Project title:	Enhancing control of the soft- and stone- fruit pest *Drosophila suzukii* (Spotted Wing *Drosophila*) by exploiting its activity patterns in the field.
Project number:	CP142
Project leader:	Michelle Fountain, NIAB EMR, KENT. Herman Wijnen, University of Southampton
Report:	Annual report. Year 2, October 2017
Previous report:	Annual report. Year 1, October 2016
Key staff:	Bethan Shaw
Location of project:	NIAB EMR, Kent and University of Southampton
Industry Representative:	Harriet Duncalfe
Date project commenced:	October 2015
Date project completed (or expected completion date):	September 2018

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Bethan Shaw

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[Organisation]

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CONTENTS

GROWER SUMMARY

Headline

- Daily locomotion rhythms of *Drosophila suzukii* are dictated by light primarily and then by temperature
- Groups of *D. suzukii* display different locomotion patterns compared to individuals housed alone
- Locomotion and oviposition rhythms under natural conditions can be predicted in the lab when the correct temperature and light profiles are used
- Oviposition occurs in the day time
- Oviposition reduces when temperatures exceed 30°C
- *D. suzukii* emergence is reduced when in competition with *D. melanogaster* for egg laying sites

Background

Daily behavioural and physiological rhythms, such as activity and sleep, are exhibited by a wide range of organisms as a result of the interaction between environmental cycles and an internal timekeeping mechanism known as the circadian clock. The clock can be regulated by environmental cues including temperature and day light and enables synchronicity to dynamic daily conditions.

D suzukii is an invasive species that attacks ripening fruit, unlike other *Drosophila* species which only feed on overripe or spoiled fruit (Rota-Stabelli *et al.*, 2013). *D. suzukii* was found for the first time in the UK in wild blackberry in Kent in August 2012 (Harris and Shaw, 2014) and is now a serious pest in cherry orchards and protected and outdoor strawberry, raspberry blueberry and blackberry. Female *D. suzukii* are able to insert eggs into the skin of ripening fruits with a serrated ovipositor. Once the eggs hatch, the larval stages consume the fruit from within causing the fruit to collapse making fruit unmarketable.

In recent years there has been a surge in the numbers of studies (in the US and Europe) on the behaviour and control of *D. suzukii*. However, little is known about the daily and seasonal rhythmicity in the behaviour and physiology of *D. suzukii*. On-going studies in the UK (e.g. AHDB project SF 145) are demonstrating peaks of seasonal activity. However methods being used to manage the pest primarily rely on research done in other countries under different environmental conditions with different approvals and devices for control.

Further insight in the daily behavioural and physiological rhythms of *D. suzukii*, as determined by its internal circadian clock and environmental cues, may help predict the times of day when *D. suzukii* poses the greatest threat to crops and when they would be most vulnerable to control measures. The research that would be invaluable to British soft- and stone-fruit growers would include exploitation of the behaviour and physiology of *D. suzukii* to enable more effective control within the UK growing season.

Hamby *et al.* (2013) described diurnal fluctuations in *D. suzukii* locomotor activity under laboratory conditions mimicking summer and winter days. Evans *et al.* (2017) investigated the oviposition rhythms of *D. suzukii* in outdoor field studies. However, both these examples are performed under temperature and light cycles that we would not experiences in the UK and use unrealistic social grouping to reach their conclusions. More expansive studies of clock-controlled daily rhythms have been done on the *D. suzukii* sister species, *Drosophila melanogaster*. The latter exhibits not only circadian locomotor behaviour, gene expression, and metabolism, but also daily clock-controlled oscillations in processes such as feeding, egg laying and eclosion (adult emerging from pupae) (Xu *et al.*, 2008). Behavioural patterns and rhythms have been observed in *D. suzukii* in constant conditions and so the influence of environmental and social conditions have not been evaluated (Lin *et al.*, 2014).

Aims and methods

To investigate daily and seasonal rhythms of *Drosophila suzukii* locomotion activity and egg laying and formulate recommendations for UK growers in regard to field detection, trapping, and crop protection

Locomotion activity was investigated using an electronic device that monitors movement of drosophila under various environmental conditions in the laboratory. Individual males, individual females, groups of males, groups of females and mixed sex groups were monitored under different seasonal conditions to determine an average locomotion pattern. The effects of removing environmental cues on the locomotion activity were investigated to understand the mechanisms that drive the internal circadian clock. Locomotion behaviour was also observed under natural light and temperature conditions in a semi-field setting. These environmental conditions were re-created in the lab to confirm whether lab locomotion patterns would correlate with those collected under natural conditions in the field when under the same temperature and light cycles.

Oviposition patterns of wild *D. suzukii* populations were determined within a strategic cherry orchard in Kent. Traps were baited with fresh fruit and were changed every two hours from sun rise to sun set for three days. The emergence of the next generation was counted to identify peaks in egg laying. The environmental conditions collected during the field oviposition trial were re-created in the lab and the oviposition pattern of lab strains recorded using the same method as in the field. Locomotion was also investigated under these environmental conditions to determine if there was a correlation between locomotion activity and oviposition.

Reproductive competition between *D. suzukii* and *D. melanogaster* was investigated by presenting each species with a substrate pre-inoculated with the opposing species' eggs or a blank substrate. Emergence of the next generation was counted.

Summary

In the first year of this PhD it was demonstrated that the standard laboratory conditions for investigating circadian rhythms were not appropriate to predict behaviour in the field. Constant temperatures and 12:12 hour light: dark cycles produced very different locomotion patterns to fluctuating temperatures and seasonal day length. Locomotion patterns for individual flies differed from single sex and mixed sex groups. Locomotion activity was mainly driven by light and then influenced by temperature cycles. Within the second year of this PhD, a wider range of semi-field and lab conditions were investigated to see if lab

strains would exhibit the same locomotion activity as those under the natural cycles. When these conditions were re-created in the laboratory, behavioural patterns of mixed sex groups and female groups correlated with those collected in the field. As this was successful, the results confirm that predictions of locomotion activity under natural conditions can be made from lab based assays if the correct environmental conditions are used.

Oviposition was investigated in August and October and in both trials more eggs were laid in the day time than at night. In August, oviposition fell when temperatures exceeded 30°C and peak egg laying occurred in the mid-morning and evening when temperatures were between 25-29°C. In October, peak egg laying occurred at peak temperature each day. When the environmental patterns were re-created in the lab, lab strains displayed the same oviposition pattern as their wild counter parts. As with the locomotion activity, oviposition patterns of lab strains, when exposed to the re-created environmental conditions, mirrored those collected in the field. Meaning oviposition rhythms can be predicated for specific times of the year if an average light and temperature cycle is known. The locomotion of mix sex groups, when exposed to the oviposition environmental conditions, correlated with the patterns of oviposition.

In the reproductive competition assay significantly fewer *D. suzukii* emerged from the *D. melanogaster* pre-inoculated substrate than the blank. It appears that this reduction is caused by female oviposition choice, with fewer eggs being laid initially. However, there may also be some egg predation with *D. melanogaster* larvae feeding on *D. suzukii* eggs.

Financial Benefits

The project will meet the vital requirements of the UK soft and stone fruit industry, filling gaps that are not being addressed in research programmes in other countries. It is essential for maintaining the viability and profitability of the UK's important soft and stone fruit industries. SWD seriously threatens the sustainability of production of these crops in the UK. By understanding the Chronophysiology of the pest we can provide growers with a better understanding of how to target the control of *D. suzukii*.

Action Points

There are no grower action points at this early stage of the project.

SCIENCE SECTION

Introduction

Drosophila suzukii (Matsumura) is a relatively new invasive pest affecting British fruit production, being first identified in Kent in an area of wild blackberry in August 2012 (Harris and Shaw, 2014). Since its arrival in the UK it has caused commercial losses in cherry and some soft fruit crops, with some very small growers abandoning cherry orchards due to extensive fruit damage (pers. comms. T. Hulme). The exact cost of economic loss in unclear as some growers were not aware of the pest and attributed losses to other pest or pathogens. *D. suzukii* is threatening soft- and stone-fruit production as it is one of only two species of Drosophila that are able to lay eggs in intact, healthy and unripe fruit (Goodhue *et al.*, 2011). Female *D. suzukii* have serrated oviscapts which are used to cut into ripe and ripening soft- and stone-fruit to insert eggs under the fruits epicarp (Kanzawa, 1935). The other species, *D. subpulchrella*, is not currently a significant threat to the global fruit market and is unable to lay eggs in thicker skinned fruits such as grapes (Takamori *et al.*, 2006; Atallah *et al.*, 2014).

The egg insertion hole made by *D. suzukii* exposes fruit to further attacks from pathogens and other insects that would not have been able to enter the undamaged fruit (Goodhue *et al*., 2011). Eggs hatch within the fruit and then develop through three larval instars (Walsh *et al.*, 2011) which feed on the fruit flesh causing it to collapse and, in some cases, cause a melting appearance making fruit unmarketable. The larvae typically pupates in the fruit, but can leave the fruit to pupate before emerging as an adult. The cycle from egg to adult can be as rapid as 10 days in constant 30°C in laboratory conditions (Tochen *et al.*, 2014). It is calculated that between 3 and 9 generations a year are realistic for the UK when estimated against the average seasonal temperature.

Many organisms have an internal clock that controls behaviours in daily (circadian) and/or annual (circannual) cycles (Bollinger and Schibler, 2014). The circadian clock is a system that controls daily physiological behaviours including activity/locomotion, feeding, sleeping and mating and is seen in organisms across all classifications. The clock regulates many functions which are triggered by external cues or zeitgebers ‘time givers’ in response to daily environmental cycles (Hardin, 2005) such as light, which is the strongest (Schmal *et al.*, 2015) and temperature. By having a clock that is entrained or synchronised to environmental cues, individuals of the same species become synchronised in behaviours and processes such as courtship and reproduction which then occur at the same time. Hamby *et al.* (2013) described diurnal fluctuations in *D. suzukii* locomotor activity, malathion toxicity, and gene expression under laboratory conditions mimicking summer and winter

days. More expansive studies of clock-controlled daily rhythms have been done on the *D. suzukii* sister species, *Drosophila melanogaster*. The latter exhibits not only circadian locomotor behaviour, gene expression and metabolism, but also daily clock-controlled oscillations in processes such as feeding, egg laying and eclosion (emerging from pupal case or hatching from egg) (Gruwez *et al*., 1971; Konopka and Benzer, 1971; Xu *et al*., 2008). Lin *et al.* (2014) investigated reproductive behavioural patterns of both *D. suzukii* and *D. melanogaster* when held under constant temperatures and found that rhythms could be identified in several behaviours including oviposition, egg hatch and adult eclosion. However, on commencing this project little was unknown about the rhythms of *D. suzukii* behaviour under natural conditions. Since 2015 few articles of interest have been published in regard to behavioural rhythms of *D. suzukii*. Evans *et al.* (2017) investigated the oviposition of *D. suzukii* in outdoor semi-field studies in Alabama USA and also how location i.e. shaded v exposed, affected egg laying behaviour. However there were limitations with this study as it did not look at oviposition rhythms in depth but at day verses night egg laying and egg survival. The temperatures experienced during the day in assay range between 30-37°C which have been found to be well above the threshold and optimum ranges for *D. suzukii* (Ryan et al., 2016).

Further insight in the daily behavioural and physiological rhythms of *D. suzukii*, as determined by its internal clock and environmental cues, may help predict the times of day when *D. suzukii* poses the greatest threat to crops and when they would be most vulnerable to control measures. If we are able to understand the mechanisms that control behavioural outputs we may be able to predict when key behaviours will occur at different times in the British growing season. It is intended that we may be able to exploit the behaviour and physiology of *D. suzukii* to enable more effective control with precision monitoring devices and chemical and biochemical controls.

Project aim

To investigate daily and seasonal rhythms of *Drosophila suzukii* locomotion activity and egg laying and formulate recommendations for UK growers in regard to field detection, trapping, and crop protection.

Objectives

1) Determine how temperature, photoperiod, and internal time keeping mechanisms affect rhythms in *D. suzukii* including locomotion activity and oviposition (Years 1-2)
2) Validate predicted *D. suzukii* daily and seasonal activity patterns in the field (Years 1-2)
3) Test trapping devices and approved chemical and biochemical plant protection products on the most vulnerable life stages, including optimal field temperatures for control of *D. suzukii* (Years 2-3)
4) Formulate recommendations for optimizing *D. suzukii* detection and control strategies for susceptible UK crops (Years 2-3)

Materials and methods

The materials and methods used within this project are currently under review and have been classed as confidential until they reach the final stages of publication. For further information please contact Bethan Shaw.

Results and discussion

Experiment 1: Investigating the locomotion of D. suzukii under different social conditions and temperature and light cycles

Activity profiles

Typically locomotion assays work on individual, virgin flies to prevent larvae moving within the cuvettes which may disrupt the results. However as we were interested in wild population behaviour, we were interested in how mixed sex groups would interact. Also in preliminary experiments it was clear that not only did mated females rarely oviposit within the agar media provided in the assay, when they did the eggs either didn't hatch or larval didn't develop past stage 1. This was probably due to the low nutritional content of the media, which consists of only agar, sugar and water and is lacking in protein and yeasts which are needed for larval development. In a previous assay by (Ferguson *et al.*, 2015) it was concluded that mated females were 4 times more active than virgin females and displayed significantly different locomotion patterns. For these reason it was decided to use mated individuals not only in the group housed flies but in the individual locomotion collections too.

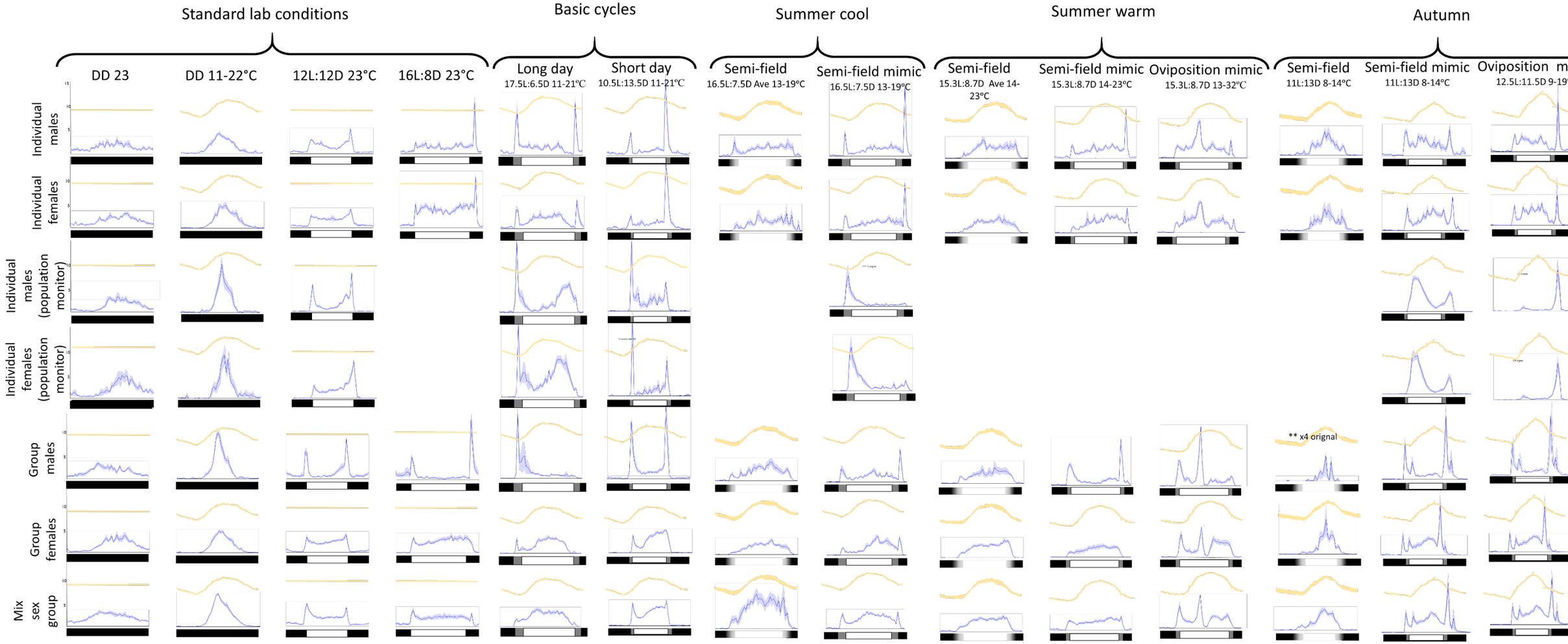
Although using wild caught *D. suzukii* would have been preferable, collecting the quantities needed to obtain a good average was not possible. It is possible that wild *D. suzukii* will behave differently to lab reared strains. For this reason we accept that the activity level of wild verses lab populations may differ but the molecular clock and entrainment process, that are responsible for the behaviour rhythms, should be genetically conserved across lab and wild strains.

From the range of social situations evaluated across all environmental conditions, sex composition, space and setting influence locomotion behaviour as seen in locomotion profiles (Figure 2.1.1). Activity profiles are visual representations of locomotion activity, monitored over a period of days is then averaged into a 24 hour period. When individual flies were housed in the narrow cuvettes they frequently displayed a different locomotion profile than those in the large population vials. Typically in the lab-based assays, individual male and female flies as well as group-housed males displayed behavioural profiles with more prominent spikes in activity at lights-on and lights-off than groups of females or mix sex groups under the same environmental conditions. The mix sex groups and female group's activity peaks follow rise and fall corresponding to temperature cycle. Groups of males resemble individual males in general activity pattern.

The effect of environmental conditions also has a great impact on Drosophila behavioural output with previous groups already highlighting the variation in activity when exposed to 'natural' conditions (Vanin *et al.*, 2012; De *et al.*, 2013; Green *et al.*, 2015). When collected in semi-field conditions and exposed to natural light and temperature cycles all social housings displayed very similar profiles, unlike the laboratory assayed flies which is discussed above. In the laboratory collected profiles (i.e all but the semi-field), when a light cycle was used, most activity occurred during the photo phase and very little occurred during the dark phase. When lighting was removed activity level followed the temperature cycle if present.

When both light and temperature cycles were removed, both individual males and females monitored in small cuvette appear to become arrhythmic after 24 hours based on the profile of the actogram (Figure 2.1.2). However, individual males and females monitored in population vials appeared to remained rhythmic for 5 days. Within the male groups rhythmicity is maintained for 5 days before the amount of activity through the 'night' increases. In groups of females and mixed sex groups, although there is an increase in night activity, they continue to have peak activity in the 'day time' and rhythmicity pattern is lost after 9 days. The ability for flies held in constant condition to remain rhythmic in social groups has been observed in D. melanogaster in previous investigations and so is not unexpected to occur in *D. suzukii* (Bloch *et al.*, 2013)

In the semi-field and their corresponding lab mimic collections the female groups and the mix sex groups are most visually similar in activity profile. Meaning that if appropriate environmental conditions are used it is possible to gain an understanding of locomotion behaviours of flies exposed to natural conditions from lab based assays.



**** Indicates activity profile on different axis scale to all others.**

Figure 2.1.1. Normalised activity profiles collected under different lab and semi-field condition (columns) and in various social grouping (rows). Temperature cycle (if present) displayed by orange line at top of each activity profile. Temperature in semi field is displayed as the average temperature over the collection period. Temperature cycles are not relative to counts axis and are a representation of the cycle peaks and troughs. Temperature range is stated at the top of each column. Lab light cycle (if present) indicated by black (no light banks), grey (2 light banks) and white bar (4 light banks) at bottom of each activity profile. Light in semi-field shows night as black, grey as dusk or dawn and white as day. Each lab based individual male and female profile displays average locomotion of 50 individuals in small cuvettes. Individual males in population monitors and individual females in population monitors displays average locomotion of a minimum of 10 individuals in population monitors. Group males and group females displays average locomotion of 5 groups of 10 males or 10 females in population monitors. Mix sex groups displays average locomotion of 5 groups of 10 males and 10 females in population monitors. All semi-field individual males and females are batch analysed on minimum of 29 individuals. All three semi-field collections are batch analysed on minimum of 5 mix sex groups. Both summer warm and summer cool are batch analysed on a minimum of 5 groups for group males and group females. Autumn collections are analysed on 1 group male and batch analysed on 3 group females due to low survival. All activity profiles display an average profile of locomotion collected over a 6-day period.

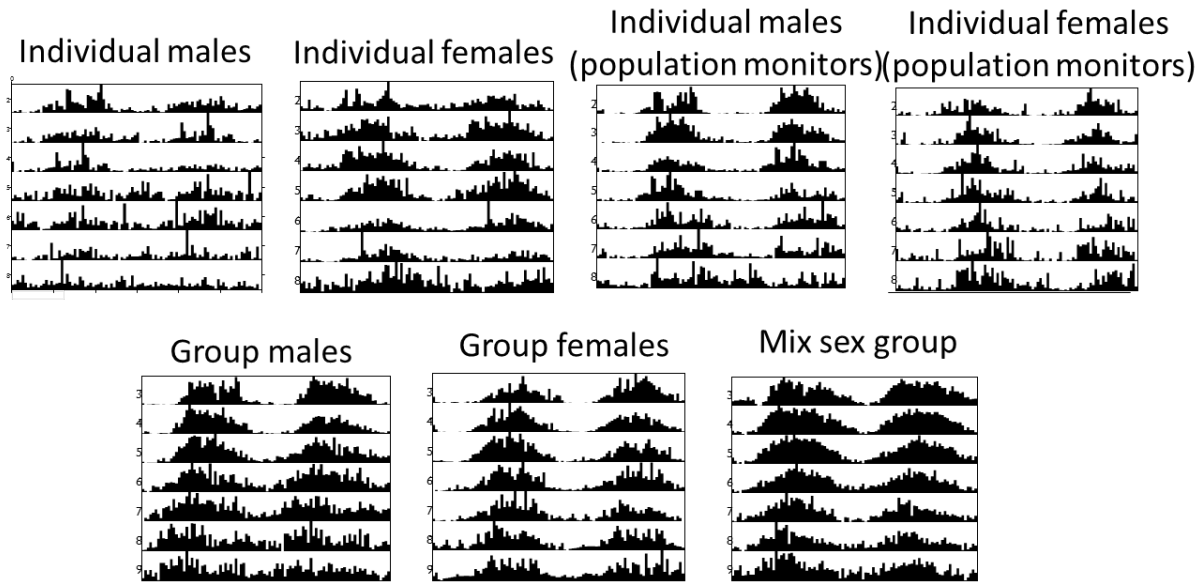


Figure 2.1.2 Actogram of locomotion of different social housing collected under constant darkness (DD) and constant temperature 23ºC. Each line represents 48 hours. The last 24 hours on each line is repeated as the first 24 hours on the line below.

Hierarchical clustering

The hierarchical clustering that encompasses all social conditions and environmental conditions typically grouped semi-field collected data with the lab semi-field mimic collections (Figure 2.1.3) with mix sex groups of both being found close together (highlighted). The standard lab conditions (DD 23 ºC, DD 11-22 ºC, 16:8 23 ºC and 12:12 23ºC) appear to cluster with those conditions that either have roughly the same photo phase or temperature cycle which is not surprising. However, this highlights the fact that not all wild behavioural predictions can be made on a standard constant temperature and light cycle. There does not seem to be a consistent effect of social grouping on clustering. When reduced to smaller groups, relevant to each of the three semi-field collections, the clustering of semi-field and the lab semi-field mimic and oviposition mimic is much closer with only autumn semi-field collections having a wider spread (Figure 2.1.4-2.1.6).

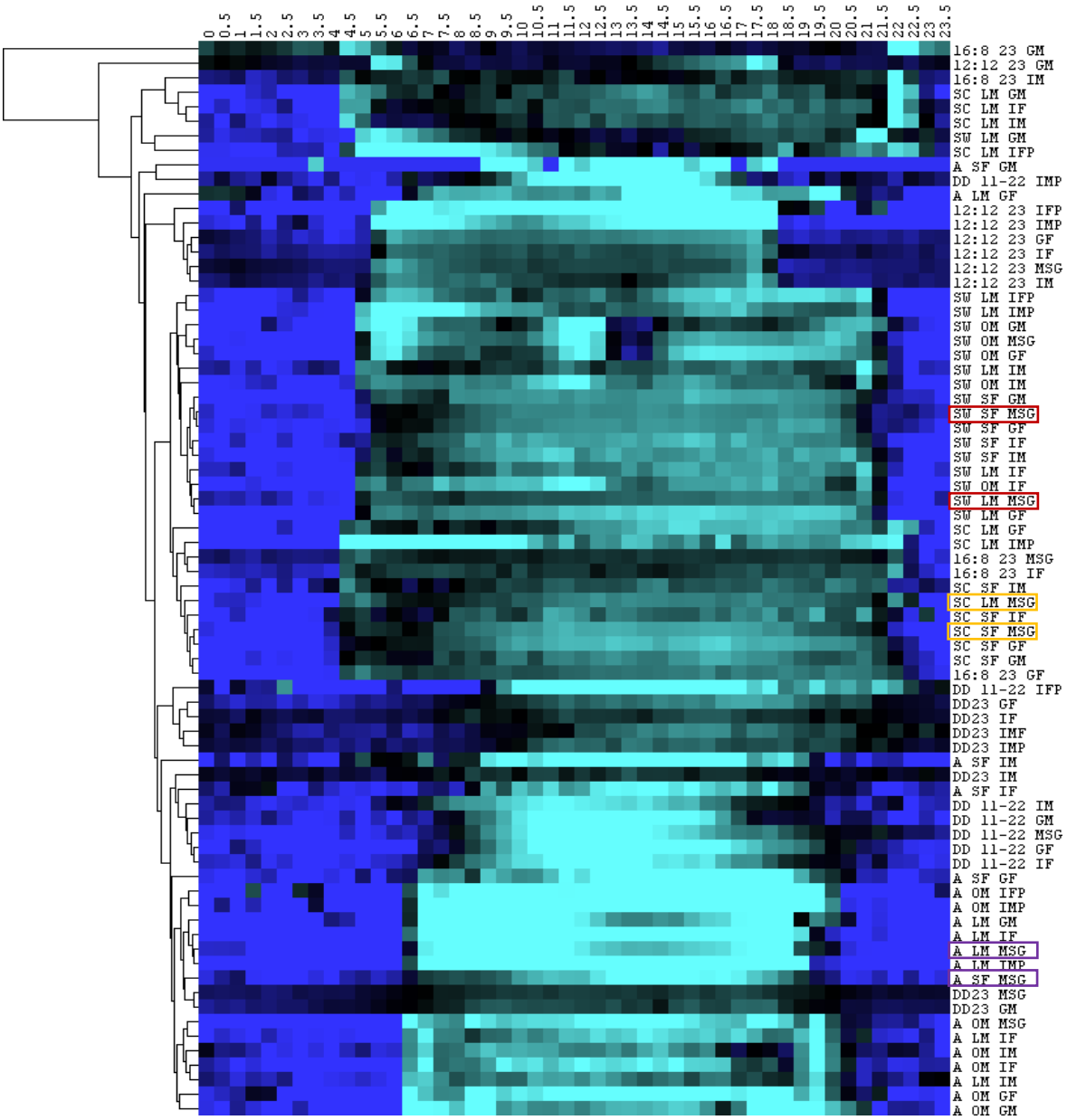


Figure 2.1.3. Hierarchical clustering of average activity counts collected over 6 days in half hour periods for all social groupings and all environmental conditions. Light blue indicated positive values. Dark blue indicates negative values. X axis denotes time in 0.5 hours. Y axis represents single social setting in a specific environmental condition. Standard lab conditions are abbreviated DD, constant darkness. 23, constant 23°C. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22°C. The first letter(s) represents the environmental condition (A, autumn. SW, summer warm. SC, summer cool). Second letters represent location (SF, semi-field. LM, semi-field locomotion mimic. OM semi-field oviposition mimic). Social grouping is indicated in the third set of letters (IM, individual males. IF, individual females. IMP, individual males in population monitors. IFP, individual females in population monitors. GM, group males. GF, group females. MSG, mix sex groups).

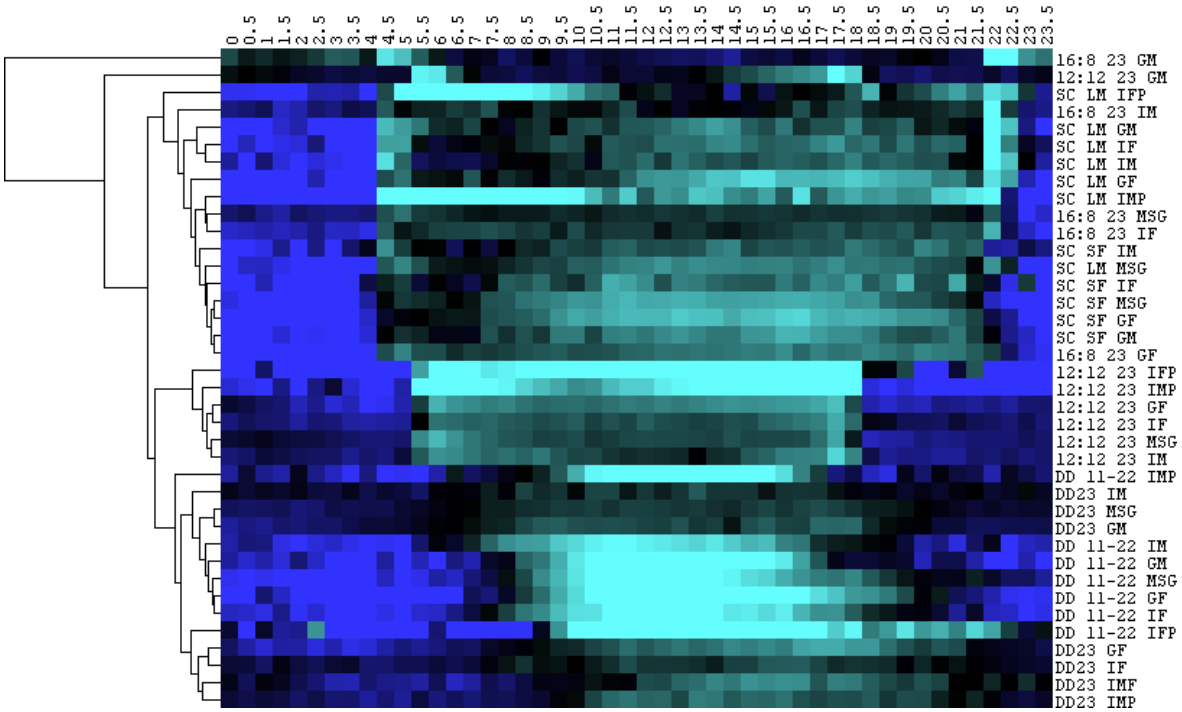


Figure 2.1.4. Summer Cool standard and basic cycle conditions hierarchical clustering. The first letter(s) represents the environmental condition (SC, summer cool). Second letters represent location (SF, semi-field. LM, semi-field mimic). Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23^{o}C. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22^{o}C.

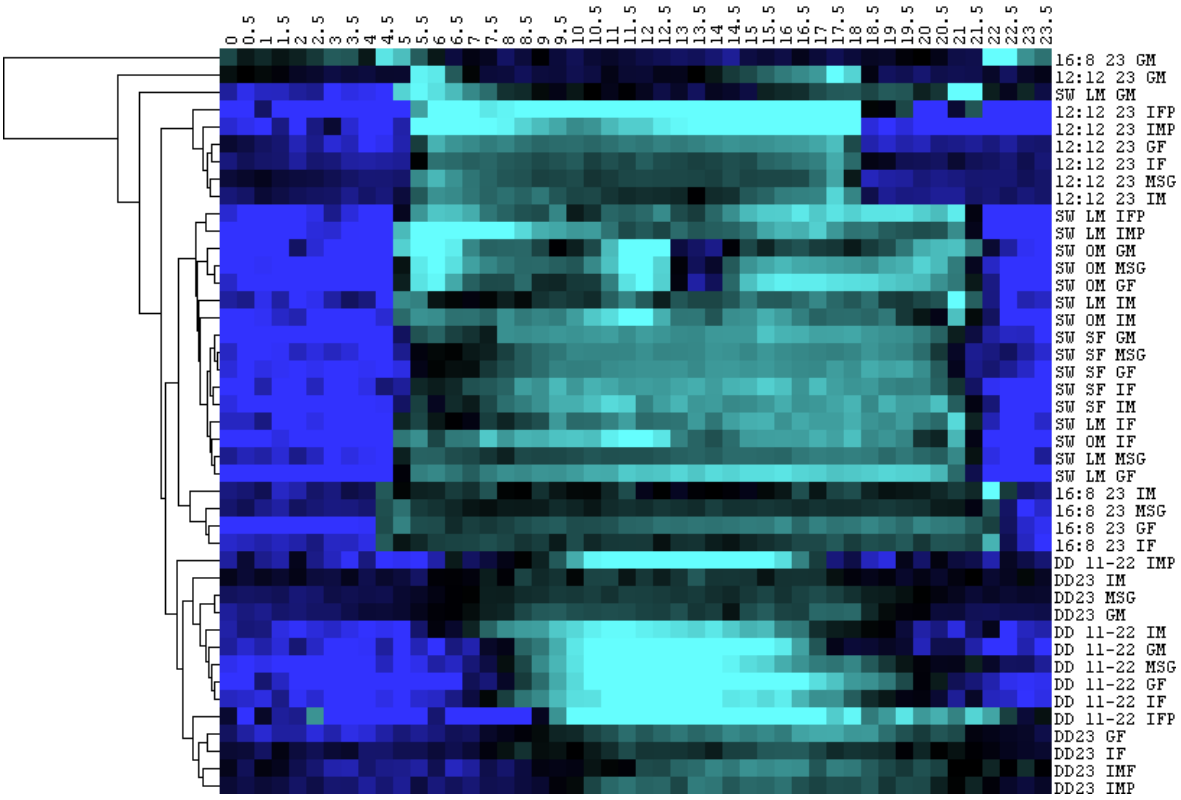


Figure 2.1.5. Summer warm standard and basic cycle conditions in hierarchical clustering. The first letter(s) represents the environmental condition (SW, summer warm). Second letters represent location (SF, semi-field. LM, semi-field mimic. OM semi-field oviposition mimic). Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23ºC. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22ºC.

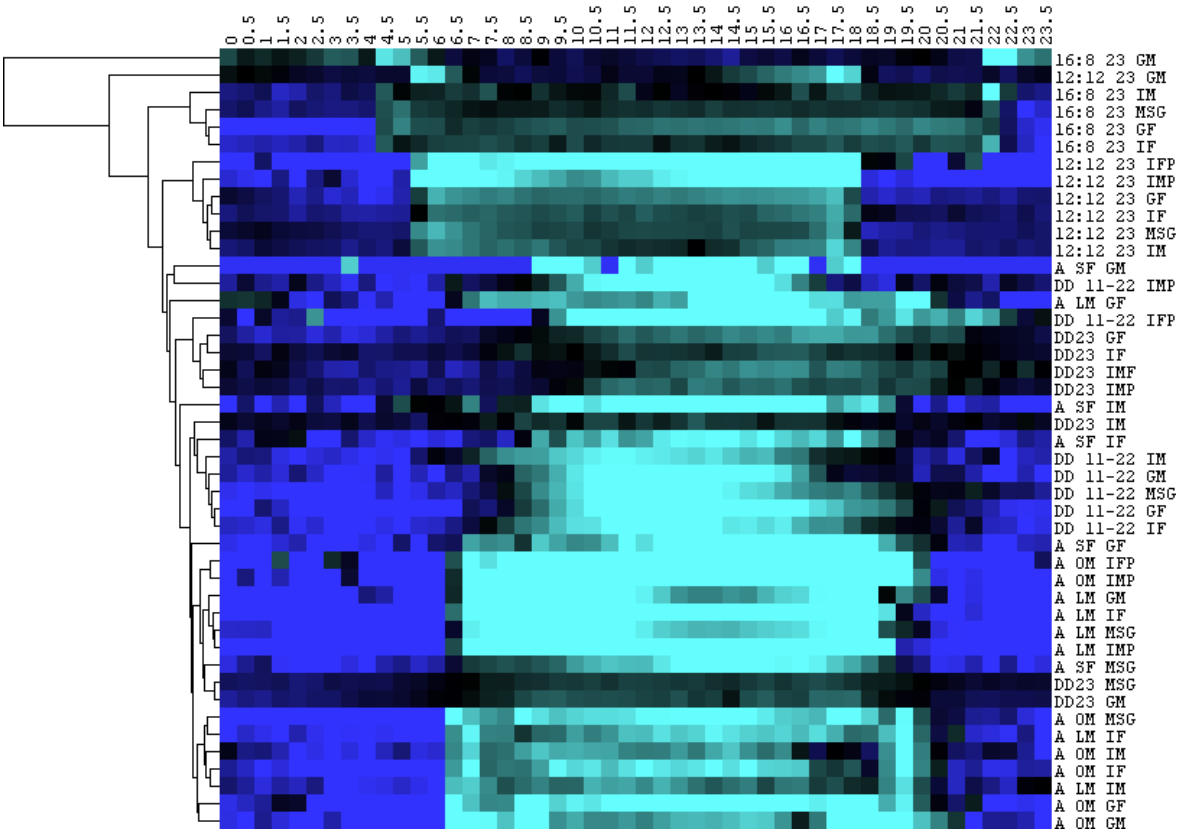


Figure 2.1.6. Autumn standard and basic cycle conditions in hierarchical clustering. The first letter(s) represents the environmental condition (A, autumn). Second letters represent location (SF, semi-field. LM, semi-field mimic. OM semi-field oviposition mimic). Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23^{o}C. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22^{o}C.

Principal component analysis

Principal component analysis (PCA) is a process of multi-variation analysis and can be used to simplify large volumes of data based on interactions of these variants (Wold *et al.*, 1987). When these PCA values are converted into a scatter graph the data can be visually interpreted, with those data points of a similar nature being grouped together on a scatter graph. The one of the aims of this experiment was to determine if laboratory conditions could be used to produce wild-like locomotion patterns and therefore used to make predations about behavioural patterns in the field. The standard laboratory conditions were compared with the semi-field, semi-field locomotion and semi-field oviposition mimic cycles to establish whether standard laboratory temperature and light conditions that are commonly used were appropriate for these predictions. When looking at the data as a

whole the PCA appears to group those with a similar photoperiod together with those of a longer photoperiod towards the bottom left of the chart and those with a shorter photo period towards the top right (Figure 2.1.7). Those collected under constant darkness are found primarily in the top left section of the chart including the DD 23ºC and DD 11-22ºC where photoperiod is not a factor. When divided into smaller groups based on each of the three semi-field collections the corresponding locomotion mimic and, where applicable, oviposotion mimic collections were grouped closest together on the scatter graph (Figure 2.1.8-2.1.10). However, some of the standard laboratory conditions overlap with the semi-field and mimic cycles but only if there are similarities in either the light and/or temperature cycles. Within all environment groupings the common anomalous social settings were individual males, individual males in population monitors and groups of males being found on the edges of the cluster. Mix sex groups and female groups were typically found within the centre of each environmental cluster. This suggests that groups that contain females could be used as a reliable indicator of what an average population's locomotion behaviour is.

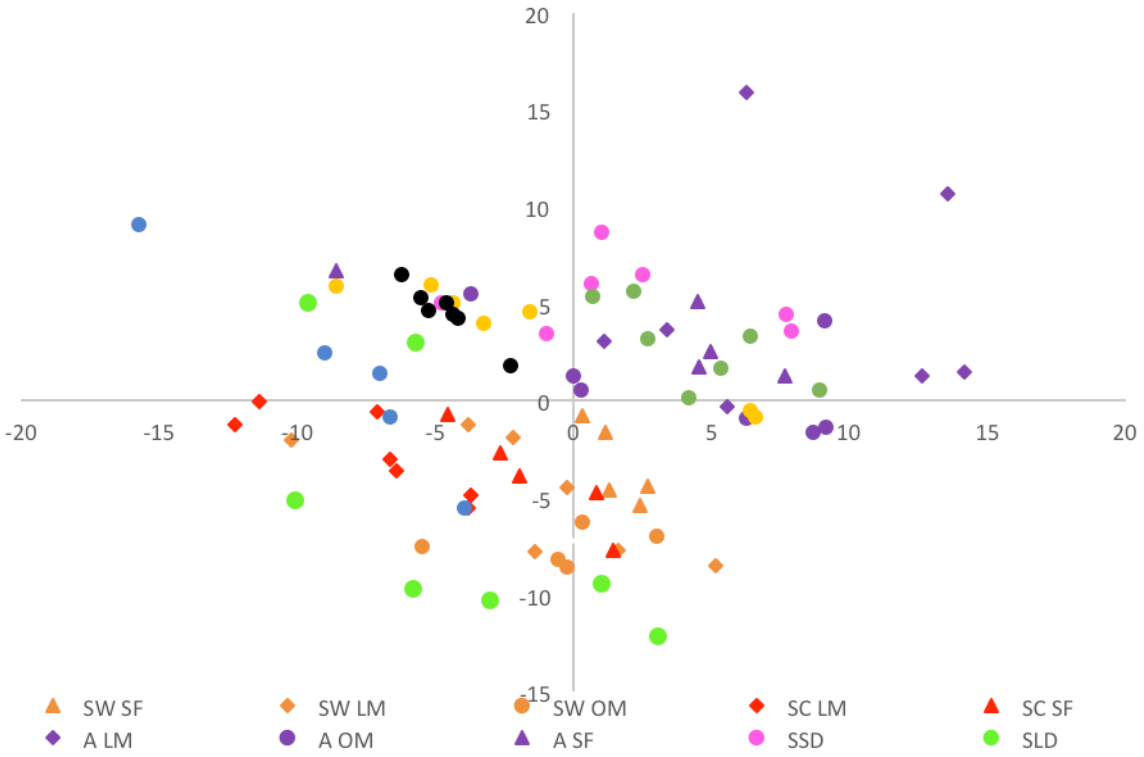


Figure 2.1.7. Principle component analysis of all environmental conditions and social housing. Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23ºC. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12

hours dark. 11-22, temperature cycle ranging from 11-22ºC. SSD, standard short day cycles 10 hours light 14 hours dark, 11-21ºC. SLD, standard long day cycles 16 hours light 8 hours dark, 11-21ºC. The first letter(s) represents the environmental condition (SC, summer cool. SW, summer warm. A, autumn). Second letters represent location (SF, semi-field. LM, semi-field mimic).

Figure 2.1.8. Principle component analysis of summer cool standard and basic laboratory condition and social housing. Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23ºC. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22ºC. The first letter(s) represents the environmental condition (SC, summer cool). Second letters represent location (SF, semi-field. LM, semi-field mimic). Social housing is denoted next to data point. IM, individual male. IF, individual female. IMP, individual male in population monitor. IFP, individual female in population monitor. GM, group male. GF, group female. MSG, mixed sex group.

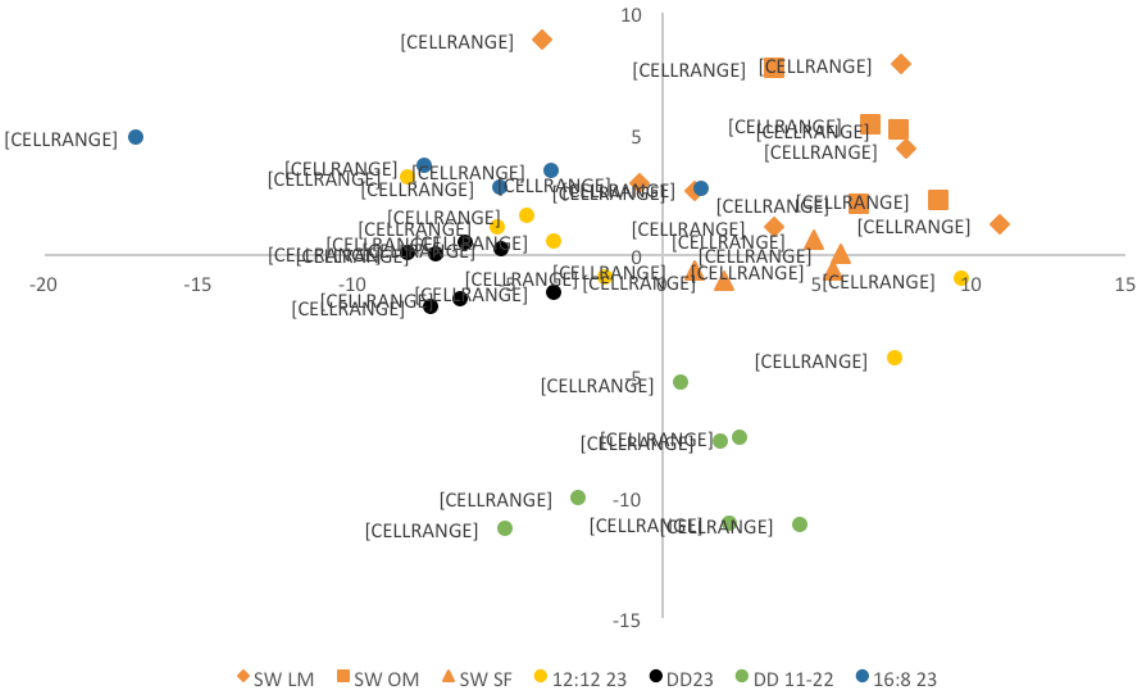


Figure 2.1.9. Principle component analysis of summer warm standard and basic laboratory condition and social housing. Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23ºC. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22ºC. The first letter(s) represents the environmental condition (SW, summer warm). Second letters represent location (SF, semi-field. LM, semi-field mimic). Social housing is denoted next to data point. IM, individual male. IF, individual female. IMP, individual male in population monitor. IFP, individual female in population monitor. GM, group male. GF, group female. MSG, mixed sex group.

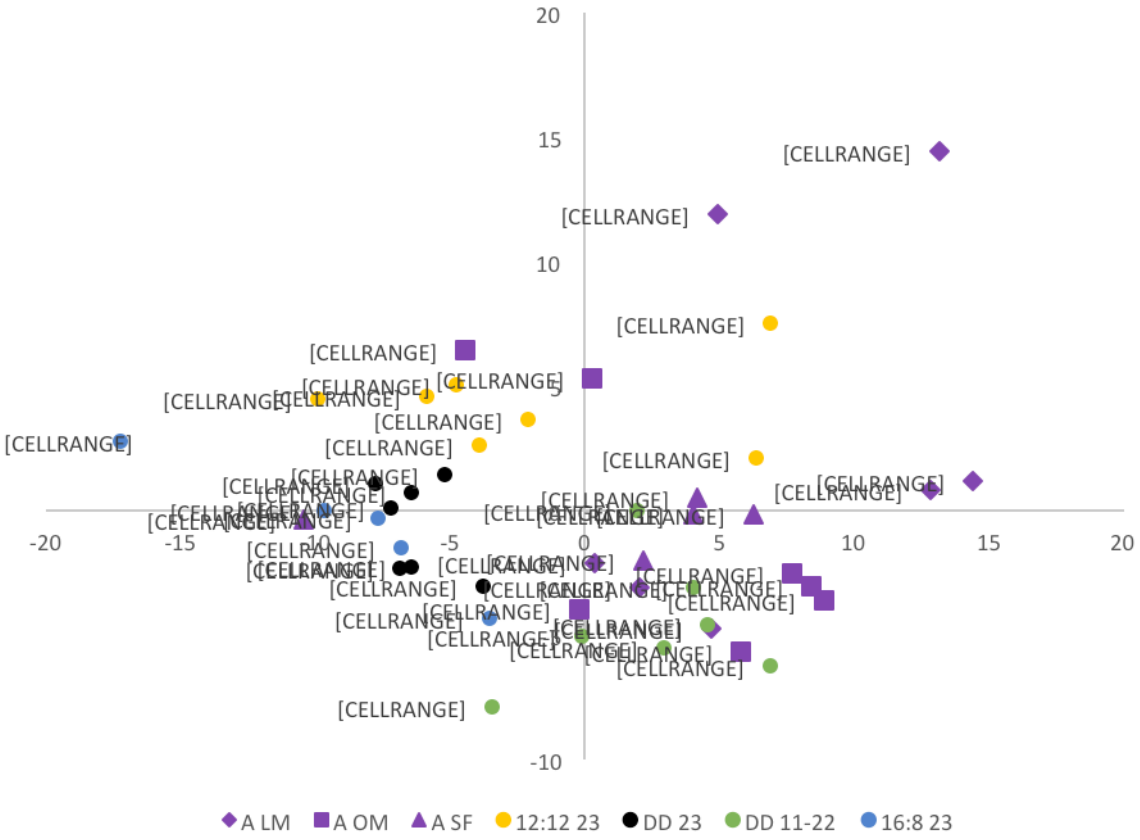


Figure 2.1.10. Principle component analysis of autumn standard and basic laboratory condition and social housing. Standard lab conditions are abbreviated to represent cycles. DD, constant darkness. 23, constant 23ºC. 16:8, 16 hours light: 8 hours dark. 12:12, 12 hours light: 12 hours dark. 11-22, temperature cycle ranging from 11-22ºC. The first letter(s) represents the environmental condition (A, autumn). Second letters represent location (SF, semi-field. LM, semi-field mimic). Social housing is denoted next to data point. IM, individual male. IF, individual female. IMP, individual male in population monitor. IFP, individual female in population monitor. GM, group male. GF, group female. MSG, mixed sex group.

Experiment 2: Can we predict oviposition rhythms in the field in laboratory based experiments?

Field oviposition

In both the summer warm and autumn oviposition trials more emergence occurred from fruit deployed during the day than at night. More emergence occurred in the summer warm assay than in the autumn overall, which could be due to the lower population numbers found at this time of the year in cropping situations (Fountain *et al.*, 2016). In the summer warm when temperatures exceeded 30°C egg laying falls (Figure 2.2.1). In the autumn the amount of emergence followed the temperature cycle and rose and fell with it through the day (Figure 2.2.2). When averaged into 24 hours, the emergence of *D. suzukii* corresponds with the activity profile of mix sex groups, collected in Experiment 1, when subject to the oviposition mimic cycle recreated in the lab in both summer warm (Figure 2.2.3) and autumn (Figure 2.2.4). Emergence was not compared to the semi-field collections as oviposition assays were performed under polytunnels which resulted in an increase in temperature compared to the semi-field locomotion conditions. In the summer warm oviposition assay, there was a reduction in emergence from fruit that was subject to temperatures of 30°C in the field. As the number of eggs were not counted on collection we do not know whether this reduction is due to a reduction in the number of eggs being laid initially or a reduction in egg survival. However, when compared to the mix sex group activity profile collected under the oviposition mimic conditions in the lab, the amount of activity greatly increases as the temperature rises to 30°C. As the temperature starts to fall, so does the activity. As Drosophila are ectotherms they do not maintain a constant body temperature and use the environment to regulate it, i.e.by moving into the sunlight when cold and moving to shade when hot (Abram *et al.*, 2016). The increase seen in the mix sex group activity as temperatures reach 30°C could be the result of the flies increasing movement to try and locate a cooler location. The resulting effect may be seen in the oviposition field trial as a reduction in emergence as females are focusing on their own survival rather than egg laying. In the lab, optimum temperatures for egg to adult survival and oviposition rate of *D. suzukii* were found to be ~28.2°C and 22.9°C respectively (Tochen *et al.*, 2014; Ryan *et al.*, 2016). As eggs are immobile and unable to escape high temperatures, the reduction in emergences could also be due to the females not depositing eggs in unfavourable environmental conditions (Dillon *et al.*, 2009).

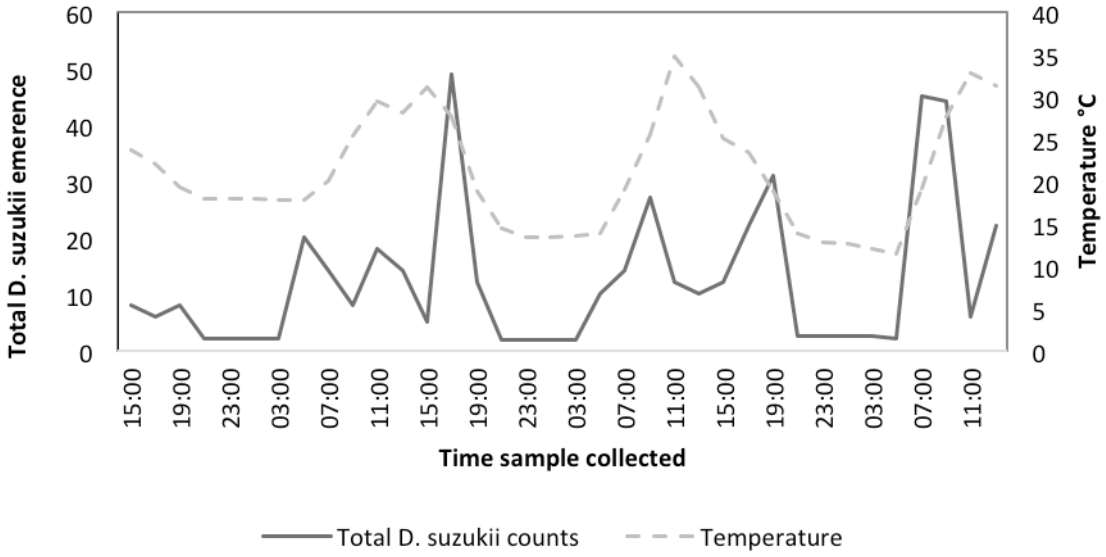


Figure 2.2.1 Emergence of *D. suzukii* from fruit deployed in an orchard in August at timed intervals (dark grey) in relation to temperature (light grey dashed).

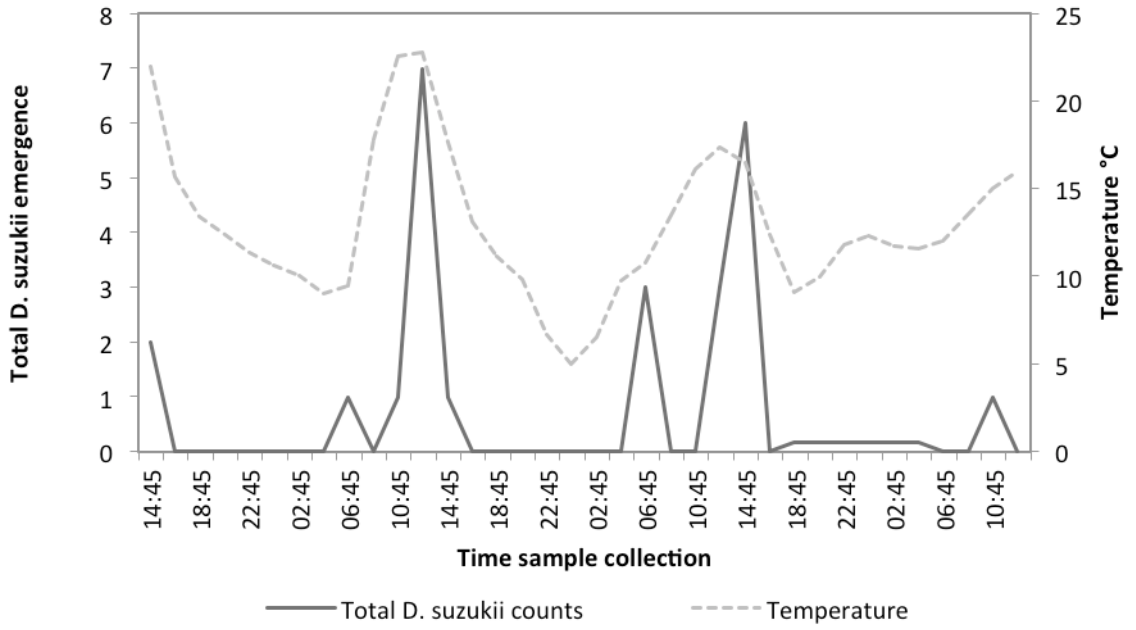


Figure 3.2.2 Emergence of *D. suzukii* from fruit deployed in an orchard in October at timed intervals (dark grey) in relation to temperature (light grey dashed).

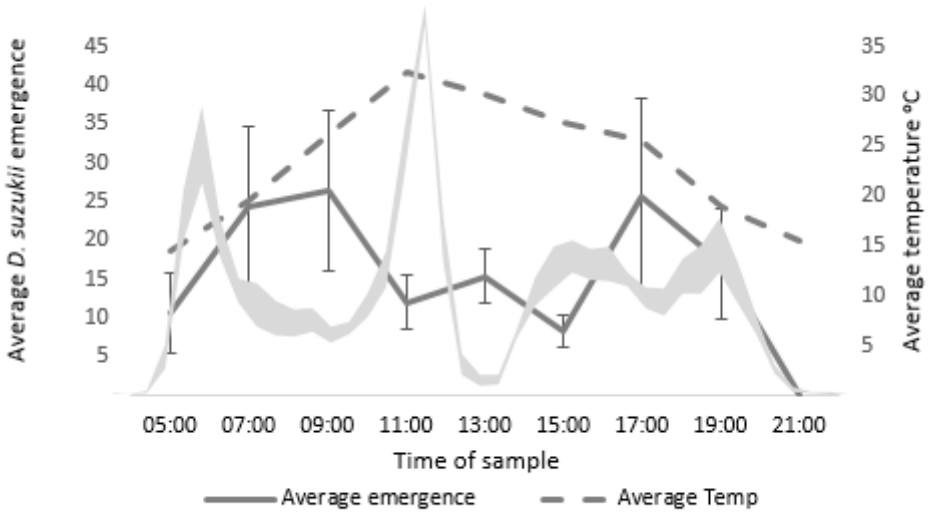


Figure 2.2.3 Average emergence of *D. suzukii* from fruit deployed in a cherry orchard (dark grey) during summer warm in comparison with average locomotion of mix sex groups when exposed to the same environmental cycle in the lab (light grey) from sun rise to sun set. Dashed line indicates average temperature.

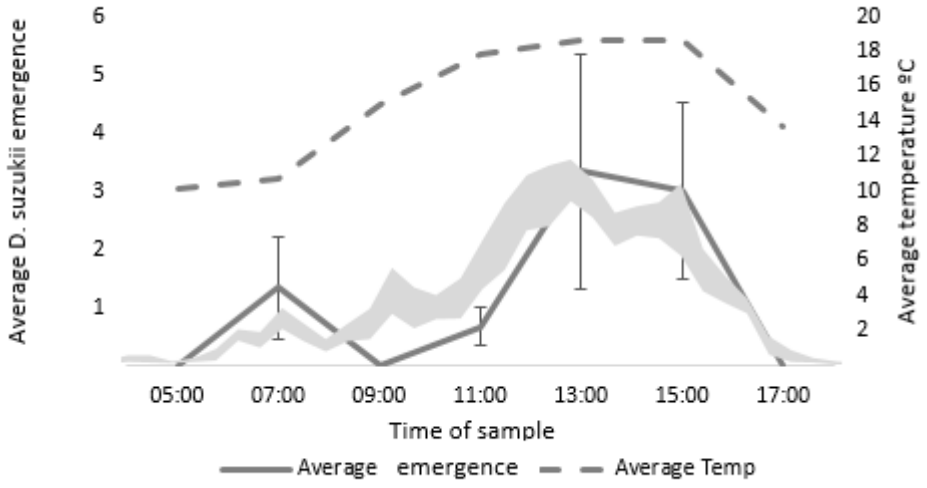


Figure 2.2.4 Average emergence of *D. suzukii* from fruit deployed in a cherry orchard (dark grey) during autumn in comparison with average locomotion of mix sex groups when exposed to the same environmental cycle in the lab (light grey) from sun rise to sun set. Dashed line indicates average temperature.

Lab oviposition

A lab bases oviposition assay was performed to see if the number of eggs laid in the lab correlated with the amount of emergence in the field. In the lab under the summer warm oviposition once temperatures exceeded 30°C egg laying stopped (Fig. 2.2.5). This would also suggest that the reduction in emergence in the summer warm field experiment was due to a reduction in initial egg laying rather than egg survival at temperatures above 30°C. However overall egg laying was low in the lab which may be due to the flies not having enough time to adapt to the high temperatures which were reached during the assessment period, which the field populations would have (Wallingford and Loeb, 2016). When the three assessment days are averaged into one, the average number laid in the lab correlates with the emergence from the field trial on wild populations (Fig. 2.2.6) suggesting that like the locomotion activity, predictions of behavioural patterns of flies in natural conditions can be promoted in the lab as long as realistic environmental conditions are used.

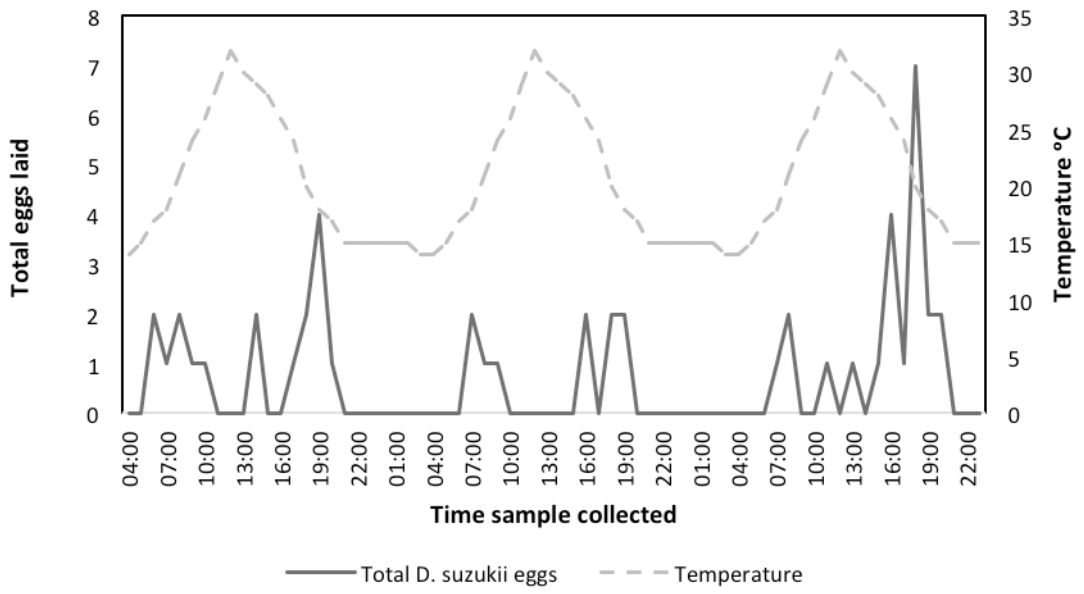


Figure 2.2.5 Total number of *D. suzukii* eggs laid under simulated summer warm conditions in the lab at timed intervals (dark grey) in relation to temperature (light grey dashed) over a three day period.

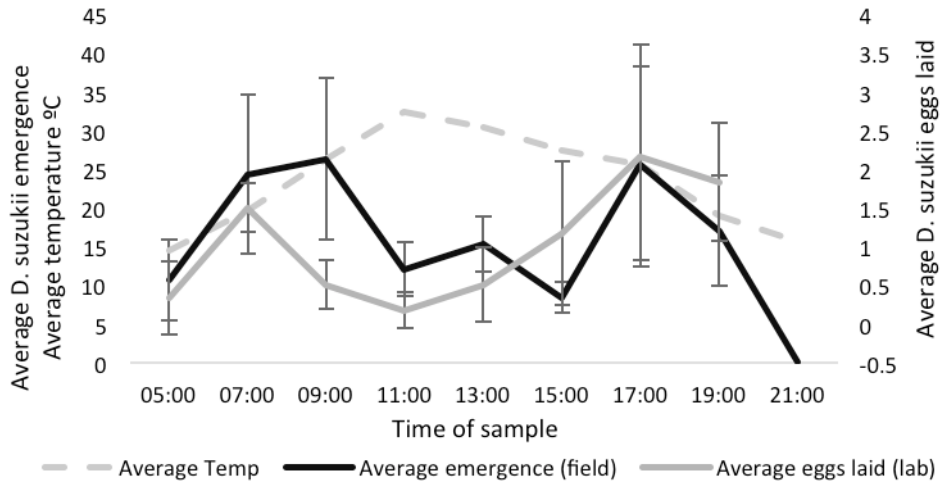


Figure 2.2.6 Average number of *D. suzukii* eggs laid under simulated summer warm conditions in the lab at timed intervals (light grey) in comparison with emergence from summer warm field assay (black line) and average temperature (light grey dashed).

Experiment 3: How will the presence of another species affect oviposition behaviour in D. suzukii?

Significantly more *D. suzukii* adults emerged from blank media that contained no eggs initially than media pre-inoculated with *D. melanogaster* (Figure 2.3.1) (Experiment 1, $F_{1,8}$ = 8.06; P = 0.022. Experiment 2, $F_{1,10}$ = 159.26; P = < 0.001. Experiment 3, $F_{1,10}$ = 14.66; P = 0.03). However, we cannot confrim whether this was due to fewer eggs being laid initally or if egg to adult survival was lower. It had been suggested that this could be the results of cannibalistic tendencies of some *Drosophila* species which occurs when nutrition is restricted (Ahmad *et al.*, 2015). Morphological defects can be a visual indication that cultures have been maintained on diets lacking nutrition (Vijendravarma *et al.*, 2010). Although no quantitative measurements were taken, there was no noticeable reduction in body and wing size in our competition experiment to indicate diet restriction which promotes cannibalism. However it has been found that a 'basal level of cannibalism' does occur in *D. melanogaster* cultured on a standard yeast/sugar diet (Bhattacharyya, 2015b, 2015a). In *D. melanogaster* the younger mobile larval stages hunt immobile larvae preparing to pupate (Vijendravarma *et al.*, 2013). We would therefore expect to see a reduction in emergence in the first inoculation species and not the second. This would not explain why *D. suzukii* emergence was lower from media pre-inoculated with *D. melanogaster*.

There was no significant difference between the numbers of *D. melanogaster* that emerged from blank media or the *D. suzukii* pre-inoculated media (Figure 2.3.2). As with many native *Drosophila* species, *D. melanogaster* utilise damaged and decomposing fruit for egg laying and may not perceive the presence of another species as detrimental. Other groups have also found *D. suzukii* oviposition wounds expose fruit that would have otherwise been immune to other *Drosophila* specie searching for egg laying sites.

There was no significance between the numbers of *D. suzukii* that emerged from blank media compared to media that had previously been exposed to other *D. suzukii* (Figure 2.3.3). If significantly more *D. suzukii* emerged from the pre-inoculated media, it could have indicated oviposition aggregation pheromones as found in some *Drosophila* species (Wertheim, 2001; Symonds and Wertheim, 2005). However, in wild populations, if given a choice, *D. suzukii* eggs are typically deposited either singly into fruit or in very small clutches (Mitsui *et al.*, 2006) indicating *D. suzukii* preference for oviposition sites low in egg counts or free from both *D. melanogaster* and conspecifics.

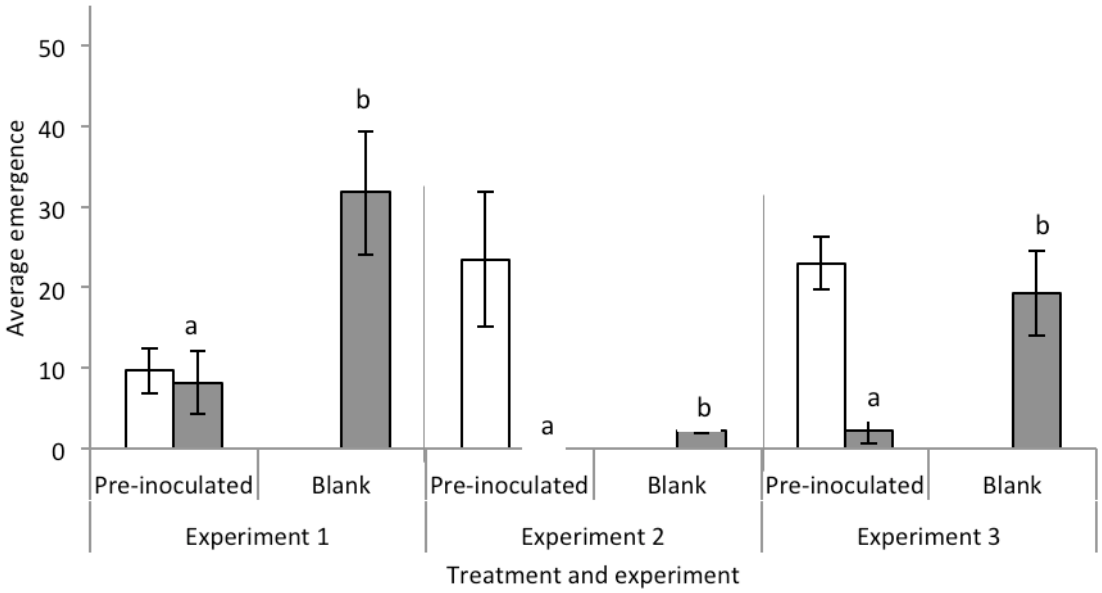


Figure 2.3.1. Average emergence (±S.E.) of *D. suzukii* adult offspring indicated by grey bars, from either cornmeal media (experiment 1) or raspberry fruits (experiment 2 and 3) pre-inoculated with *D. melanogaster* eggs or blank media. White bars display emergence of *D. melanogaster* pre-inoculation. Different lower case letters indicate significant difference between average *D. suzukii* emergence from inoculated and blank media for each experiment

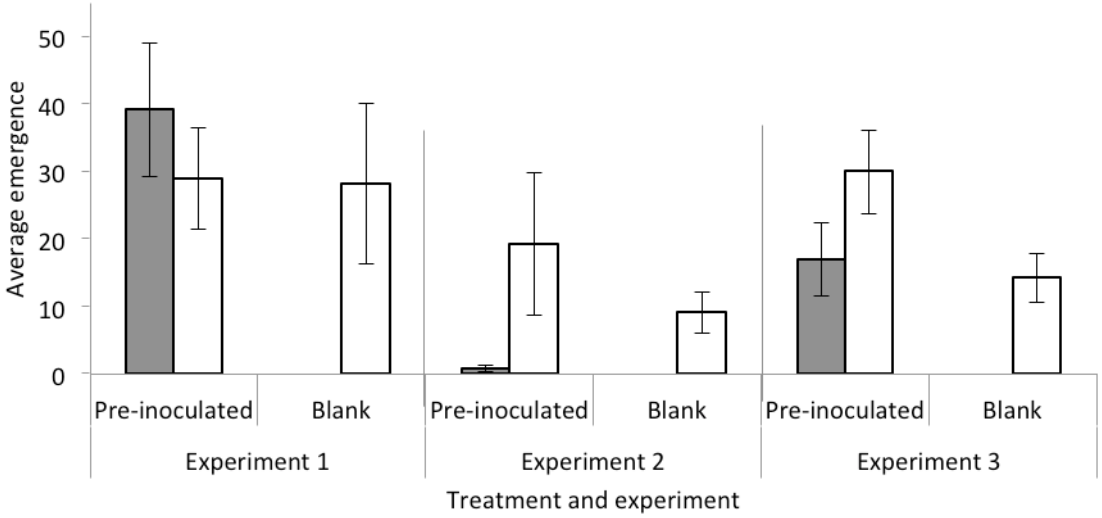


Figure 2.3.2. Average emergence (±S.E.) of *D. melanogaster* adult offspring indicated by white bars, from either cornmeal media (experiment 1) or raspberry fruits (experiment 2 and 3) pre-inoculated with *D. suzukii* eggs or blank media. Grey bars display emergence *D.*

suzukii of pre-inoculation. NSD between treatments in average *D. melanogaster* emergence from inoculated and blank media for each experiment

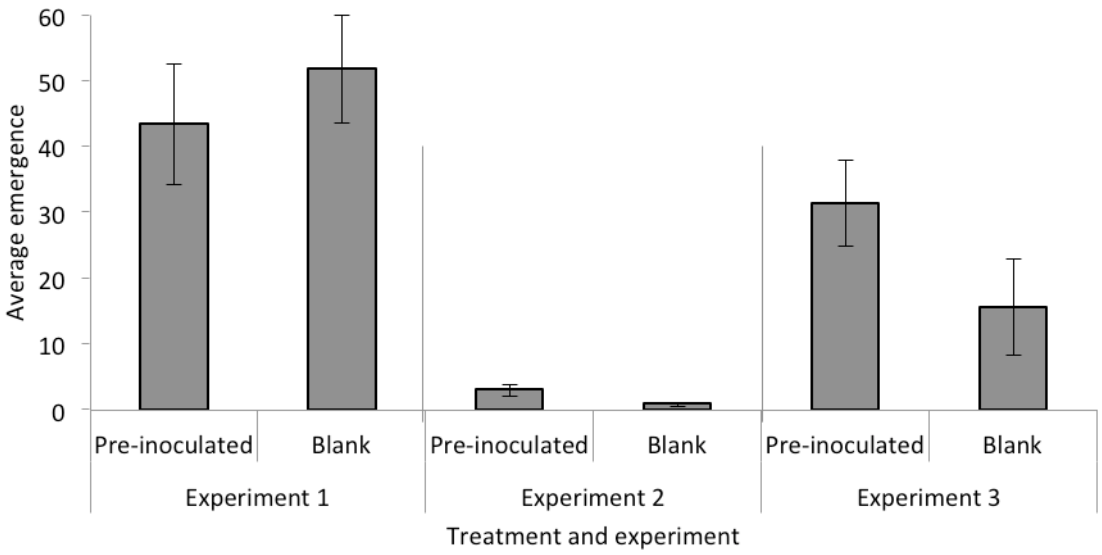


Figure 2.3.3. Average emergence (±S.E.) of *D. suzukii* adult offspring from either cornmeal media (experiment 1) or raspberry fruits (experiment 2 and 3) pre-inoculated with *D. suzukii* eggs or blank media. NSD between treatments in emergence from inoculated and blank media for each experiment

Conclusion

The main aim of this PhD is to investigate daily and seasonal rhythms of *Drosophila suzukii,* which will lead on to formulate recommendations for UK growers to aid combating this pest. In order to do so, understanding what is influencing the patterns of behaviour is key. By identifying the determinants of daily rhythms of *D. suzukii* activity this may help predict field behaviour.

By comparing lab based and semi-field locomotion assays, we have investigated a wide range of environmental conditions to identify those that would be most appropriate for making behavioural predations in the lab. At the beginning of this investigation the mix sex groups were presumed to be the optimum to investigate 'wild-like' locomotion patterns. However, in the semi-field and the corresponding lab semi-field mimic it was the female groups that displayed the fewest differences in activity profiles. From across all environmental conditions is have been evident that individual males and females are not representative of mix sex or single sex group locomotion.

The semi-field conditions were deemed the optimum environmental condition to investigate wild-like behavioural rhythms. However, performing these assays outdoors is not always possible. As the lab semi-field mimic conditions displayed the fewest differences in activity to the semi-field, it seems that with a realistic temperature and light cycle you are able to promote wild-like behaviour in lab conditions. These findings were also confirmed in the hierarchical clustering and the PCA analysis, with the semi-field and semi-field mimic mix sex group data being located together on both. In the PCA, individual males were typically found on the peripheral of each environmental group with mix sex groups and female groups at the centre. Individual males are usually used to investigate locomotion rhythms. It is clear from these results that individual males are not an appropriate social housing to use when predicting average behaviour and mix sex and female groups would be more representative.

In the field oviposition assays it is clear that temperature has a great influence on egg laying and that rhythms are noticeable. Egg laying is primarily occurring during the day time with very few, if any, being laid at night. In the oviposition field assay the similarities between the locomotion of mix sex groups under the oviposition mimic cycles corresponds with the amount of emergence and so the locomotion activity of mix sex groups could be being influenced by egg laying females. The following lab based oviposition assay also supported the rhythm of egg laying even when in a lab based setting. From this results, providing a

realistic temperature and light cycles used, it would be possible to make predictions of oviposition rhythms of wild *D. suzukii* populations from a lab based experiment.

In the competition experiments next generation *D. suzukii* emergence was reduced from media pre-inoculated by *D. melanogaster*. As we do not know the extent of larval and egg mortality we cannot conclude whether this reduction was due to female oviposition choice or larval competition. *D. melanogaster* emergence was not reduced by *D. suzukii* presence and so concerns for the reduction in wild populations of this species are currently unwarranted.

By understanding how behaviour of *D. suzukii* is influenced we can begin to make predictions about when the pest can be targeted. From both the locomotion and oviposition experiments it is clear that there are rhythms in both behaviours and they are influenced by not only environmental but also by social conditions.

Knowledge and Technology Transfer

Poster presentation:

- The UK clock club winter conferences, Edinburgh, December 2015
- The UK clock club summer conference, Coventry, July 2016
- SCI young researchers in crop sciences, Berkshire, United Kingdom, July 2016
- IOBC International Conference on Integrated Fruit Production, Thessaloniki, Greece September 2016
- The UK clock club winter conference, Oxford, December 2016
- The UK clock club summer conference, Bristol, July 2017
- University of Southampton Post-graduate day, July 2017

Flash presentation and poster:

- AHDB Tomato Conference, Warwickshire, 29th September 2016
- AHDB The Studentship Conference, Warwickshire 16-17th November 2016

Oral presentation:

- SWD working group meeting, September 2016
- AHDB Soft fruit day November 2016
- AHDB Tree Fruit panel presentation November 2016
- AHDB Tree fruit day March 2017
- International Horticultural Research Conference July 2017

Industry tours

- AHDB studentship industry tour July 2017

Successful grant applications

- GCRI travel grant, September 2016, £500.
- Worshipful company of fruiterers, September 2016, £300
- GCRI travel grant, May 2017, grant returned due to conference not being attended

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