Control of daily locomotor activity patterns in *Drosophila suzukii* by the circadian clock, light, temperature and social interactions

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Control of daily locomotor activity patterns in *Drosophila suzukii* by the circadian clock, light, temperature and social interactions

Running title: Locomotion patterns of *Drosophila suzukii*

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Key words: semi-field; diurnal rhythm; spotted wing *Drosophila*; seasonal morph

Abstract

Understanding behavioural rhythms in a pest species can contribute to improving the efficacy of control methods targeting that pest. However, in some species the behavioural patterns recorded in artificial conditions contrast greatly with observed 'wild-type' behavioural rhythms. In this study we identify the determinants of daily activity rhythms of the soft and stone fruit pest *Drosophila suzukii*. The impact of gender, space, social housing, temperature, light, fly morph and the circadian clock on *D. suzukii* locomotor rhythms were investigated. Assays were performed under artificial laboratory conditions or more natural semi-field conditions to identify how these factors impacted daily locomotor behaviour.

Daily locomotor activity patterns collected under semi-field conditions varied very little between the various sex and social condition combinations. However, in lab-based assays, individual and group-housed males often exhibited divergent activity patterns with more prominent hyperactivity at light/dark transitions. In contrast, hyperactivity responses were suppressed under lab protocols mimicking summer conditions for groups of females and mixed sex groups. Moreover, when environmental cues were removed, flies held in groups displayed stronger rhythmicity than individual flies. Thus, social interactions can reinforce circadian behaviour and resist hyperactivity responses in *D. suzukii*. Fly morph appeared to have little impact on behavioural pattern, with winter and summer morph flies displaying similar activity profiles under 'April' semi-field and laboratory mimic environmental conditions.

In conclusion, separate and combined impacts of light, temperature, circadian clock function and social interactions were apparent in the daily activity profiles of *D. suzukii*. When groups of female or mixed sex flies were used, implementation of matching photoperiods and realistic daily temperature gradients in the lab was sufficient to recreate behavioural patterns observed in summer semi-field settings. The ability to leverage lab assays to predict *D. suzukii* field behaviour promises to be a valuable asset in improving control measures for this pest.

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Introduction

Behavioural and physiological processes are regulated in a wide range of organisms by an internal pacemaker: the circadian clock (Allada and Chung, 2010). Light and temperature, along with other biotic and abiotic factors, entrain the clock which is vital in synchronising organisms to their environment (Dubruille and Emery, 2008). Observations of model organisms, within a laboratory setting, are often made under simplified environmental conditions. While this approach has expanded knowledge and provided an invaluable understanding of the circadian clock (Young, 2018) there is a need for complementary studies involving more realistic environmental conditions. Behavioural and physiological rhythms in a natural setting remain relatively unexplored for many species, but their analysis could provide valuable insights into the most relevant phenotypic outcomes of daily timekeeping and highlight opportunities to exploit such rhythms in pest species (Miyata, 2011).

Drosophila suzukii (Matsumura) is a global pest of soft-, stone- and ornamental-fruit and has dispersed from its native range in Japan, aided by the importing and exporting of contaminated fruit (Cini et al., 2014). Female *D. suzukii* are equipped with serrated oviscapts which enable them to cut into the epicarp of ripening, otherwise healthy soft fruits to deposit eggs (Atallah et al., 2014). After hatching, the larvae consume the fruit from within which causes collapse resulting in yield and economic losses (Walsh et al., 2011, Lee et al., 2011), and a wide range of Integrated Pest Management (IPM) options are emerging to tackle this pest (Landolt et al., 2012, Cha et al., 2014, Cha et al., 2017, Haye et al., 2016, Gabarra et al., 2015, Wiman et al., 2016). A greater understanding of behavioural patterns in *D. suzukii* would enable specific targeting of rhythms which could result in an increased efficacy of these methods and higher level of control (Ferguson et al., 2015).

It is not always possible to perform behavioural assays under natural conditions as limiting factors, such as providing reliable power sources, often restrict the ability to monitor specimens in a remote, semi-field

location. This has driven groups to investigate behavioural patterns within laboratory settings, instead; often using simplified environmental cycles (i.e., 12:12 L:D, 23°C) or constant environmental conditions for convenience and ease of interpretation (Lin et al., 2014). However, successful field and semi-field observations of *Drosophila* have highlighted the large variation in behavioural responses between laboratory and more natural settings (Green et al., 2015, Vanin et al., 2012, De et al., 2013, Menegazzi et al., 2012, Prabhakaran et al., 2013). Mammals also exhibit discrepancies between the two settings with some species switching from diurnal to nocturnal when housed within a laboratory (Daan et al., 2011, Gattermann et al., 2008). More realistic environmental cycles can be introduced in the laboratory to reduce the divergence between natural and artificial conditions and has been explored in *Drosophila* diapause (Nagy et al., 2018) and, to a limited extent, locomotion (Hamby et al., 2013) For observations of oviposition (Shaw et al., 2018b) and emergence (Kannan et al., 2012) the success of laboratory-based simulation of semi-natural behavioural profiles was addressed directly. Such an investigation of the parameters needed to reproduce semi-natural behaviour in the laboratory has, to date, not yet been conducted for the locomotor behaviour of *D. suzukii*. Thus, the aim of this research was to identify the determinants of daily rhythms of *D. suzukii* locomotor activity that may help predict field behaviour. In the experiments presented here, the impact on *D. suzukii* locomotor rhythms was assessed for gender, space, social housing, temperature, light and the circadian clock. Fly morph was also a parameter. Within wild populations, winter morph *D. suzukii* are found through the colder months once temperatures drop below 10°C. Winter morph flies are better able to withstand cooler temperatures and have a longer life span than summer morph flies when exposed to low temperatures (Dalton et al., 2011, Jakobs et al., 2015, Kacar et al., 2016, Plantamp et al., 2016, Ryan et al., 2016). To test whether there were quantitative or qualitative differences in daily activity patterns between winter or summer morph flies, a limited number of comparative analyses including winter morph flies was run. All of these factors were assessed in a controlled manner in the laboratory, while the impact of gender and social housing setting was also addressed at semi-field conditions sampled at four different

times of year. Understanding how environmental factors affect key behaviours in *D. suzukii* could enable us to target specific behaviours and improve pest control measures.

Materials and Methods

1.1 *Drosophila* culturing

D. suzukii cultures were established at the University of Southampton from a wild strain collected in 2013, Trento, Italy. Populations of *D. suzukii* were housed in glass 25 x 95 mm Opticlear vials (Kimble Chase, Fisher Scientific Loughborough, UK) at 23°C in a 12:12 L:D cycle at a constant 65% relative humidity. Cultures were maintained on standard yeast cornmeal diet (6g agar, 48g table sugar, 73g yellow maize meal, 17g yeast, 10g soya flour, 46g malt extract, 0.2% propionic acid, 0.07% methyl-4-hydroxybenzoate, distilled H2O to 1 litre) and were transferred into new vials every week to promote egg laying as *D. suzukii* prefer less densely colonised oviposition sites (Mitsui et al., 2006). After the transferal of the flies, the vials were returned to the prior conditions to await the subsequent generation emergence. Once emergence began the vials were kept for a week before the offspring were transferred to new vials. At this point the existing vials were discarded. This ensured that overlapping aged populations were established. Cultures were kept genetically diverse by randomly mixing offspring between multiple populations to reduce inbreeding at each transferal. Summer morph flies were held within the conditions stated above. Winter morph flies were produced by transferring stage 3 larvae to the same diet within a 10°C incubator under constant darkness until a minimum of 200 adults of each sex emerged. Under these conditions, *D. suzukii* emerge as a larger and darker fly able to withstand cooler temperatures than the summer morph (Dalton et al., 2011, Shearer et al., 2016, Stephens et al., 2015, Toxopeus et al., 2016, Wallingford and Loeb, 2016). Prior to commencing the assays involving winter morph flies a sample of 10 females were removed from the culture and dissected to determine their reproductive state. All females were categorised as stage 1 using the scale proposed by Gerdeman and Tanigoshi (2012), corresponding to 'no distinguishable ovaries'. We would not expect a

switch to 'summer reproductive state' during the experiments as temperatures used in the behavioural assays with the winter morph flies (8-15°C) were within the range required to produce winter morph *D. suzukii* (Dalton et al., 2011, Hamby et al., 2016, Stockton et al., 2018, Wallingford et al., 2016) and winter morph females have been found to delay reproduction under comparable conditions (Zhai et al., 2016, Wallingford et al., 2016).

1.2 *Drosophila* activity monitoring

The TriKinetics *Drosophila* Activity Monitoring (DAM) (TriKinetics Inc, Waltham, MA, USA) system was used to record activity by analysing the number of infrared beam breaks in 5 minute bins (Pfeiffenberger et al., 2010). *D. suzukii* were taken from mixed sex cultures and were three to seven days old at the start of the assay and presumed mated. Flies were immobilised and held on a CO_2 pad before being transferred by the wing with soft forceps to either cuvettes (for individuals, 5 mm x 65 mm glass cuvettes; volume≈ 1.28 cm^3) or vials (for groups, 25 mm x 95 mm glass vials; volume≈ 44.8 cm^3). Tubes and vials were monitored within Trikinetics DAM2 and LAM25 monitors for narrow cuvettes and population vials, respectively. Both contained a basic sugar agar food (100% dH_2O, 1% agar, 5% table sugar, 0.07% Tegosept). Cuvettes and vials were filled with the sugar agar food up to a height of ~10 and ~30 mm, respectively, and left to set for 24 hours. Cuvettes were loaded with individual males or females. Population vials were loaded with either individual males or females, single sex groups of 10 males or 10 females or mixed sex groups of 10 males and 10 females. Both vessels were sealed by a breathable cotton bung. Males were identified by the presence of sex combs and a single spot on each forewing and females by the oviscapt morphology. Both vessel types were held horizontal while the flies became active. Both vials and cuvettes were monitored for a minimum of six days after a 24 hour 'settling' period.

1.3 Semi-field locomotion

Individual and populations of flies were exposed to natural environmental conditions in a sheltered, semi-field setting in the south-east of England at four different times in the year; April, June, August and October (Table 1). In this location, artificial light was absent. The DAM systems were held within a well-ventilated waterproof housing unit to protect the equipment from rain and direct sunlight. Environmental monitors (DEM Model DEnM, TriKinetics Inc, Waltham, MA, USA) were deployed alongside the DAM to record temperature and humidity conditions. The average sunrise and sunset times through the assay period were recorded (Table 1). Summer morph flies were used in June, August and October collections. Winter morph and summer morph fly locomotor activity was collected in parallel during April conditions to mimic the variation in morph in the field at this time of the year in the UK (Fountain et al., 2017). For winter morph assays', only individual males, individual females and mixed sex groups were assayed due to the difficultly in culturing large populations winter morph flies in the laboratory.

Table 1. Environmental conditions for semi-field locomotor assays. Average daily range for temperature, humidity and illuminance taken from environment monitor throughout the assay. For photoperiod average day length and civil sunrise/sunset (time at which there is no need of artificial lights) are provided. *Illuminance under April semi-field conditions could have been underestimated due to suboptimal placement of the environment monitor in the weatherproof enclosure.

	Temperature	Humidity	Photoperiod	Illuminance
April	8-15°C	42-98%	13.5h 06:15-20:00	0-161* Lux
June	13-19°C	51-91%	16.5h 04:00-22:00	0-1235 Lux
August	14-23°C	50-91%	15.35h 04:45-21:20	0-2746 Lux
October	8-14°C	63-96%	11h 06:45-18:45	0-2722 Lux

1.4 Laboratory locomotion

A range of environmental conditions were established in Percival DR-36VL environmental chambers within the insectary facility at the University of Southampton, UK. Conditions were divided into three categories; simple cycles, combined temperature/light cycles and semi-field mimic (Table 2). Humidity

was kept at a constant 65% relative humidity in all laboratory conditions. The semi-field mimic environmental conditions were extrapolated from data loggers deployed alongside the DAM system in the semi-field assays. Average daily temperature and light cycles were established from the data. To replicate dawn stepping light were used in which half the lighting banks came on initially, followed half an hour later by the remaining half (full illuminance: 2670 lux, half illuminance: 1386 lux). This was reversed for dusk. We also tested whether an increase in space for individual flies affected locomotor behaviour by comparing the behaviour of individual males and individual females in narrow cuvettes versus population vials for the simple and combined environmental cycles in the laboratory.

Table 2. Environmental conditions used in Percival DR-36VL environmental chambers.

Category	Name	Temperature	Photoperiod
Simple	DD 23°C	Constant 23°C	Constant darkness
Simple	12:12 23°C	Constant 23°C	12:12 light: dark
Simple	16:8 23 °C	Constant 23°C	16:8 light: dark On 04:00/ Off 20:00
Simple	DD 11-22°C	Ramping 11-22°C Min: 04:00 Max: 12:00	Constant darkness
Combined cycles	Long day	Ramping 11-22°C Min: 04:00. Max: 12:00	17.5:6.5 Stepping lights Half-light: 04:30/ Full light: 05:00 Half-light: 21:30/ Darkness: 22:00
Combined cycles	Short day	Ramping 11-22°C Min: 04:00. Max: 12:00	10.5:14.5 Stepping lights Half-light: 06:30/ Full light: 07:00 Half-light: 16:30/ Darkness: 17:00
Semi-field mimic	April mimic	Ramping 8-15°C Min: 05:30 Max: 14:00	13.5:10.5 Stepping lights Half-light: 05:30/ Full light: 06:00 Half-light: 18:30/ Darkness: 19:00
Semi-field mimic	June mimic	Ramping 13-19°C Min: 04:00 Max: 14:00	17.5:6.5 Stepping lights Half-light: 04:30/ Full light: 05:00 Half-light: 21:30/ Darkness: 22:00
Semi-field mimic	August mimic	Ramping 14-23°C Min: 05:00	16:8 Stepping lights Half-light: 05:00/ Full light: 05:30

		Max: 14:00	Half-light: 20:30/ Darkness: 21:00
Semi-field mimic	October mimic	Ramping 8-14°C	12.5:11.5 Stepping lights
		Min: 06:45	Half-light: 06:45/ Full light: 07:15
		Max: 14:00	Half-light: 18:45/ Darkness: 19:15

Table 3. Number of each fly sample recorded within each environmental condition and used for analysis. IM (individually-housed males); IF (individually-housed females); IMP (individually-housed males in populations vials); IFP (individually-housed females in population vials); VGM (virtual groups of males made up of 10 IM sets each); VGM (virtual groups of females made up of 10 IF sets each); VMSG (virtual mixed sex groups made up of 10 IM + 10 IF sets each); GM (group-housed populations of 10 males); GF (group-housed populations of 10 females); MSG (group-housed mixed sex groups of 10 males + 10 females each); WM IM (individually-housed winter morph males); WM IF (individually-housed winter morph females); WM MSG (group-housed winter morph populations of 10 males + 10 females).

	DD 23°C	12:12 23°C	16:8 23°C	DD 11-22°C	17.5:6.5 11-22°C	10.5:13.5 11-22°C	April SF	April Mimic	June SF	June Mimic	Aug SF	Aug Mimic	Oct SF	Oct Mimic
IM	50	50	50	50	50	50	42	50	50	50	50	49	26	50
IF	47	46	47	49	50	50	42	50	50	50	48	50	28	50
IMP	15	15	10	10	9	6								
IFP	15	16	9	10	7	7								
VGM	5	5	5	5	5	5	4	5	5	5	5	5	2	5
VGF	5	5	5	5	5	5	4	5	5	5	5	5	1	5
VMSG	5	5	5	5	5	5	4	5	5	5	5	5	1	5
GM	10	10	5	10	10	10	9	5	10	5	10	5	1	5
GF	10	10	5	10	10	10	10	4	9	5	10	5	3	5
MSG	10	8	5	12	10	10	10	5	9	5	10	6	10	5
WM IM							44	32						

WM IF	41	37
WM MSG	10	5

Analysis

Only flies that survived the whole assessment period were included in all analysis resulting in varying replication (Table 3). Groups in which any mortality occurred were also discarded. The CLOCKLAB software package (Actimetrics, Inc.) was used to analyse locomotor activity counts for the generation of actograms, chi square periodograms and daily activity profiles. The number of beam breaks in 5-minute intervals, were collected for 6 days and this data was used to generate the activity profiles. The beam break counts were then 'binned' into 30-minute intervals for further analysis.

To calculate anticipation, the method suggested by Zhang et al 2013 was performed on data collected from the simple and combined laboratory cycles (12:12 23°C, 16:8 23°C, long day and short day cycles). In brief, average activity counts of each social grouping was measured in 30 minute bins, over a 2.5 hour period during the middle of the dark phase for each day. This was subtracted from the average activity counts measured in 30 minute bins over the 2.5 hours prior to lights on. This was also performed for the evening anticipation by subtracting average activity counts during 2.5 hours during the middle of the light phase from the average of 2.5 hours prior to lights off. The data was tested for normality with the Shapiro-Wilk test based on the results (normality was confirmed in only 2 out of 8 data sets) the Kruskal-Wallis non-parametric alternative to the one-way ANOVA was conducted with Dunn's post hoc tests for the analysis with different days as replicates (7 days).

To measure normalised hyperactivity at daily light/dark transitions, the 30-min interval which commenced with the lighting event was identified for both dawn and dusk and its number of counts were then normalised by dividing it by the daily average activity (per 30 min). The dawn and dusk periods were each defined as

a two-hour period which encompassed 30-minutes before a lighting event and, 1.5 hours after the event. In those conditions that experienced stepping light events (i.e. the semi-field mimic cycles within the laboratory) the 2-hour period commenced 30-minutes before the first event. For profiles collected under constant darkness, subjective dawn/dusk was defined by daily phases corresponding to the most recent dawn and dusk periods. To ensure the best signal-to-noise estimates normalised hyperactivity was determined subsequent to averaging over both the available replicate days and flies. Prior to analyses addressing the impact of environmental or social housing conditions on dawn- or dusk-associated hyperactivity, data sets were tested for the normality of their distribution using the Shapiro-Wilk test. Based on these results (instances of non-confirmed normality in all data sets) non-parametric statistical tests (Kruskal-Wallis) were employed with an additional Dunn's multiple comparison post hoc tests in GraphPad Prism 7.03 on these results. Only summer morph flies were used in this analysis.

To determine the impact of social housing on time keeping ability in constant conditions (DD 23ºC) the period length and the strength of the rhythm (relative rhythmic power or RRP) of locomotor behaviour was identified by the CLOCKLAB software. The RRP was calculated from chi-square periodogram analyses in CLOCKLAB by dividing the 'power' by the 'significance' threshold values (using a threshold of p=0.01) manually in Excel (Goda et al., 2011). To explore the impact of social interactions on activity, data from group-housed flies was compared to that of individually housed flies both directly and after the creation of 'virtual fly groups' by summing the activity data from matching numbers of individually-housed flies (10 individual males, 10 individual females or 10 individual males and 10 individual females, respectively). Circadian activity patterns were categorized as arrhythmic (in which period length could not be identified), weakly rhythmic (RRP 1-1.5) and rhythmic (RRP >1.5) and the distribution of observed behavioural patterns across these categories was compared for the different social groupings. Significant differences in the distribution of flies across the three rhythmicity categories as a function of social setting was explored

using pairwise Fisher's exact tests and the results were annotated relative to a multiple-testing corrected (Bonferroni) p-value threshold.

For social groupings in which a period length could not be identified, data were not included within the overall analysis or figures in which statistical comparisons of RRP and period length were made. Subsequently, based on the outcome of Shapiro-Wilk tests for normality, a Kruskal-Wallis test with Dunn's post hoc tests was performed in GraphPad Prism 7.03 on both these parameters. Only summer morph flies were used for this experiment.

Average activity counts per hour per fly were calculated by dividing hourly activity by the number of flies in the sample group to enable comparison between all social groupings (including virtual populations) and environments. This was done to ensure that activity counts based on equal numbers of individually versus group-housed flies were compared. Non-parametric Kruskal-Wallis tests with post hoc pairwise comparison of average activity counts per hour per fly (using Mann-Whitney tests with multiple testing correction) was performed in SPSS. In October semi-field collections survival was low and so, in some cases, fewer replicates were included and are indicated in figure legends or tables. In addition to the summer morph flies used across all conditions, winter morph flies were included for a subset of these analyses as annotated.

Results

1.5 Impact of environment and social conditions on daily locomotor activity

Daily locomotor activity profiles exhibited clear differences across combinations of environment and social setting (Figure 1a, b). Profiles collected in semi-field conditions (exposed to natural light and temperature

cycles) (Figure 1b, columns 1,3,5,7 and Figure 1c, column 1, 3) and those collected in the laboratory under constant darkness (Figure 1a, columns 1, 2), showed less variation in locomotor pattern between social groupings than those collected in the laboratory with a light/dark cycle. In the presence of light/dark cycles increased activity was observed during the light phase relative to the dark phase. In the laboratory, individual males (Figure 1a, b, c row 1), individual females (Figure 1a, b, c row 2) and group-housed males (Figure 1a, row 5 and 1b, row 3) displayed behavioural profiles featuring prominent bouts of hyperactivity, immediately following lights-on and lights-off transitions. This response was, in most cases, more subdued for group-housed females (Figure 1a, row 6 and 1b row 4) or summer morph mixed sex groups (Figure 1a, row 7 and 1b row 5) under the same environmental conditions. Flies monitored under semi-field conditions (Figure 1b, columns 1, 3, 5, 7 and Figure 1c, column 1, 2) did not show these bursts of activity at dawn and dusk to the same extent, suggesting this behavioural response may be an artefact caused by abrupt changes in light levels, associated with the use of fluorescent lights.

In the presence of a temperature cycle, locomotor behaviour of flies, under semi-field and some laboratory conditions, tended to rise and fall along with the temperature; this was especially clear for flies maintained in the presence of a daily temperature gradient in the dark within the laboratory. Dramatic changes in the daily activity profile were observed when a long- or short-day exposure to fluorescent lights was integrated with a daily temperature gradient for the combined cycles (Figure 1a, column 2 versus 5, 6). Again, hyperactivity was prominently observed in conjunction with the turning on or off fluorescent lights. The alignment between environmental temperature cycle and daily locomotor activity pattern appeared to be well-preserved for all social conditions tested under June, August and October semi-field conditions. However, peak activity was delayed relative to peak temperature for the April semi-field conditions that featured long days combined with somewhat cooler temperature cycles (Figure 1b, column 1, 2). The peak in locomotor activity in the April semi field conditions occurred between 19:00-19:30 whereas the peak in temperature occurred, on average between 15:00-16:00. Light transition-associated hyperactivity for June,

August, and October lab mimic conditions was relatively subdued in groups of females or mixed gender flies compared to individual male or female flies or group-housed males (Figure 1b, columns 4, 6). Divergent results were, however, obtained for the April lab mimic conditions, which featured more prominent lights-on hyperactivity for group-housed flies (Figure 1b, column 2 rows 3, 4, 5) and more pronounced lights-off hyperactivity in individual flies (Figure 1b, column 2, rows 1, 2). Finally, the October lab mimic profiles differed from the corresponding semi-field originals by lights-on-associated hyperactivity as well as either acute lights-off-associated hyperactivity in the case of individual flies (Figure 1b, column row 1,2) or a delay in the main activity peak in the case of group-housed flies (Figure 1b, column row 3-5).

Average daily activity profiles for individual males and females in population vials showed higher circadian locomotor activity peaks under constant conditions than individuals in narrow cuvettes (Figure 1a, column 1). The behaviour of individual flies in population vials in other simple and combined cycles in the laboratory was also suggestive of improved circadian rhythmicity in the wider vial environment as behavioural anticipation of the lights-off transition appeared more prominent (Figure 1, column 3-6).

A limited analysis of possible behavioural differences between winter and summer morph flies was included for April semi-field and corresponding laboratory mimic conditions based on the rationale that a mixture of summer and winter morph flies may be present at this time of year within the UK. Moreover, as differences in *Drosophila* female reproductive state have been reported to affect locomotor activity (Ferguson et al., 2015, Isaac, 2019), we hypothesised that analogous differences might be observed between reproductively active summer morph flies and winter morph flies that have entered reproductive diapause. As described above (Materials and Methods), reproductive quiescence was, indeed, confirmed for the winter morph flies prior to their use in our behavioural analyses. Due to the difficulty in culturing the large numbers needed to assay all social groupings, only individual males, individual females and mix sex groups

were monitored in winter morph form. The resulting activity profiles collected for both morphs under the April each of the conditions displayed very similar daily locomotion patterns, with the winter morph flies under semi-field conditions being representative of semi-field summer morph activity (Figure 1c, column 1-4). However, regardless of morph, laboratory mimic profiles diverged from their semi-field counterparts, as described above.

[Insert Figure 1a.]

[Insert Figure 1b.]

[Insert Figure 1c.]

1.6 Anticipation

The anticipation of lighting events varied with social parameters. In the simple and combine laboratory cycles involving a light cycle (12:12 23°C, 16:8 23°C, long day and short day combined cycles), both morning and evening anticipation was evaluated. Kruskal-Wallis tests indicated a significant association between social grouping and anticipation was found for all conditions except morning anticipation during the long day combined cycles (see Figure 2). Dunn's post hoc tests revealed further pairwise significant differences as indicated in Figure 2. In general terms, more significant differences were observed in evening versus morning anticipation and the impact of social setting on anticipatory behaviour diverged for the single short day condition that was included. Under 12h or longer photoperiods single males in population monitors showed a significant increase in evening anticipation, while mix sex groups showed a significant decrease in evening anticipation. Furthermore, the above-noted trend for individual flies to exhibit higher

levels of evening anticipation when housed in population vials rather than narrow vials did not result in statistically significant differences in these analyses.

[Insert Figure 2.]

1.7 Social and environmental determinants of hyperactivity at light/dark transitions

The daily activity profiles, discussed above, showed that acute behavioural responses at light/dark transitions were much more prominent under laboratory than semi-field conditions (Figure 1, supplemental Figure S1). The impact of environmental conditions and social groupings on light transition-associated hyperactivity was examined quantitatively. First, normalised hyperactivity at (subjective) dawn and dusk was determined from the average daily activity profile per environmental/social grouping combination (Figure 3a, b). Next, the resulting normalised hyper activity values were compared across environmental conditions (Figure 3c, d) and social groupings (Figure 3e, f). As flies in constant dark conditions and in semi-field conditions showed little to no hyperactivity at (subjective) dusk or dawn across different social or environmental settings, these conditions were used as controls for the detection of significant changes in associated laboratory cycles with light/dark transitions. Separate Kruskal-Wallis tests for dawn- and dusk-associated hyperactivity each showed highly significant impacts ($p<0.0001$) of both environment and social grouping. The impact of environmental conditions and social settings on dawn- and dusk-associated hyperactivity was then assessed at the level of pairwise post hoc tests. Notably, the addition of a photoperiod to a DD condition resulted in an increase in normalised hyperactivity at dawn and dusk with statistically significant differences detected in the context of an 11-22ºC cycle in DD, in comparison to long- and short-day photoperiods with essentially the same temperature cycle (Figure 3c, d). Furthermore,

laboratory-based protocols designed to mimic semi-field conditions inadvertently produced increases in average normalised hyperactivities at light/dark transitions and a significant result was found for the April and October semi-field mimic at dawn (Figure 3c).

The effect of social housing on hyperactivity at light/dark transitions was illustrated when data was collated by social, rather than environmental, conditions (Figure 3e, f) resulting in considerably more significant pairwise differences (Table 4). Typically, more differences occurred in comparison with DD and semi-field hyperactivity at dawn than at dusk. Single male and single female flies in narrow cuvettes and group males, showed significantly increased hyperactivity at both dawn and dusk in laboratory-based photocycles compared with either DD or semi-field conditions. Differences were less consistent for other social settings.

Table 4. Table of significant differences in dawn- or dusk-associated hyperactivity for lab assays using various social settings. The probabilities represent Dunn's post hoc tests relative to control data sets under constant darkness (DD) and semi-field conditions. Non-significant p-values are indicated between parentheses.

	Dawn		Dusk	
Social grouping	**DD**	**Semi-field**	**DD**	**Semi-field**
IM (Individual males)	$p< 0.001$	$p<0.003$	$p<0.001$	$p<0.001$
IMP (Individual males in population monitors)	$p<0.001$	$p<0.0001$	($p=1$)	($p=1$)
IF (Individual females)	$p<0.036$	($p=0.1$)	$p<0.003$	($p=0.05$)
IFP (Individual females in population monitors)	$p<0.001$	$p<0.0001$	($p=1$)	($p=1$)
GM (Group males)	$p<0.001$	$p<0.0001$	$p<0.048$	($p=0.47$)
GF (Groups of females)	$p<0.003$	$p<0.010$	($p=1$)	($p=1$)
MSG (Mix sex groups)	$p<0.0001$	$p<0.002$	($p=1$)	($p=1$)

A more detailed representation of laboratory lighting-induced hyperactive episodes across different social settings and semi-field paradigms is provided by the scatter plot in Figure 3g. Social setting/environmental cycle combinations were plotted according to observed differences in normalised hyperactivity at dawn (x axis) and dusk (y axis) between semi-field and matching laboratory mimic conditions, and so more

successful recreations of semi-field conditions would be found closest to the origin of the graph. For June, August and October laboratory mimic conditions group female and mixed sex group conditions performed best in this regard, while the profile for individual females was closest to the semi-field profile observed in April. Typically, individual males and groups of males were found on the periphery indicating more divergence between laboratory and semi-field behaviour (Figure 3g).

[Insert Figure 3.]

1.8 Impact of social housing on time keeping ability in constant conditions

Monitoring *D. suzukii* in the absence of light and temperature cycles revealed a significant impact of social condition on daily locomotor activity patterns, rhythmic strength and period length. Actograms displaying daily activity patterns vary greatly between social groupings with the weakest rhythmicity in individual males and the strongest in group-housed females and mix sex groups (Figure 4). Individual males and individual females assayed in narrow tubes had high percentages of arrhythmic flies and weakly rhythmic flies (Figure 4b). In fact 0 out of 50 individual males in this condition were classified as 'rhythmic'. Since the number of flies contributing to a single behavioural profile for group-housed individuals was 10-fold or 20-fold larger than that of individual flies, we considered that pooling the data from matching numbers of individually-housed flies might improve the subsequently detected circadian rhythmicity. However, when virtual groups of males and females were created from individually-housed fly data to address this point, no significant improvement in rhythmicity was observed relative to the original data sets (Figure 4b). Significantly increased levels of rhythmicity were found for group-housed flies relative to individually housed flies (Figure 4b, d). Circadian period length was also affected and showed a much wider variation in measurements from individual rather than groups of flies (Figure 4c). Moreover, the weakly rhythmic individual males showed a circadian period that was longer than that of group-housed males or females (Figure 4c). The bulk of individual fly assays was conducted in narrow vials and it was, therefore, of interest

to determine whether the difference in vial size might have contributed to the observed differences in rhythmicity between individually and group-housed flies. This was addressed by conducting experiments using individual males and females in population-sized vials. Of note, individual males in larger, population-sized vials exhibited a significant shift in their distribution across categories of rhythmicity (Figure 4b), indicating that confinement to narrow vials negatively impacted circadian behaviour in individual males. However, this was not the case for female flies and group-housed females showed increased rhythmicity versus their individually-housed counterparts regardless of the vial size used for the latter (Figure 4b, d).

[Insert Figure 4a.]

[Insert Figure 4b.]

[Insert Figure 4c.]

[Insert Figure 4d.]

Table 5. Mean (±SEM) of relative rhythmic power (RRP) and period length (hours) for social groupings held under constant darkness and constant temperature conditions (DD 23ºC). Only those in which a period length could be identified were included in this analysis

Overall significance	***p=0.0003***	***p<0.0001***
Social grouping (N)	**RRP (±SEM)**	**Period (hours) (±SEM)**
IM (24)	1.17 ±0.02	27.7 ±0.93
IF (25)	1.29 ±0.05	25.8 ±0.76
IMP (12)	1.36 ±0.08	25.8 ±1.31
IFP (11)	1.27 ±0.08	23.6 ±0.67
GM (10)	1.57 ±0.09	23.3 ±0.18
GF (10)	2.13 ±0.11	23.6 ±0.14
MSG (10)	1.90 ±0.09	23.7 ±0.13

1.9 Impact of social setting and environment on activity rate

There were significant differences in the average activity counts per hour per fly in relation to environmental condition and social housing (Table 6). Group females displayed the highest activity counts per hour per fly in 5 of the 14 environmental conditions; the highest of any social grouping. Individual females in population monitors displayed the highest counts in 3 out of the 6 environments in which they were monitored. Social conditions containing females showed, typically, higher than average activity counts per hour per fly. Individual females, groups of females and mix sex groups had higher than average activity counts per hour per fly in 9, 10 and 8 of the 14 conditions, respectively. Individual males and group

males displayed activity counts lower than the mean in 11 and 9 of the 14 environmental conditions, respectively. Virtual groups were created to define whether variation in activity counts was attributed to fly density, and the result of flies disturbing one another within the assay or whether individual flies could be analysed as groups and gain the same results as real groups. This would mean that if only individual fly data was available it could be converted to give an indication of how groups of flies would behave. Different social groupings exhibited significant differences in average activity per hour per fly for almost all environmental conditions, the only exception being DD 23ºC. Further, average activity levels observed for winter versus summer morph flies showed no significant difference in individual male behaviour and individual female behaviour. However, mix sex group activity counts differed between morph in the April semi-field conditions with summer morph flies displaying higher activity counts.

Table 6. Mean (±S.E.M) *D. suzukii* activity counts per hour per fly as a function of social setting (rows) and environmental condition (columns). Sample sizes are found in Table 3, above. Overall median (±S.E.M) activity count within each environmental condition is shown at the top of each column. Kruskal-Wallis *p* values of the impact of environment on activity rate within each social setting or the impact of social setting on activity rate within each environment are displayed in the row and column headings, respectively. The pattern of multiple-testing-corrected significant *p* values for pairwise Mann-Whitney tests within social settings or environments are annotated below the numerical values in each cell with upper-case letters across the rows and the lower case letters across the columns. * Indicates that only one replicated occurred for this condition/social grouping. NSD indicates no significant difference.

			DD 23°C	12:12 23°C	16:8 23°C	DD 11-22°C	17.5:6.5 11-22 °C	10.5:13.5 11-22 °C	April SF	April Mimic	June SF	June Mimic	Aug SF	Aug Mimic	Oct SF	Oct Mimic
		Median	**9.1±0.4**	**13.1±0.6**	**11.1±0.7**	**10.5±0.5**	**7.3±0.6**	**7.2±0.6**	**5.0±0.3**	**4.3±0.3**	**6.6±0.4**	**4.6±0.3**	**8.3±0.6**	**11.2±0.5**	**2.1±0.2**	**4.8±0.3**
	Median	**Overall *p***	**NSD**	***p* <0.001**	***p* <0.001**	***p* <0.002**	***p* <0.001**	***p* <0.007**	***p* <0.001**	***p* <0.001**	***p* <0.001**	***p* <0.001**	***p* <0.001**	***p* <0.004**	***p* <0.008**	***p* <0.001**
IM	**6.3±0.2**	***p* <0.001**	7.8±0.7	13.6±0.9 d	7.4±0.7	11.2±0.9	4.9±0.6 ab	4.7±0.3 abc	4.7±0.8 a	1.9±0.2 a	5.8±0.7 ab	4.8±0.4 Abcd	3.9±0.3 ade	9.5±0.8 abc	1.5±0.2 a	3.8±0.3 ab
IF	**7.7±0.2**	***p* <0.001**	9.4±0.7	15±1.2 d	14.2±1.4	11.5±0.9	4.9±0.6 ab	5.7±0.5 abc	4±0.4 a	2.5±0.3 ab	5.3±0.7 b	5.4±0.4 Abcd	8.3±0.7 bf	11.8±0.9 abc	1.6±0.1 a	5.3±0.5 ab
IMP	**11.7±1.1**	***p* <0.001**	8.2±0.8 A	3.9±0.7 abA	18.8±3.4 B	12.5±2.4 AB	18.2±3.2 abcA	16.9±3.4 abcA								
IFP	**14.4±1.5**	***p* <0.001**	13.5±2.1 ABC	5.8±2.0 abcA	17.5±1.1 C	6.3±1.5 AB	22.7±2.4 bcBC	34.8±6.0 aBC								
VGM	**6.3±0.4**	***p* <0.001**	7.8±0.6 A	14.6±1.2 abcdA	7.4±0.5 A	11.3±0.4 A	3.5±0.1 abcA	4.7±0.5 abcA	4.8±1.4 abcA	1.9±0.4 abA	5.8±1.2 abA	4.8±0.4 abA	3.9±0.1 efghA	10.2±0.6 abA	1.5±0.3 abA	3.8±0.3 aA
VGF	**8.9±0.6**	***p* <0.001**	13.2±2.3 A	18.2±1.4 abcdA	16.9±2.9 A	12.8±1.9 A	5.5±0.8 abcA	5.7±0.4 abcA	4±1.0 abcA	2.5±0.2 abA	5.3±0.8 abA	5.4±0.8 abA	8.9±1.4 abcdA	11.8±0.9 abA	1.7*	5.3±0.5 abA
VMSG	**7.6±0.5**	***p* <0.001**	10.5±1.0 A	16.4±1.4 abcdA	12.1±1.4 A	12.1±1.2 A	4.5±0.4 abcA	5.2±0.4 abcA	4.4±0.8 abcA	2.2±0.2 abA	5.6±0.8 abA	5.1±0.4 abA	6.4±0.7 abcdA	11±0.3 abA	1.8*	4.5±0.4 abA

GM	**7.2±0.4**	***p* <0.001**	7.5±1.2 ABC	15±1.7 cdC	2.6±0.5 ABC	8.2±0.7 BC	5.8±1.3 aAB	3.4±0.2 bA	8.6±0.7 abcC	11.3±0.6 abABC	7.4±0.5 aB	0.9±0.2 dABC	6.1±0.6 abhABC	8±1.3 abABC	0.1*	8.7±1.2 bABC
GF	**13.7±0.8**	***p* <0.001**	8.1±1.5 CDE	19.7±2.0 dABCD	5.5±0.7 ABCDE	9±0.6 E	17.7±2.0 cABCD	9.3±1.4 abcCDE	13.5±0.7 cCDE	13.6±0.5 abABCDE	17.1±1.6 bCDE	1.4±0.1 dABCDE	32.2±1.4 dfAB	25.7±3.5 cABCDE	3.3±1.6 abABCDE	5.8±0.7 abABCDE
MSG	**8.8±0.5**	**p <0.001**	8.8±0.6 CDE	15±0.8 dA	3.6±0.5 ABCDE	6.6±0.8 CE	7.2±1.8 abcABCDE	6.8±0.4 cC	13.9±0.8 cAD	7.2±0.5 abABCDE	10.3±1.2 abABCD	1.7±0.1 cABCDE	16.5±1.6 cgAB	12.1±1.4 abcABCDE	3.5±0.3 bE	8±0.8 bABCDE
WM IM	**3.6±0.3**								3.3±0.3 ab	3.8±0.6 ab						
WM IF	**5.3±0.7**								3.6±0.4 ab	7.2±1.3 b						
WM MSG	**7.4±0.8**								6.4±0.9 ab	9.3±0.6 ab						

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Discussion

We examined the impact of environmental and social parameters on the daily locomotor activity profile of the horticultural pest species *Drosophila suzukii* with a particular interest in recreating and predicting behavioural patterns encountered in the field. The behaviour of mated female flies was of particular interest given their role in mediating damage to fruit and the relative scarcity of virgin females in the field (Revadi et al., 2015). Thus, in spite of the fact that the mating state of *D. suzukii* influences patterns in locomotor behaviour in *D. suzukii* (Ferguson et al., 2015), all observations made in this study used mated flies, in both individual and social housing groups. Prior analyses of *D. suzukii* locomotor activity have been reported for laboratory and 'semi-natural light' conditions (Hamby et al., 2013, Ferguson et al., 2015), while two more studies inferred diurnal activity in the field based on trapping results (Evans et al., 2017) or visual detection (Van Timmeren et al., 2017). However, the present study is, to our knowledge, the first for this species to both document high resolution daily activity profiles in a semi-field setting as well as systematically address the contributions of temperature, light, and social groupings on daily locomotor activity.

In both laboratory- and field-collected profiles, most activity occurred during the day time and very little occurred during the night. This also matches observations for oviposition rhythms in the laboratory and field (Shaw et al., 2018b). Moreover, 'subjective day-active' patterns were found under constant dark conditions. However, it is important to note that the temperature cycles applied in this study did not include noxiously warm temperatures. In a study that did include high (>30°C) temperature maxima, Ferguson et al. (2015) reported crepuscular behaviour for *D. suzukii* faced with 'simulated summer conditions' in the laboratory, while field observations made on similarly warm days likewise suggested a crepuscular activity pattern (Evans et al., 2017, Van Timmeren et al., 2017). Therefore, we hypothesise that *D. suzukii* daily locomotor activity patterns, may seasonally switch from unimodal diurnal profiles to crepuscular, as

temperature maxima reach noxiously high levels. Indeed, this hypothesis is consistent with previously observed temperature-disrupted, *D. suzukii* oviposition patterns (Shaw et al., 2018b), and seasonal variation of activity peaks in *Drosophila melanogaster* Meigen under semi-natural conditions (Menegazzi et al., 2013).

Although anticipatory behaviour is seen as a key indicator of the presence of a functioning circadian clock (Stoleru et al., 2004), in our assays we found that social settings that favoured increased evening anticipation under 12h or longer photoperiod (individual males or females in population vials), showed weaker rhythms under constant conditions than groups of females and mix sex groups that showed very little to no anticipation of lighting evenings in laboratory conditions.

The impact of daily temperature cycles on *D. suzukii* locomotor activity was apparent from the increased amplitude in rhythm upon the introduction of a temperature gradient in constant darkness. Moreover, the daily activity profiles of flies under June, August and October semi-field conditions, appeared to track the daily temperature profiles. However, the April temperature cycle, which featured a longer interval at or near peak temperature, resulted in a delay of maximal daily activity by an average of 4 hours across all the social groups. The same was also true for the winter morph flies under the April semi-field conditions. and as *Drosophila* are ectothermic, it may be a form of behavioural thermoregulation used to heighten physiological performance in the colder conditions (Dillon et al., 2009). These features were recapitulated relatively well when the semi-field temperature and light cycles were mimicked in the laboratory, although the artificial lighting conditions triggered acute bursts of activity following the light/dark transitions. This hyperactivity at light transitions, termed 'masking' or 'startle response', has been observed previously in *D. melanogaster* (e.g. Sheeba et al, 2010) and is thought to represent behavioural responses to changes in illuminance that shroud or 'mask' underlying circadian behaviour (Mrosovsky, 1999, Allada and Chung, 2010). We found that the hyperactivity responses observed in lab assays of *D. suzukii* were clearly influenced by the social housing condition, with female and mixed sex groups displaying reduced

hyperactivity, and showing the most accurate behavioural representations of June and August semi-field conditions when subjected to the laboratory mimic conditions. The absence of similar bouts of acute hyperactivity in semi-field conditions suggests that the abrupt changes in illuminance in the lab are responsible for this phenomenon. While both lab and semi-field conditions featured comparable maximum illuminance levels (2670 lux in the lab versus 2746 lux in the August semi-field setting) the changes in illuminance were much more gradual in the field.

Our analyses across environmental conditions provided evidence that gender, space constraints and social interactions all influenced locomotor behaviour. Apart from their impact on hyperactivity at light/dark transitions, social settings also affected behavioural rhythmicity under constant conditions for group-housed flies. Within this project individual males and females had the highest percentage of arrhythmic counts in constant conditions and had a much lower relative rhythmic power (RRP) than group housed flies. The impact of social interactions on rhythm-maintenance, may be related to prior findings in *D. melanogaster* (Bloch et al., 2013), where social olfactory cues were found to be capable of regulating or resetting circadian rhythms in the same way environmental cues do (Levine et al., 2002). In *D. melanogaster* Lone and Sharma (2011) concluded that social grouping resulted in an increase of phase synchrony in comparison to individual flies or pairs of flies under constant darkness after prior entrainment to a L:D cycle. They also found that under constant darkness, housing loss-of-olfaction-function mutant flies in groups did not result in rhythm maintenance, supporting the idea that it is an olfactory signal that is driving this response. It will be interesting to probe the relevant sensory input and neural pathways in this regard. The broader relevance of observing behaviours under social conditions has been promoted by several groups who observed that interaction between individuals will affect behaviour, not only in species that have complex social structure, such as the honeybee, but also in less socially complex systems, including *Drosophila melanogaster* (Bloch et al., 2013, Fujii et al., 2007). In particular, prior studies have established an impact of social interactions on activity rhythms in honey bee (Beer et al., 2016, Moore et al., 1998) and both activity rhythms and

mating behaviour in *Drosophila melanogaster* ((Fujii et al., 2007, Krupp et al., 2008, Levine et al., 2002)). Mating and courtship in *D. suzukii* involve mobile rituals of wing flashing and circling and would surely increase activity levels in populations of flies (Revadi et al., 2015). De et al. (2013) modified lighting factors 'under otherwise semi-natural' conditions and monitored locomotor behaviour of *D. melanogaster*, to determine how activity was affected by changes in light. They also hypothesised that there would be changes in activity due to courtship and concluded that the morning peak of activity in *D. melanogaster* is due to 'courtship-related locomotion'. Within our assay, individual flies housed in the narrow cuvettes frequently displayed a different locomotor profile than groups in the large population vials. Although, unlike De et al. (2013), we do not see variation in morning activity peaks between individual and group-housed flies under the semi-field collected locomotor profiles in *D. suzukii.* However, we did see this variation in the laboratory collected profiles.

Comparison of the behaviour of individual flies housed in narrow tubes versus population vials in the lab revealed a number of differences. These included changes in the balance between hyperactivity at lights-on- versus lights-off. Moreover, circadian rhythmicity under constant conditions was reduced for males in narrow tubes. Virtual groups were also created from matching numbers of individual fly data sets to demonstrate that the increased circadian rhythmicity of group-housed flies was not simply due to an increased signal-to-noise ratio in multi-fly records. Instead social interactions rather than vial size or pooled activity levels were implicated as driving increased circadian rhythmicity in *D. suzukii*.

For each social grouping, changes in environmental conditions triggered changes in the activity rate per fly. Conversely, social groupings affected the activity rate per fly for all environmental conditions, save one: constant 23°C in DD. When evaluating social housing alone, typically individual males and groups of males had lower than average counts per hour per fly than any housing that contained females; both as individuals or in single and mixed sex groups. We considered that having a higher density of flies in mixed sex groups

(20 individuals) would result in disturbances and therefore higher activity counts than in the single sex groups (10 individuals) or the individual flies. This hypothesis was investigated in an extensive examination of social interactions and its effect on sleep in *D. melanogaster*, conducted by Liu et al. (2015). Patterns of individual mated flies were compared with single sex groups of various densities and variations in sleep habits were detected. They found that total sleep decreased drastically as population density increased. In our assay, groups of females, which contained 10 individuals, displayed the highest activity counts per hour per fly in 5 out of the 14 conditions. This was higher than any other social grouping, including the mixed sex groups containing 20 individuals per vial, which, based on the interpretation by Liu et al. (2015), would have been expected to exhibit the highest activity counts. The increased activity per fly per hour in group females may be due to females searching for additional egg laying sites as *D. suzukii* prefer less dense sites compared to *D. melanogaster* (Mitsui et al., 2006). *D suzukii* and to also reduce oviposition competition (Shaw et al., 2018a).

The present study also included a limited comparison between the behaviour of summer and winter morph flies. The locomotor behaviour of flies exposed to April semi-field or April laboratory mimic conditions displayed minor shifts in average activity level between winter and summer morph flies, with winter morph flies relatively less active in the field, but more active in the laboratory. Perhaps an environmental difference between the two settings such as the gradual light/dark cycle or the presence of a relative humidity cycle in the field condition might explain these relatively modest differences. Nevertheless, the wave forms of daily activity patterns observed for summer morph versus winter morph flies were similar. Thus, under the conditions tested, summer morph behaviour was also representative of winter morph behaviour. From a practical approach this is a welcome result as culturing significant numbers of winter morph flies is time consuming and logistically challenging. As winter morph flies are in reproductive diapause (Rossi-Stacconi et al., 2016, Toxopeus et al., 2016, Wallingford and Loeb, 2016), cultures are not continuous and need frequent replenishment.

Within this research, we have investigated locomotion under both semi-field and laboratory settings. Ideally, to predict natural behaviours observations should be made under (semi-)field conditions (Kannan et al., 2012). However, there are obvious limitations and restrictions to working in natural conditions. The reproducible control environment provided by the laboratory enables researchers to replicate parameters and provides second chances, which would not occur in the field as no two days are the same. Also, being able to manipulate conditions enables investigations at any time opposed to waiting for environmental conditions to occur naturally in the field. Where semi-field-based assays are not appropriate, controlled environment chambers with gradual changes in light and temperature are a good substitute for investigating physiological behaviours such as diapause (Nagy et al., 2018). We did not attempt to manipulate relative humidity within the semi-field environments, whilst in the laboratory, it was maintained at 65%. As a result, we were able to dissociate effects of light and temperature from those of humidity, while promoting a high level of survival (Tochen et al., 2016, Hamby et al., 2016). Yet, despite this artificial feature, and the use of fluorescent rather than dimmable lights, we were able to successfully mimic locomotor behaviour found for 'summer' semi-field conditions in the laboratory.

Conclusion

The main aim of this paper was to identify the determinants of daily rhythms of *D. suzukii* activity that may help predict field behaviour. We demonstrated clear separable impacts of the circadian clock, light, temperature, and social housing on *D. suzukii* behavioural rhythms. By comparing laboratory-based and semi-field locomotor assays, we have investigated a wide range of environmental conditions to identify those that would be most appropriate for making behavioural predictions in the laboratory. *Drosophila* locomotor activity assays are most commonly conducted for individual male flies in laboratory conditions. However, in our analyses mixed sex or mated female groups showed stronger circadian rhythmicity and were more amenable to the recreation of semi-field behaviour in the laboratory. Using these social settings

'summer' semi-field conditions were successfully reproduced in incubators with fluorescent lights programmed to provide a realistic daily temperature cycle and photoperiod at constant humidity. As the behaviour of mixed gender populations is also of more direct concern to integrated pest management strategies aimed at minimizing harm to soft and stone fruit cultures by wild *D. suzukii* populations, we propose that future studies of natural locomotor behaviour should preferentially focus on flies in this social setting.

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Figure legends

Figure 1. Normalised activity profiles of summer morph *D. suzukii* adults under (a) simple and combined laboratory conditions, (b) semi-field and laboratory-based mimic conditions and (c) winter and summer morph flies under April semi-field and laboratory-based mimic conditions. For ease of comparison data for summer morph flies in (c) is duplicated from (b). Profiles were collected under different laboratory and semi-field condition (columns) and in various social grouping (rows) collected over a 6 consecutive-day period. The blue line and shaded area for each profile represent the average activity counts per half hour ± S.E.M. across these 6 days. Temperature regime is displayed by orange line at top of each activity profile and is not relative to axis counts. The temperature cycle for semi-field conditions represents the average daily cycle temperature over the collection period. Temperature maxima and minima are indicated in the column captions, with additional information provided in Table 1 and 2; The Y axes solely refer to average activity counts. Laboratory light regime is 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. All flies were kept in a 12:12 LD 23°C laboratory environment prior to experimental conditions and this is particularly relevant as the entraining condition for the laboratory DD 23°C conditions (as in column 3). The number of replicates contributing to each behavioural profile are annotated in Table 3. In all cases, group-housed sets contained either 10 males or 10 females or 10 males + 10 females. Due to low survival, October semi-field activity collections were based on 1 group of male flies and 3 groups of females. ** Indicates activity profile on different axis scale to all others.

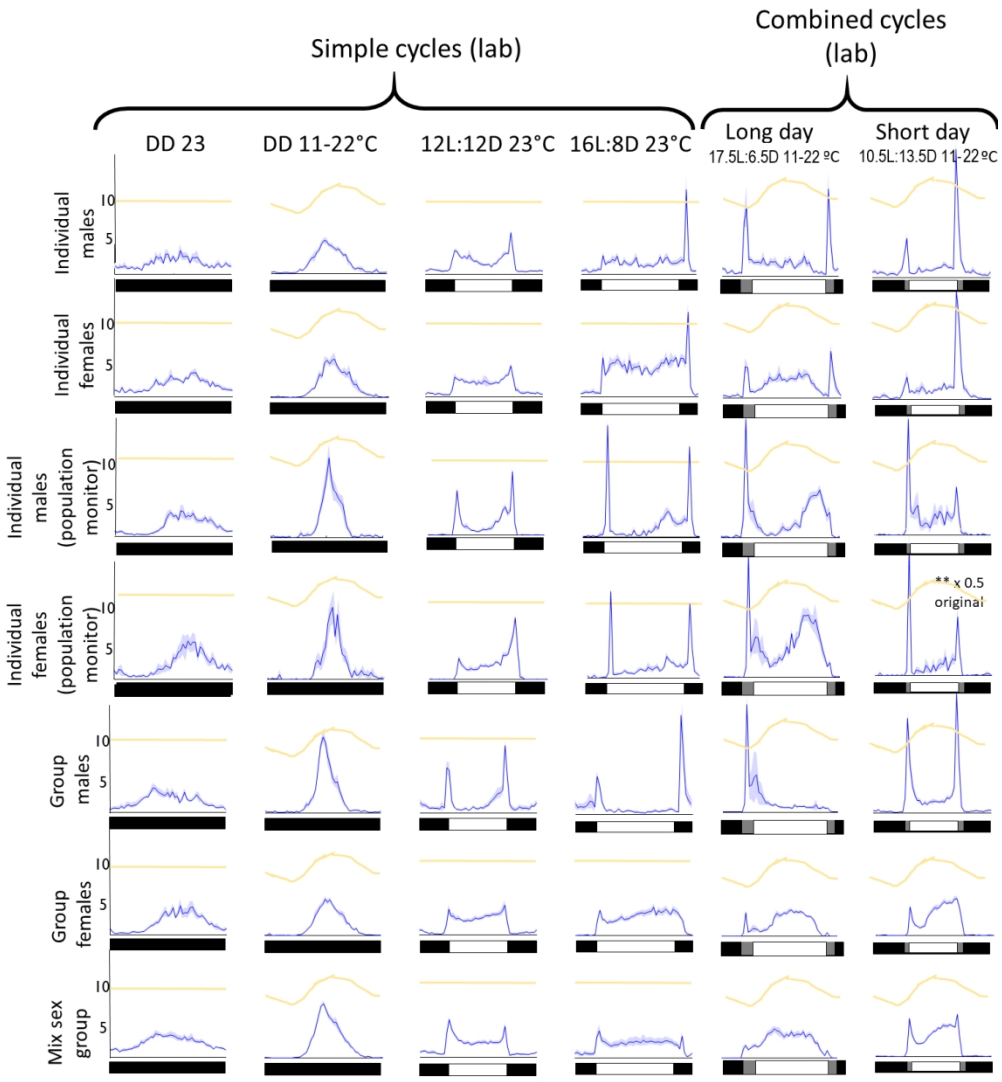
Figure 2. Morning (left) and evening (right) anticipation of various *D. suzukii* social groupings under 12:12 and 16:8 light cycles at 23°C as well as long day and short day combined light/temperature cycle conditions. IM= individual males. IF= individual females. IMP= individual males in population monitors. IFP= individual females in population monitors. GM= group males. GF= groups of females. MSG= mix sex

groups. Group sizes and replicate numbers are as described above. Anticipation analysis from 2.5 hours prior to lighting events, not including the event itself. Kruskal-Willis p-values are indicated above each panel and pairwise Dunn's post hoc test differences are annotated on the graphs.

Figure 3. Impact of environmental and social conditions on normalised *D. suzukii* dawn and dusk hyperactivity. (a, b) Heat maps representing normalised hyperactivity at (subjective) dawn (a) or dusk (b) across different environmental and social settings. Missing conditions are indicated in grey. (c-f). Normalized hyperactivity at (subjective) dawn (c.e) or dusk (d, f) plotted as a function of environmental protocol (c, d) or social setting (elf). Graphs are labelled with Kruskal-Wallis p-values indicating the statistically significant effect of environment or social setting. Pairwise comparisons using Dunn's post hoc tests in (c) and (d) are annotated for matching LD versus DD protocols as well as for associated semi-field versus laboratory mimic conditions, while pairwise comparisons in (e) and (f) use either the lab DD or collective semi-field data sets as control in comparison with data for non-DD lab conditions with the specified social settings. IM= individual males. IMP= individual males in population monitors. IF= individual females. IFP= individual females in population monitors. GM= group males. GF= groups of females. MSG= mix sex groups. * $p<0.05$, ** $p<0.01$, ***$p<0.001$, **** $p<0.0001$. Black and grey typeface for the * annotation in (e) and (f) indicate significant difference relative to DD and semi-field conditions, respectively. (g) Distribution of different social setting/lab mimic protocol combinations based on their divergence from matching semi-field data in normalised hyperactivity at light/dark transitions. Colours indicate environmental condition, symbols indicated social setting with groups indicated by larger symbols as annotated. Only summer morph flies were used in this analysis.

Figure 4. Impact of social setting on *D. suzukii* circadian behaviour under constant conditions. Prior to the analysed DD 23°C condition flies were entrained to a 12:12 LD 23°C cycle. (a) Double-plotted actograms collected over a 7-day period in 30-minute bins. (b) Top panel: distribution of DD rhythmicity for each social setting across three categories: arrhythmic, weakly rhythmic and rhythmic (see Materials & Methods). Bottom panel: heat map of $-\log_{10}$ (*p*-value) scores representing the outcome of pairwise 2x3 Fisher's exact tests for differences in distribution across rhythmicity categories between different social settings. Values representing non-significant and significant p-values are indicated in grey and black typeface, respectively with values representing significant differences after Bonferroni multiple testing correction indicated in bold. (c, d) Graph of circadian period length and relative rhythmic power, respectively, as a function of different social settings. Dashed line denotes 24 hour length and 1.5 relative rhythmic power. Kruskal-Wallis p-values for the effect of social setting on each of these parameters are printed on the graphs and significant pairwise differences in post hoc tests are annotated as well. IM= individual males. IF= individual females. IMP= individual males in population monitors. IFP= individual females in population monitors. VGM= virtual group males. VGF=virtual group females. VMSG= virtual mix sex groups. GM= group males. GF= groups of females. MSG= mix sex groups. Only summer morph flies were included in this analysis.

Supplemental Figure S1. Replotted example activity profiles (from Figure 1b) at 5 and 30 min resolution to illustrate the nature of acute hyperactivity in lab conditions featuring fluorescent lights versus activity profiles from semi-field conditions.



Normalised activity profiles of summer morph *D. suzukii* adults under (a) simple and combined laboratory conditions, (b) semi-field and laboratory-based mimic conditions and (c) winter and summer morph flies under April semi-field and laboratory-based mimic conditions. For ease of comparison data for summer morph flies in (c) is duplicated from (b). Profiles were collected under different laboratory and semi-field condition (columns) and in various social grouping (rows) collected over a 6 consecutive-day period. The blue line and shaded area for each profile represent the average activity counts per half hour ± S.E.M. across these 6 days. Temperature regime is displayed by orange line at top of each activity profile and is not relative to axis counts. The temperature cycle for semi-field conditions represents the average daily cycle temperature over the collection period. Temperature maxima and minima are indicated in the column captions, with additional information provided in Table 1 and 2; The Y axes solely refer to average activity counts. Laboratory light regime is 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. All flies were kept in a 12:12 LD 23°C laboratory environment prior to experimental conditions and this is particularly relevant as the entraining condition for the laboratory DD 23°C conditions (as in column 3). The number of replicates contributing to each behavioural profile are annotated in Table 3. In all cases, group-housed sets contained either 10 males or 10 females or 10 males + 10 females. Due to

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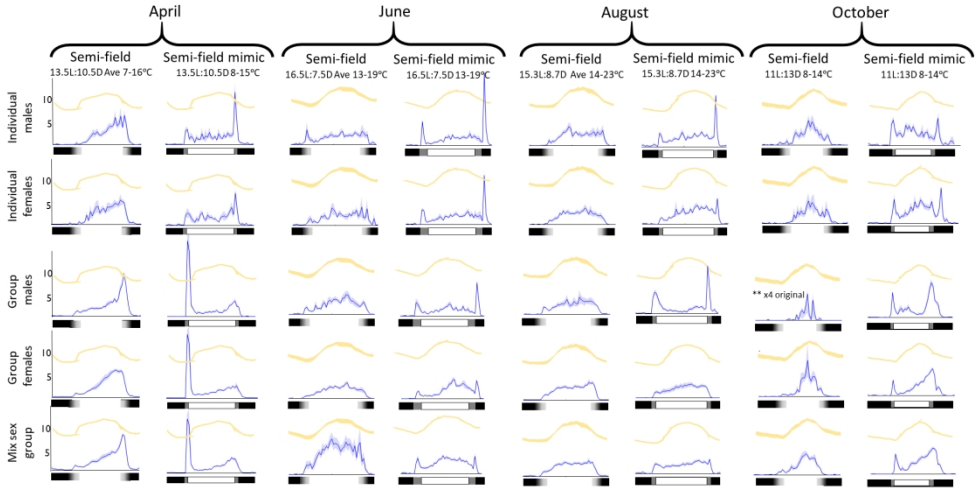

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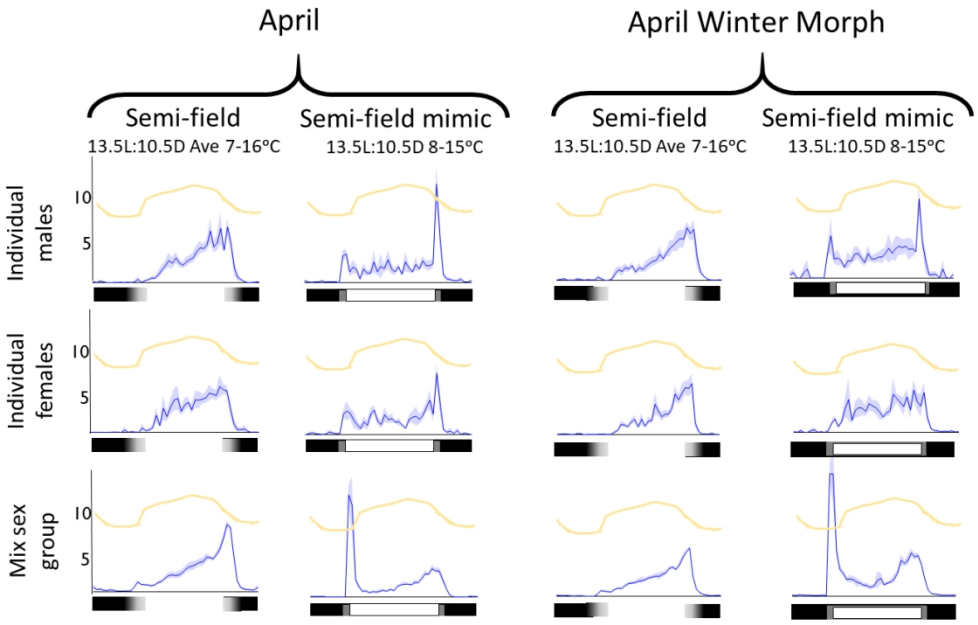

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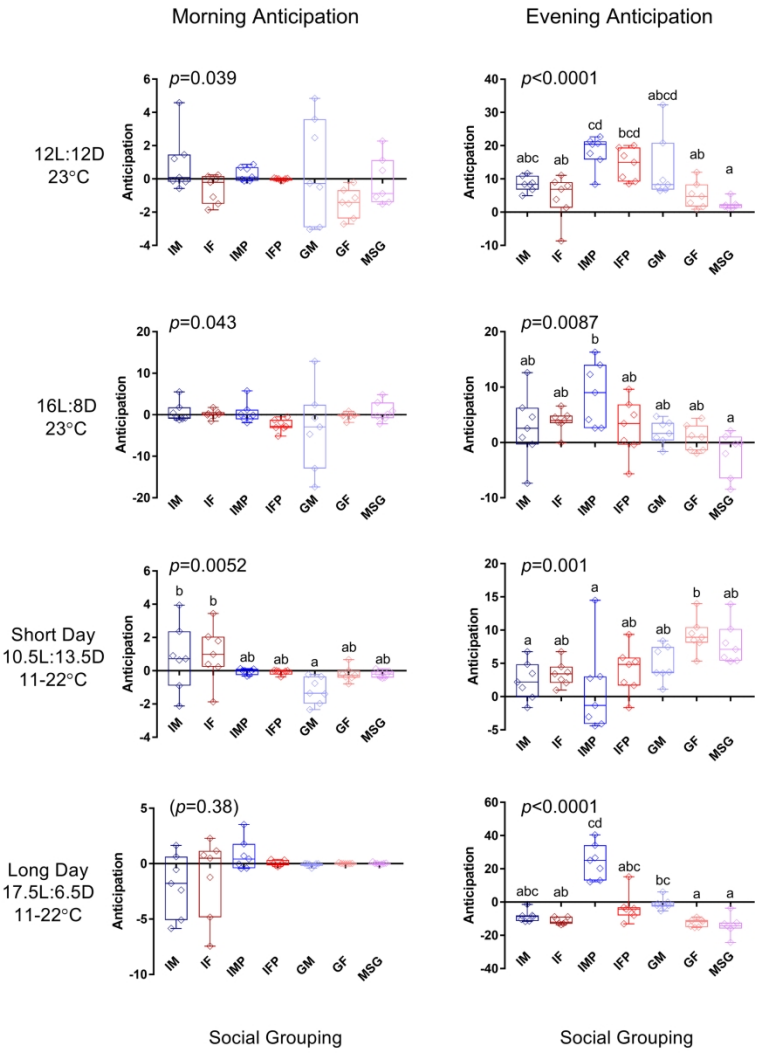

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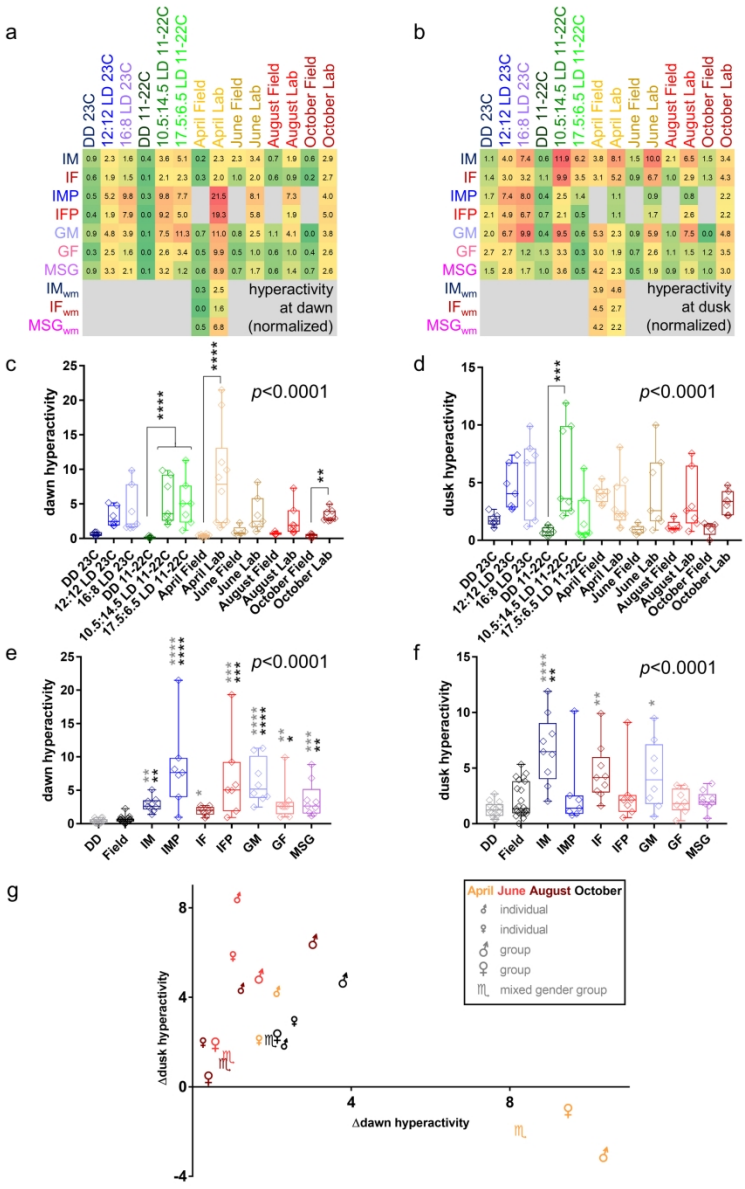

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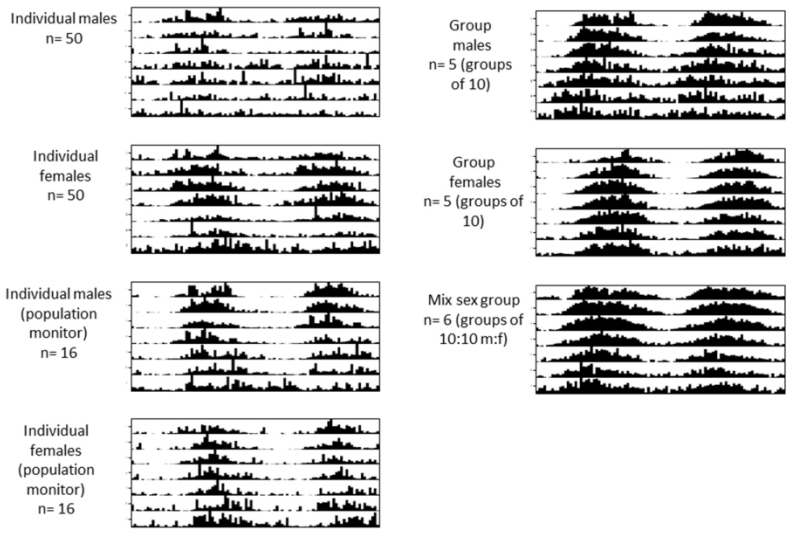

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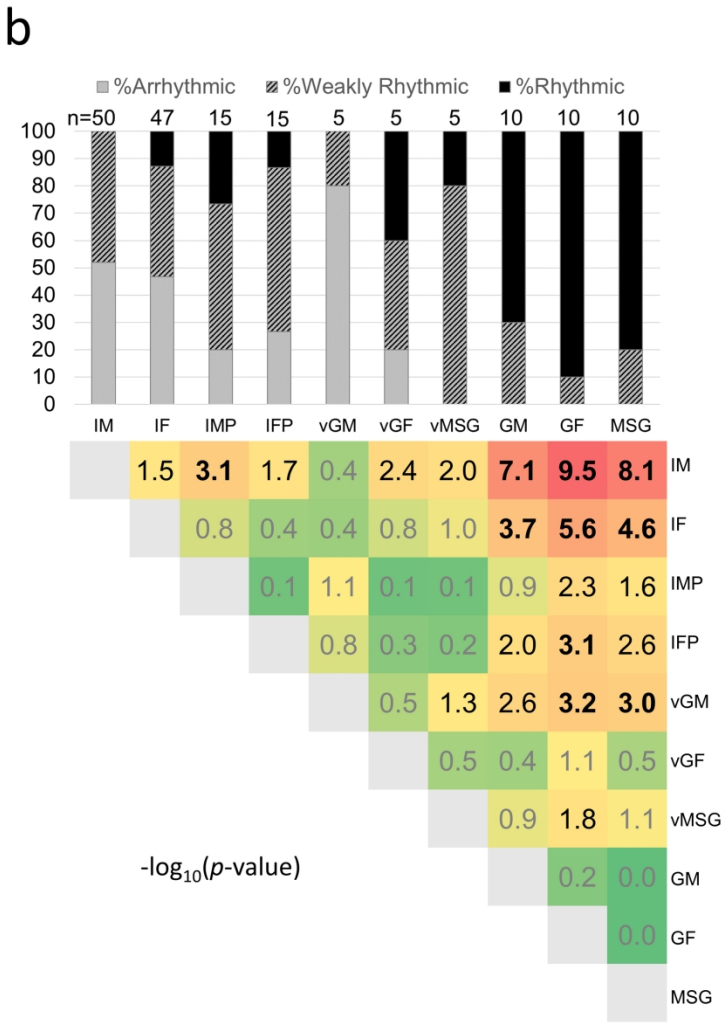
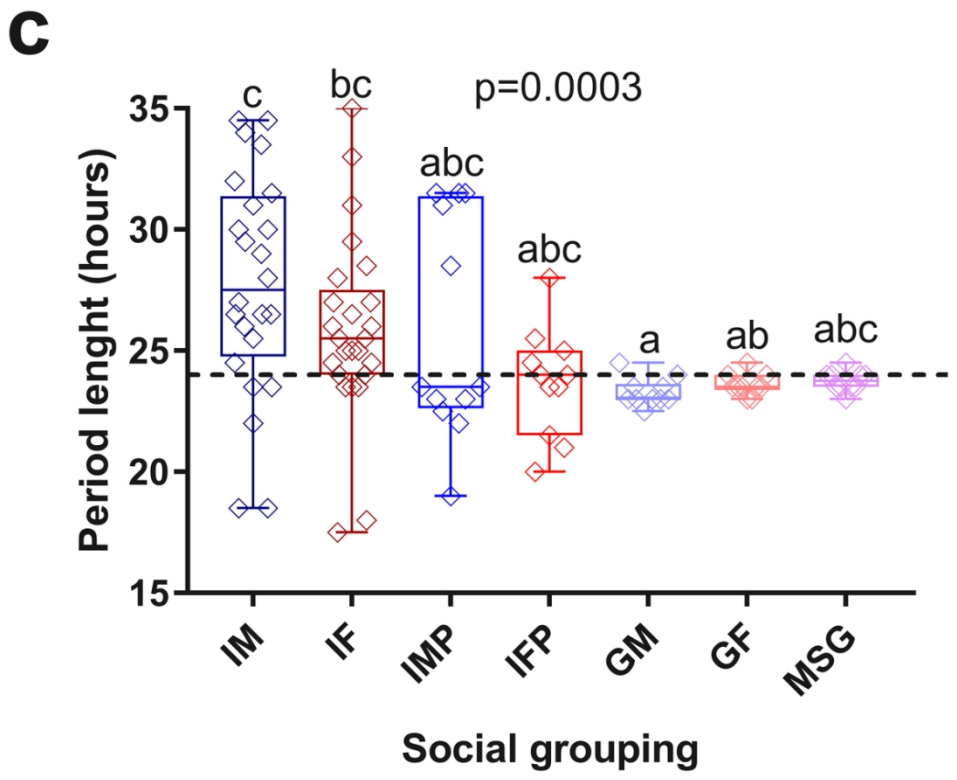
190x275mm (300 x 300 DPI)



Impact of social setting on *D. suzukii* circadian behaviour under constant conditions. Prior to the analysed DD 23°C condition flies were entrained to a 12:12 LD 23°C cycle. (a) Double-plotted actograms collected over a 7-day period in 30-minute bins. (b) Top panel: distribution of DD rhythmicity for each social setting across three categories: arrhythmic, weakly rhythmic and rhythmic (see Materials & Methods). Bottom panel: heat map of -log10 (p-value) scores representing the outcome of pairwise 2x3 Fisher's exact tests for differences in distribution across rhythmicity categories between different social settings. Values representing non-significant and significant p-values are indicated in grey and black typeface, respectively with values representing significant differences after Bonferroni multiple testing correction indicated in bold. (c, d) Graph of circadian period length and relative rhythmic power, respectively, as a function of different social settings. Dashed line denotes 24 hour length and 1.5 relative rhythmic power. Kruskal-Wallis p-values for the effect of social setting on each of these parameters are printed on the graphs and significant pairwise differences in post hoc tests are annotated as well. IM= individual males. IF= individual females. IMP= individual males in population monitors. IFP= individual females in population monitors. VGM= virtual group males. VGF=virtual group females. VMSG= virtual mix sex groups. GM= group males. GF= groups of

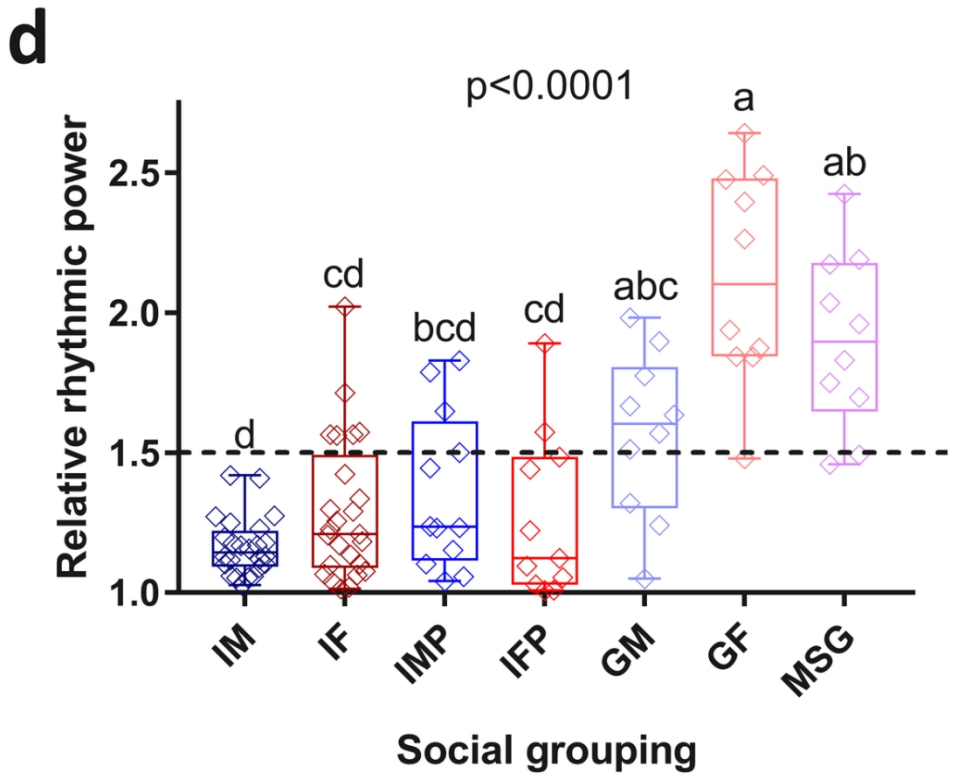
females. MSG= mix sex groups. Only summer morph flies were included in this analysis.

190x275mm (300 x 300 DPI)



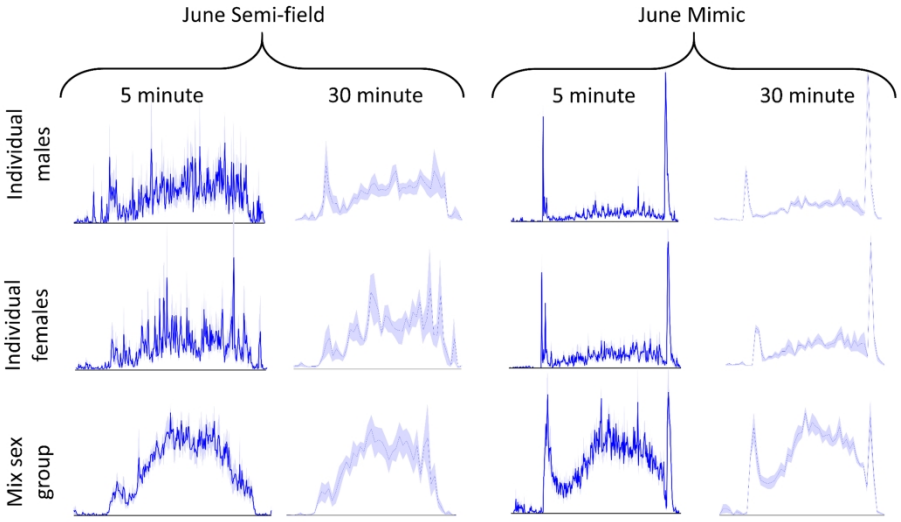
Impact of social setting on *D. suzukii* circadian behaviour under constant conditions. Prior to the analysed DD 23°C condition flies were entrained to a 12:12 LD 23°C cycle. (a) Double-plotted actograms collected over a 7-day period in 30-minute bins. (b) Top panel: distribution of DD rhythmicity for each social setting across three categories: arrhythmic, weakly rhythmic and rhythmic (see Materials & Methods). Bottom panel: heat map of -log10 (p-value) scores representing the outcome of pairwise 2x3 Fisher's exact tests for differences in distribution across rhythmicity categories between different social settings. Values representing non-significant and significant p-values are indicated in grey and black typeface, respectively with values representing significant differences after Bonferroni multiple testing correction indicated in bold. (c, d) Graph of circadian period length and relative rhythmic power, respectively, as a function of different social settings. Dashed line denotes 24 hour length and 1.5 relative rhythmic power. Kruskal-Wallis p-values for the effect of social setting on each of these parameters are printed on the graphs and significant pairwise differences in post hoc tests are annotated as well. IM= individual males. IF= individual females. IMP= individual males in population monitors. IFP= individual females in population monitors. VGM= virtual group males. VGF=virtual group females. VMSG= virtual mix sex groups. GM= group males. GF= groups of females. MSG= mix sex groups. Only summer morph flies were included in this analysis.

104x90mm (300 x 300 DPI)



Impact of social setting on *D. suzukii* circadian behaviour under constant conditions. Prior to the analysed DD 23°C condition flies were entrained to a 12:12 LD 23°C cycle. (a) Double-plotted actograms collected over a 7-day period in 30-minute bins. (b) Top panel: distribution of DD rhythmicity for each social setting across three categories: arrhythmic, weakly rhythmic and rhythmic (see Materials & Methods). Bottom panel: heat map of -log10 (p-value) scores representing the outcome of pairwise 2x3 Fisher's exact tests for differences in distribution across rhythmicity categories between different social settings. Values representing non-significant and significant p-values are indicated in grey and black typeface, respectively with values representing significant differences after Bonferroni multiple testing correction indicated in bold. (c, d) Graph of circadian period length and relative rhythmic power, respectively, as a function of different social settings. Dashed line denotes 24 hour length and 1.5 relative rhythmic power. Kruskal-Wallis p-values for the effect of social setting on each of these parameters are printed on the graphs and significant pairwise differences in post hoc tests are annotated as well. IM= individual males. IF= individual females. IMP= individual males in population monitors. IFP= individual females in population monitors. VGM= virtual group males. VGF=virtual group females. VMSG= virtual mix sex groups. GM= group males. GF= groups of females. MSG= mix sex groups. Only summer morph flies were included in this analysis.

103x88mm (300 x 300 DPI)



Replotted example activity profiles (from Figure 1b) at 5 and 30 min resolution to illustrate the nature of acute hyperactivity in lab conditions featuring fluorescent lights versus activity profiles from semi-field conditions.

275x190mm (300 x 300 DPI)