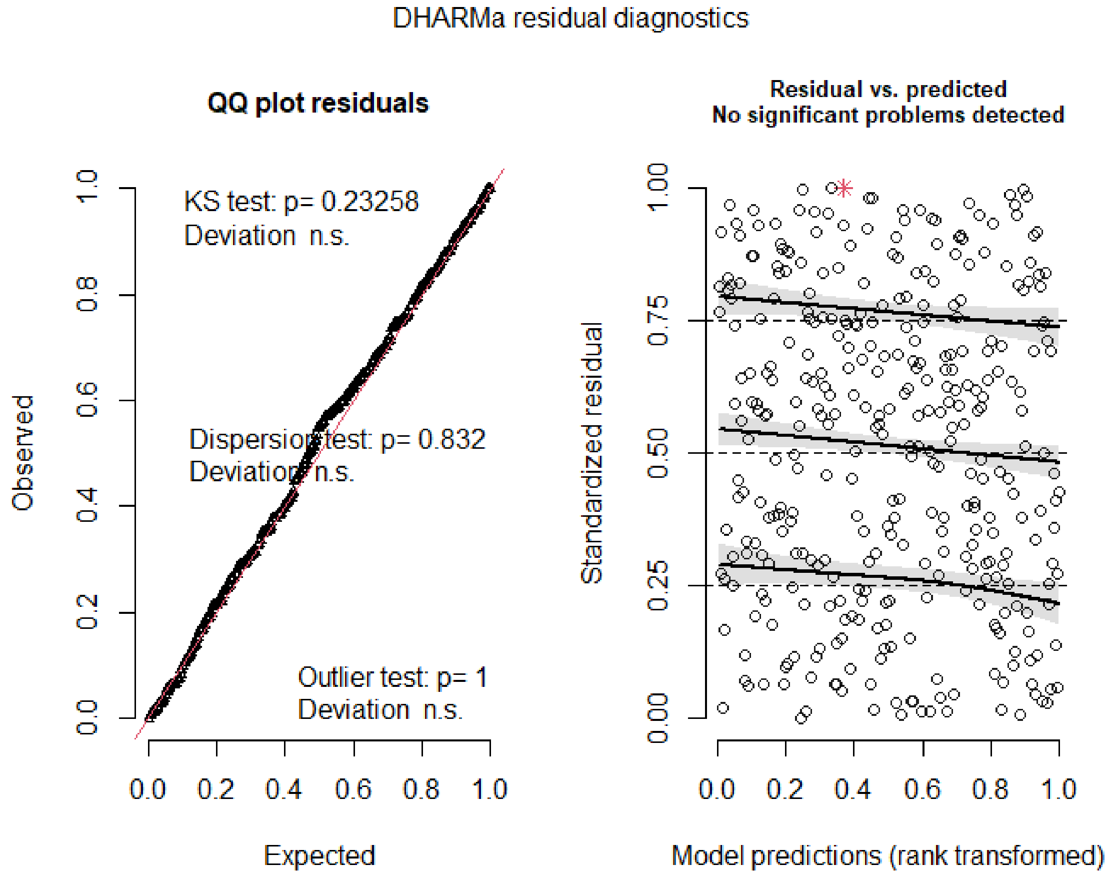

Figure E.1 Q-Q plot of and the residuals versus the predicted for the hurdle model for *Lucilia* spp.

Appendix E.2

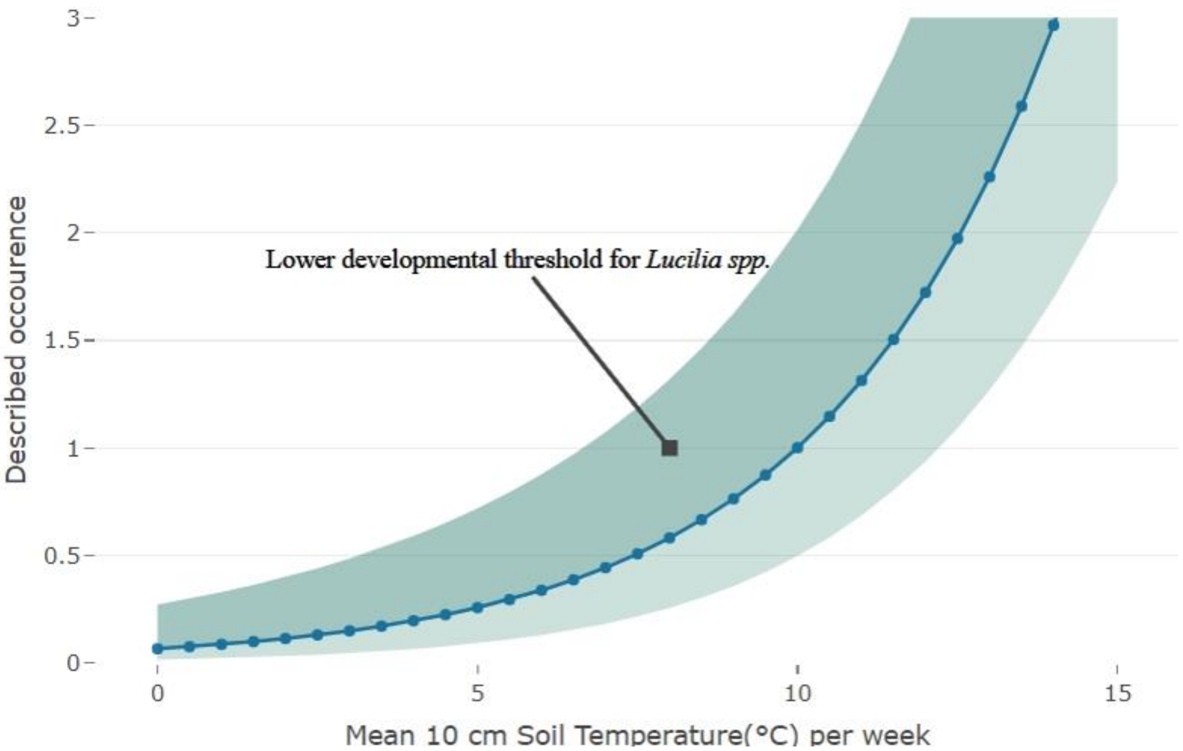

Figure E.2 A simulation of the described occurrence of *Lucilia* spp. on each farm due to the effect of a mean ten 10 soil temperature at a lag of three weeks from 0 – 15℃ with a lower developmental threshold of 10℃.

Appendix E.3

```
# packages used #
library(lme4)
library(glmmTMB)
library(performance)
library(lmerTest)
library(dplyr)
library(sjmisc)
library(sjPlot)
library(sjstats)
library(tidyverse)
library(effects)
library(ggeffects)
library(Rtools)
library(insight)
library(parameters)

# Data set up
DataV12_1<-DataV12 %>% group_by(Farm)%>%
  mutate(Tencm_Soil_Temperature1 = lag(Tencm_Soil_Temperature,1, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature2 = lag(Tencm_Soil_Temperature,2, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature3 = lag(Tencm_Soil_Temperature,3, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature4 = lag(Tencm_Soil_Temperature,4, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature5 = lag(Tencm_Soil_Temperature,5, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature6 = lag(Tencm_Soil_Temperature,6, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature7 = lag(Tencm_Soil_Temperature,7, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature8 = lag(Tencm_Soil_Temperature,8, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature9 = lag(Tencm_Soil_Temperature,9, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature10 = lag(Tencm_Soil_Temperature,10, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature11 = lag(Tencm_Soil_Temperature,11, order_by = Week))%>%
  mutate(Tencm_Soil_Temperature12 = lag(Tencm_Soil_Temperature,12, order_by = Week))%>%
  mutate(Sum_Rainfall1 = lag(Sum_Rainfall,1, order_by = Week))%>%
  mutate(Sum_Rainfall2 = lag(Sum_Rainfall,2, order_by = Week))%>%
  mutate(Sum_Rainfall3 = lag(Sum_Rainfall,3, order_by = Week))%>%
  mutate(Sum_Rainfall4 = lag(Sum_Rainfall,4, order_by = Week))%>%
  mutate(Sum_Rainfall5 = lag(Sum_Rainfall,5, order_by = Week))%>%
  mutate(Sum_Rainfall6 = lag(Sum_Rainfall,6, order_by = Week))%>%
  mutate(Sum_Rainfall7 = lag(Sum_Rainfall,7, order_by = Week))%>%
  mutate(Sum_Rainfall8 = lag(Sum_Rainfall,8, order_by = Week))%>%
  mutate(Sum_Rainfall9 = lag(Sum_Rainfall,9, order_by = Week))%>%
  mutate(Sum_Rainfall10 = lag(Sum_Rainfall,10, order_by = Week))%>%
  mutate(Sum_Rainfall11 = lag(Sum_Rainfall,11, order_by = Week))%>%
  mutate(Sum_Rainfall12 = lag(Sum_Rainfall,12, order_by = Week))%>%
  mutate(Average_Temperature1 = lag(Average_Temperature,1, order_by = Week))%>%
  mutate(Average_Temperature2 = lag(Average_Temperature,2, order_by = Week))%>%
  mutate(Average_Temperature3 = lag(Average_Temperature,3, order_by = Week))%>%
  mutate(Average_Temperature4 = lag(Average_Temperature,4, order_by = Week))%>%
  mutate(Average_Temperature5 = lag(Average_Temperature,5, order_by = Week))%>%
  mutate(Average_Temperature6 = lag(Average_Temperature,6, order_by = Week))%>%
  mutate(Average_Temperature7 = lag(Average_Temperature,7, order_by = Week))%>%
  mutate(Average_Temperature8 = lag(Average_Temperature,8, order_by = Week))%>%
  mutate(Average_Temperature9 = lag(Average_Temperature,9, order_by = Week))%>%
  mutate(Average_Temperature10 = lag(Average_Temperature,10, order_by = Week))%>%
  mutate(Average_Temperature11 = lag(Average_Temperature,11, order_by = Week))%>%
  mutate(Average_Temperature12 = lag(Average_Temperature,12, order_by = Week))%>%
  mutate(Max_High_Temperature1 = lag(Max_High_Temperature,1, order_by = Week))%>%
  mutate(Max_High_Temperature2 = lag(Max_High_Temperature,2, order_by = Week))%>%
  mutate(Max_High_Temperature3 = lag(Max_High_Temperature,3, order_by = Week))%>%
  mutate(Max_High_Temperature4 = lag(Max_High_Temperature,4, order_by = Week))%>%
```

```
  mutate(Max_High_Temperature5 = lag(Max_High_Temperature,5, order_by = Week))%>%
  mutate(Max_High_Temperature6 = lag(Max_High_Temperature,6, order_by = Week))%>%
  mutate(Max_High_Temperature7 = lag(Max_High_Temperature,7, order_by = Week))%>%
  mutate(Max_High_Temperature8 = lag(Max_High_Temperature,8, order_by = Week))%>%
  mutate(Max_High_Temperature9 = lag(Max_High_Temperature,9, order_by = Week))%>%
  mutate(Max_High_Temperature10 = lag(Max_High_Temperature,10, order_by = Week))%>%
  mutate(Max_High_Temperature11 = lag(Max_High_Temperature,11, order_by = Week))%>%
  mutate(Max_High_Temperature12 = lag(Max_High_Temperature,12, order_by = Week))%>%
  mutate(Min_Low_Temperature1 = lag(Min_Low_Temperature,1, order_by = Week))%>%
  mutate(Min_Low_Temperature2 = lag(Min_Low_Temperature,2, order_by = Week))%>%
  mutate(Min_Low_Temperature3 = lag(Min_Low_Temperature,3, order_by = Week))%>%
  mutate(Min_Low_Temperature4 = lag(Min_Low_Temperature,4, order_by = Week))%>%
  mutate(Min_Low_Temperature5 = lag(Min_Low_Temperature,5, order_by = Week))%>%
  mutate(Min_Low_Temperature6 = lag(Min_Low_Temperature,6, order_by = Week))%>%
  mutate(Min_Low_Temperature7 = lag(Min_Low_Temperature,7, order_by = Week))%>%
  mutate(Min_Low_Temperature8 = lag(Min_Low_Temperature,8, order_by = Week))%>%
  mutate(Min_Low_Temperature9 = lag(Min_Low_Temperature,9, order_by = Week))%>%
  mutate(Min_Low_Temperature10 = lag(Min_Low_Temperature,10, order_by = Week))%>%
  mutate(Min_Low_Temperature11 = lag(Min_Low_Temperature,11, order_by = Week))%>%
  mutate(Min_Low_Temperature12 = lag(Min_Low_Temperature,12, order_by = Week))%>%
  mutate(Average_Humidity1 = lag(Average_Humidity,1, order_by = Week))%>%
  mutate(Average_Humidity2 = lag(Average_Humidity,2, order_by = Week))%>%
  mutate(Average_Humidity3 = lag(Average_Humidity,3, order_by = Week))%>%
  mutate(Average_Humidity4 = lag(Average_Humidity,4, order_by = Week))%>%
  mutate(Average_Humidity5 = lag(Average_Humidity,5, order_by = Week))%>%
  mutate(Average_Humidity6 = lag(Average_Humidity,6, order_by = Week))%>%
  mutate(Average_Humidity7 = lag(Average_Humidity,7, order_by = Week))%>%
  mutate(Average_Humidity8 = lag(Average_Humidity,8, order_by = Week))%>%
  mutate(Average_Humidity9 = lag(Average_Humidity,9, order_by = Week))%>%
  mutate(Average_Humidity10 = lag(Average_Humidity,10, order_by = Week))%>%
  mutate(Average_Humidity11 = lag(Average_Humidity,11, order_by = Week))%>%
  mutate(Average_Humidity12 = lag(Average_Humidity,12, order_by = Week))%>%
  mutate(Daylength1 = lag(Daylength,1, order_by = Week))%>%
  mutate(Daylength2 = lag(Daylength,2, order_by = Week))%>%
  mutate(Daylength3 = lag(Daylength,3, order_by = Week))%>%
  mutate(Daylength4 = lag(Daylength,4, order_by = Week))%>%
  mutate(Daylength5 = lag(Daylength,5, order_by = Week))%>%
  mutate(Daylength6 = lag(Daylength,6, order_by = Week))%>%
  mutate(Daylength7 = lag(Daylength,7, order_by = Week))%>%
  mutate(Daylength8 = lag(Daylength,8, order_by = Week))%>%
  mutate(Daylength9 = lag(Daylength,9, order_by = Week))%>%
  mutate(Daylength10 = lag(Daylength,10, order_by = Week))%>%
  mutate(Daylength11 = lag(Daylength,11, order_by = Week))%>%
  mutate(Daylength12 = lag(Daylength,12, order_by = Week))
DataV11_1
```

```
# model code
variables removed using a backward selection method first, then by the variable with the highest VIF
rating

# daylength 1
model_x2_29 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
              zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                  Daylength1 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                  family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_29)
r2_zeroinflated(model_x2_29,method = "correlation")
AIC(model_x2_29)
check_collinearity(model_x2_29)
plot(check_collinearity(model_x2_29))

# daylength 2
model_x2_30 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
              zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                  Daylength2 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                  family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_30 )
r2_zeroinflated(model_x2_30 2,method = "correlation")
AIC(model_x2_30 )
check_collinearity(model_x2_30 )
plot(check_collinearity(model_x2_30 ))

# daylength 3
model_x2_31 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
              zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
```

```
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                  Daylength3 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                  family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_31)
r2_zeroinflated(model_x2_31,method = "correlation")
AIC(model_x2_31)
check_collinearity(model_x2_31)
plot(check_collinearity(model_x2_31))

# daylength 4
model_x2_32 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
              zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                  Daylength4 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                  family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_32)
r2_zeroinflated(model_x2_32,method = "correlation")
AIC(model_x2_32)
check_collinearity(model_x2)
plot(check_collinearity(model_x2))

# daylength 5
model_x2_33 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
              zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                    Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                  Daylength5 +
                                  Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                  family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_33)
r2_zeroinflated(model_x2_33,method = "correlation")
AIC(model_x2_33)
check_collinearity(model_x2)
plot(check_collinearity(model_x2))

# daylength 6
model_x2_34 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
```

```
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
                zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                        Daylength6 +
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                        family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_34)
r2_zeroinflated(model_x2_34,method = "correlation")
AIC(model_x2_34)
check_collinearity(model_x2_34)
# daylength 7
model_x2_35 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
                zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                        Daylength7 +
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                        family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_35)
r2_zeroinflated(model_x2_35,method = "correlation")
AIC(model_x2_35)
check_collinearity(model_x2_35)


# daylength 8
model_x2_36 <- glmmTMB(LCLS_Combined ~ Tencm_Soil_Temperature1 +
Tencm_Soil_Temperature2 + Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 +
Tencm_Soil_Temperature5 +
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5  +(1|Farm),
                zi = ~     Tencm_Soil_Temperature1 + Tencm_Soil_Temperature2 +
Tencm_Soil_Temperature3 + Tencm_Soil_Temperature4 + Tencm_Soil_Temperature5 +
                        Max_High_Temperature1 + Max_High_Temperature2 + Max_High_Temperature3
+ Max_High_Temperature4 + Max_High_Temperature5   +
                                        Daylength8 +
                                        Sum_Rainfall1 + Sum_Rainfall2 + Sum_Rainfall3 + Sum_Rainfall4
+ Sum_Rainfall5,
                                        family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_36)
r2_zeroinflated(model_x2_36,method = "correlation")
AIC(model_x2_36)
check_collinearity(model_x2_36)

# AIC, BIC and Anova
```

```
AIC   (model_x2_29,model_x2_30, model_x2_31, model_x2_32, model_x2_33, model_x2_34,
model_x2_35, model_x2_36)
BIC   (model_x2_29,model_x2_30, model_x2_31, model_x2_32, model_x2_33, model_x2_34,
model_x2_35, model_x2_36)
anova (model_x2_29,model_x2_30, model_x2_31, model_x2_32, model_x2_33, model_x2_34,
model_x2_35, model_x2_36)
compare_performance (model_x2_29,model_x2_30, model_x2_31, model_x2_32, model_x2_33,
model_x2_34, model_x2_35, model_x2_36)

# model_x2_34 had the lowest AIC, BIC

#######
# final model -
model_x2_34 <- glmmTMB(LCLS_Combined ~   Tencm_Soil_Temperature2    +
                                                 Sum_Rainfall5 +
                         Max_High_Temperature1  + Max_High_Temperature3 + Max_High_Temperature4
+(1|Farm),
                zi = ~

                                               Daylength6
                                                 ,
                                               family = truncated_nbinom2, data = DataV11_1)
summary(model_x2_34)
r2_zeroinflated(model_x2_34,method = "correlation")
AIC(model_x2_34)
check_collinearity(model_x2_34)
plot(check_collinearity(model_x2_34))
model_parameters(model_x2_34, effects = "all")
tab_model(model_x2_34)
tab_model(model_x2_34,transform = NULL, auto.label = FALSE)
performance_mse(model_x2_34)
performance_rmse(model_x2_34)

library(DHARMa)
set.seed(123)
simulationOutput <- simulateResiduals(fittedModel = model_x2_28,plot = TRUE)
residuals(simulationOutput,quantileFunction = qnorm)
testResiduals(simulationOutput)
plot(simulationOutput, quantreg = T)
testUniformity(simulationOutput = simulationOutput)
simulationOutput <- simulateResiduals(fittedModel = model_x2_28, refit = T)
simulationOutput <- simulateResiduals(fittedModel = model_x2_28, n = 250, use.u = T)


# prediction
predict(model_x2_34, newdata = DataV11_1)
ls26_v1<-predict(model_x2_34,newdata = DataV11_1,type = "conditional",zitype =
NULL,allow.new.levels=TRUE)
ls26_v1<-data.frame(ls26_v1)
ls26_v1

#
library(sjplot)
# to get model estimates
get_model_data(model_x2_34, type = "est")
# data used to create table 7.2


# grab data from the predictions
# change max print option to above 1,000
```

```
getOption("max.print")
options(max.print = 10000)

##### Simulation predictions
# Rainfall
Rainfall<-ggpredict(model_x2_34, terms = "Sum_Rainfall5" , type = "sim")
str(Rainfall)
Rainfall <-data.frame(Rainfall)
Rainfall


# 10cm soil
TencmSoil<-ggpredict(model_x2_34, terms = "Tencm_Soil_Temperature2 ",  type = "sim")
TencmSoil <-data.frame(TencmSoil)
TencmSoil

#Max_High_Temperature1
MaxTemp1<-ggpredict(model_x2_34, terms = "Max_High_Temperature1", type = "sim")
MaxTemp1 <-data.frame(MaxTemp1)
MaxTemp1

#Max_High_Temperature2
MaxTemp3 <-ggpredict(model_x2_34, terms = "Max_High_Temperature3", type = "sim")
MaxTemp3 <-data.frame(MaxTemp3)
MaxTemp3

#Max_High_Temperature4
MaxTemp4<-ggpredict(model_x2_34, terms = "Max_High_Temperature4", type = "sim")
MaxTemp4 <-data.frame(MaxTemp4)
MaxTemp4

#Daylength6
DaylengthSix <-ggpredict(model_x2_34, terms = "Daylength6", type = "sim")
DaylengthSix <-data.frame(DaylengthSix)
DaylengthSix

###
 # Values from the count model
 # 10cm Soil temperatuee
TencmSoil_2<-ggpredict(model_x2_34, terms = "Tencm_Soil_Temperature2 [0:30]", type = "fe")
TencmSoil_2 <-data.frame(TencmSoil_2)
TencmSoil_2

TencmSoil_2<-ggpredict(model_x2_34, terms = "Tencm_Soil_Temperature2 [0.5:30]", type = "fe")
TencmSoil_2 <-data.frame(TencmSoil_2)
TencmSoil_2

TencmSoil_2<-ggpredict(model_x2_34, terms = "Tencm_Soil_Temperature2 [all]", type = "fe")
TencmSoil_2 <-data.frame(TencmSoil_2)
TencmSoil_2


# Rainfall
Rainfall_2<-ggpredict(model_x2_34, terms = "Sum_Rainfall5 [0:130]", type = "fe")
Rainfall_2 <-data.frame(Rainfall_2)
Rainfall_2

Rainfall_2<-ggpredict(model_x2_34, terms = "Sum_Rainfall5 [0.5:130]", type = "fe")
Rainfall_2 <-data.frame(Rainfall_2)
Rainfall_2
```

```
# Max_High_Temperature1 - fixed effects from model
MaxTemp1_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature1 [0:40]", type = "fe")
MaxTemp1_2 <-data.frame(MaxTemp1_2)
MaxTemp1_2

MaxTemp1_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature1 [0.5:40]", type = "fe")
MaxTemp1_2 <-data.frame(MaxTemp1_2)
MaxTemp1_2

# Max_High_Temperature2 - fixed effects from model
MaxTemp3_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature3 [0:40]", type = "fe")
MaxTemp3_2 <-data.frame(MaxTemp3_2)
MaxTemp3_2

MaxTemp3_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature3 [0.5:40]", type = "fe")
MaxTemp3_2 <-data.frame(MaxTemp3_2)
MaxTemp3_2


# Max_High_Temperature4 - fixed effects from model
MaxTemp4_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature4 [0:40]", type = "fe")
MaxTemp4_2 <-data.frame(MaxTemp4_2)
MaxTemp4_2

MaxTemp4_2<-ggpredict(model_x2_34, terms = "Max_High_Temperature4 [0.5:40]", type = "fe")
MaxTemp4_2 <-data.frame(MaxTemp4_2)
MaxTemp4_2


# Values from logistic model
Day6_2<-ggpredict(model_x2_34, terms = "Daylength6 [all]", type = "zi_prob")
Day6_2 <-data.frame(Day6_2)
Day6_2

Day6_2<-ggpredict(model_x2_34, terms = "Daylength6 [0:16]", type = "zi_prob")
Day6_2 <-data.frame(Day6_2)
Day6_2

Day6_2<-ggpredict(model_x2_34, terms = "Daylength6 [0.5:16]", type = "zi_prob")
Day6_2 <-data.frame(Day6_2)
Day6_2
```

Appendix F.

Appendix F.1

The following outlines the model diagnostics for the final model presented in Chapter Six

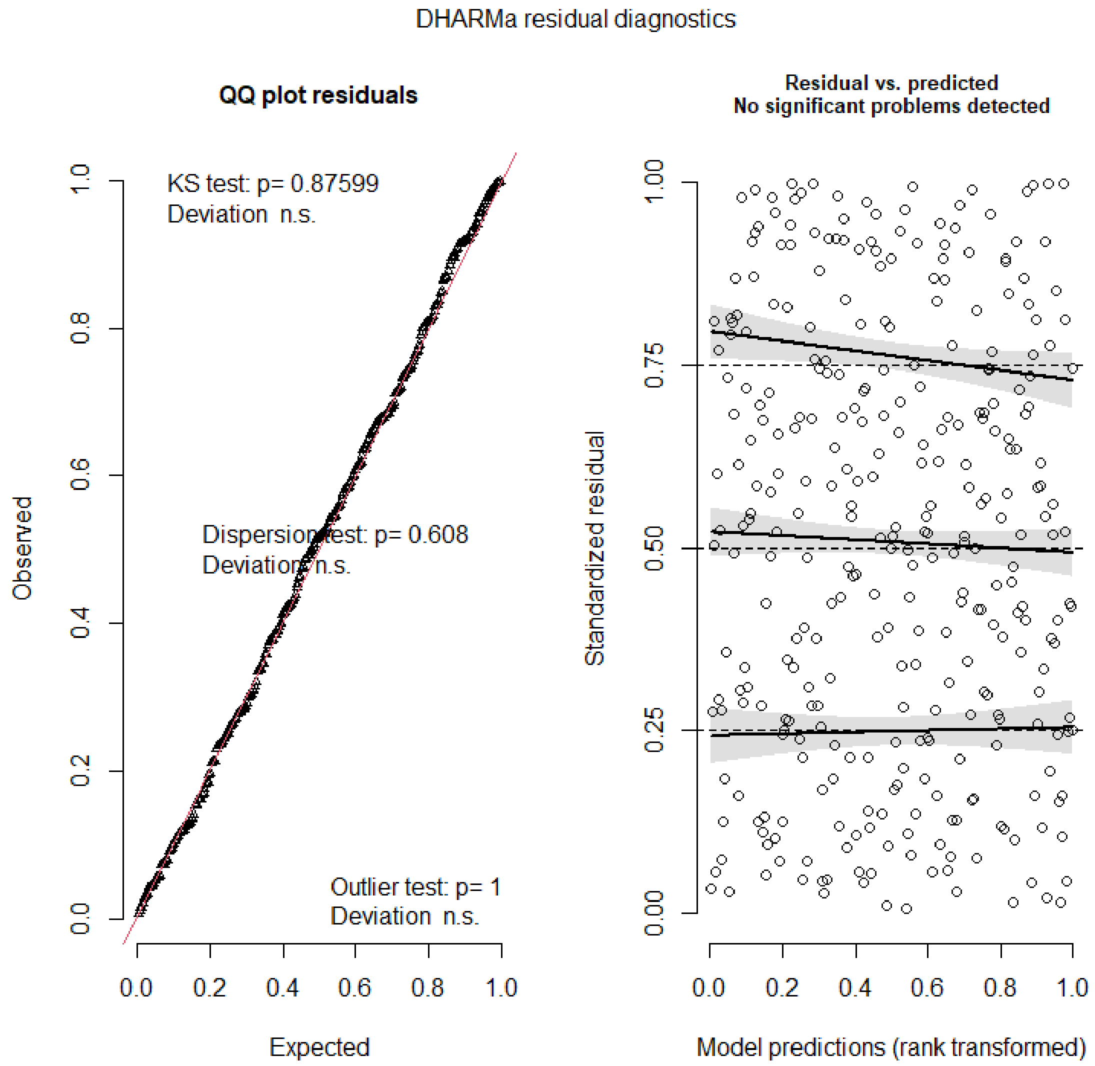

Figure F.1. A Q-Q plot and simulated residuals plotted against observed data both were found to be normally distributed

Appendix F.2

The following outlines the packages and code used in R studio for the analysis in Chapter Six

```
# packages #
library(lme4)
library(sjmisc)
library(performance)
library(glmmTMB)
library(ggeffects)
library(tidyverse)
library(gridExtra)
library(grid)
library(ggplot2)
library(lattice)
library(ROCR)
library(caTools)
library(MASS)
library(dplyr)

#################
# 10cm soil + Avg soil moisture
set.seed(7279)
model_1 <- glmer( LCLS ~ Average_Tencm_Soil_Temperature  + Average_Soil_Moisture  + (1|Farm) ,
data = DataV6_Training_2, family = binomial, , nAGQ = 0)
summary(model_1)
r2_zeroinflated(model_1,method = "correlation")
cor.test(model_1)
vif(model_1)
check_collinearity(model_1)
plot(check_collinearity(model_1))
tab_model(model_1,transform = NULL, auto.label = FALSE)
check_model(model_1)
check_collinearity(model_1)
model_performance(model_1)
compare_performance(model_1)
performance_hosmer(model_1)
r2(model_1)

# 10cm soil + Sum_Rain
model_2 <- glmer( LCLS ~ Average_Tencm_Soil_Temperature  + Sum_Rain  + (1|Farm) , data =
DataV6_Training_2, family = binomial, , nAGQ = 0)
summary(model_2)

# Rainfall not signficant
cor.test(model_2)
vif(model_2)
check_collinearity(model_2)
plot(check_collinearity(model_2))
tab_model(model_2,transform = NULL, auto.label = FALSE)
check_model(model_2)
check_collinearity(model_2)
model_performance(model_1,model_2)
compare_performance(model_1,model_2)
performance_hosmer(model_2)
r2(model_2)
```

```
# 10cm soil + Avg_SWET
model_3 <- glmer( LCLS ~ Average_Tencm_Soil_Temperature  + Avg_SWET  + (1|Farm) , data =
DataV6_Training_2, family = binomial, , nAGQ = 0)
summary(model_3)
cor.test(model_3)
vif(model_3)
check_collinearity(model_3)
plot(check_collinearity(model_3))
tab_model(model_3,transform = NULL, auto.label = FALSE)
check_model(model_3)
check_collinearity(model_3)
model_performance(model_1,model_3)
compare_performance(model_1,model_3)
performance_hosmer(model_3)
r2(model_2)

#####
Soil_1<-ggpredict(model_1, terms = "Average_Tencm_Soil_Temperature  [0:30]", type = "zi_prob")
Soil_1 <-data.frame(Soil_1)
Soil_1

Soil<-ggpredict(model_1, terms = "Average_Tencm_Soil_Temperature [0.5:30]", type = "zi_prob")
Soil <-data.frame(Soil)
Soil

Soilm_1<-ggpredict(model_1, terms = "Average_Soil_Moisture  [-150:30]", type = "zi_prob")
Soilm_1 <-data.frame(Soilm_1)
Soilm_1

Soilm<-ggpredict(model_1, terms = "Average_Soil_Moisture [-150.5:30]", type = "zi_prob")
Soilm <-data.frame(Soilm)
Soilm

#####
# To generate values for the 3d graph

# to create data set for model
grid.lines = 50
x.pred <- seq(min(DataV6_Test_2$Average_Soil_Moisture),
max(DataV6_Test_2$Average_Soil_Moisture), length.out = grid.lines)
y.pred <-
seq(min(DataV6_Test_2$Average_Tencm_Soil_Temperature),max(DataV6_Test_2$Average_Tencm_So
il_Temperature), length.out = grid.lines)
xy <- expand.grid( Average_Soil_Moisture = x.pred, Average_Tencm_Soil_Temperature= y.pred)
head(xy)
xy$log <-exp(-7.165239 +  0.445807*xy$Average_Tencm_Soil_Temperature +-0.031515
*xy$Average_Soil_Moisture)
xy$probability <-xy$log/(1+xy$log)
head(xy)

# Graph V1
# First manually set the colours for the probability - adding 100 works for some reason
colfunc <- colorRampPalette(c("red","orange","yellow","green"))
wireframe(probability ~ Average_Tencm_Soil_Temperature * Average_Soil_Moisture, data = xy,scales
= list(arrows = FALSE),
  xlab = "Mean 10 cm Soil Temperature (°C) per week",
  ylab = "Mean Soil Moisture (mm) per week",
  zlab = "Probability",
  fontfa
  drape = TRUE,colorkey = TRUE,screen = list(z = 30, x = -60),
```

```
  col.regions = colfunc(100))

# Plotly Version of 3d graph - not used in chapter
# Note this is too hard to change, the issue with the graph itself - cannot get the eye part of code to be
correct to generate a plot to use in chapter
library (plotly)

fig <-plot_ly(data=xy, x=~xy$Average_Tencm_Soil_Temperature, y=~xy$Average_Soil_Moisture,
z=~xy$probability, color=~xy$probability, type = "scatter3d", mode="markers")
axx <- list(title = "Mean 10 cm Soil Temperature (°C)")
axy <- list(title = "Mean Soil Moisture (mm)")
axz <- list(title = "Probability")
fig <- fig %>% layout(scene = list(xaxis=axx,yaxis=axy,zaxis=axz), eye = list (x = 1.95, y = -1.25, z =
0.5))
fig

######
library(sjmisc)
sjp.glmer(model_1,type = "fe",sort.coef = TRUE)
plot_model(model_1, type = "pred")

#####
set.seed(100)
# Roc Curve code
library(ROCR)
library(caTools)
library(MASS)
library(dplyr)

pred_test <- predict(model_1,Test_DataV6,type="response")
ROCR_pred_test <- prediction(pred_test,Test_DataV6$LCLS)

perf <- performance(ROCR_pred_test, "auc") # to get auc value
perf$y.values

# generate Roc curve
ROCR_perf_test <- performance(ROCR_pred_test,'tpr','fpr')
plot(ROCR_perf_test,colorize=TRUE,abline(a=0,b=1),print.cutoffs.at=seq(0.1,by=0.1))

#####
#    Confusion Matrix output
set.seed(100)
library(caret)
ls_S4 = ifelse(ls_S4 > 0.7261757, "1", "0")
ls_S4
confusionMatrix(factor(ls_S4), factor(Test_DataV6$LCLS))

## GGplot verions of Roc Curve
ROC_perf <- performance(ROCR_pred_test,"tpr","fpr")
ROC_sens <- performance(ROCR_pred_test,"sens","spec")
ROC_auc <- performance(ROCR_pred_test,"auc")

# Make plot data
plotdat <-
data.frame(FP=ROC_perf$x.values[[1]],TP=ROC_perf$y.values[[1]],CUT=ROC_perf$alpha.values[[1]],
POINT=NA)
plotdat[unlist(lapply(seq(0,1,0.1),function(x){which.min(abs(plotdat$CUT-x))})),"POINT"] <-
seq(0,1,0.1)
plotdat
```

```
# Plot the curve
library(ggplot2)
Roc_2  <-ggplot(plotdat, aes(x=FP,y=TP)) +
  geom_abline(intercept=0,slope=1) +
  geom_line(lwd=1) +
  geom_point(data=plotdat[!is.na(plotdat$POINT),], aes(x=FP,y=TP,fill=POINT), pch=21, size=3) +
  geom_text(data=plotdat[!is.na(plotdat$POINT),], aes(x=FP,y=TP,fill=POINT), label=seq(1,0,-0.1),
hjust=1.75, vjust=0) +
  scale_x_continuous("False Positive Rate", limits=c(0,1)) +
  scale_y_continuous("True Positive Rate", limits=c(0,1)) +
  scale_fill_gradientn("Threshold Cutoff",colours = terrain.colors(10)) +
  theme(legend.justification = "top",text=element_text(family="Times New Roman", size=16)) +
  annotate("text",x=0.675,y=0.25, label = paste("AUC = 0.89")) +
  theme_bw()
Roc_2
Roc_2 + annotate("text",x=0.785,y=0.20, label = paste("Optimum cutoff point = 0.73")
```

Digital Appendices

The following are available as digital appendices:

Appendix G

This appendix contains the hourly climate data from the onsite weather stations on each farm for the following years 2018, 2019, 2020 and 2021.

Appendix H

This appendix contains the 10 cm Soil Temperature Data from the probes on each farm for the following years 2018, 2019 and 2020.

Appendix I

This appendix contains the full fly collection data set from the North and South Island farms for each of the seasonal catches (2018/2019, 2019/2020 and 2020/2021 seasons) referred to in Chapters Three and Four.

Appendix J

This appendix contains the daily Virtual climate station data for each farm for the following years 2018, 2019, 2020 and 2021.

DRC 16

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Paul Brett
Name/title of Primary Supervisor:	William Pomroy
Name of Research Output and full reference:	
ustralian Trap with Three Different Baits Types for Monitoring Lu	
In which Chapter is the Manuscript /Published work:	Chapter Nine
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	85%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate contributed towards the conceptualization, methodology, data collection, data analysis, data visualization and the writing of drafts of this manuscript.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Paul Brett Digitally signed by Paul Brett DN: cn=Paul Brett, c=NZ, o=Massey Univeristy, email=p.t.j.brett@massey.ac.nz Location: Palmerston North Date: 2022.05.10 15:10:00 +12'00'
Date:	
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Date:	

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

GRS Version 4– January 2019