University of Huddersfield Repository

Bostock, Esta, Green, Edward W., Kyriacou, Charalambos P. and Vanin, Stefano

Chronobiological studies on body search, oviposition and emergence of Megaselia scalaris (Diptera, Phoridae) in controlled conditions

Original Citation

Bostock, Esta, Green, Edward W., Kyriacou, Charalambos P. and Vanin, Stefano (2017) Chronobiological studies on body search, oviposition and emergence of Megaselia scalaris (Diptera, Phoridae) in controlled conditions. Forensic Science International, 275. pp. 155-159. ISSN 0379-0738

This version is available at http://eprints.hud.ac.uk/id/eprint/31953/

Chronobiological studies on body search, oviposition and emergence of *Megaselia scalaris* (Diptera, Phoridae) in controlled conditions.

Bostock E.[1], Green E.W.[2], Kyriacou C.P[2], Vanin S.[1$].

1 Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, HD1 3DH, UK.

2 Department of Genetics, University of Leicester, LE1 7RH, UK.

$ Corresponding author: stefano.vanin@hud.ac.uk.

Abstract

Circadian clocks have evolved to synchronize physiology, metabolism and behaviour to the 24-h geophysical cycles of the Earth. Understanding the circadian clock mechanism could play an important role in forensic entomology because it temporally gates behaviour such as locomotor activities, feeding, mating, egg laying and adult emergence which could provide useful information for crime reconstruction. The scuttle fly *Megaselia scalaris* colonises both exposed and buried bodies, whether indoors or outdoors. Locomotor activity, oviposition and adult emergence of this species have been investigated using technologies employed in many previous *Drosophila* circadian studies. The results reported here clearly highlight the underlying role of the circadian clock in regulating the behaviour of males and females of *M. scalaris*, and show the role of light as a "zeitgeber" for clock resetting. In contrast to Calliphoridae, *M. scalaris* can reach the oviposition site and lay eggs in darkness both during the day and the night, although the number of ovipositing females is lower under subjective darkness. The number of eggs laid shows a clear circadian rhythm with much higher numbers laid during the day than during night or subjective night. In conclusion, locomotor activity and oviposition rate of *M. scalaris* is under circadian clock control with significant forensic implications.

Key words: *Megaselia scalaris*, locomotor activity rhythm, nocturnal oviposition, emergence

Introduction

Circadian clocks have evolved to synchronize physiology, metabolism and behaviour to the 24-h geophysical cycles of the Earth [1]. Though the chronobiology of diverse organisms from bacteria to humans has been studied, the preeminent circadian model both in the laboratory and under natural and semi-natural conditions is the fruit fly *Drosophila melanogaster* (e.g. [1-3]). Understanding the circadian clock mechanism could play an important role in forensic entomology (where insects are used to obtain useful information for crime reconstruction) because it temporally gates behaviour such as locomotor activities, feeding, mating, egg laying and adult emergence.

Colonisation of human cadavers by insects (mainly flies and beetles) allows a minimum post mortem interval (mPMI) to be estimated. The prevailing opinion is that flies - the first colonizers of a cadaver - are not active during the night time period, and therefore do not oviposit during this time, despite some sporadic observations of nocturnal oviposition or larviposition among Calliphoridae and Sarcophagidae [4-7]. Determining flies' ability to reach a body in dark conditions and the prevalence – if any – of nocturnal oviposition therefore becomes fundamental for the accurate estimation of the mPMI.

In addition to blow (Calliphoridae), house (Muscidae) and flesh flies (Sarcophagidae), scuttle flies (Phoridae) are also reported in cadavers. In particular, *Megaselia scalaris* Loew, 1866 (Diptera, Phoridae)*,* is known to have an important role in forensic entomology due to its ability to colonise both exposed and buried bodies, whether indoors or outdoors [8-12]. The species is widely distributed in warmer regions of the world [8], and it has been reported to be shifting its distribution to Northern regions, possibly as a result of global warming [13,14]. Although there is a wealth of information regarding the biology of this species, including non-forensic contexts [8], there is only a single field observation [15] and indoor experiment [16] reporting nocturnal activity and oviposition in *M. scalaris*. Lacking are data concerning the night-time behaviour and chronobiological profile of this species.

In contrast to previous studies which employed subjective, observational data, we have used the same infrared activity monitors (TriKinetics Inc, MA) employed in many previous *Drosophila* studies to quantify circadian locomotor profiles and pupal emergence time. The activity rhythms of *M. scalaris* were monitored over several days in LD 12:12 cycles (12 hours of light followed by 12 hours of dark) at 21°C – conditions corresponding to those found in the species' original distribution area of the Mediterranean and North America during spring and summer [8]. These observations were complemented with experiments performed for over a week in constant darkness (DD), conditions

which may occur in the forensic context, and which assay the endogenous free-running period of the *M. scalaris* clock.

Material and Methods

Megaselia scalaris flies were collected from a wild population in Pisa (Italy) in 2011 and bred in an incubator (LMS 410XAL) under LD 12:12 and a constant temperature of $21 \pm 0.5°C$.

All experiments were recorded in an incubator at $21 \pm 0.5°C$. Experiments were illuminated either with white cold LEDS under a photoperiod of 12:12 LD, or were recorded under complete darkness (DD) or constant light (LL).

Fly locomotor activity was recorded using a TriKinetics DAM5 unit (*Drosophila* activity monitor) using tubes with a length of 65 mm and a diameter of 5 mm, which allowed the flies free movement without flying (*M. scalaris* being of a size comparable to or smaller than *D. melanogaster*). Individual flies (male and virgin female) were introduced into tubes. Food was positioned at one end and the other was sealed with cotton allowing some air flux. Temperature, humidity and light intensity were recorded by a TriKinetics DEM (*Drosophila* environmental monitor). Flies were entrained to a 12:12 LD cycle for 3 days and then subjected to free running DD conditions for 7 days.

Beam crossing events were recorded in 30 min bins, and DD data were trimmed such that the final 24 hours of activity from any individual not surviving the experiment were excluded. DD data were analysed using cosinor analysis [17] to scan the best fitting period with a length between 8 and 32 hours in 0.2 hour steps. Significance was assessed by F-ratio where a significant F-value means the amplitude was significantly >0. This was achieved by randomising activity data 1000 times using a Monte Carlo approach, and accepting as significant any period that had an F-value within the upper 5% of the distribution of maximum F-values. F-value plots and double plotted actograms for each fly were also manually inspected.

Emergence was investigated using the same DAM5 monitors but with shorter tubes (5 mm width, 30 mm long) in which individual pupae were placed at the midpoint of the tubes underneath the IR beams. When the fly emerged it would activate the beam.

To determine the time of day during which *M. scalaris* oviposits, 120 mated females were placed individually into glass tubes containing food at one end and left for 12 hours, either during the day, night, or subjective day under DD (i.e when the lights would normally be on). The experiment was repeated three times.

Oviposition experiments were performed in light boxes where flies were introduced following the LD phases. Males and females flies were maintained for one day in a breeding jar before females being selected for the experiments. Commercial cat food, previously tested as a suitable substratum

for oviposition and larval growth, was used in all experiments. Eggs were counted on the deposition medium under a Leica M60 stereomicroscope. Percentage of deposition was calculated counting the number of females laying eggs divided by the total number of females. All of the experiments were triplicated.

Statistical analysis was performed using SPSS statistical software V.20. Data reported on plots are presented as average ± standard error of the mean (SEM).

Results

To understand the potential for flies to colonise a body, it is fundamental to know when individuals of either sex are active. The general pattern of activity for both sexes indicates a persistent nocturnal activity with an average of 11.08 ± 0.73 counts 30 min^{-1} for males and 10.75 ± 0.64 counts 30 min^{-1} for females (Fig. 1). During the light phase, activity increased significantly in both sexes (17.17 ± 0.44 for males and 17.04 ± 0.46 for females) but with no significant sex differences (ANOVA Time $F_{1,1004} = 62.4$, p 0.000; Sex $F_{1,1004}=0.08$, p = 0.77). In addition the locomotor activity profiles for males and females showed bimodal peaks: a well-defined Evening (E) peak in both sexes, and a Morning (M) peak that was clearer in males than in females. Both sexes responded to the light transitions with a clear burst of activity termed 'environmental masking' that reflects a startle response.
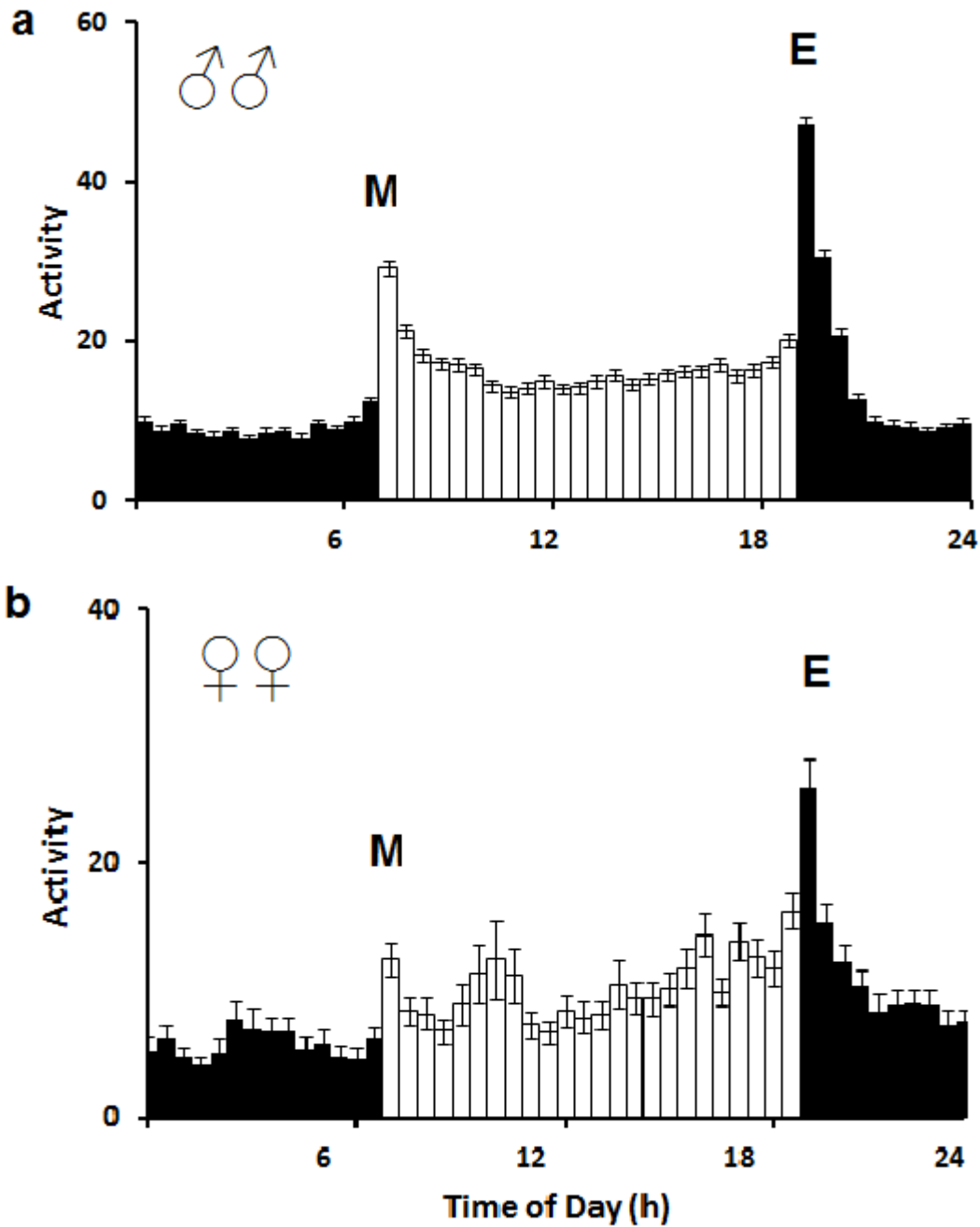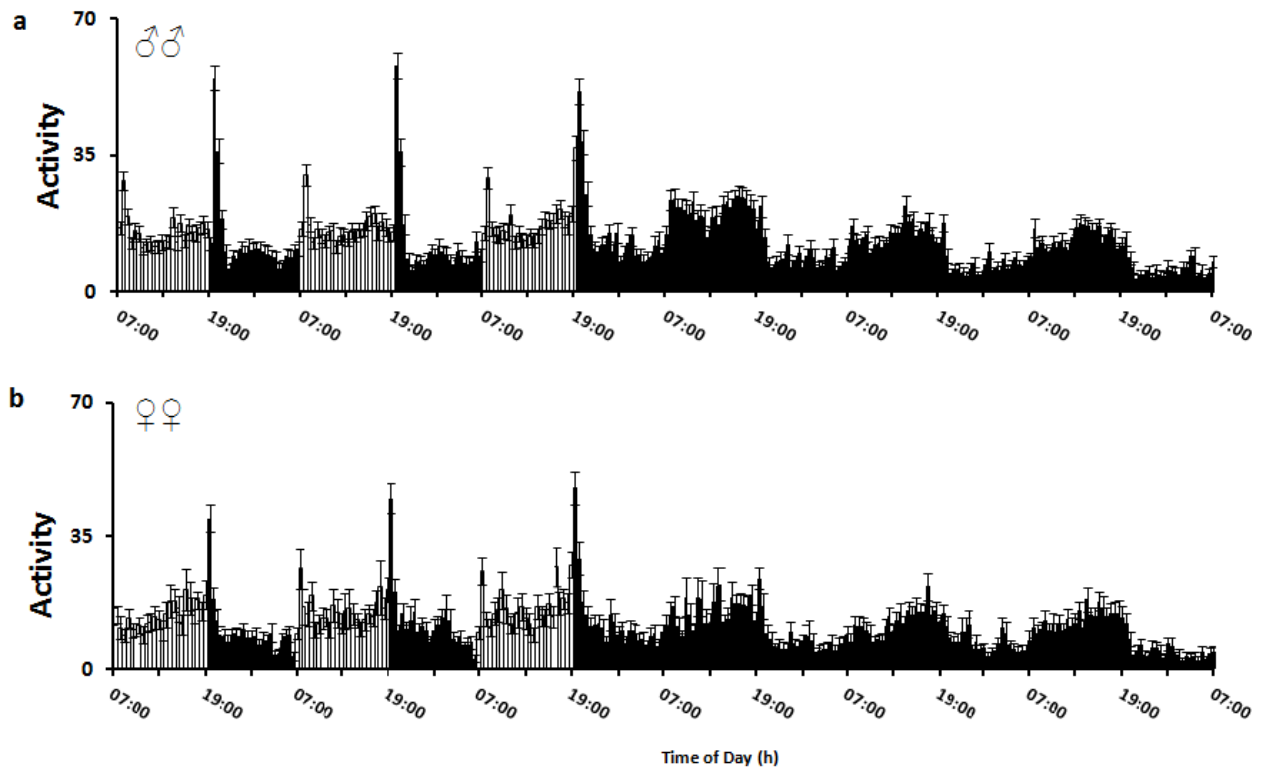
Figure 1 Average activity profiles (±SEM) of male (N = 593) (a) and female (N = 205) (b) flies under 12:12 LD photoperiod and constant temperature (20 ± 0.5°C). The activity profiles (white bars white during day and black during the night) showed bimodal peaks at morning (M) and at evening (E). The bimodal pattern is more evident in males. A persistent nocturnal activity is present in both sexes.

In both sexes, the anticipatory increase in activity before lights on was observed for males rather more than females, and both sexes show a gentle anticipation of lights off, and certainly not as dramatic as that usually observed for *D. melanogaster* (eg. [18]). As this anticipation of light transitions is driven by the circadian clock in *Drosophila*, we sought to evaluate whether the locomotor rhythms in LD cycles are under (circadian) clock regulation. Flies that had been entrained in 12:12 LD for 3 days,

were subsequently maintained in constant darkness (DD) for several days (Fig 2a, b). The activity pattern under DD revealed the presence of M and E peaks without the masking burst of activity associated with the light transition. 86.3% of male flies and 73.5% of females demonstrated a statistically significant ~24 h rhythmic pattern of activity (Fig 3).


Figure 2 Mean activity profiles (±SEM) (number of counts 30 mins^{-1}) of male (N=113) (a) and female (N=104) (b) flies under three days of 12:12 LD and three days in DD under constant temperature (21°C) (white bars during day and black during the night or in constant darkness conditions). The activity bimodal profile (M and E peaks) is maintained for both sexes in complete darkness.
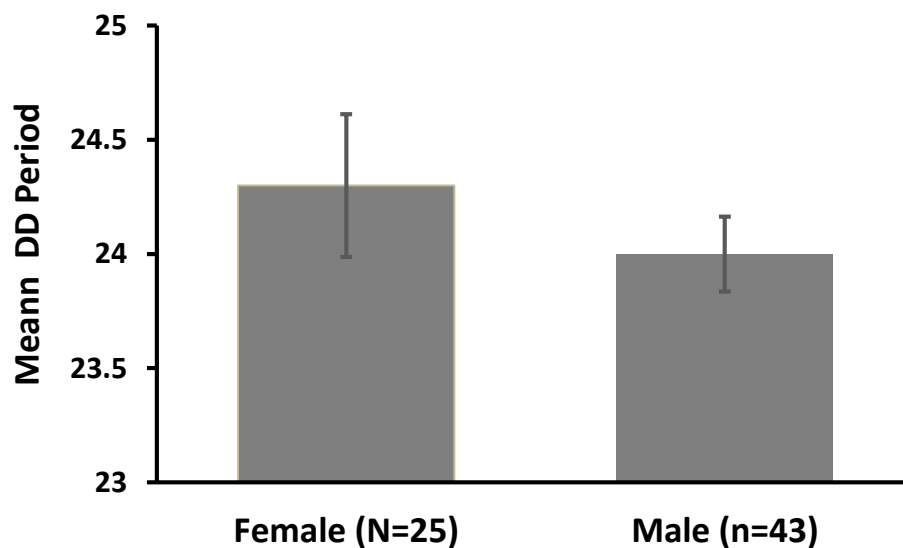
Figure 3 Average period of activity (±SEM) of males and females derived from the experiments in Fig 2. Both sexes have a period of circa 24 hours (males: 24.00 h ± 1.1; females: 24.3 h ± 1.5) with no statistical differences between males and females (t = 1.2, df = 67, p = 0.24).

We found statistically significant difference in the percentage of ovipositing females when comparing day (37.2%), night (34.5%) or subjective day (14%) (Yates' χ^2 = 10.265, df = 2, p = 0.006) (Fig. 4a).

In addition, the number of eggs per tube showed a significant difference in the three conditions (ANOVA, F = 8.55, df = 2, p = 0.01): in light condition, flies laid more eggs (17.5 +/- 0.1 egg per tube) than in dark (6.7+/-0.3 and 7.9 +/- 0.3 respectively in dark during the subjective day and dark during the night) (Tukey HSD *post hoc* p = 0.02 (LD) and 0.02 (DD). No difference were detected comparing the objective and subjective dark conditions (Tukey's HSD p = 0.90) (Fig 4b).
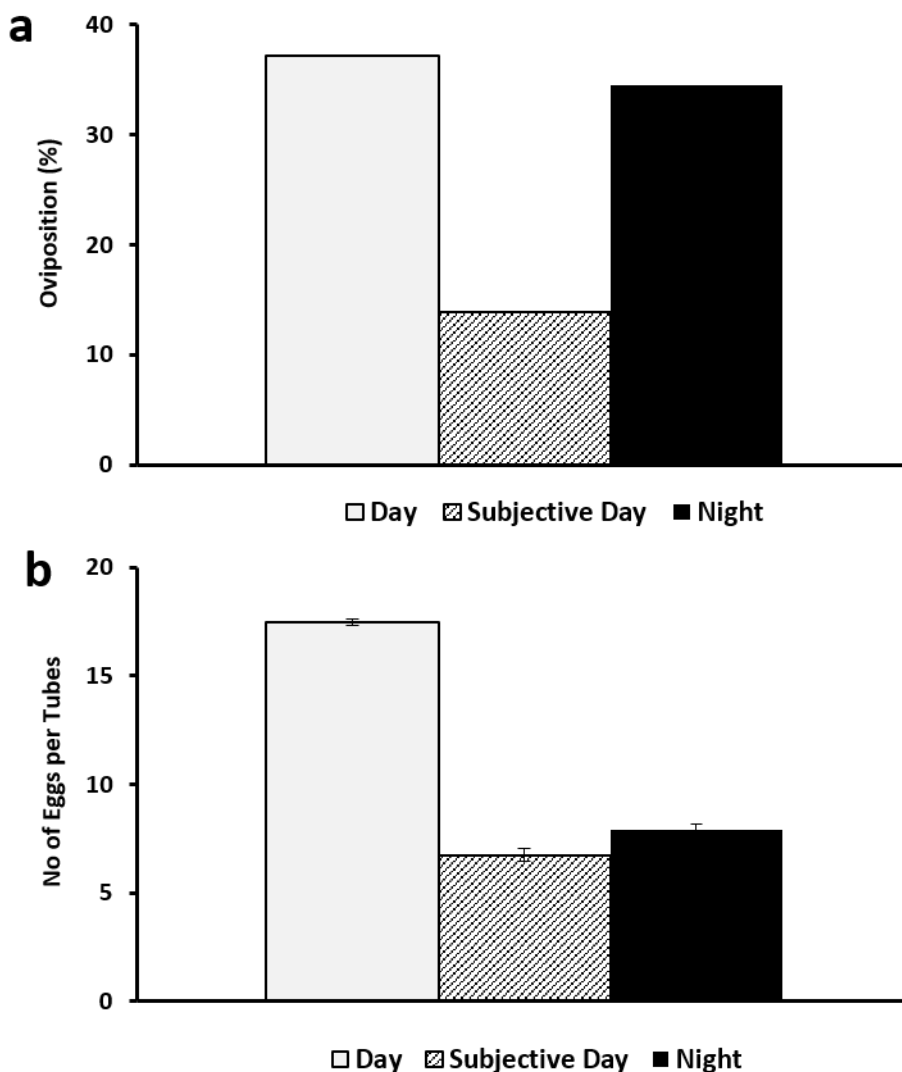
Figure 4 a) Average percentage of tubes with eggs. Flies laid eggs in all the tested conditions, a significant statistical difference was observed when comparing the number of ovipositions (Yates' χ^2 = 10.265, df = 2, p = 0.006). b) Number of eggs per tube. Flies laid eggs in all the conditions but with a statistically significant difference when comparing the number of eggs laid in light and dark conditions (p = 0.013).

To evaluate when emergence occurs, pupal emergence experiments were carried out in three different conditions: 12:12 LD, DD and LL. Pupal emergence experiments in LD 12:12 showed a significant difference between light and dark phases (Fig 5) (N = 80, p = 0.00) with the majority of the flies emerging in the light. In contrast, pupa emergence experiments in DD or LL did not show any significant differences between the subjective day and night phases (N = 88, p = 0.24 and N = 79, p = 0.26 respectively).
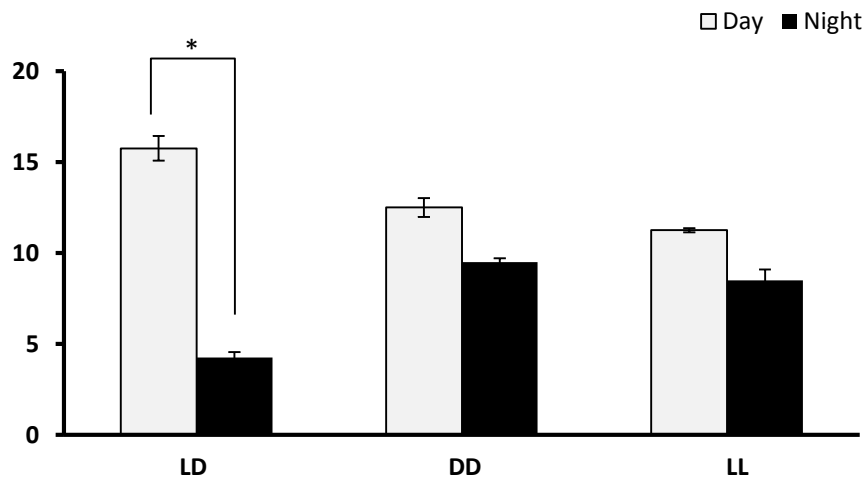
Figure 5 Mean Emergence +/- SEM (LD = 12:12, DD = continuous darkness, LL = continuous light). Asterisk indicates a statistical difference (p = 0.00) between day and night.

Discussion

The ability of insects of various taxa to colonise a body during the night or in dark conditions has been discussed and challenged by several authors (e.g.: [4,19,20]); indeed whether flies can oviposit during the night is fundamental for improving the accuracy of the mPMI estimation. We therefore set out to address if a circadian clock underlies the locomotor behaviour of *M. scalaris* – particularly under periods of prolonged darkness such as in caves [21] or coffins [9] – and finally whether *M. scalaris* will oviposit in darkness.

The time of day when an insect is active depends on its 24 hours circadian rhythm and on the environmental stimuli or "zeitgebers" (temperature and light) that entrain the clock. Among taxa of forensic interest this parameter has been investigated in species belonging to the families Calliphoridae (*Calliphora vicina, Cochliomya macellaria, Lucilia caesar, L. cuprina* and *L. sericata*) [22-28] and Sarcophagidae (*Sarcophaga argyrostoma, S. crassipalpis*) [29,30]. In all of these species locomotor activity and other characters such as adult emergence from the pupal case are reported to be clock-regulated, with higher levels of activity observed during the day, suggesting this is the time when these species are able to colonize exposed bodies. However there remain many genera that are often collected from buried remains in the forensic and archaeological context where one would assume the ability to be active in dark conditions plays an important role, but which have not yet been characterised - including the genera *Hydrotaea* and *Muscina* from the Muscidae, or genera *Megaselia*, *Conicera* and *Metopina* among the Phoridae [31].

Our results clearly show that *M. scalaris* is active both during the day and – to a lesser extent – at night, and responds directly to sudden changes in illumination (Fig 1). Furthermore, flies of this species have a functional circadian clock capable of driving rhythmic activity with a period of 24 hours after entrainment to 12:12 LD conditions and in the absence of thermal and day/night cues (Fig 2).

The ability of flies and other insects to move and reach food sources in darkness is well known not only in the forensic context (with several taxa troglophilous or troglobious), but also in many other species that show clearly nocturnal activity such as several Lepidopteran species. In the genus *Megaselia*, *M. scalaris* was reported as being active in the dark environments of coffins [9] and caves [21], presumably relying on their well-developed sensory organs to detect environmental stimuli. While many insects can both walk and fly, flies associated with carrion seem to have an altered flight or navigation ability [32] as in *C. vomitoria* and *L. sericata* (Calliphoridae).

Although flies have been reported to "enter dark places, such as cellars and chimneys, in search of carcasses during the daytime when they are normally active" [33], several authors have questioned whether oviposition can occur in darkness, suggesting that oviposition might require some degree of artificial light [19] or that nocturnal oviposition occurs as a result of unusually high night time temperatures in the presence of gravid females [4]. Because temperature and light are two of the main "zeitgebers" that influence insect metabolism, physiology and behaviour, the conclusions of Amendt et al. [4] and Zurawski et al. [19] concerning night-time can justify that light exposure or a temperature changes might encourage nocturnal oviposition.

In contrast to the data reported for Calliphoridae, we observed that the same proportion of *M. scalaris* females oviposit in darkness as in the light, but fewer eggs are laid during darkness. Emergence experiments carried out with *M. scalaris* also revealed that this character is under circadian control as already highlighted for other fly species such as *Sarcophaga argyrostoma* [29]. Clearly the circadian clock regulates several aspects of *Megaselia* behaviour even though this insect may spend a large component of its life cycle in darkness.

Conclusion

The locomotor activity of *M. scalaris* is under circadian clock control as evidenced by the maintenance of circadian locomotor cycles under constant darkness. Males and females show similar patterns of activity. However, in contrast to the data reported for Calliphoridae, *M. scalaris* can reach the oviposition site and lay eggs in complete darkness both during the day and the night, but with many more eggs laid during the day. Furthermore, even in constant darkness, the number of eggs laid at night is very similar to that laid in the dark under LD conditions, so in environments where prolonged periods of darkness are common, the number of eggs laid is sufficient to maintain the population.

The accuracy of the mPMI estimation is affected by several factors such as precocious development, light-induced variability, promptness arrival on the body, diapause and circadian rhythms. Among these factors nocturnal oviposition may affect the mPMI estimation of 10-14 hours at most latitudes. However this inaccuracy is reduced when nocturnal oviposition is more likely to happen [34]. In this contest our results allow a reduction of the inaccuracy in the mPMI estimation when *M. scalaris* is involved in body colonization, especially in indoor cases, where the species can be one of the first colonizers. In addition the demonstration that some features of *M. scalaris'* behaviour and development are under circadian clock control plays an important role in the evaluation of the mPMI

accuracy estimation. In fact, as reported by Villet et al [34], the internal clock re-synchronising the development of the insect in several points of the life cycle, reduces or eliminates the accumulating variation due to other factors.

References

[1] S. Vanin, S. Bhutani, S. Montelli, P. Menegazzi, E.W. Green, M. Pegoraro, F. Sandrelli, R. Costa, C.P. Kyriacou, Unexpected features of *Drosophila* circadian behavioural rhythms under natural conditions, Nature 484 (2012) 371-375.

[2] T. Yoshii, S. Vanin, R. Costa, C. Helfrich-Förster, Synergic entrainment *Drosophila*'s circadian clock by light and temperature, J. Biol. Rhyth. 24 (2009): 452-464.

[3] E.W. Green, E.K. O'Callaghan, C.N. Hansen, S. Bastianello, S. Bhutani, S. Vanin, J.D. Armstrong, R. Costa, C.P. Kyriacou, *Drosophila* circadian rhythms in seminatural environments: summer afternoon component is not an artifact and requires TrpA1 channels, Proc. Natl. Acad. Sci. USA 112 (2015) 8702-8707.

[4] J. Amendt, R. Zehner, F. Reckel, The nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae) in Central Europe and its forensic implications, Forensic Sci. Int.175 (2008) 61–64.

[5] B. Greenberg, Nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae), J. Med. Entomol. 27 (1990) 807–810.

[6] D. Singh, M. Bharti, Further observations on the nocturnal oviposition behaviour of blow flies (Diptera: Calliphoridae), Forensic Sci. Int. 120 (2001) 124–126.

[7] D. Singh, M. Bharti, Some notes on the nocturnal larviposition by two species of *Sarcophaga* (Diptera: Sarcophagidae), Forensic Sci. Int. 177 (2008) e19–20.

[8] R.H.L. Disney, Natural history of the scuttle fly, *Megaselia scalaris*, Annu Rev Entomol 53 (2008) 39–60.

[9] C.P. Campobasso, R.H.L. Disney, F. Introna, A case of *Megaselia scalaris* (Loew) (Dipt., Phoridae) breeding in a human corpse, Aggrawal's Internet J. Forensic Med. Toxicol. 5 (2004) 3–5.

[10] V. Bugelli, D. Forni, L.A. Bassi, M. Di Paolo, D. Marra, S. Lenzi, C. Toni, M. Giusiani, R. Domenici, M. Gherardi, S. Vanin, Forensic Entomology and the estimation of the minimum time since death in indoor cases, J. Forensic Sci. 60 (2015) 525-531.

[11] S. Reibe, B. Madea, Use of *Megaselia scalaris* (Diptera: Phoridae) for post-mortem interval estimation indoors. Parasitol. Res.;106 (2010) 637–640.

[12] M.L. Goff, Comparison of insect species associated with decomposing remains recovered inside dwellings and outdoors on the island of Oahu, Hawaii, J. Forensic Sci. 36 (1991) 748–753.

[13] M. Turchetto, S. Vanin, Forensic Entomology and Climatic Change, Forensic Sci. Int.146S (2004) 207-209.

[14] M. Turchetto, S. Vanin, Climate change and Forensic entomology. In: Amendt, J.; Campobasso, C.P.; Goff, M.L.; Grassberger, M. (Eds.)., Current Concepts in Forensic Entomology. Springer, 2010, pp. 327-351.

[15] A.M. Young, Distribution and abundance of Diptera in flypaper traps at *Theobroma cacao* L. (Sterculiaceae) flowers in Costa Rican cacao plantations. J. Kansas Entomol. Soc.;59 (1986) 580–587.

[16] A.B.S Zulaikha, R.M. Zuha, Nocturnal oviposition of the forensic scuttle fly, *Megaselia scalaris* (Loew) (Diptera: Phoridae), indoors. Egypt. J. Forensic Sci. (2017) http://dx.doi.org/10.1016/j.ejfs.2016.02.002

[17] R. Refinetti, G.C. Lissen, F. Halberg, Procedures for numerical analysis of circadian rhythms. Biol. Rhythm. Res. 38(2007) 275-325.

[18] B. Grima, E. Chélot, R. Xia, F. Rouyer, Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. Nature.431 (2004) 869-873.

[19] K.N.Zurawski, E.M.Benbow, J.R. Miller, R.W. Merritt, Examination of nocturnal blow fly (Diptera: Calliphoridae) oviposition on pig carcasses in Mid-Michigan, J. Med. Ent. 46 (2009) 671-679.

[20] T. Stamper, P. Davis, R.W. DeBry, The nocturnal ovipositing behavior of carrion flies in Cincinnati, Ohio, J. Forensic Sci. 54 (2009) 1450-1452.

[21] R.H.L. Disney, Cave Phoridae (Diptera) of Trinidad, G. Ital. Entomol. 6 (1995) 417-436.

[22] J.W. Tessmer, C.L. Meek, V.L. Wright, Circadian patterns of oviposition by necrophilous flies (Diptera: Calliphoridae) in southern Louisiana, Southwestern Entomol. 20 (1995) 439-445.

[23] P.H. Smith, Responsiveness to light of the circadian clock controlling eclosion in the blowfly, *Lucilia cuprina,* Physiol. Ent. 10 (1985) 323-336.

[24] E. Pyza, B. Cymborowski, Circadian rhythms in the behaviour and in the visual system of the blow fly, *Calliphora vicina*, J. Insect. Physiol. 47 (2001) 897–904.

[25] D.S. Saunders, B. Cymborowski, Light-induced behavioural effects on the locomotor activity rhythm of the blow fly, *Calliphora vicina* (Diptera: Calliphoridae) Eur. J. Ent. 105 (2008) 585-590.

[26] B. Cymborowski, Serotonin modulates a photic response in circadian locomotor rhythmicity of adults of the blow fly, *Calliphora vicina*, Physiol. Entomol. 23 (1998) 25-32.

[27] D.S. Saunders, S.-F. Hong, Effects of temperature and temperature steps on circadian locomotor rhythmicity in the blow fly *Calliphora vicina*, J. Insect Physiol. 46 (2000) 289-295.

[28] R.A. Ring, Photoperiodic control of diapause induction in the larva of *Lucilia caesar* L. (Diptera: Calliphoridae) J. Exp. Biol. 46 (1967) 117-122.

[29] D.S. Sauders, The circadian eclosion rhythm in *Sarcophaga argyrostoma*: some comparisons with the photoperiodic "clock", J. Comp. Physiol.110 (1976) 111–133.

[30] K.H. Joplin, D. Moore, Effects of environmental factors on circadian activity in the flesh fly, *Sarcophaga crassipalpis*, Physiol. Ent. 24 (1999) 64-71.

[31] K.G.V. Smith, A Manual of Forensic Entomology, Trustees of the British Museum (Natural History), London, 1986.

[32] J. Wooldridge, L. Scrase, R. Wall, Flight activity of the blowflies, *Calliphora vomitoria* and *Lucilia sericata*, in the dark, Forensic Sci. Int. 172 (2007) 94–97.

[33] Z. Erzinclioglu, Blowflies, Naturalists' Handbook 23, The Richmond Publishing Co. Ltd, Slough. 1996.

[34] M.H. Villet, C.S. Richards, J.M. Midgley, Limitations to the precision and accuracy of estimates of minimum post-mortem intervals using development of carrion-feeding insects. In: Amendt, J.; Campobasso, C.P.; Goff, M.L.; Grassberger, M. (Eds.). Current Concepts in Forensic Entomology. Springer, 2010, pp. 108-137.