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, October 2016
Previous report:	none
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

PhD student

NIAB EMR

Signature ..... Date .....

Michelle Fountain

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CONTENTS

GROWER SUMMARY

Headline

- New findings are being made about the daily activity of spotted wing drosophila in the UK.

Background and expected deliverables

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, blueberry, strawberry, raspberry and blackberry. Female *D. suzukii* are able to insert eggs through 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 USA 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, 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 (Gruwez *et al*., 1971; Konopka and Benzer, 1971; Xu *et al*., 2008). It is not known if similar physiological and behavioural circadian rhythms also exist in *D. suzukii*.

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

Summary of the project and main conclusions

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.

Oviposition patterns were also determined within the laboratory under the same environmental conditions as the locomotion assays. Petri dishes containing an artificial diet were changed every hour and the number of eggs laid were counted.

Oviposition in the field was explored by using the laboratory method within a highly infested cherry orchard in August. Fresh fruit was used instead of artificial diet and was changed every two hours from sun rise to sun set. The emergence of the next generation was counted as Drosophila eggs cannot be identified to species.

It was discovered 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. Oviposition was also light driven with no egg laying occurring in the hours of darkness in both the laboratory and in the field. As with locomotion, temperature influenced egg laying although the parameters have not yet been conclusively identified.

Main conclusions so far

- 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.

- Oviposition occurs in the day time, with maximum egg laying occurring at temperatures between 25-29.9°C.

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. Improving understanding of SWD behaviour is essential for developing improved management and control of SWD thereby 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 Chrono physiology of the pest we can provide growers with a better understanding of how to target *D. suzukii*.

Action points for growers

- 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.). 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 (Sasaki, 1995). 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 soft-fruit 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). It was unknown before the beginning of this project if similar physiological and behavioural circadian rhythms also exist in *D. suzukii*.

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, feeding 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, oviposition, and feeding (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

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

Locomotion profiles for *D. suzukii* were created using a Trikinetics Drosophila Activity Monitor (DAM) device. This was an electrical device that records activity by movement breaking an infra-red beam. Individual male and female activity profiles were created using a 32 channel individual DAM (Figure 1) system which held glass cuvettes 7cm long with 5mm diameter. Groups of males, females and mixed sex groups were monitored within a DAM population monitor (Figure 1.1) which held glass vials 15cm long with 30mm diameter. The DAM devices recorded within an incubator mimicking environmental conditions found at key times in the commercial year (Table 1) and under various temperature and light conditions (Table 1.1, 1.2 and Figure 1.2). Average temperature cycles were produced for each time of the year by analysing met data collected from 2013-2015 at NIAB EMR. Average sun rise, sun set and photo period ('day light length') times were produced from averages from 2013-2015 from an online database (http://www.timeanddate.com/sun/uk/maidstone) .Conditions mimicked:

- June, when cherry ripening occurs;
- October, the end of raspberry cropping;
- November, when flies move into sheltered habitats and
- April, when flies move into cropping areas.

Each channel of the DAM devices either held a glass cuvette which housed an individual fly or a vial containing groups of flies. Groups of flies consisted of either single sex groups of 10 males or 10 females, or mix sex groups of 10 males and 10 females. The devices were recording from within a Panasonic incubator capable of running a fluctuating temperature and light cycle indefinitely. Incubators were set to a constant humidity of 65%. The strain of *D. suzukii* used for experimentation were originally collected from Italy and have been cultured on a standard yeast cornmeal *Drosophila* medium including malt extract (http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/bloomfood.htm) for 3 years. Cultures were kept genetically diverse by randomly mixing offspring to prevent inbreeding. Summer morph adults were used (cultured at 23°C under 16:8 L:D) for the June and October conditions. Winter and summer morphs were used for the April and November assays to replicate the stages found in the field but the November and April results are not currently available. The variation of behavioural rhythms in constant versus fluctuating environmental cycles were used to assess the stability of *D. suzukii*'s internal time keeping. This involved

running the same locomotion assays under constant dark (D:D), constant light (L:L), constant temperatures or combinations of conditions. * See appendix for summary of conditions and combinations tested and outstanding. For each recording across all combinations of sex, group and condition, data was collected for a minimum of 7 days after 1 day acclimatisation. The ClockLab Circadian Analysis software for circadian biology (ACTi Metrics) was used to analyse the data and produce activity profiles, actograms and determine period length for each set of data. A minimum of 60 individual males or females that survived the whole assessment period were batch analysed to create one average profile per sex i.e. the average daily locomotion activity for 60 individuals collected over 7 days displayed over 24 hours.

Figure 1. Standard 32 channel Trikenetics individual Drosophila Activity Monitor. Glass cuvettes 7cm long with 5mm diameter.

Figure 1.1. Large 32 channel Trikenetics population Drosophila Activity Monitor. Glass vials 15cm long with 30mm diameter.

Table 1. Key times in the commercial year to target *D. suzukii*.

Month	Commercial crop activity	*D. suzukii* movements	Grower concerns
April	Some crops in blossom e.g. cherry, pear.	Low populations numbers that have survived winter move back into sheltered cropping areas such as orchards	Cannot apply pesticides to cropping areas in blossom
June	Early cherry varieties ripening	First commercially available cherry crop in the year ripening so targeted for egg laying	Growers want to hold back on applying pesticides, preparing for heavy infestation later in the season. Traps are not as attractive as the fruit
October	Late raspberry harvest	Large population numbers have built up over the summer and exploit waste fruit left in the crop	Growers will have applied their maximum applications of high impact pesticides, picking is less frequent and hygiene practises may be reduced Traps are not as attractive as the fruit.
November	Dormancy	High populations moving back into woodland areas for shelter over the winter	Will not spray at this time.

Table 1.1. Environmental parameters for each condition including temperature range and day length to be used in incubators

Condition	Temp range (°C)	Time highest temp first reached	Time lowest temp first reached	Full 'Sun rise'	Full 'Sun set'	Day light (Hours)
April	6-11°C	14:00	05:00	07:00	19:30	12.50

Table 1.2. Hourly temperatures used under each environmental condition.

June	11-22°C	16:00	01:00	05:00	22:00	15.00
Time	**April**	**June**		**October**	**November**	
October	12-21°C	14:00	23:00	07:15	17:00	9.25
	Spring	**Cherry**		**Late raspberry**	**Winter activity**	
November	8-12°C	13:00	20:00	07:45	16:45	9.00
	activity	**ripening**		**harvest**		

00:00	7°C	12°C	13°C	8°C
01:00	7°C	11°C	14°C	8°C
02:00	7°C	11°C	13°C	8°C
03:00	7°C	11°C	13°C	9°C
04:00	7°C	11°C	13°C	9°C
05:00	6°C	11°C	13°C	9°C
06:00	6°C	12°C°	14°C	9°C
07:00	6°C	14°C	14°C	9°C
08:00	6°C	16°C	14°C	9°C
09:00	7°C	17°C	15°C	10°C
10:00	8°C	19°C	17°C	10°C
11:00	9°C	20°C	18°C	11°C
12:00	10°C	21°C	19°C	11°C
13:00	10°C	20°C	20°C	11°C
14:00	11°C	21°C	20°C	12°C
15:00	11°C	21°C	19°C	11°C
16:00	11°C	22°C	19°C	10°C
17:00	10°C	20°C	19°C	10°C
18:00	10°C	18°C	17°C	9°C
19:00	9°C	16°C	15°C	9°C
20:00	8°C	14°C	14°C	8°C
21:00	8°C	13°C	13°C	8°C
22:00	8°C	12°C	12°C	8°C
23:00	7°C	12°C	12°C	8°C

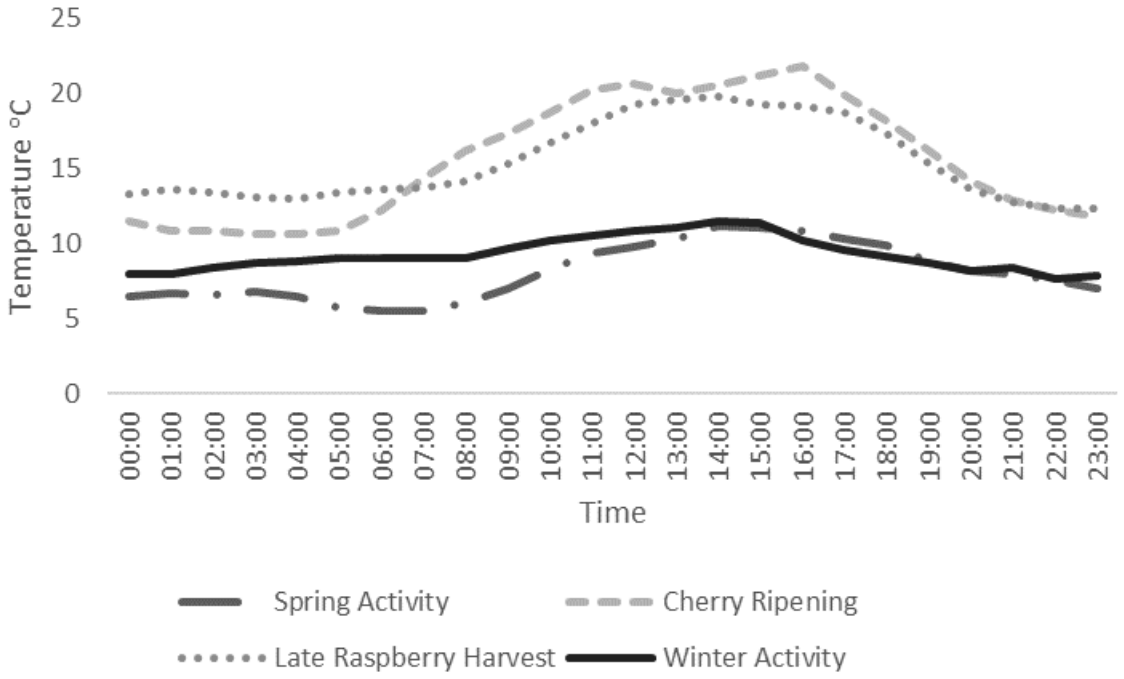

Figure 1.2. Hourly temperature profiles to be used for the four key times in the commercial year.

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

In the laboratory egg laying assays were performed within several Panasonic incubators using the environmental profiles for June (Figure 1.1, 1.2). Incubators were staggered to different time zones so 24 hours could be sampled in just 12 hours. *D. suzukii* were aged between 3 and 14 days at the beginning of the assay. Each incubator housed 4 cages each 15 x 15 x 15cm. Each cage held 20 females and 10 males. All cages were provisioned with water *ad libitum* delivered by a small plastic pot with a cotton ball soaked in distilled water which was refilled when low. A petri dish (6 cm) of standard Drosophila cornmeal diet was also provided before the assay began. On commencing the trial the petri dish of cornmeal was replaced on the hour every hour between real time 08:00 and 20:00. After each dish was removed they were inspected under a microscope (x6 magnification) and the numbers of eggs laid were counted. Eggs laid between real time 20:00-8:00 were counted but not included in analysis as the exact time of egg laying could not be identified. The results from

each replicate were combined within the incubator and the average taken for each repetition day.

In the field the oviposition experiment followed a similar method to the laboratory assay with petri dishes of fruit deployed within a cherry orchard. Dishes were changed every two hours from sun rise (5am) till sun set (9pm). Petri dishes (6 cm) were housed in modified green delta traps (Figure 1.3, 1.4). These modified traps had wire mesh entry points which reduces the amount of large bi-catch and restricted the entry of larger insects which may utilise the media. Direct egg counts were made of eggs that were within the fruit and laid underneath the skin, as other species may have deposited eggs on the fruits surface. The samples were stored at 25°C for 2 weeks to allow development and subsequent counts of emerging adults to confirm the egg count predictions, as Drosophila eggs and larvae cannot be identified to species. Ten replicates were taken over 4 replicate days. The emergence of *D. suzukii* from fruit left in the orchard from 21:00 -05:00 was counted and an hourly average taken to calculate egg laying through the night.

Figure 1.3. Modified green delta trap with new side entry point. Petri dish containing a single cherry is the egg laying site for the experiment

Figure 1.4. Modified green delta trap from side entrance covered with a 5mm wire mesh to prevent entry of larger insects and birds.

Results

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

**All data currently not statistically analysed. The results below display trends.

Maintained in 12:12 hour L:D cycle at constant 23°C, male and female *D. suzukii* exhibited rhythmic activity profiles (Figure 2.a, 2.b respectively) with peaks of activity occurring at lights off in both profiles. Under conditions found in June, when cherry ripening occurs, individual males (Figure 2.1) and females (Figure 2.2) exhibited morning and evening peaks of activity with little activity occurring in total darkness. These peaks coincided when the lights were turned on and off and could be the results of a startle response. When housed in single sex groups, males displayed a morning activity peak but no evening peak (Figure 2.3). Female groups had an activity peak at the peak temperature of 22°C and also exhibited a morning and evening activity peak (Figure 2.4). When housed in mixed sex groups the peak of activity, as with female groups, correlated to the peak in temperature and the morning and evening activity peaks were not present (Figure 2.5).

To determine whether space was a factor in the locomotion rhythms, individual males (Figure 2.6) and females (Figure 2.7) were monitored within larger population monitors. In individual smaller cuvettes individual males and females had a constant fluctuation in locomotion

throughout the photo phase. In the larger population monitors there were two prolonged periods of increased activity divided by a ‘siesta’ (period of inactivity) in the middle of the day.

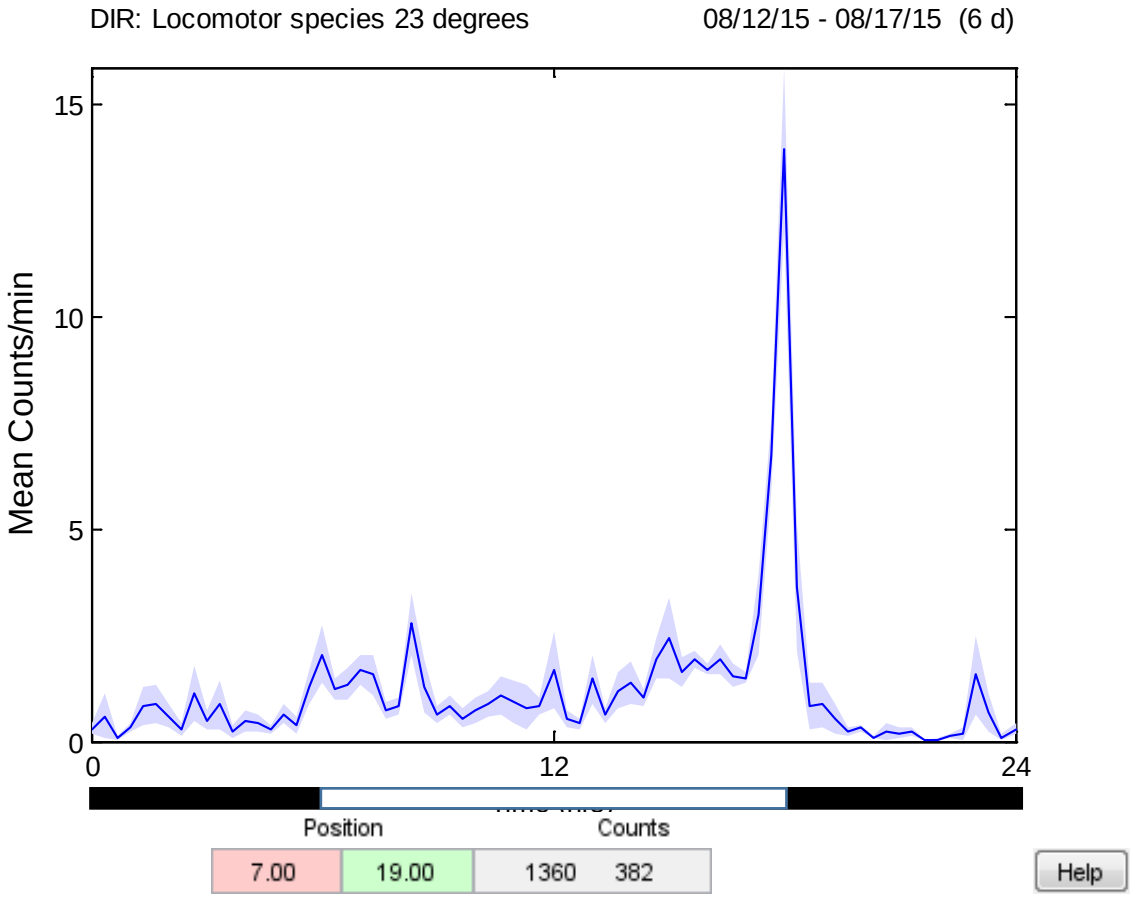

Figure 2a. Male *D. suzukii* in 12:12 hour light dark cycle at 23°C. Black bar at bottom of figure indicated darkness. White bar indicates light. Dark blue line displays average locomotion activity. Light blue area displays variability

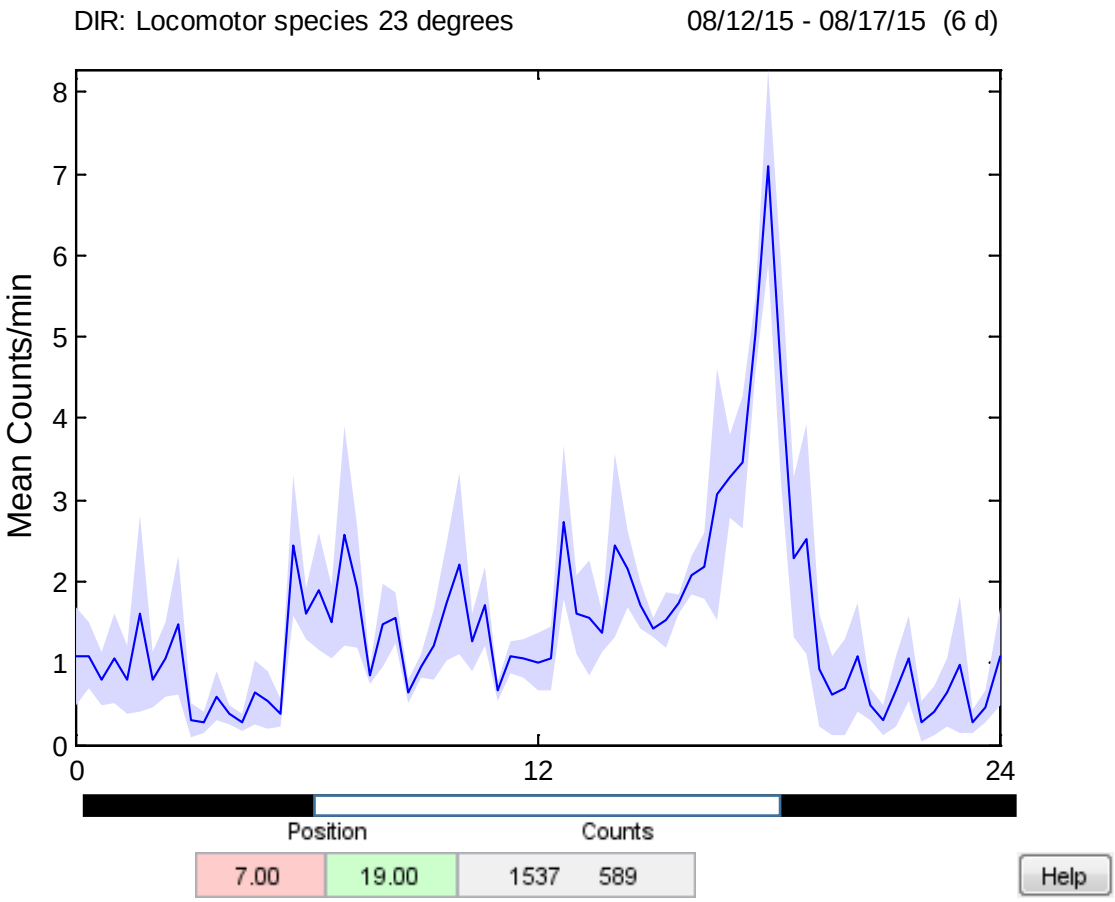

Figure 2b. Female *D. suzukii* in 12:12 hour light dark cycle at 23°C. Black bar at bottom of figure indicated darkness. White bar indicates light. Dark blue line displays average locomotion activity. Light blue area displays variability

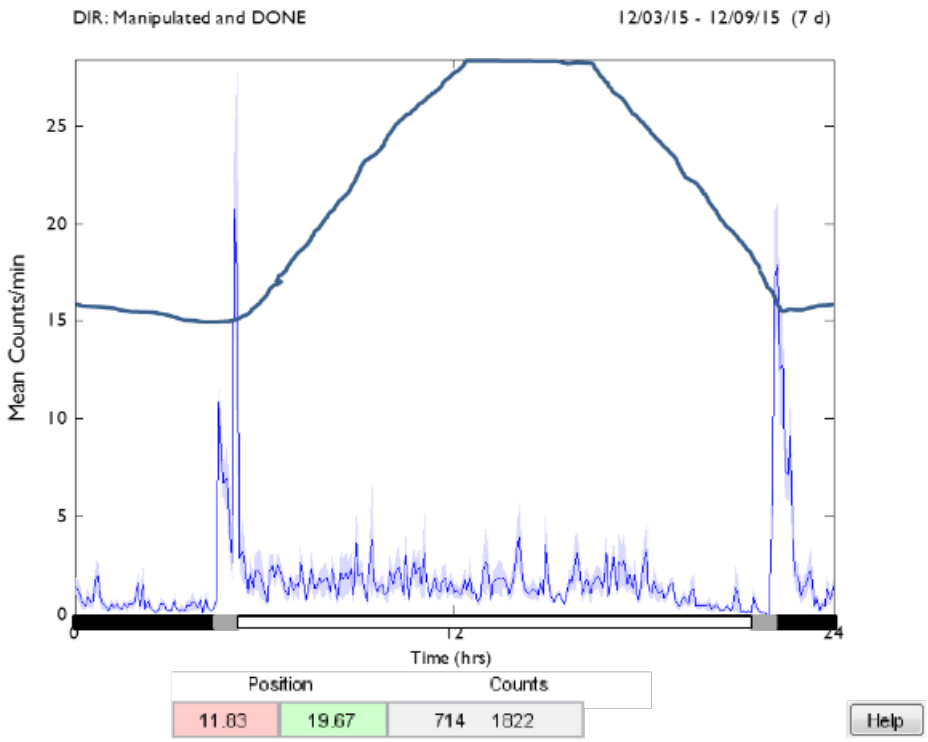

Figure 2.1. Activity profile for male *D. suzukii* with June environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing over 60 individual males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

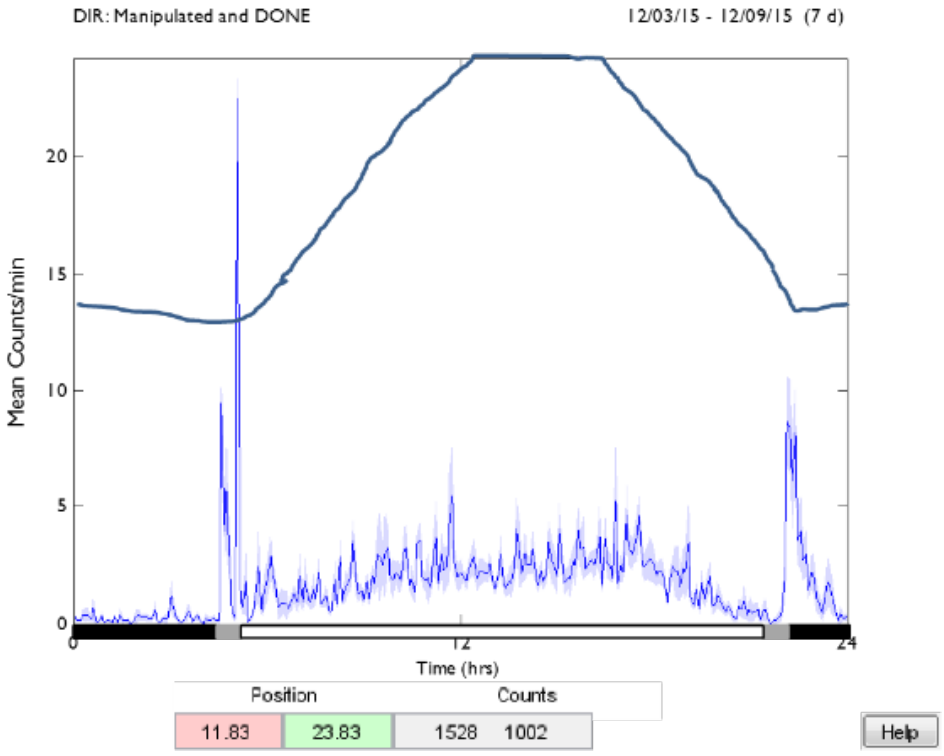

Figure 2.2. Activity profile for female *D. suzukii* with June environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing over 60 individual females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

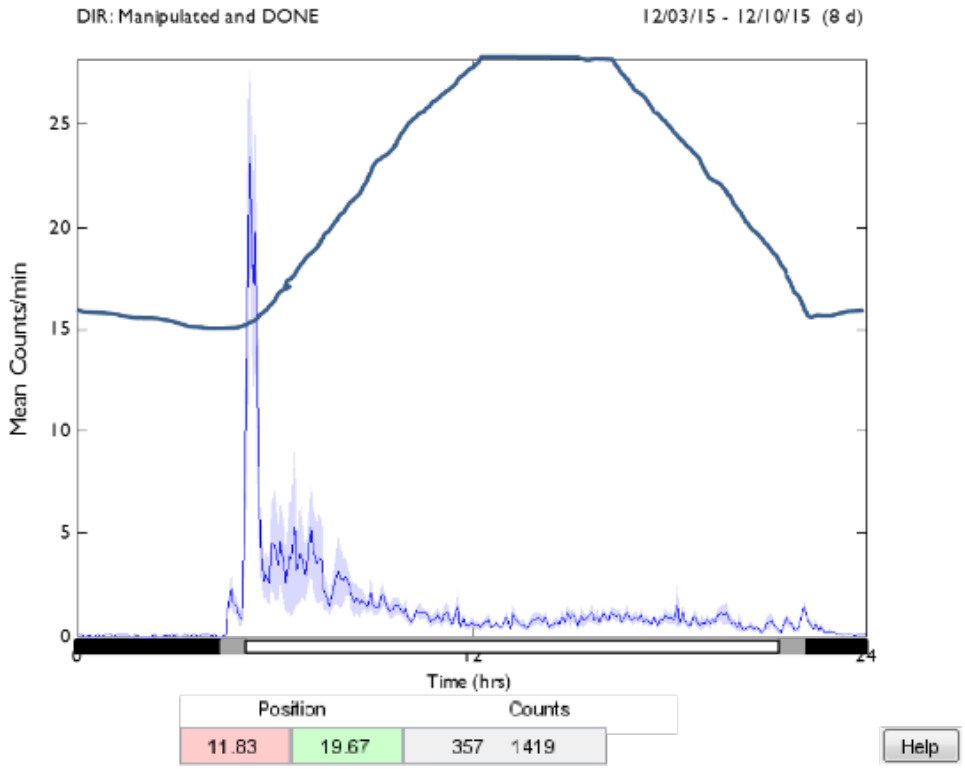

Figure 2.3. Activity profile for groups of male *D. suzukii* with June environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

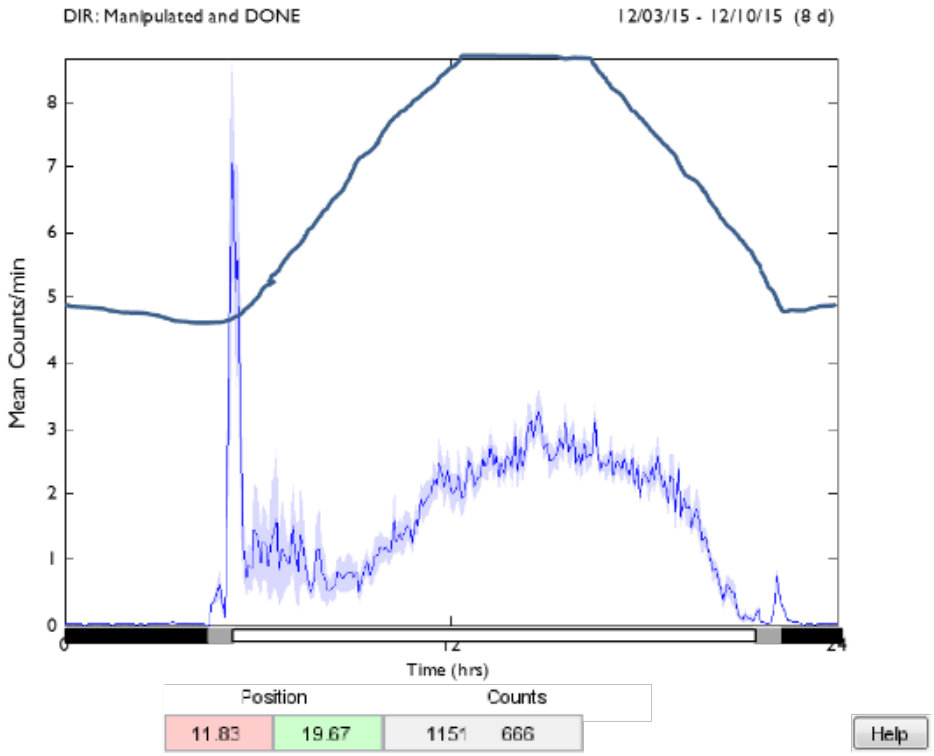

Figure 2.4. Activity profile for groups of female *D. suzukii* with June environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

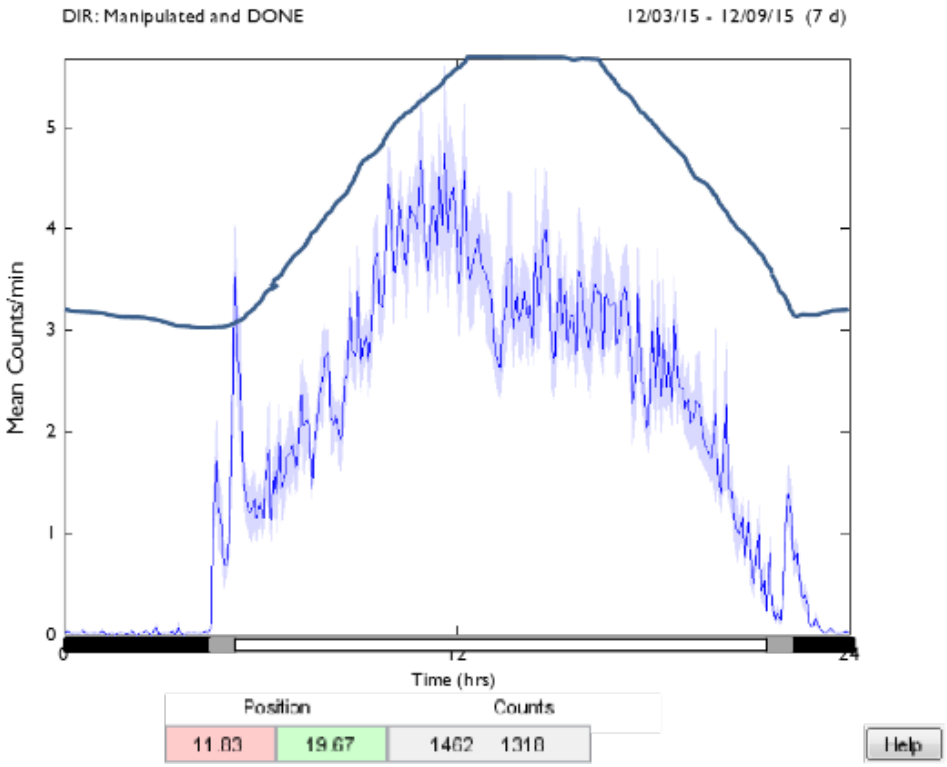

Figure 2.5. Activity profile for groups of male and female *D. suzukii* with June environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

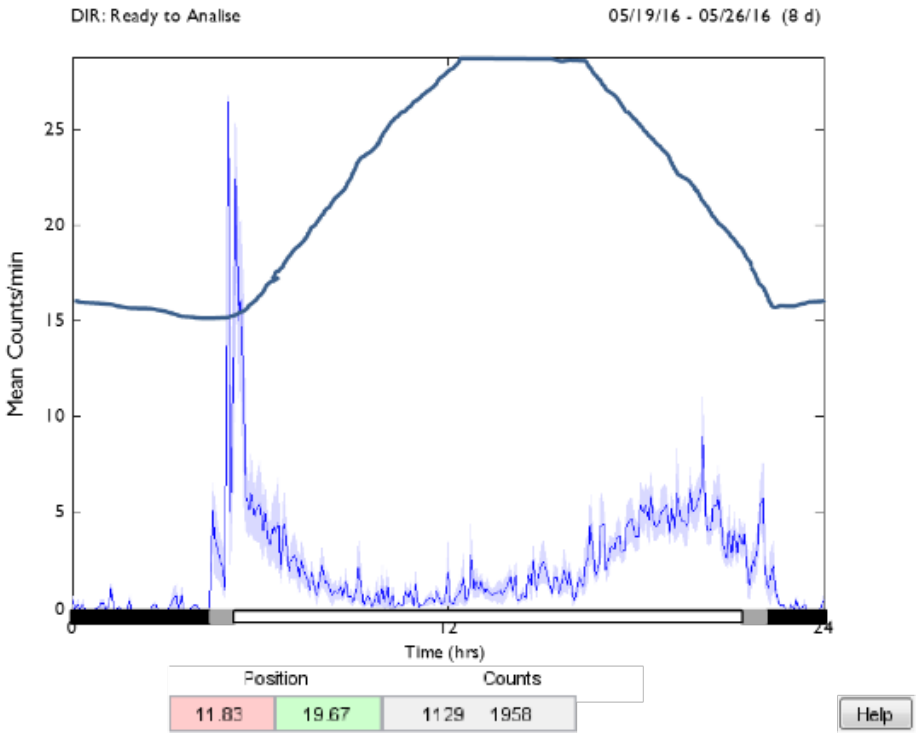

Figure 2.6. Activity profile for individual male *D. suzukii* within a population monitor with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

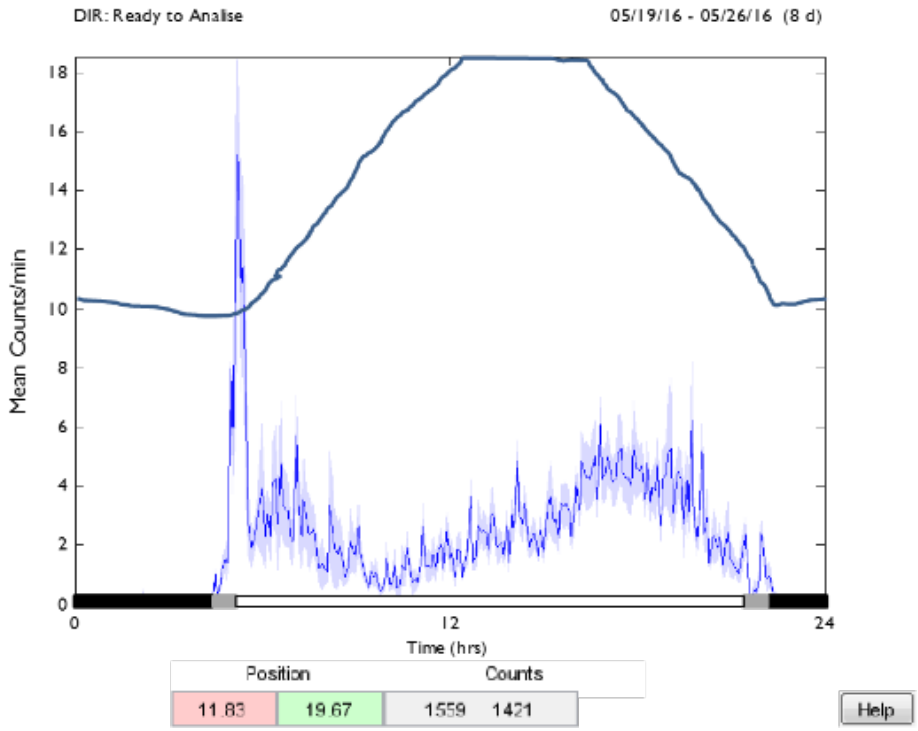

Figure 2.7. Activity profile for individual female *D. suzukii* within a population monitor with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

Under the October conditions simulating a vulnerable time in primacane raspberry production, all social situations displayed morning and evening startling peaks of activity at lights on and lights off (Figure 2.8-2.12). There was less activity in darkness hours than in light but there was more activity occurring in darkness for both individual males and individual females (Figure 2.8, 2.9 respectivily). In all group housed flies, an activity peak follows the temperature cycle but were primarily restricted to the photo phase (Figures 2.10-2.12).

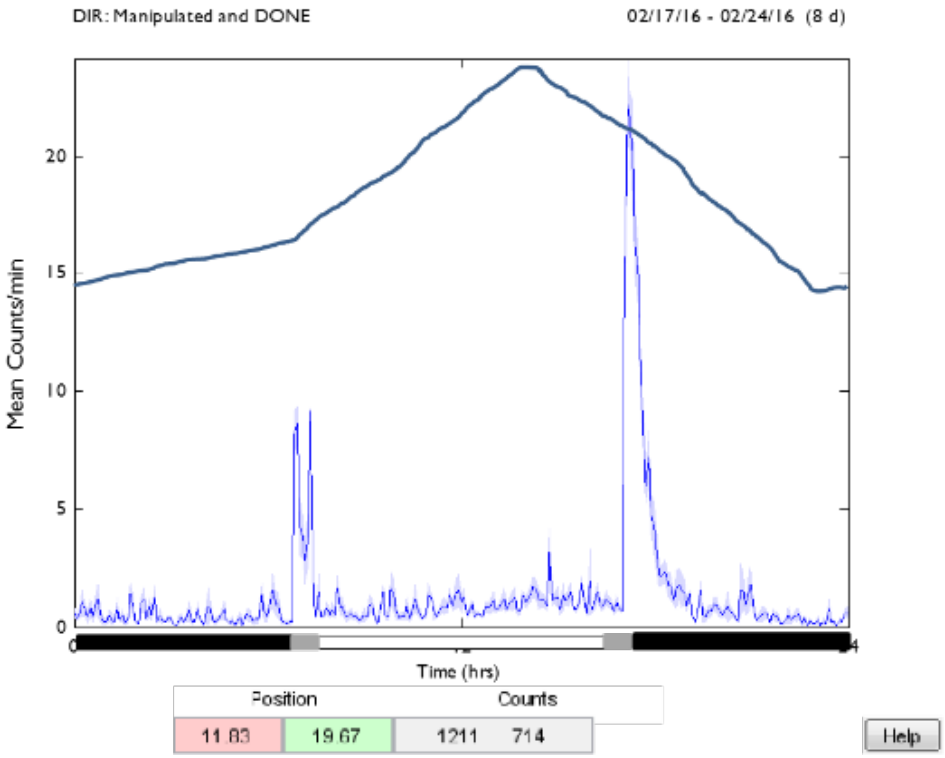

Figure 2.8. Activity profile for male *D. suzukii* with October environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing over 60 individual males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

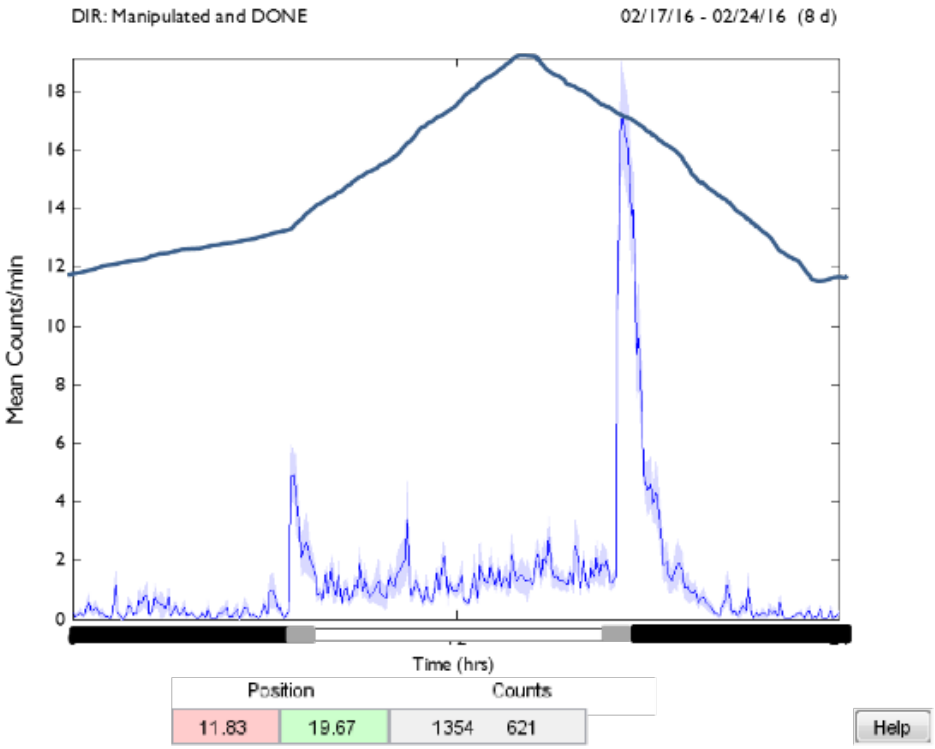

Figure 2.9. Activity profile for female *D. suzukii* with October environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing over 60 individual females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

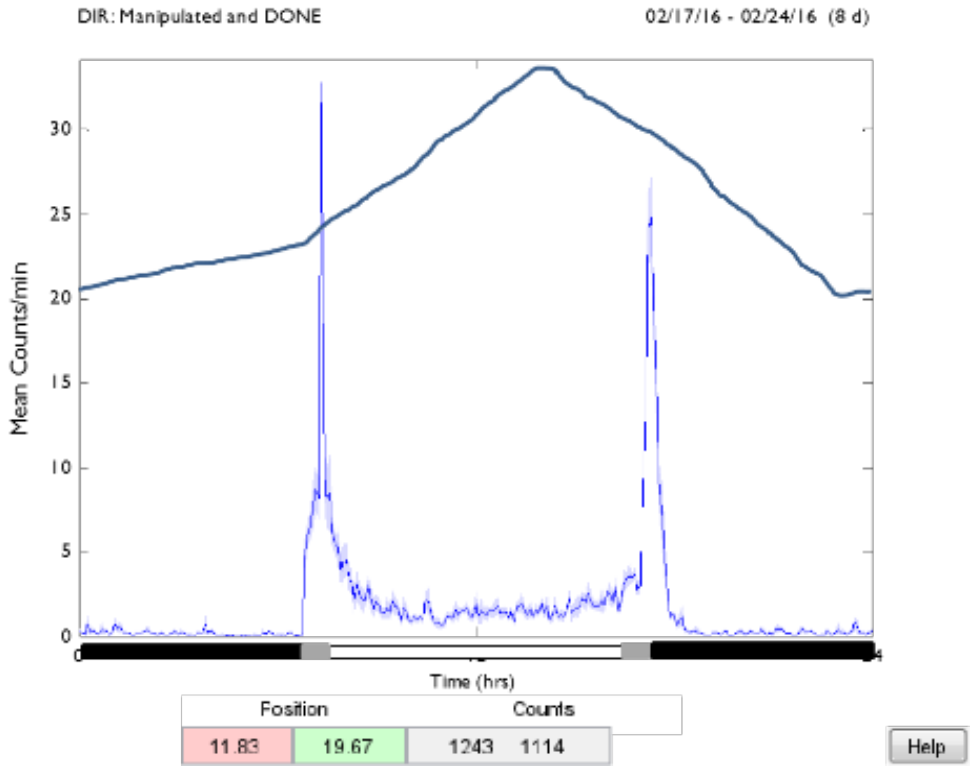

Figure 2.10. Activity profile for groups of male *D. suzukii* with October environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

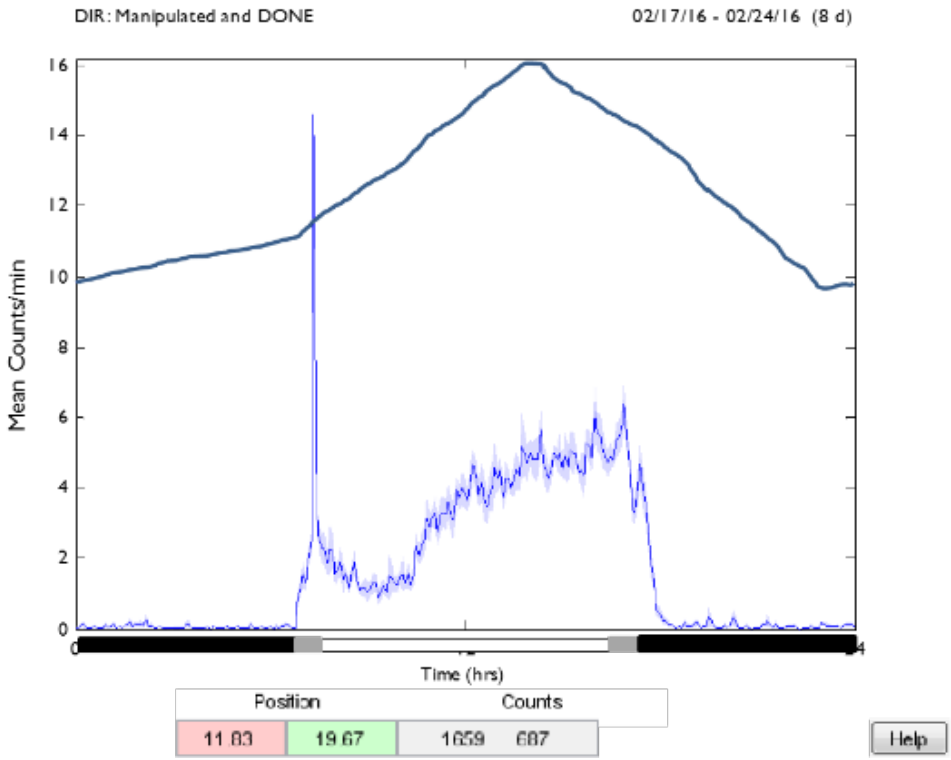

Figure 2.11. Activity profile for groups of female *D. suzukii* with October environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

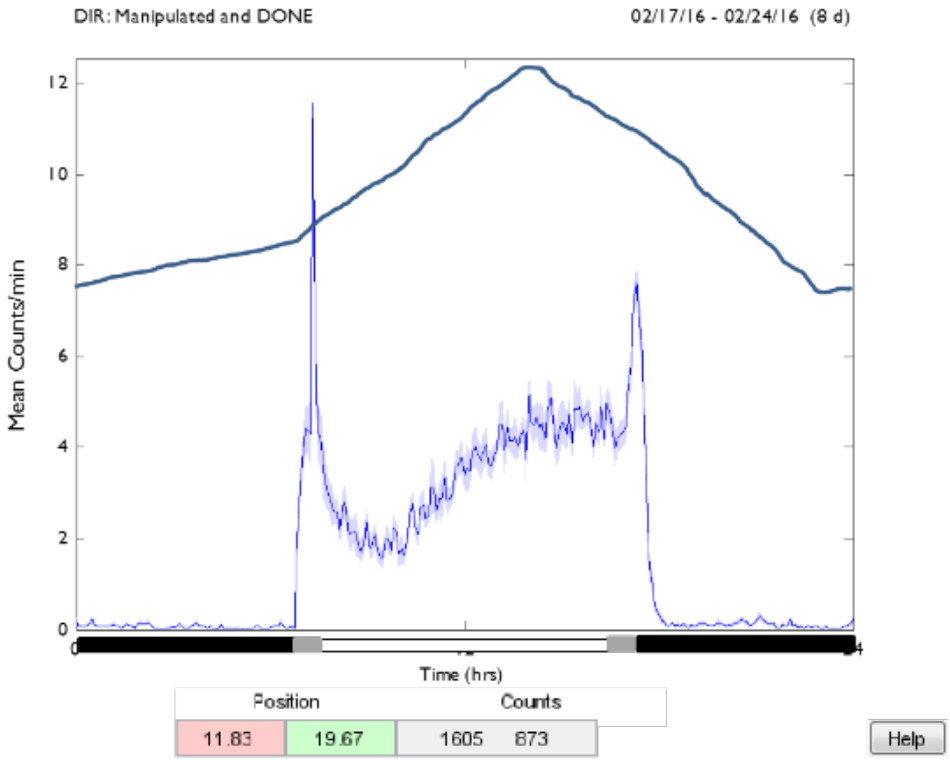

Figure 2.12. Activity profile for groups of male and female *D. suzukii* with October environmental conditions. Temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. Produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

To understand the effect of environmental cues on locomotion rhythms, assays were performed with either one or all cues removed. Using the same social situations locomotion of individual males, females, groups of males or females and mixed sex groups were first housed under the same temperature cycle as the June conditions but in constant darkness. In all of the social situations the startle response of the lights coming on and going off has been removed (Figures 2.13-2.17). With the light cue removed, the activity profiles for each situation followed the increase and decrease in temperature cycle. Although there was no light cue there was significantly less activity at ‘night time’ in comparison to the ‘day time’.

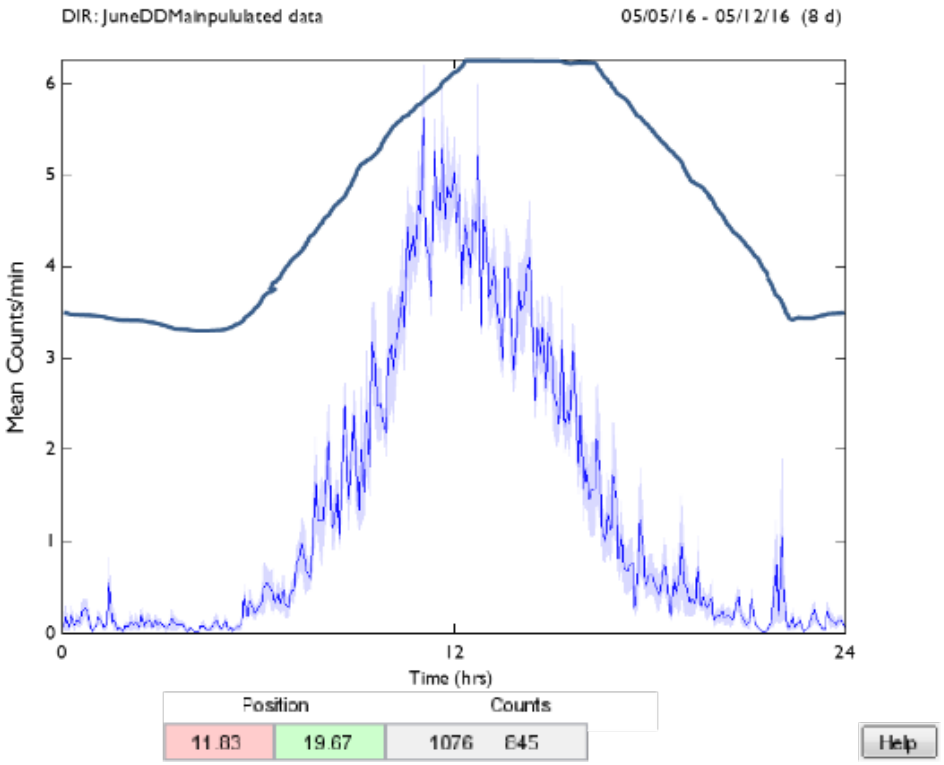

Figure 2.13. Activity profile for individual male *D. suzukii* under constant darkness with a June temperature cycle indicated by blue line at top of figure. Produced by batch analysing over 60 individuals that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

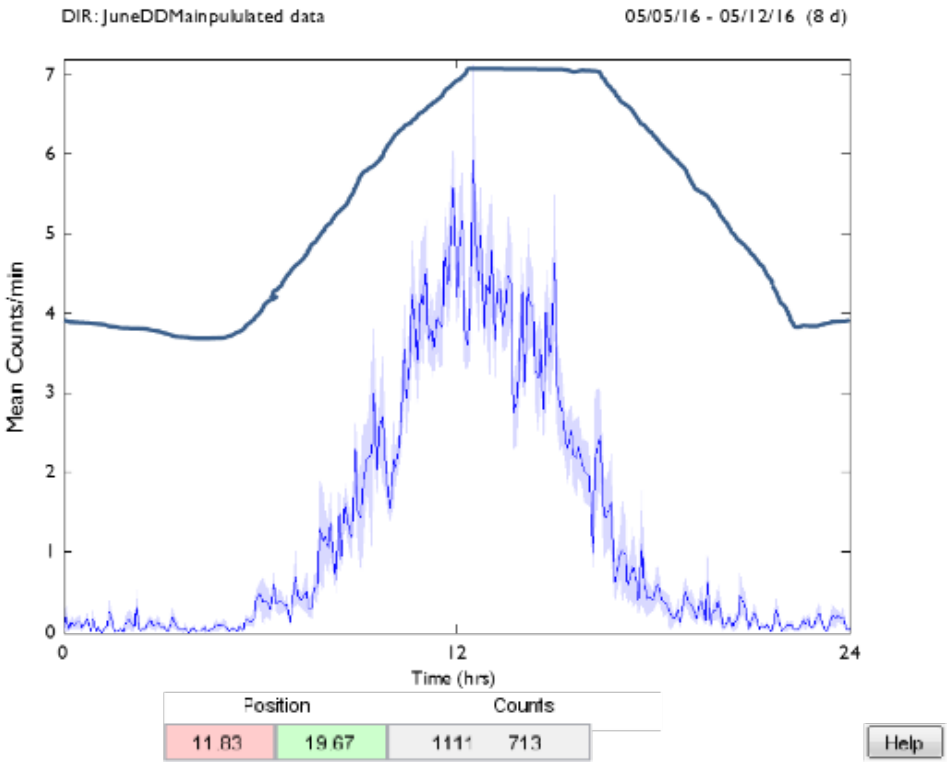

Figure 2.14. Activity profile for individual female *D. suzukii* under constant darkness with a June temperature cycle, indicated by blue line at top of figure. Produced by batch analysing over 60 individuals that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

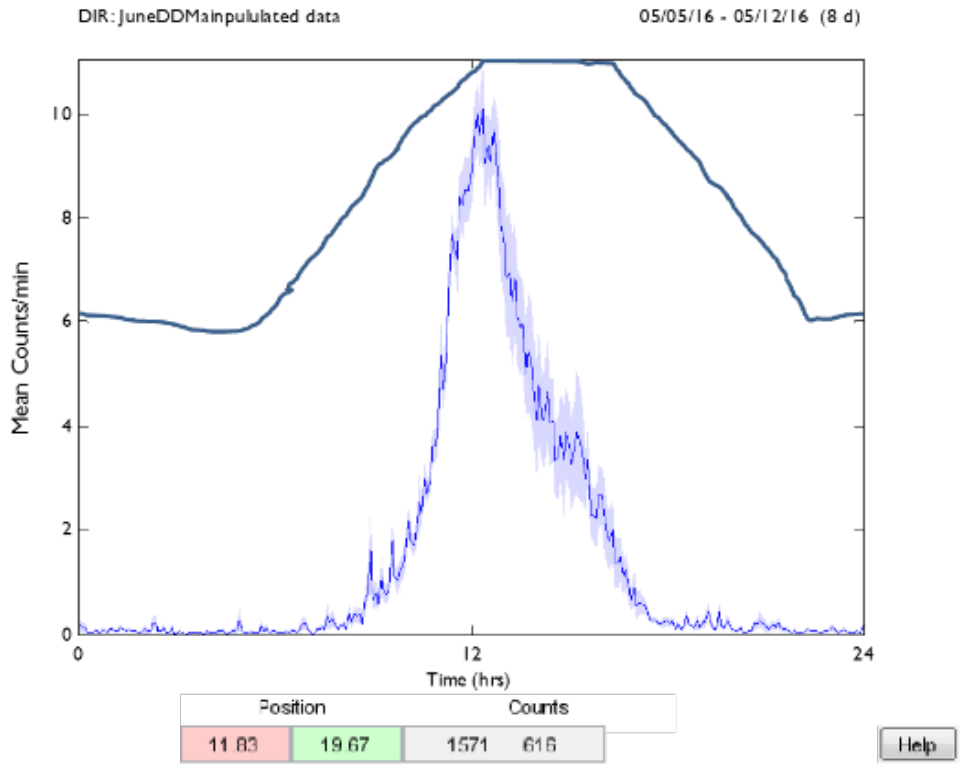

Figure 2.15. Activity profile for groups of male *D. suzukii* under constant darkness with a June temperature cycle, indicated by blue line at top of figure. Produced by batch analysing 10 groups of 10 males that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

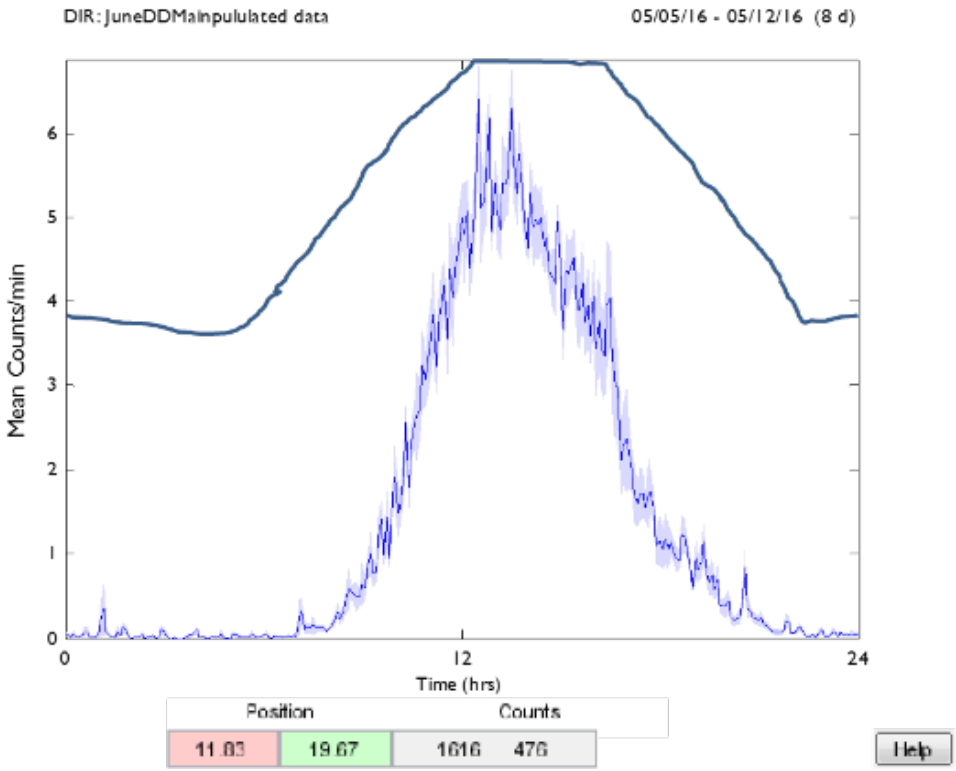

Figure 2.16. Activity profile for groups of female *D. suzukii* under constant darkness with a June temperature cycle, indicated by blue line at top of figure. Produced by batch analysing 10 groups of 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

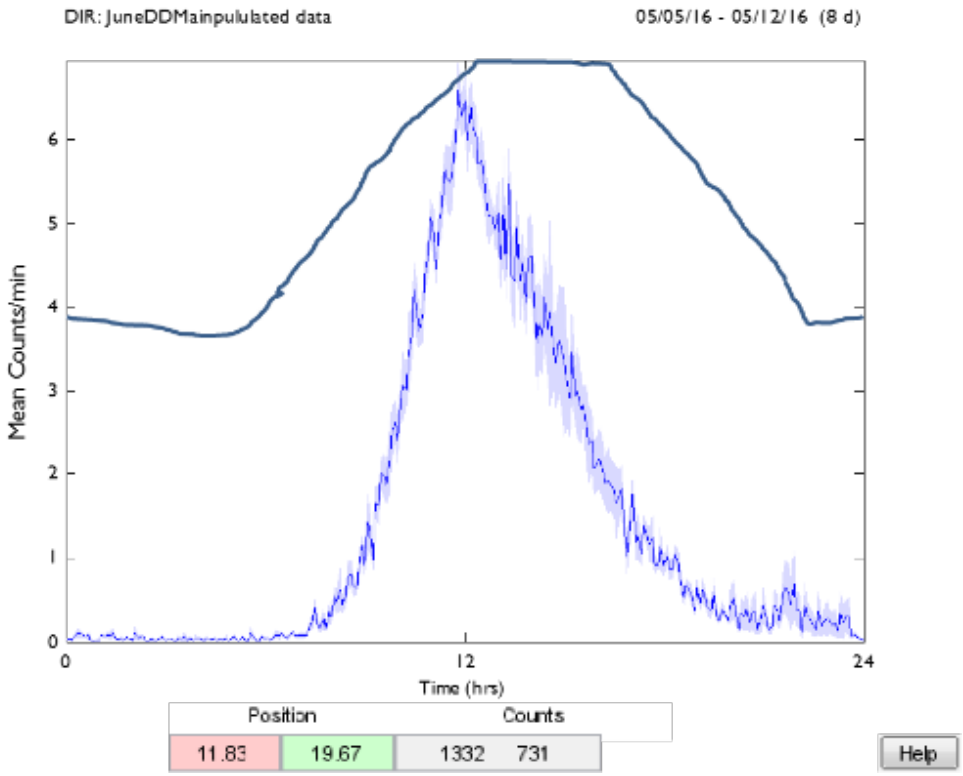

Figure 2.17. Activity profile for groups of male and female *D. suzukii* under constant darkness with a June temperature cycle, indicated by blue line at top of figure. Produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period. Dark blue line displays average locomotion activity. Light blue area displays variability.

To investigate the internal molecular oscillator both light and temperature cues were removed. Experiments were conducted under constant darkness and 23°C with the same social situations. In both individual males (Figure 2.18) and females (Figure 2.19) became arrhythmic after 24 hours without environmental cues. Within the male groups (Figure 2.20) and mix sex groups (Figure 2.21) rhythmicity is maintained for 5 days before the day peak is reduced and the night peak increased. Groups of females, although there is an increase in night activity, continue to have peak activity in the 'day time' and only disappears after 9 days (Figure 2.22).

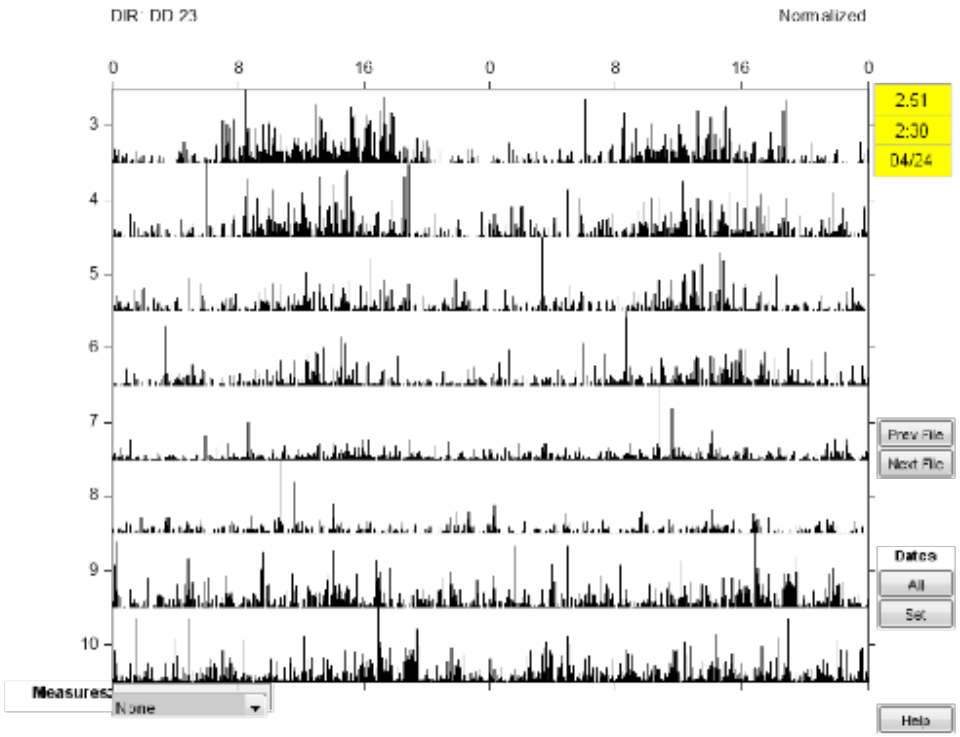

Figure 2.18. Actogram of individual male *D. suzukii* activity over a 7 day period under 23°C in constant darkness. X axis displays days monitored (3-10 = 7 days), Y axis displays hours. Second 0 on top axis denotes the start of activity of the following day, this is then repeated on the line below starting at the first 0.

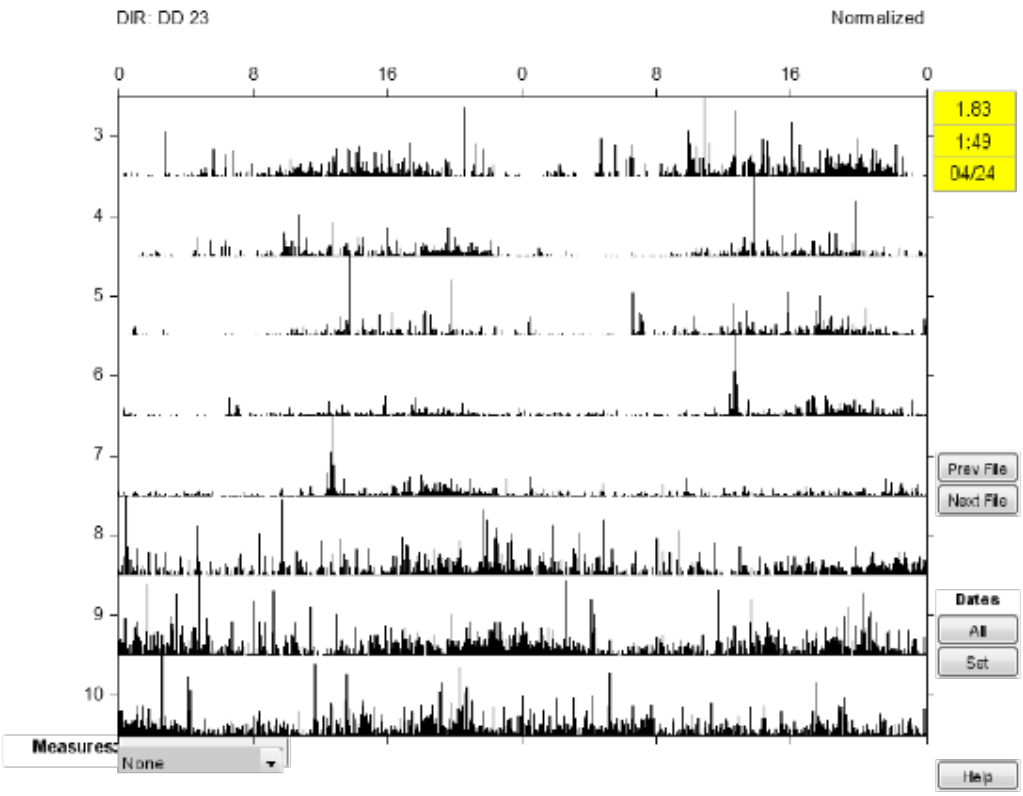

Figure 2.19. Actogram of individual female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. X axis displays days monitored (3-10 = 7 days), Y axis displays hours. Second 0 on top axis denotes the start of activity of the following day, this is then repeated on the line below starting at the first 0.

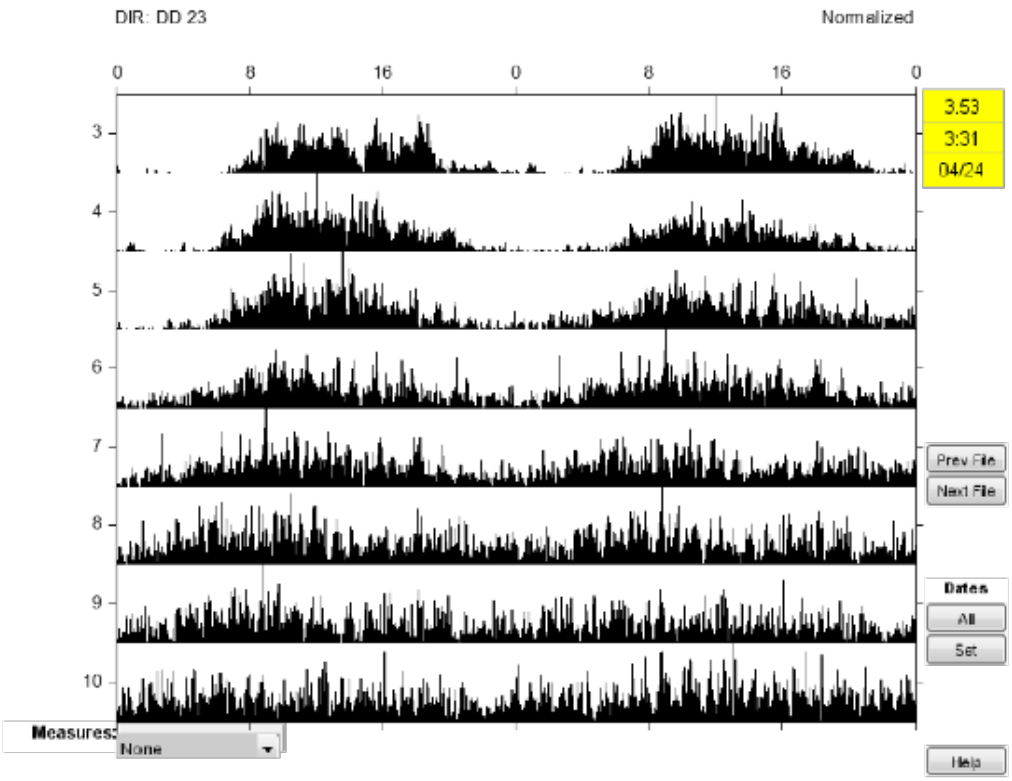

Figure 2.20. Actogram of groups of male *D. suzukii* activity over a 7 day period under 23°C in constant darkness. X axis displays days monitored (3-10 = 7 days), Y axis displays hours. Second 0 on top axis denotes the start of activity of the following day, this is then repeated on the line below starting at the first 0. Produced by batch analysing 10 groups of 10 males that survived the whole assessment period

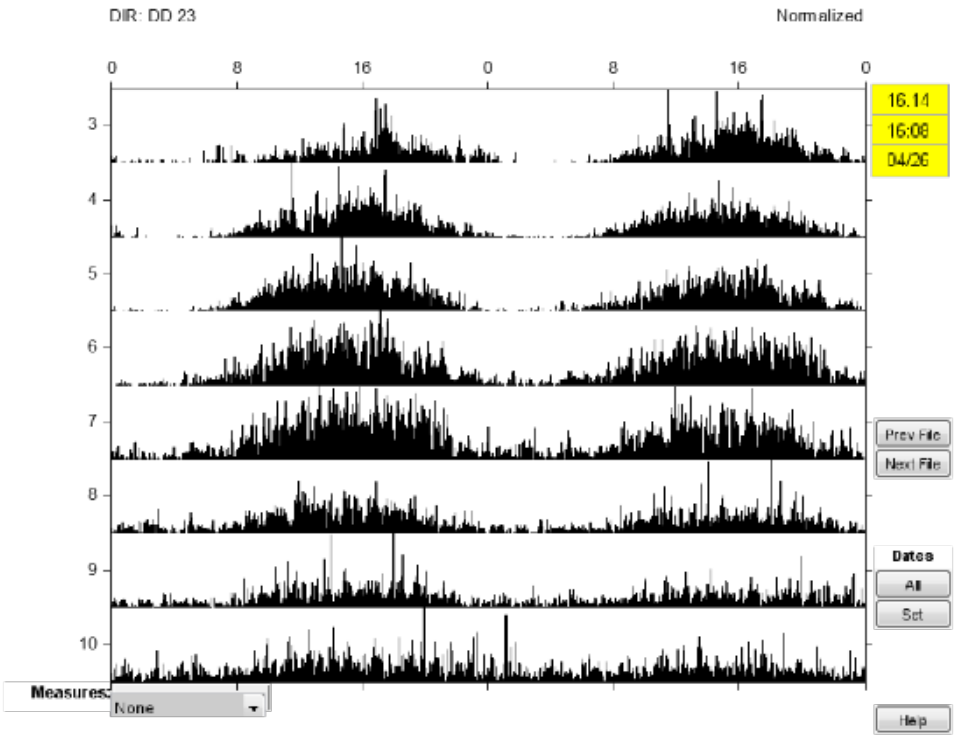

Figure 2.21. Actogram of groups of female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. X axis displays days monitored (3-10 = 7 days), Y axis displays hours. Second 0 on top axis denotes the start of activity of the following day, this is then repeated on the line below starting at the first 0. Produced by batch analysing 10 groups of 10 females that survived the whole assessment period.

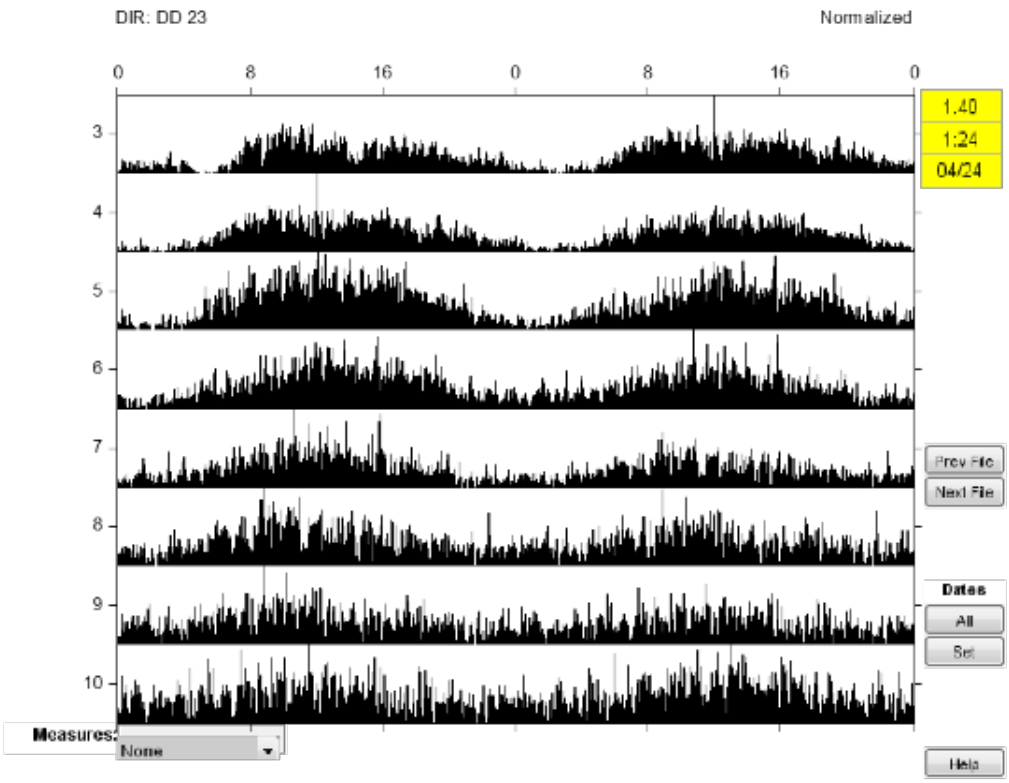

Figure 2.22. Actogram of groups of male and female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. X axis displays days monitored (3-10 = 7 days), Y axis displays hours. Second 0 on top axis denotes the start of activity of the following day, this is then repeated on the line below starting at the first 0. Produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period.

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

From the laboratory oviposition experiment (Figure 3.1) only one egg was laid in the hours of darkness over the 3 day assessment period. The majority of eggs were laid from 14:00 to 18:00. In the field based assessment (Figure 3.2) very few eggs were laid over night. For each of the three assessment days peaks of egg laying occurred in the morning and evening generally divided by a cessation in egg laying which occurred at peak temperature each day. The majority of egg laying occurred in periods where temperatures averaged between 25-29.9°C with the fewest eggs laid at 10-14.9°C (Figure 3.3). The majority of eggs were laid in periods with a humidity between 50-59.9% with fewest eggs laid between 90-95% (Figure 3.4). The interaction between humidity and temperature has not yet been analised.

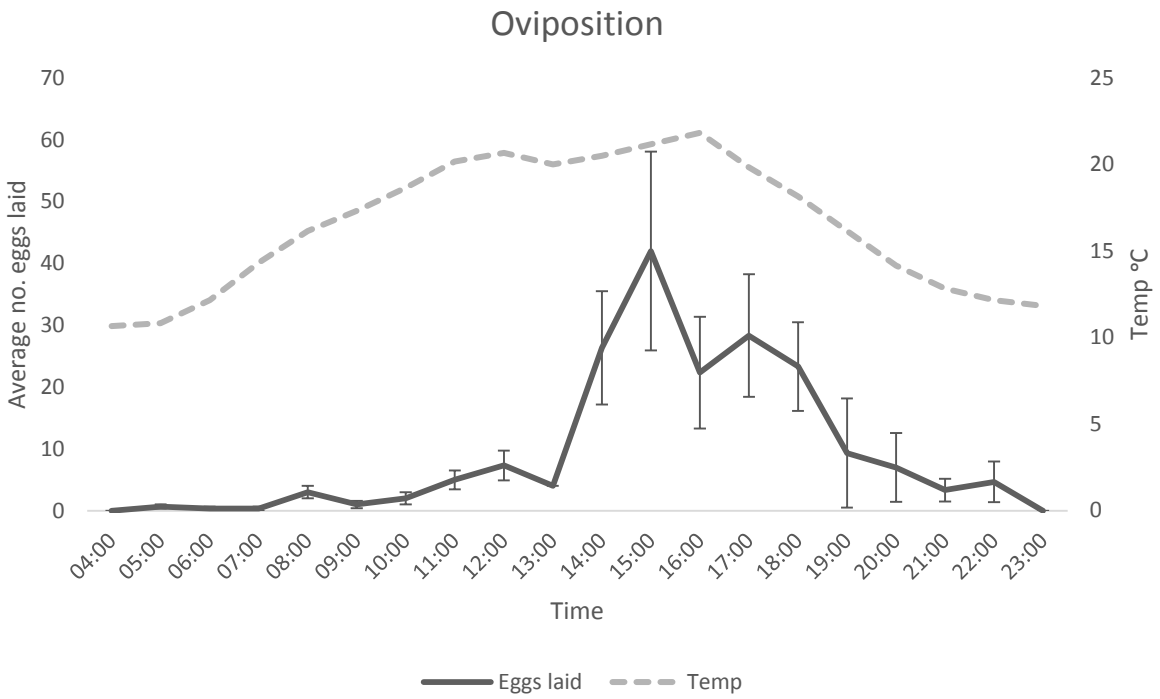

Figure 3.1. Laboratory based oviposition experiment. Average number of *D. suzukii* eggs laid per hour over a 3 day assessment period. Temperature indicated by dash line.

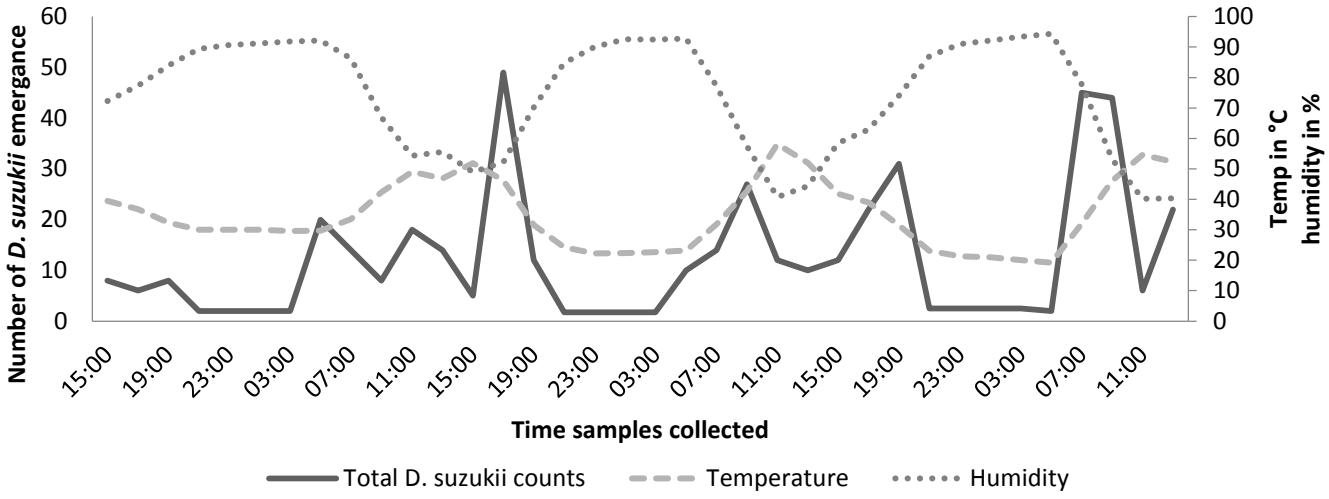

Figure 3.2. Total emergence of *D. suzukii* over a 3 day period from samples collected at two hour intervals. Temperature is indicated by dash line, humidity is indicated by dotted line.

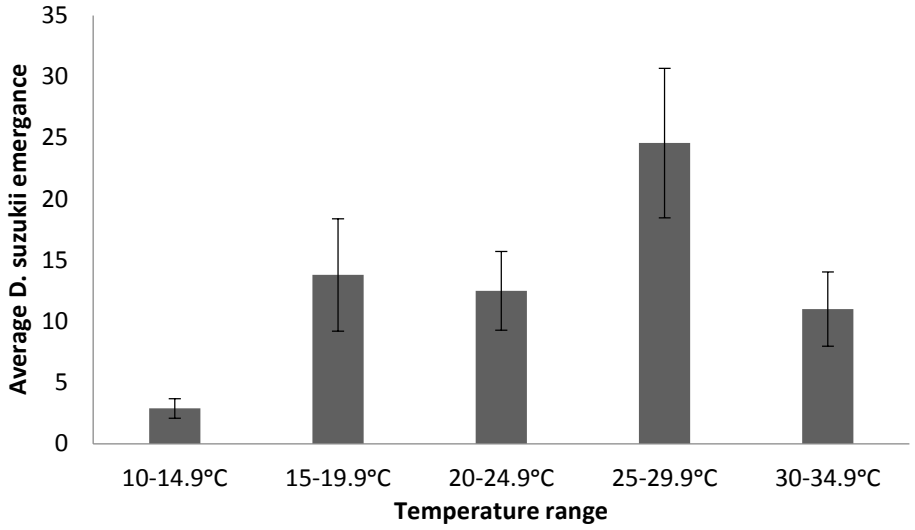

Figure 3.3. Average number of eggs laid in relation to temperature range. Error bars denote standard error

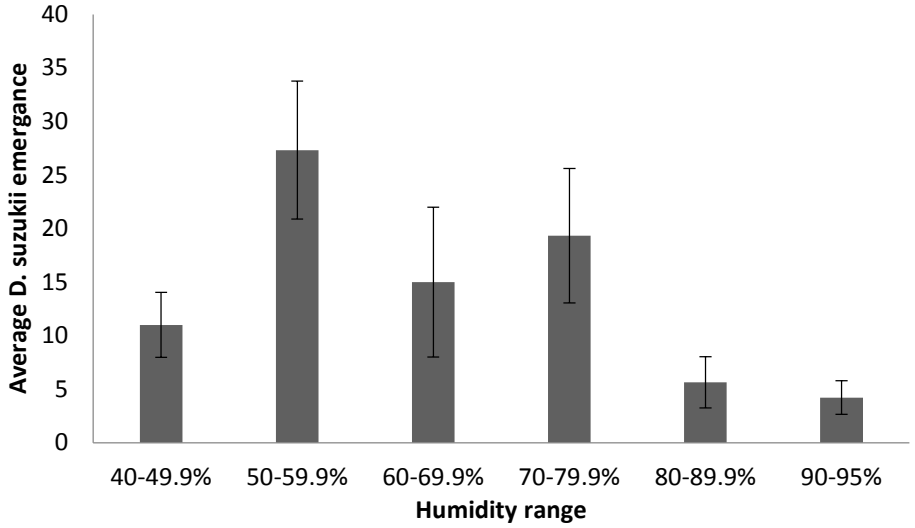

Figure 3.4. Average number of eggs laid in relation to humidity range. Error bars denote standard error

Discussion

In June and October condition locomotion assays the peaks of activity occurred when the lights were on, even though there was variation in 'daylight' time from 15 hr in June to 9.25 hr October. In both June and October conditions the period length was 24 hours suggesting that *D. suzukii* were able to regulate behaviour by entraining to the light cues. We would, therefore, expect *D. suzukii* display this behaviour under the daylength found in April (12.5 hr) and November activity (9.0 hr) conditions (to be tested). There were differences in the activity profiles produced for individual flies and mixed sex groups in both conditions assayed. The activity peak in groups that contained females (mixed sex groups and groups of females) generally followed the temperature profile. The individual male, female and groups of males displayed activity peaks at lights on and off with low but constant activity through the 'day'. However groups of females displayed higher activity at peak temperature. This was also obvious in the mixed sex groups. It could be that the groups containing females display these activity peaks as the increase in locomotion could be a result of searching for egg laying sites. If this is the case we would expect to see peaks of egg laying to occur at these times.

In a temperature only cycle with no lighting cues, all social situations displayed locomotion profiles that follow the temperature cycle. All situations also showed less activity in the 'night-time' when temperatures were lower. This means that although light was more dominant cue for entrainment, temperature was capable of entraining the circadian clock in *D. suzukii*.

When both light and temperature cues were removed, individual flies became arrhythmic within 48 hours. In group housed flies rhythmicity was maintained for 5 days in male groups and mixed sex groups, and 8 days in female groups. In other Drosophila species, other insects (Bloch *et al.*, 2013) and other organisms (Rajaratnam and Redman, 1998) it has been observed that social interactions are able to entrain individuals through volatile detection and it seems that *D. suzukii* are also able to use this as a cue.

Within these locomotion assays only mated females and males were used. Typically in circadian rhythm assays virgin flies are used to prevent egg laying and larval development within the cuvettes which may disrupt the results. However, 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. To keep situations as natural as possible mated females were used as it is the females that are causing damage by egg laying. 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 (Ferguson *et al.*, 2015). For these reason it was decided to use mated individuals only. It is possible that wild *D. suzukii* will behave differently to lab reared strains but it is difficult to catch live adult specimens for use in these assays. For this reason we accept that the activity level of wild verses lab populations may differ but the molecular clock and entrainment process should be genetically conserved across lab and wild strains.

In laboratory based oviposition assays under June conditions egg laying occurred in the daytime with no eggs being laid at night. The peak of egg laying occurred at peak temperature and gradually decreased through the afternoon until dusk. This supported the theory that the increase in locomotion in the mixed sex groups and the female groups at peak temperature could be females looking for egg laying sites. Egg laying in April and October conditions will also be investigated in both the lab and the field in future experiments. Several oviposition studies of *D. suzukii* have been published in recent years all with the aim of identifying the ideal conditions under which maximum egg laying occurs. As many researchers have realised once the conditions of egg laying has been identified growers can be informed when crops will be vulnerable. The problem with these studies is they are either focused on the range of conditions such as extreme heat or cold (Kinjo *et al.*, 2014; Ryan *et al.*, 2016) and did not focus on the rhythm of egg laying. Lin *et al.* (2014) investigated the rhythm of egg laying but did not use realistic temperature or light cycles. As with the locomotion results, as we predict oviposition to be under circadian regulation, variations in these environmental conditions would result in differences in times of egg laying.

Due to a mild winter and a warm spring the development of cherries was delayed by three weeks from the predicted dates for the oviposition field trial. The field trial was conducted from 02/08/16 to 05/08/16. As there was a delay in starting the assessments the temperature range was different to that predicted and the photo phase was shorter by two hours. Over the 72 hours of the assessment very few eggs were laid in the hours of darkness which supported the results of the laboratory experiment. It is possible that some egg laying did occur in the ‘night’ on bright, moon lit nights as there was not complete darkness. Peaks of egg laying occurred mid-morning and mid-evening. These peaks seem to sit either side the peak in locomotion activity. The average amount of egg laying in relation to temperature showed that the majority of eggs were laid between 25-29.9°C. The least amount of eggs were laid in the lower temperature range 10-14.9°C. The temperature involvement in egg laying should become clearer once it is investigated under October conditions. Although in the lab trial the peak in egg laying occurred at peak temperature (22°C), in the field the peak temperature was 34°C, 12°C above the predicted maximum temperature and that which was used in the lab In a paper by Ryan *et al.* (2016) the optimum temperature for maximum number of eggs per female per day was 22.9°C but as this was a constant temperature under a 16:8 L:D cycle

this experiment is looking at optimum conditions and not the circadian rhythm of egg laying and so cannot be compared.

When looking at the relationship between humidity and the average number of eggs laid, the majority occurred between 50-59.9% humidity. The least amount of eggs were laid between 90-95% relative humidity. These results do not support the finding of Tochen *et al.* (2016) who concluded that the highest net reproductive rate, highest intrinsic rate of population increase and the reproductive success of females were at their greatest above 82%. However in this paper they do not look at oviposition preferences and egg laying in relation to humidity and so cannot be exclusively compared.

Conclusions

From the initial results it is clear that light and temperature play key roles in the regulation of *D. suzukii* behavioural patterns. Locomotion and egg laying appear to be under circadian control and so behavioural pattern predictions should be possible. The ability of groups of males and females and mixed sex groups to entrain locomotion behaviour by social interaction will be investigated further by exploring the effects of social housing on *D. melanogaster*. *D. melanogaster* will be used as it has been the major model organism to investigate the circadian clock and could also display this change in behaviour under different social housing. If so it provides evidence that predictions relating to the rhythms of key behaviours such be performed on groups of flies and not individuals as has been the norm. With the oviposition assays, the laboratory and field trial results differed with peaks of egg laying occurring at different times. As the aim of the circadian clock is to entrain to environmental cues, the changes in temperature and light intensity will alter behavioural patterns from day to day. If we can understand the mechanisms that drive these patterns we can understand when they will occur. By understanding when key behaviours will occur we can provide growers with times to target specific actions of *D. suzukii* and will result in tailored pest control.

Knowledge and Technology Transfer

The student attended and presented a poster at:

- 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 student will attend the upcoming conference and provide a flash presentation and poster at:

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

Successful grant applications

- GCRI travel grant, £500.
- Worshipful company of fruiterers, £300

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Appendices

se	DONE						
Type	MSG	GM	GF	IM	IF	IM (pop)	IF (pop)
DD 23	10	10	10	29	17	NA	NA
April SM	0	0	0	13	7	NA	NA
April WM	8	0	0	15	12	NA	NA
April Field SM	18	0	0	14	33	NA	NA
April Field WM	0	0	0	0	0	NA	NA
June	10	10	10	67	67	4	4
June DD	12	10	10	74	66	NA	NA
June RL	0	0	0	0	0	NA	NA
June Light 23	10	10	10	30	30	NA	NA
June Field	0	0	0	0	0	NA	NA
October Field	0	0	0	0	0	NA	NA
October	10	10	10	156	137	NA	NA
Novemeber SM	UN	UN	UN	UN	UN	NA	NA
Novemeber WM	0	0	0	0	0	NA	NA
Novemeber Field SM	0	0	0	0	0	NA	NA
Novemeber Field WM	0	0	0	0	0	NA	NA

Figure 1.1 Number of performed and outstanding assays for locomotion in both the field and in the laboratory. UN = Collected by not yet analysed. WM = winter morphs to be used in assay. SM = summer morphs to be used in assay. DD = constant darkness. RL = red light. Light = 23 June light cycle under constant 23 degrees. Field = field based assay or semi field depending on weather conditions.

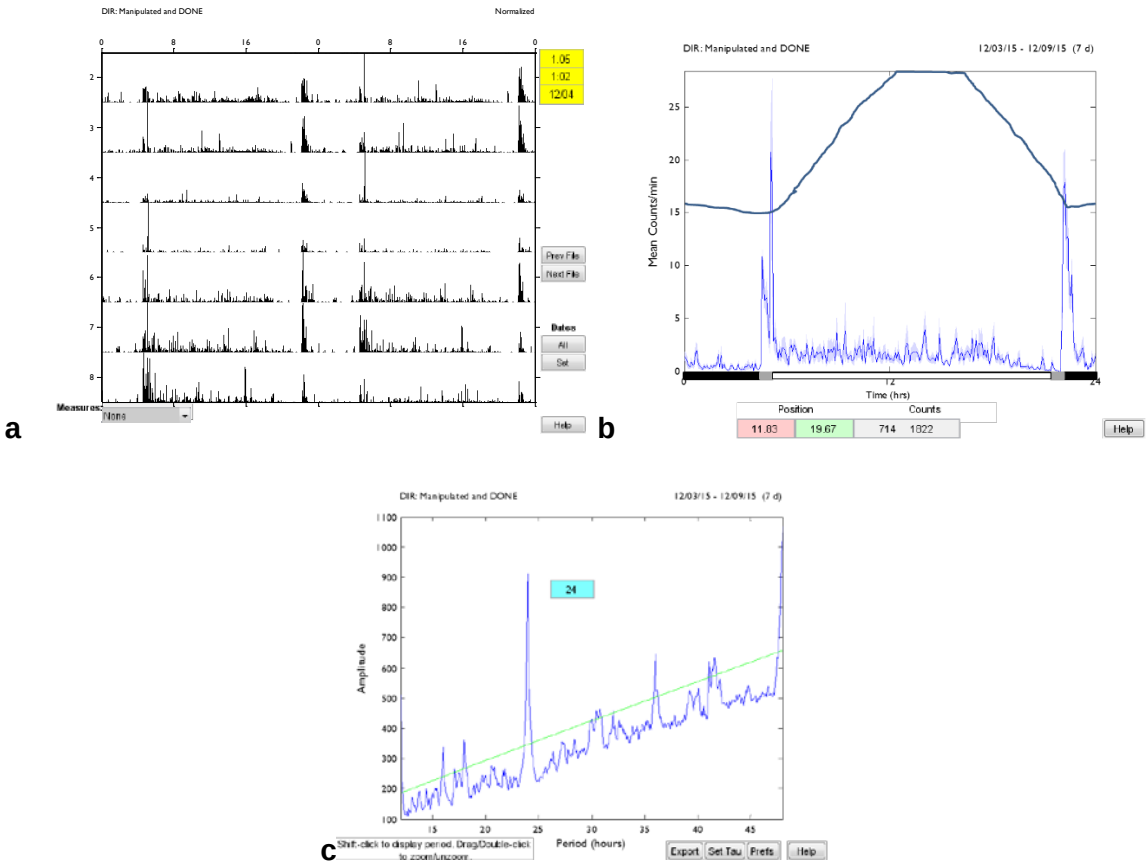

Figure 1.2a. Actogram of male *D. suzukii* activity over a 7 day period under June environmental conditions. 1.2b Activity profile for male *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.2c Chi-squared periodogram indicated average period length. 1.2a-c all produced by batch analysing 60 individual males that survived the whole assessment period.

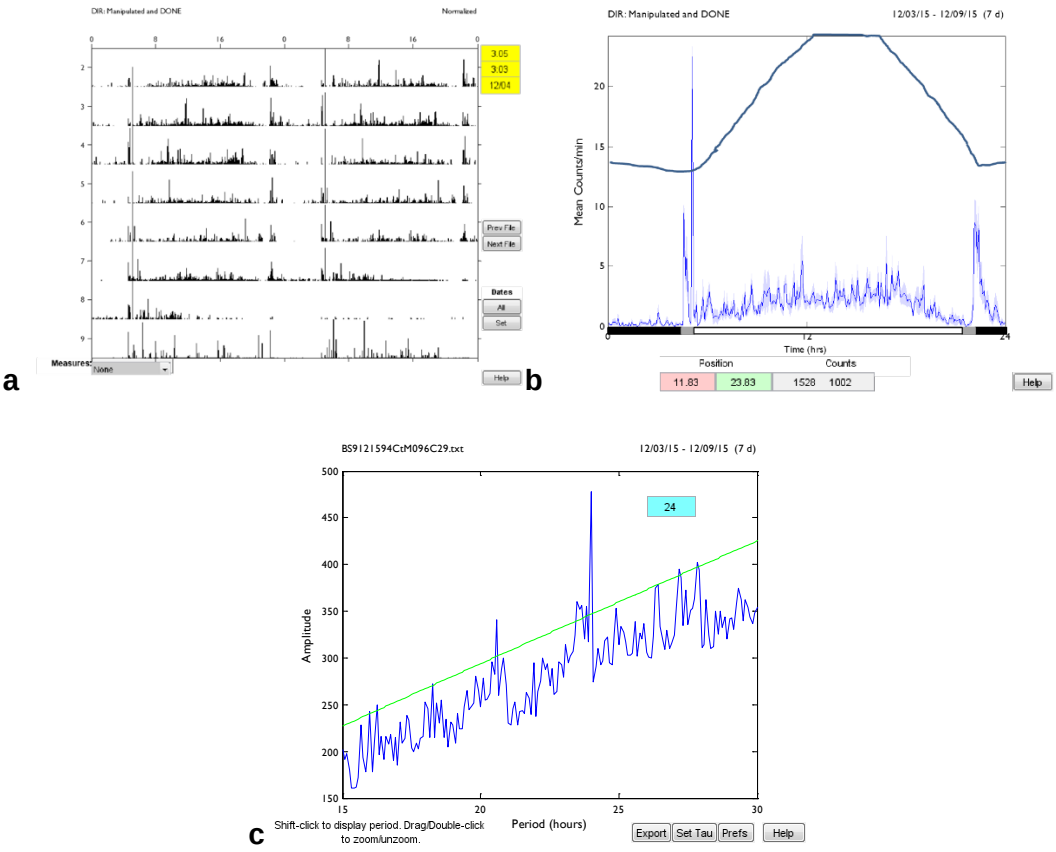

Figure 1.3a. Actogram of female *D. suzukii* activity over a 7 day period under June environmental conditions. 1.3b Activity profile for female *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.3c Chi-squared periodogram indicated average period length. 1.3a-c all produced by batch analysing 60 individual females that survived the whole assessment period.

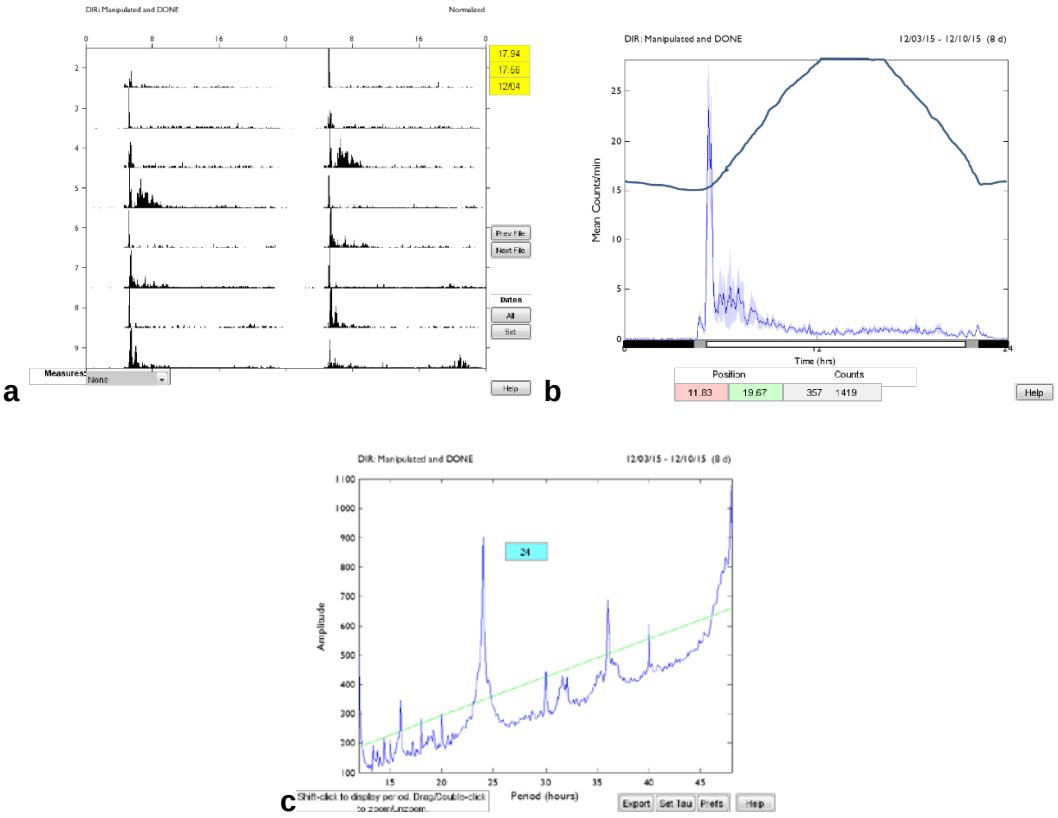

Figure 1.4a. Actogram of single sex groups of male *D. suzukii* activity over a 7 day period under June environmental conditions. 1.4b Activity profile for groups of male *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.4c Chi-squared periodogram indicated average period length. 1.4a-c all produced by batch analysing 10 groups of 10 males that survived the whole assessment period.

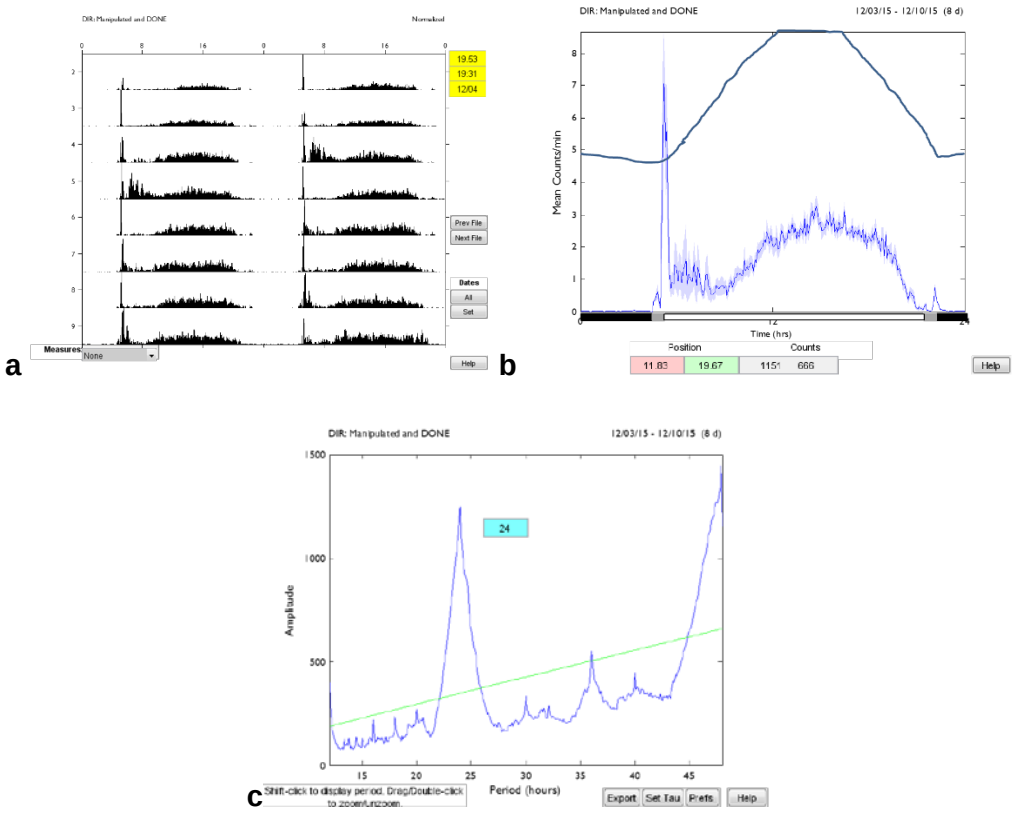

Figure 1.5a. Actogram of single sex groups of female *D. suzukii* activity over a 7 day period under June environmental conditions. 1.5b Activity profile for groups of female *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.5c Chi-squared periodogram indicated average period length. 1.5a-c all produced by batch analysing 10 groups of 10 females that survived the whole assessment period.

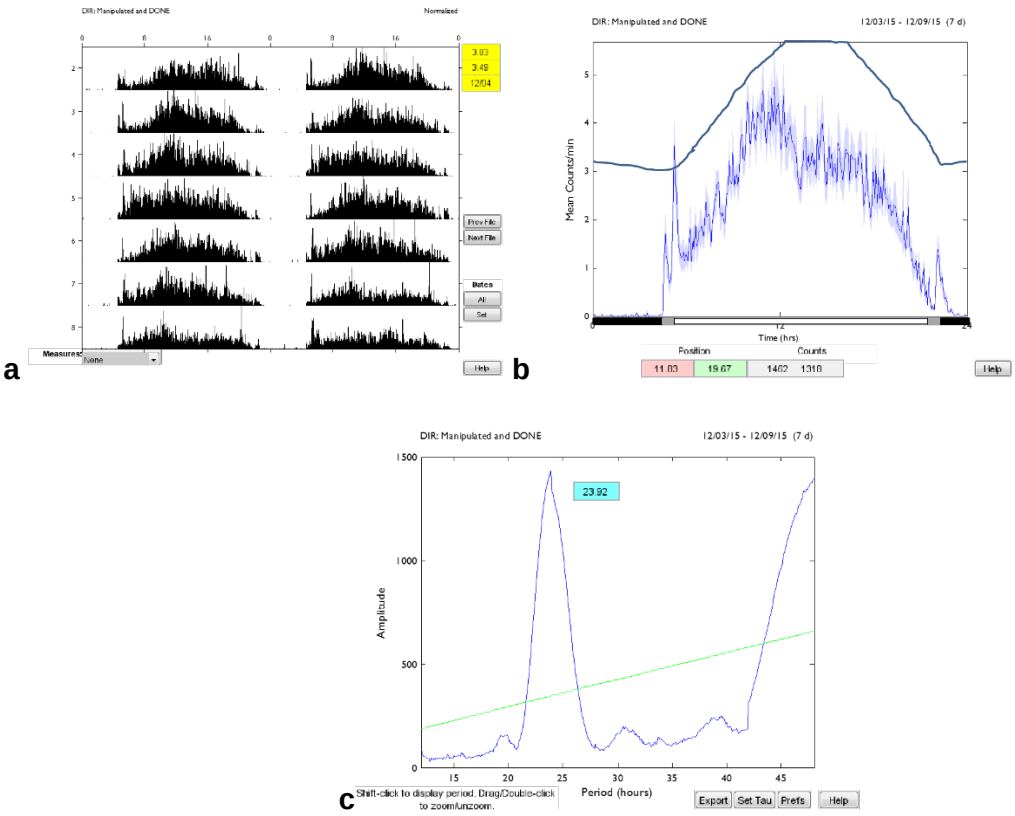

Figure 1.6a. Actogram of mix sex groups of male and female *D. suzukii* activity over a 7 day period under June environmental conditions. 1.6b Activity profile for mix sex groups of *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.6c Chi-squared periodogram indicated average period length. 1.6a-c all produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period.

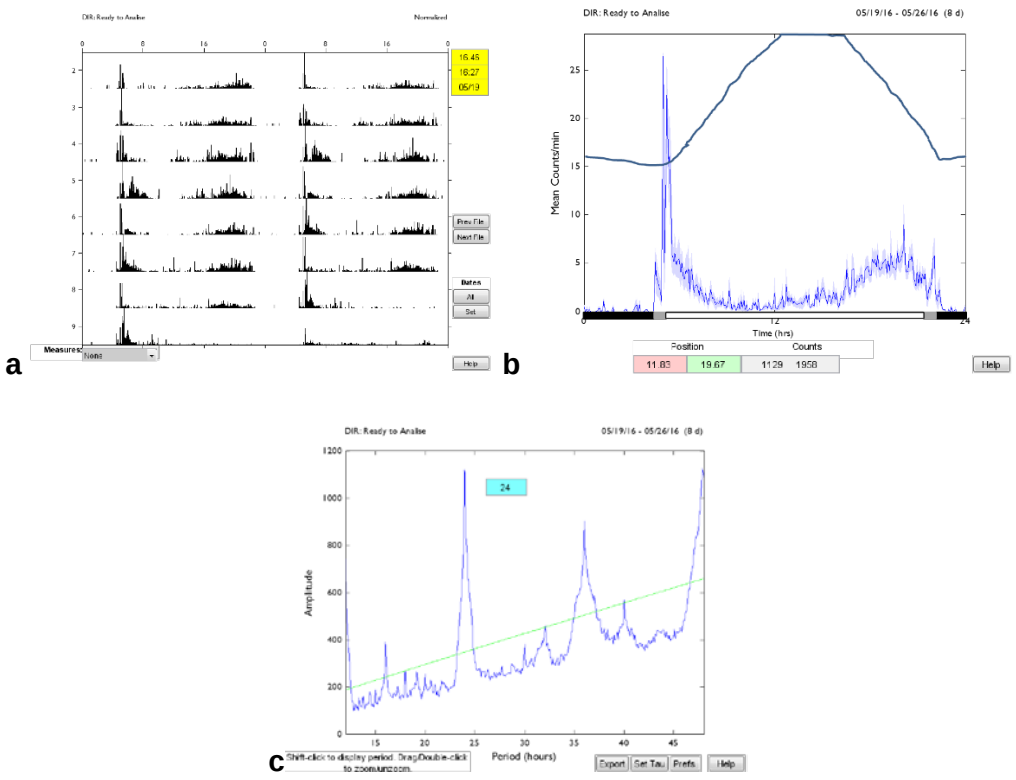

Figure 1.7a. Actogram of individual male *D. suzukii* activity within a population monitor over a 7 day period under June environmental conditions. 1.7b Activity profile for individual male *D. suzukii* within a population monitor with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.7c Chi-squared periodogram indicated average period length. 1.7a-c all produced by batch analysing 10 individual males that survived the whole assessment period.

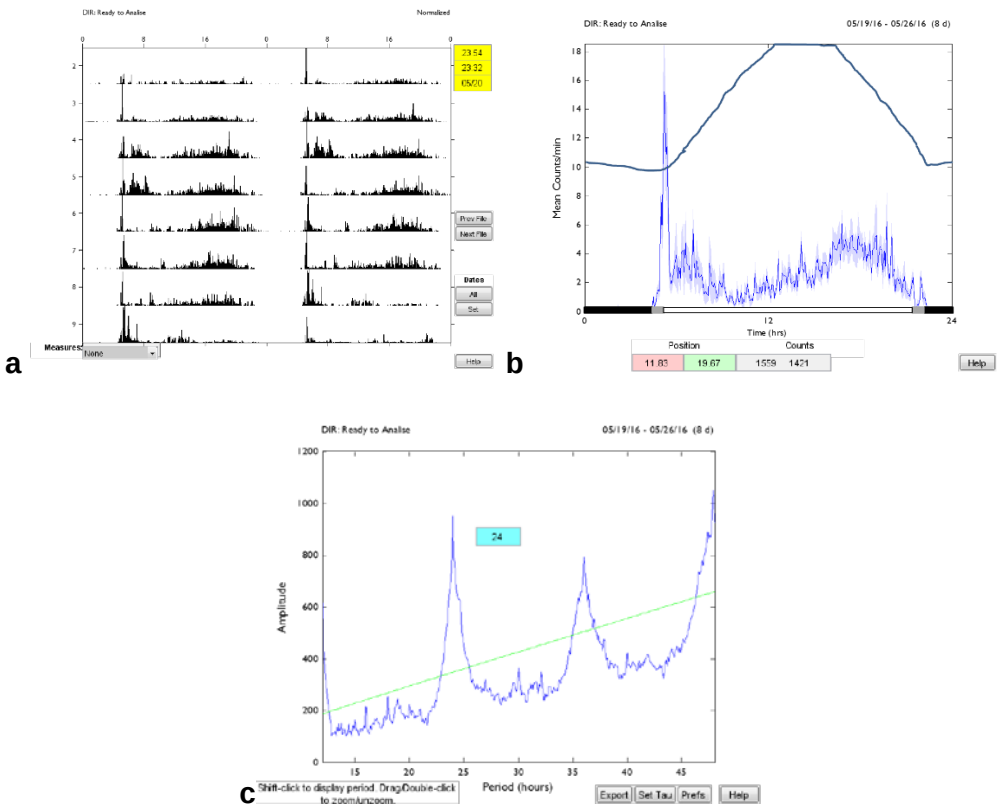

Figure 1.8a. Actogram of individual female *D. suzukii* activity within a population monitor over a 7 day period under June environmental conditions. 1.8b Activity profile for individual female *D. suzukii* within a population monitor with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.8c Chi-squared periodogram indicated average period length. 1.8a-c all produced by batch analysing 10 individual females that survived the whole assessment period.

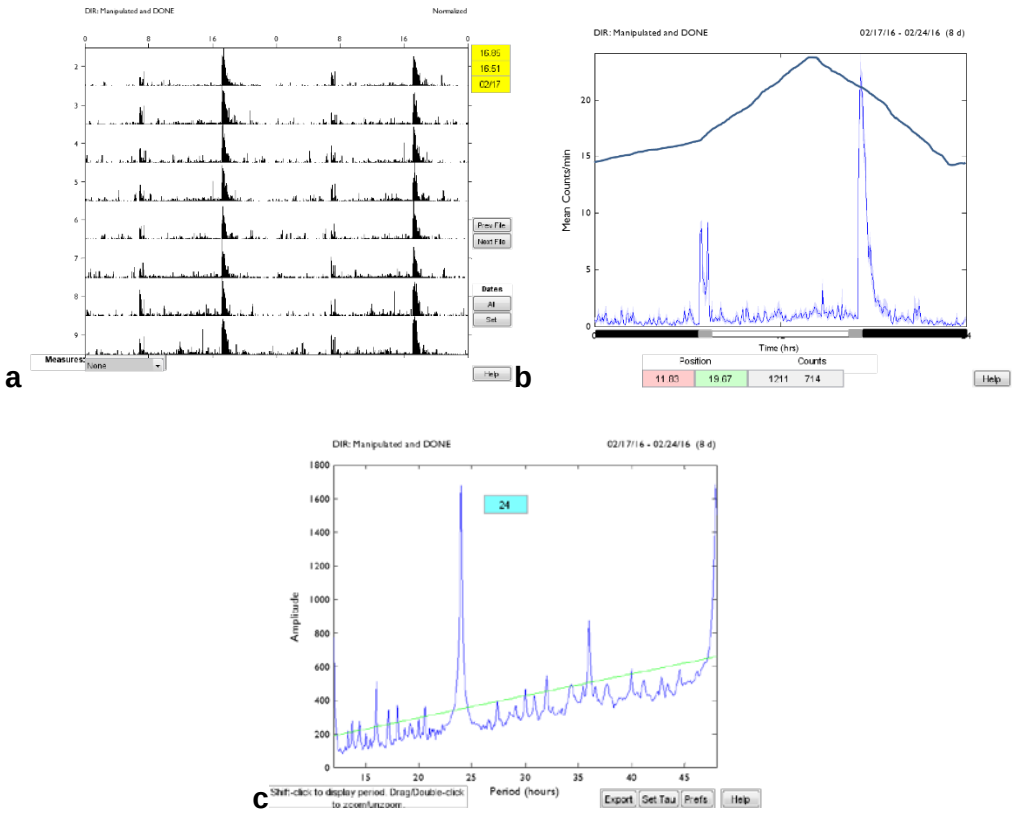

Figure 1.9a. Actogram of male *D. suzukii* activity over a 7 day period under October environmental conditions. 1.9b Activity profile for male *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.9c Chi-squared periodogram indicated average period length. 1.9 a-c all produced by batch analysing 60 individual males that survived the whole assessment period.

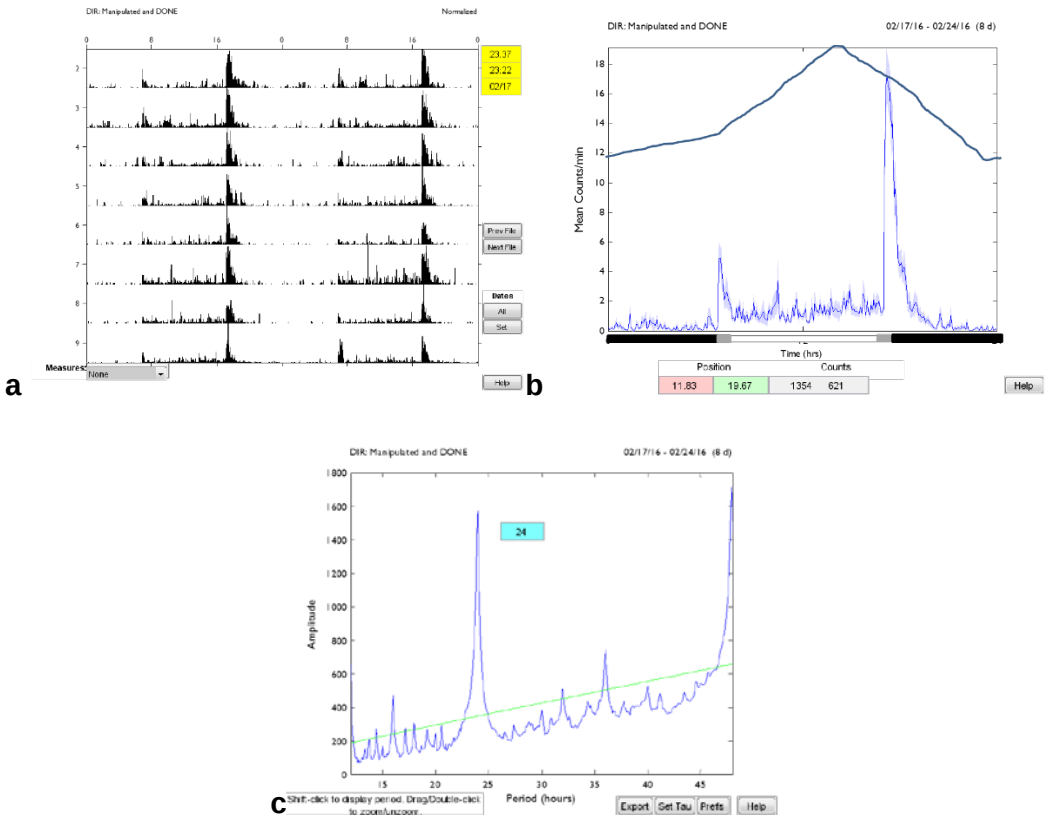

Figure 1.10a. Actogram of female *D. suzukii* activity over a 7 day period under October environmental conditions. 1.10b Activity profile for female *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.10c Chi-squared periodogram indicated average period length. 1.10 a-c all produced by batch analysing 60 individual females that survived the whole assessment period.

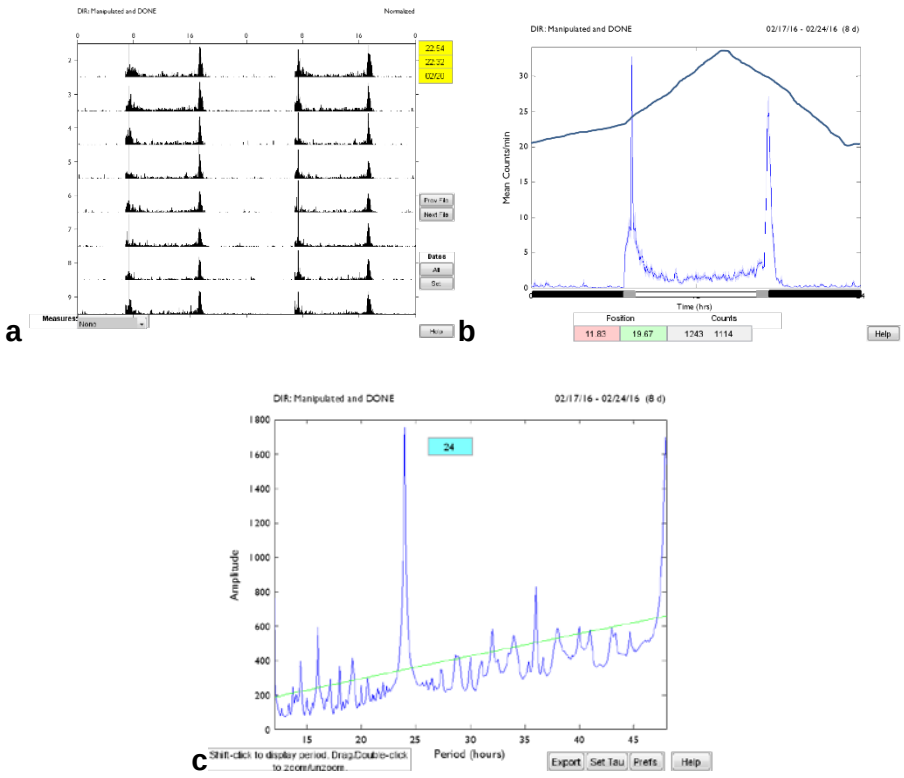

Figure 1.11a. Actogram of groups of male *D. suzukii* activity over a 7 day period under October environmental conditions. 1.11b Activity profile for groups of male *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.11c Chi-squared periodogram indicated average period length. 1.11 a-c all produced by batch analysing 10 groups of 10 males that survived the whole assessment period.

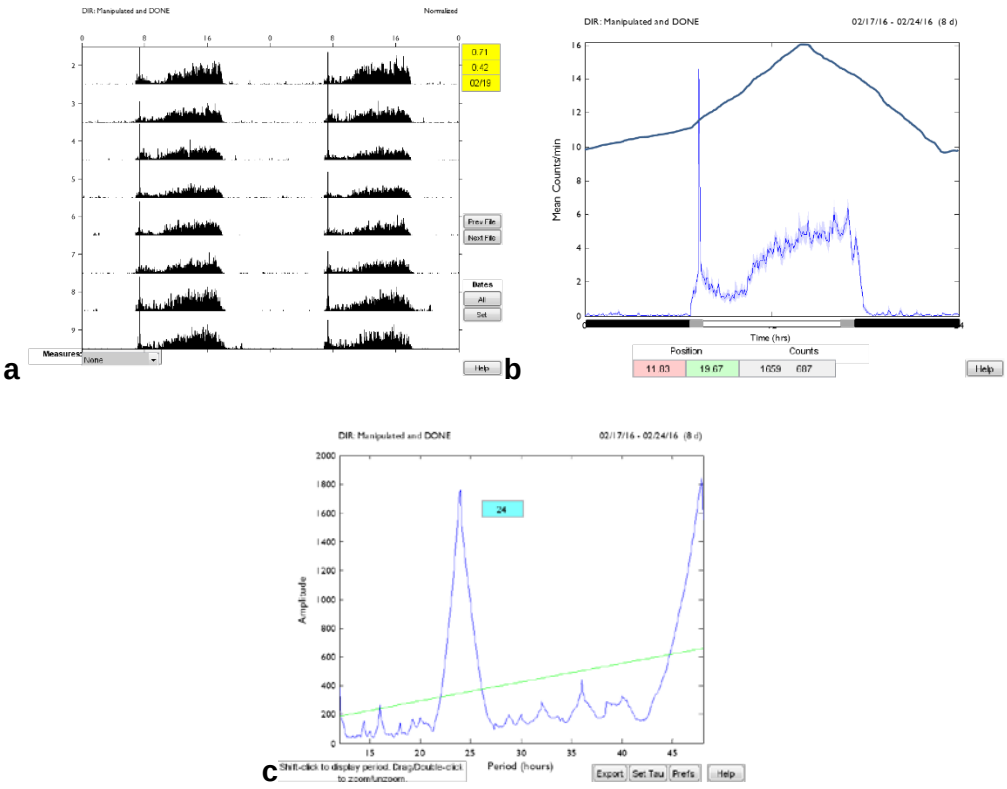

Figure 1.12a. Actogram of groups of female *D. suzukii* activity over a 7 day period under October environmental conditions. 1.12b Activity profile for groups of female *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.12c Chi-squared periodogram indicated average period length. 1.12 a-c all produced by batch analysing 10 groups of 10 females that survived the whole assessment period.

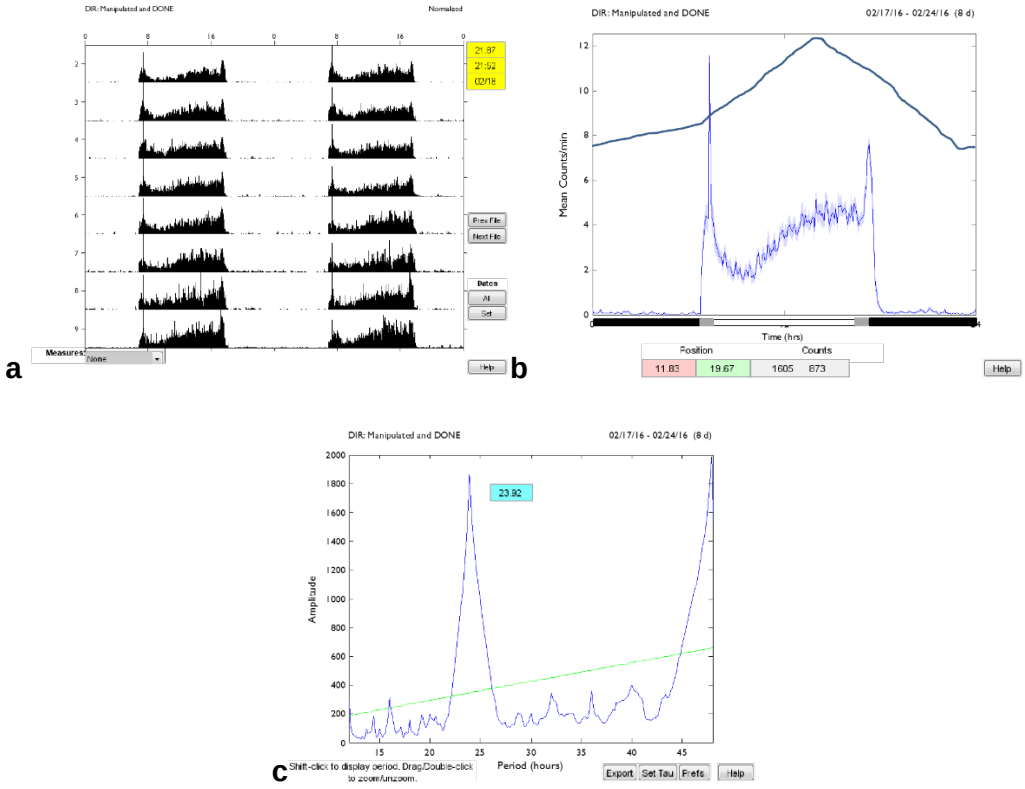

Figure 1.13a. Actogram of mix sex groups of male and female *D. suzukii* activity over a 7 day period under October environmental conditions. 1.13b Activity profile for mix sex groups of female *D. suzukii* with temperature cycle indicated by blue line at top of figure. Light profile is indicated by black grey and white bar beneath figure. 1.13c Chi-squared periodogram indicated average period length. 1.13 a-c all produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period.

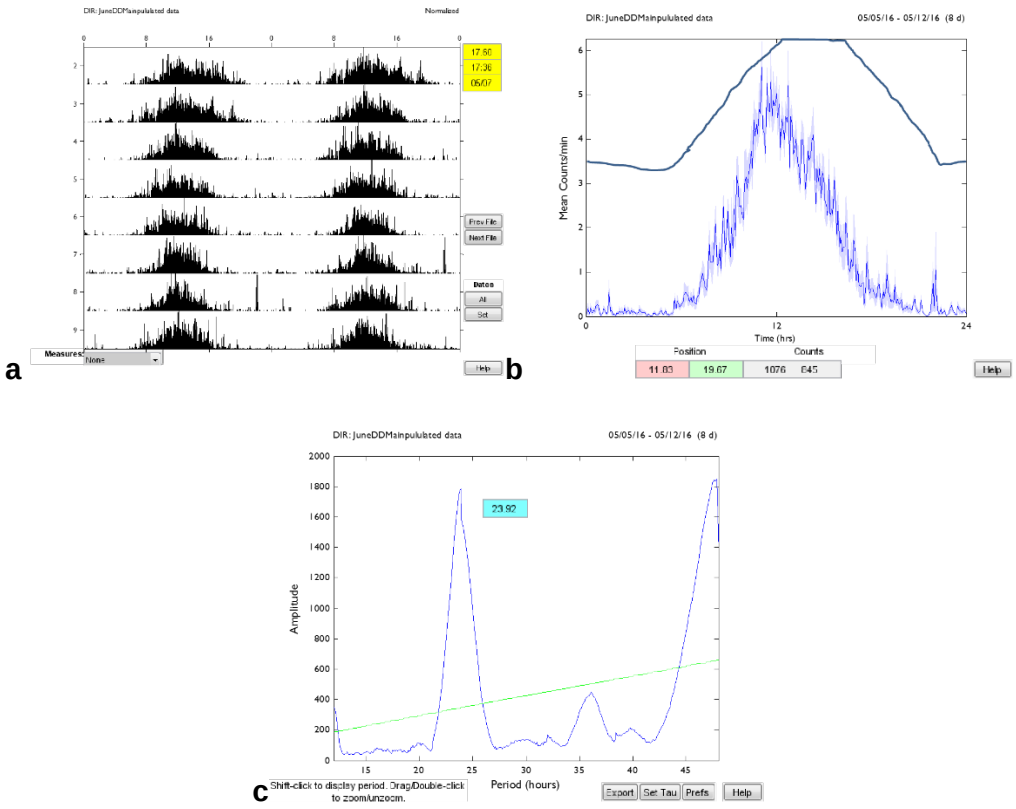

Figure 1.14a. Actogram of male *D. suzukii* activity over a 7 day period under a June temperature cycle in constant darkness. 1.14b Activity profile for male *D. suzukii* with temperature cycle indicated by blue line at top of figure. 1.14c Chi-squared periodogram indicated average period length. 1.14a-c all produced by batch analysing 75 individual males that survived the whole assessment period.

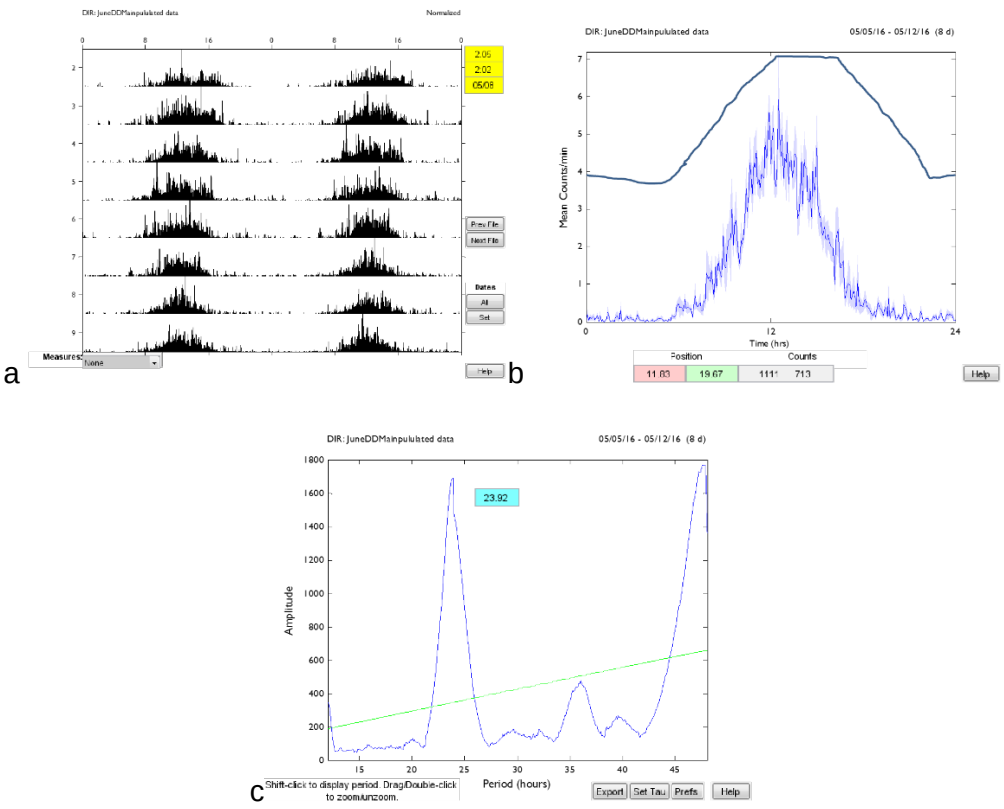

Figure 1.15a. Actogram of male *D. suzukii* activity over a 7 day period under a June temperature cycle in constant darkness. 1.15b Activity profile for male *D. suzukii* with temperature cycle indicated by blue line at top of figure. 1.15c Chi-squared periodogram indicated average period length. 1.15a-c all produced by batch analysing 65 individual females that survived the whole assessment period.

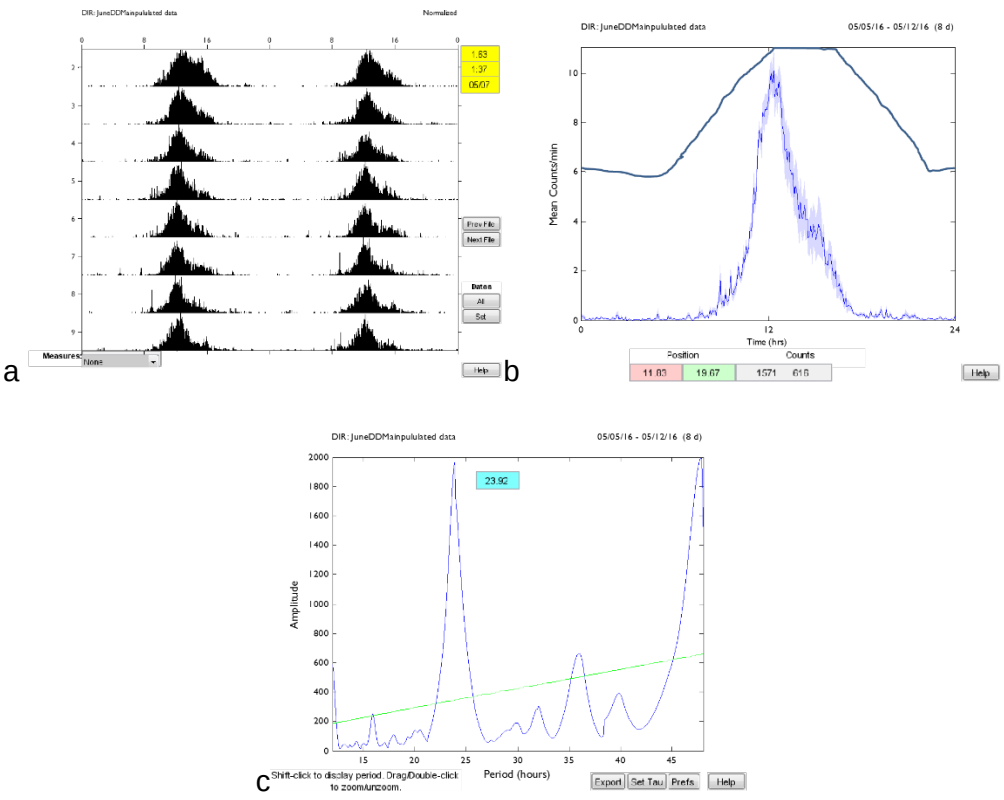

Figure 1.16a. Actogram of groups of male *D. suzukii* activity over a 7 day period under a June temperature cycle in constant darkness. 1.16b Activity profile for groups of male *D. suzukii* with temperature cycle indicated by blue line at top of figure. 1.16c Chi-squared periodogram indicated average period length. 1.16a-c all produced by batch analysing 10 groups of 10 males that survived the whole assessment period.

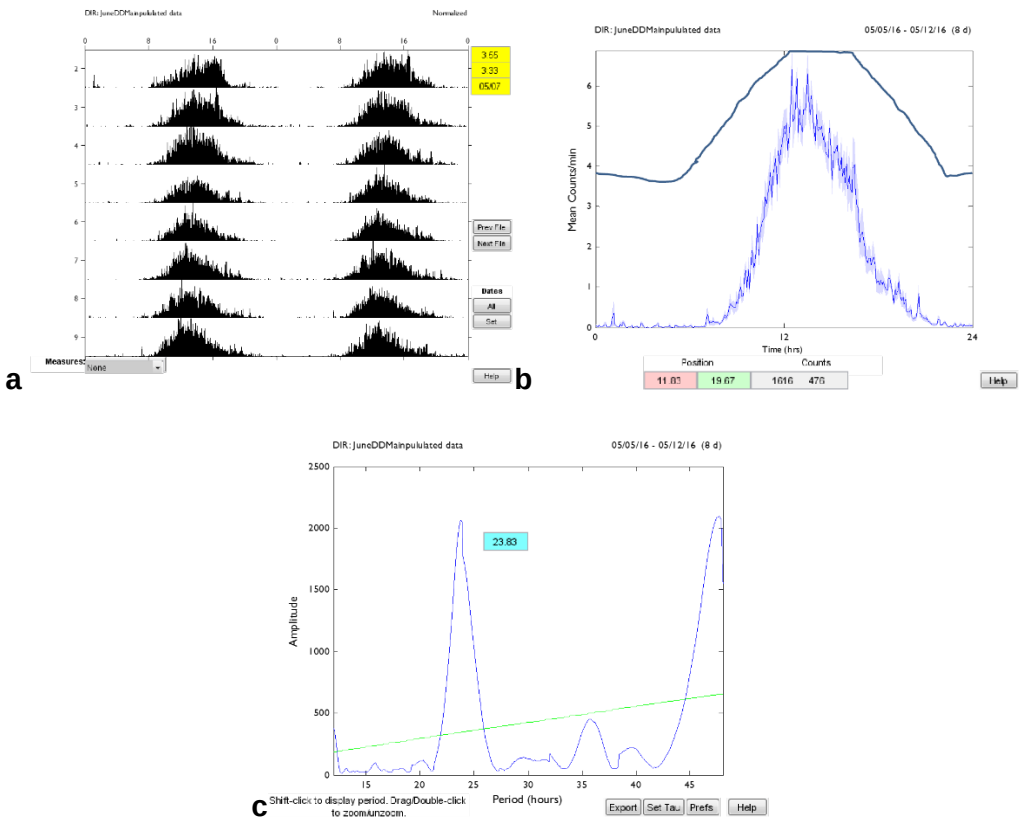

Figure 1.17a. Actogram of groups of female *D. suzukii* activity over a 7 day period under a June temperature cycle in constant darkness. 1.17b Activity profile for groups of female *D. suzukii* with temperature cycle indicated by blue line at top of figure. 1.17c Chi-squared periodogram indicated average period length. 1.17a-c all produced by batch analysing 10 groups of 10 females that survived the whole assessment period.

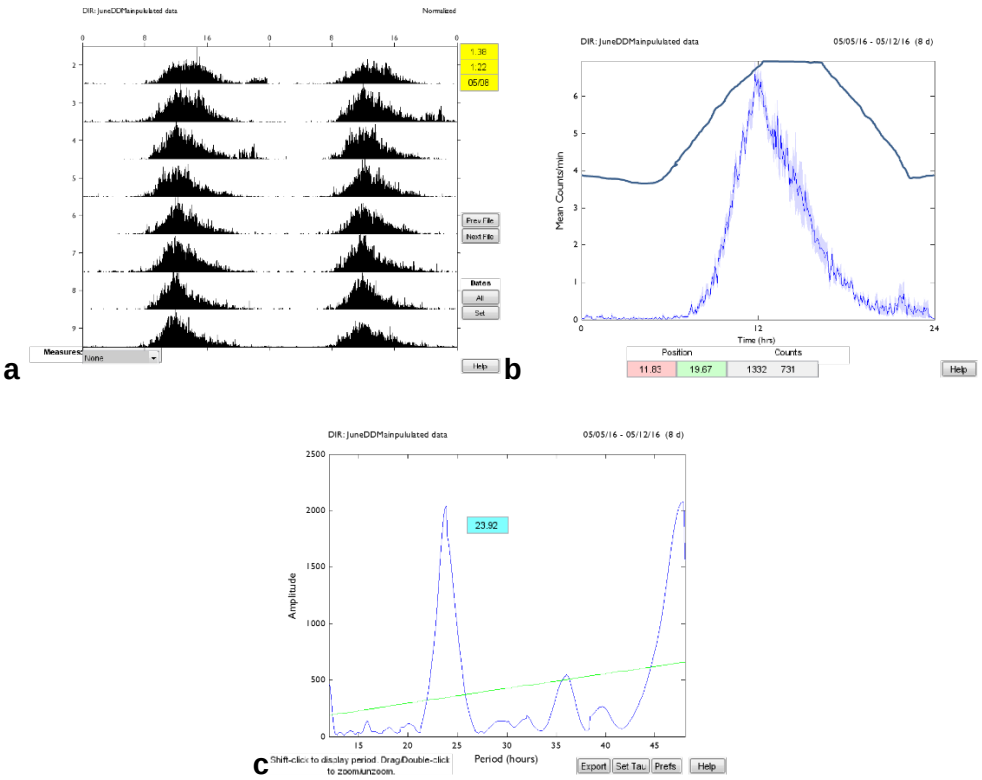

Figure 1.18a. Actogram of mix sex groups of male and female *D. suzukii* activity over a 7 day period under a June temperature cycle in constant darkness. 1.18b Activity profile for mix sex groups of male and female *D. suzukii* with temperature cycle indicated by blue line at top of figure. 1.18c Chi-squared periodogram indicated average period length. 1.18a-c all produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period.

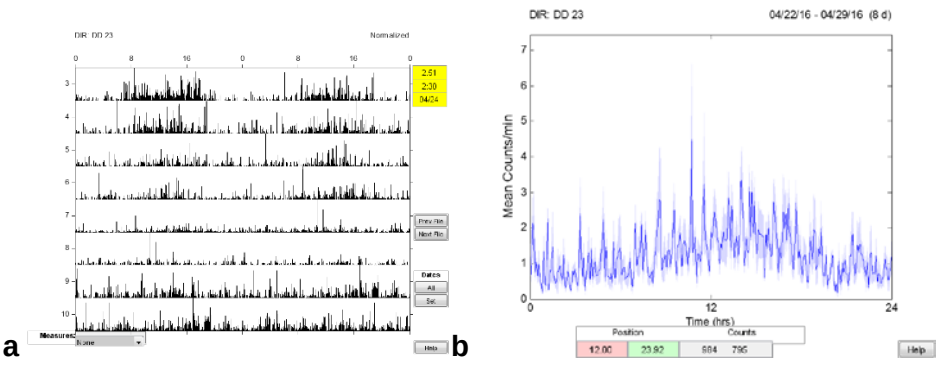

Figure 1.19a. Actogram of individual male *D. suzukii* activity over a 7 day period under 23°C in constant darkness. 1.19b Activity profile for male *D. suzukii* under 23°C in constant darkness. 1.19a and b, produced by batch analysing 29 individual males that survived the whole assessment period.

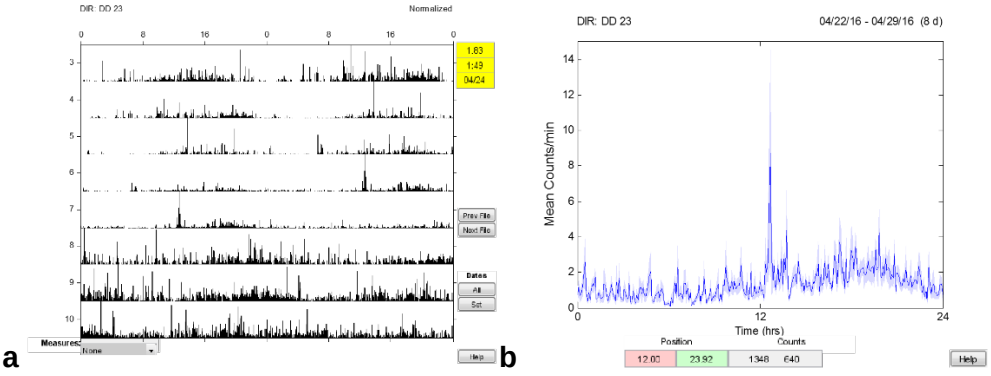

Figure 1.20a. Actogram of individual female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. 1.20b Activity profile for female *D. suzukii* under 23°C in constant darkness. 1.20a and b, produced by batch analysing 17 individual females that survived the whole assessment period.

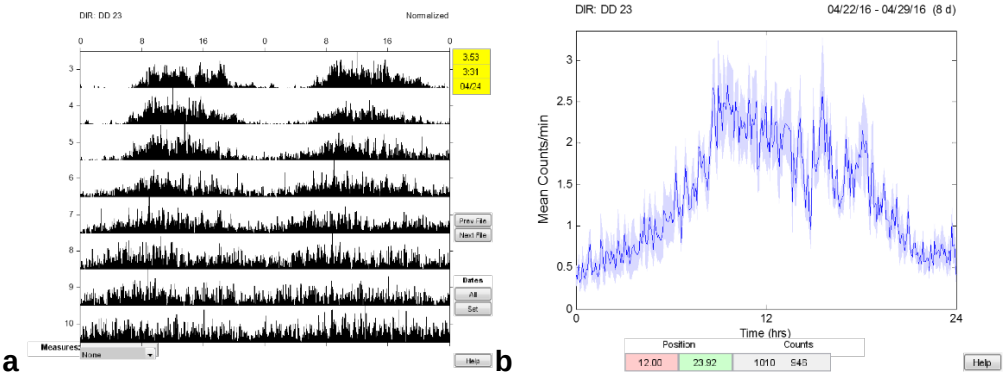

Figure 1.21a. Actogram of groups of male *D. suzukii* activity over a 7 day period under 23°C in constant darkness. 1.21b Activity profile for groups of male *D. suzukii* under 23°C in constant darkness. 1.21a and b, produced by batch analysing 10 groups of 10 males that survived the whole assessment period.

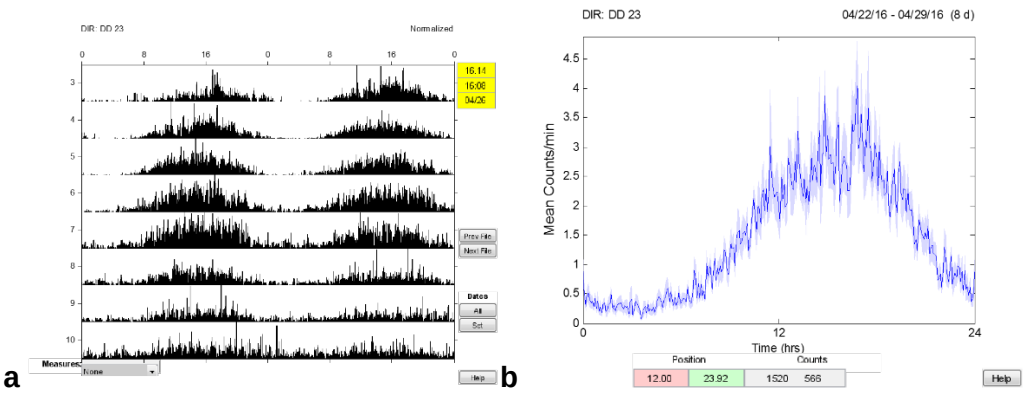

Figure 1.22a. Actogram of groups of female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. 1.22b Activity profile for groups of female *D. suzukii* under 23°C in constant darkness. 1.22a and b, produced by batch analysing 10 groups of 10 females that survived the whole assessment period.

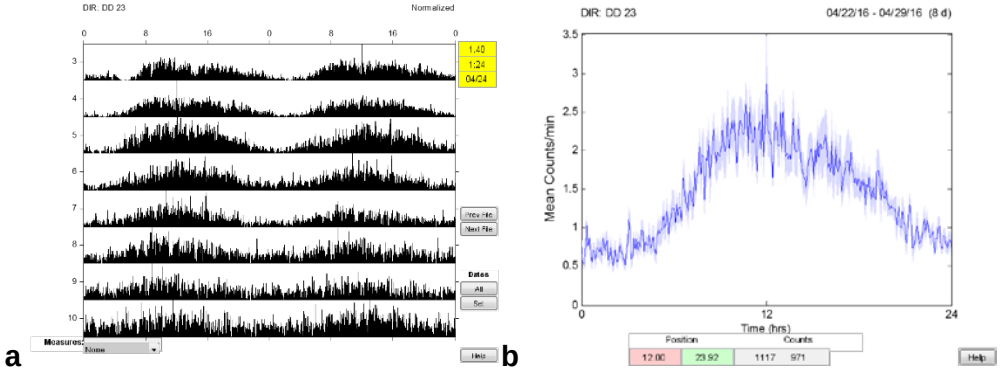

Figure 1.23a. Actogram of mix sex groups of male and female *D. suzukii* activity over a 7 day period under 23°C in constant darkness. 1.23b Activity profile for mix sex groups of male and female *D. suzukii* under 23°C in constant darkness. 1.23a, b, produced by batch analysing 10 groups of 10 males and 10 females that survived the whole assessment period.

