Post-feeding activity of *Lucilia sericata* (Diptera: Calliphoridae) on common domestic indoor surfaces and its effect on development

Abstract

Developmental data of forensically important blowflies used by entomologists to estimate minimum post mortem interval (mPMI) are established under controlled laboratory conditions for various temperature ranges throughout the stages of egg, 1st - 3rd instar, puparia, and adult fly emergence. However, environmental conditions may influence the patterns of development and behaviour of blowflies, potentially impacting on these established development rates. Previous studies investigating indoor colonisation have focused on the delay to oviposition, with behaviour during the post-feeding phase in this setting often overlooked. The environment in which third instar larvae disperse when searching for a pupariation site may vary drastically at both outdoor and indoor scenarios, influencing the activity and distance travelled during this phase and possibly affecting developmental rates. This study investigated the effect of eight common domestic indoor surfaces on dispersal time, distance travelled, and behaviour of post-feeding *Lucilia sericata* as well as any resulting variation in development. It was found that pupariation and puparia length within a pupariation medium of sawdust (often used in laboratory settings) produced comparable results with that of carpeted environments (those deemed to be 'enclosed'). Non-carpeted environments (those which were 'exposed') produced a delay to pupariation likely due to increased activity and energy expenditure in searching for pupariation sites which enabled burial. In addition, the observed speed of travel during dispersal was seen via time lapse photography to be greater within 'exposed' conditions. Larvae which dispersed upon burnt laminate flooring were observed to travel faster than in all other conditions and showed the only significant variation (P=0.04) in the day of emergence in comparison to the control condition of sawdust. This study has demonstrated that wandering phase activity is affected by the environmental surface which has potential implications for estimating both the distance travelled by

dispersing larvae in indoor conditions and with further research, may be a consideration in mPMI calculations.

Key words: mPMI; forensic entomology; blowfly development; dispersal; wandering; post-feeding

1.0 Introduction

The use of necrophagous insects to establish the period of insect activity (PIA) and in turn the minimum post mortem interval (mPMI) is a long established practice within the field of forensic entomology. Blowflies (Calliphoridae) are of particular importance as they are known to be the first colonisers of remains (Anderson, 2011; Gomes *et al*, 2006; Baqué *et al*, 2015). The mPMI is established by comparing the development stage of samples recovered at a crime scene with that of known development lifecycle data which have been established under controlled laboratory conditions. The most important factor which causes alterations to the length of the development cycle is temperature, with developmental rate increasing due to the increase in metabolic rate (Anderson, 2000). For each species minimum and maximum threshold temperatures exist with development rates slowing to negligible, or death occurring, if exceeded. In order to identify any zoogeographic variation in development cycles of species, experiments have been conducted to establish development rates in different countries under various temperatures to identify phenotypic plasticity (Tomberlin *et al*, 2011). When recovering larvae from a crime scene, estimation of mPMI can be established, for example, by calculating the accumulated degree hours (ADH) using crime scene data and established reference sets for the species recovered. However, the period of development for which this becomes more difficult occurs when larvae have finished feeding at the third instar and begin to search for a site to pupariate. This period is known as the wandering stage (also called the migratory or post-feeding stage) and is known to vary dramatically between species (Greenberg, 1990). Larvae of *Lucilia sericata* can be identified as post-feeding/dispersing third instars when they have left the source of food on

which they have been feeding, and the crop (which is visible through the cuticle of the larvae) begins to empty. When wandering, the larvae search for suitable sites away from light which ideally allow burial in order to avoid predation and desiccation (Gomes *et al*, 2007). It has also been shown that the heavier the larva, the deeper it will bury to pupariate (Gomes *et al*, 2007). It is the inability to bury at a suitable pupariation site which appears to be a key factor in the distance of dispersal (Greenberg, 1990).

Controlled laboratory testing to establish development data for species involves experimental set-ups which provide a pupariation site within close proximity to the food source. However, the same may not be true in the field where the ground may be hard and unyielding both indoors and outdoors, forcing larvae to travel a greater distance to locate a site for pupariation. Effects of variation in dispersal time/behaviour in differing environmental conditions on development time are not considered within the calculation of ADH (Arnott & Turner, 2008; Mai & Amendt, 2012) and few studies have been conducted into factors effecting the post-feeding phase. The time spent dispersing is often combined with third instar development data in ADH calculations (Arnott & Turner, 2008), however no current guidelines exist for estimating this time in relation to mPMI estimation. It has been observed that there is an energetic cost to dispersal with a greater distance of travel/time spent dispersing found to be negatively correlated with both the weight of larvae (Gomes & Von Zuben, 2005) and that of adult flies, resulting in an increase to ADH (Arnott & Turner, 2008). However, Mai and Amendt (2012) found that overall development time was only affected once larvae had been dispersing for a period >24h resulting in smaller adults due to increased energy consumption. Additionally, they noted that even when in an unfavourable environment, larvae began to pupariate after an extended period of time despite being unable to locate a suitable site, potentially due to a build-up of paralysins (Chiou, 1998).

It has been stated that a single value cannot be applied to the time spent post-feeding as this is heavily dependent on the environment (Arnott & Turner, 2008; Anderson, 2000). However, some discussion of the effects of unfavourable post-feeding conditions in indoor environments (such as hard wood floors) has been considered in relation to dispersal. Anderson (2011) studied the decomposition rates of six pig carcasses,

placing three outside and three within different rooms of an empty house which contained hard surfaced floors throughout. Dispersal distances were much greater indoors, with puparia being discovered throughout all areas of the house including the basement and heating ducts by day 32 as larvae searched for a suitable site to pupariate. This supports previous research that, depending on substrate, *Lucilia* larvae may travel 3-100 feet before beginning to pupariate (Green, 1951; Cragg, 1955, Greenberg, 1990; Turpin *et al*, 2014). There has been speculation of how particular types of surface may affect dispersal movement, for example Arnott & Turner (2008) have suggested that carpet is likely to impede movement and require greater expenditure of energy. However, observations of blowfly development on carrion in indoor environments have usually been focused on the delay in colonisation of remains, seen to be between 1-4 days (Reibe and Madea, 2010a; Pohjoismäki *et al*, 2010) rather than effects on dispersal time.

To the best of our knowledge, there have been no publications relating to the effects of indoor surfaces on dispersing larvae and consequent effects on development. If the environment leads to larvae spending more time in the post-feeding stage this could impact on the mPMI, particularly when considering indoor scenarios. Additionally, adjustments to isomorphen-diagrams may need to be considered, as although the post-feeding stage is difficult to measure against linear models (Baqué *et al,* 2015) and samples should be allowed to pupariate before measuring (Grassberger & Reiter, 2001), any factor which may considerably increase the time spent in the post-feeding stage may have an effect upon these estimations. Consideration may also need to be taken with regard to the search radius for entomological evidence, currently recommended as 2-10m including the search of nearby rooms for indoor locations (Amendt *et al*, 2007) with the potential for this to increase to 20-25m (Lewis & Benbow, 2011). Quantifying the variation in the time spent dispersing and dispersal behaviour due to surface characteristics will help support or modify these recommendations for a wider range of scenarios and provide further information regarding larval behaviour in a domestic setting.

2.0 Methods

99 *2.1 Species of study*

100 *Lucilia sericata* (Meigen, 1826) has been widely studied and appears to be one of the most active species in
101 the wandering phase, often moving away from a food source or remaining active for a period of time despite
102 a suitable pupariation medium being available (Greenberg, 1990; Mai & Amendt, 2012). It was noted by
103 Anderson (2000) that wandering behaviour was so strong, larvae climbed the walls of a glass jar and were
104 difficult to restrain within an experimental environment. Given that this species has an active wandering
105 phase (Cragg, 1955; Greenberg, 1990) and has been shown to colonise remains discovered indoors
106 (Pohjoismäki *et al*, 2010; Reibe & Madea, 2010a; Reibe and Madea, 2010b; Anderson, 2011) it was chosen as
107 a suitable species to investigate the effects of indoor post-feeding conditions on larval behaviour and the
108 occurrence of pupariation.

109

110 *2.2 Selection of post-feeding larvae*

111 The colony of *L. sericata* were obtained from Blades Biological Ltd as 3rd instar larvae and emergence of
112 adults within a temperature controlled laboratory (21^{o}C) was observed between 19-21 days from oviposition
113 in accordance with Anderson (2000). Rearing occurred within a 60x60x60cm BugDorm-2120 Insect Rearing
114 Tent (obtained from www.bugdorm.com) containing pig's liver for oviposition and a water/sugar mix.
115 Development occurred within this colony until larvae had passed the third instar and had entered the post-
116 feeding stage. Confirmation that larvae had entered this stage was performed using the recommended
117 factors suggested by Mai & Amendt (2012). Post-feeding was therefore demonstrated by larvae reaching the
118 minimum age of development according to Greenberg & Kunich (2002) (observed through primary
119 environment testing), the offering of an additional food source which was untouched, and the display of a
120 visibly empty crop. As dispersal is known to begin during the night (Green, 1951), checks for post-feeding
121 stage were made in the mornings. Gomes *et al,* (2007) showed that differences in burial behaviour could
122 occur as a result of variation in larval mass and Berrigan & Pepin (1995) found larval mass to be correlated
123 with speed and subsequent distance travelled. Therefore selected larvae were individually measured to

ensure all were the same starting mass of 0.05g (weighed individually) and measured 12mm in length (when expanded) at commencement of study.

2.3 Experimental set-up

Larval dispersal can be studied in two ways, radial or unidirectional, of which radial more closely mimics the behaviour encountered in a natural environment (apart from occasional occurrences of en masse migration such as that described by Lewis & Benbow (2011) in which radial migration is delayed). However, for simplicity and to enable multiple surfaces to be studied at the same time, a linear arrangement was adopted for each environment. Eight lanes each measuring 1m in length with a width of 40mm and 40mm high were constructed within a single framework (Fig. 1). This structure was composed of unplasticised polyvinyl chloride (uPVC) with the base constructed from two laminate flooring panels of which one was placed face up showing a wood grain effect and the second placed face down to display the smooth backing. Tape measures were fixed to the top of the outer and centre lane walls to allow rapid measurements of distances. The surface of each lane was composed of one of eight common domestic surface types; short pile (10mm) carpet (SP), long pile (20mm) carpet (LP) of which both were composed of synthetic fibres, ribbed matting (RM) similar to that used for doormats and entrance ways into buildings with a 6mm tread, smooth flooring (SF) created using the reverse side of a laminate panel, laminate flooring (LF) composed of fibre board materials and melamine resin, laminate flooring containing obstacles (LO) which were made of a synthetic rubber compound for moulding into the lane measuring 20mm in height, burnt laminate flooring (BL) to mimic surfaces at possible fire scenes and laminate with sawdust (LS) to a 10mm depth as the control. Laminate flooring was burnt using a blowtorch to cause charring damage to the surface (to a depth of approximately 1mm) without breaking up the wood into pieces and no ash/residue remaining in the lane after burning. Due to the known persistence of *L. sericata* to escape from housed conditions, the experimental environment was covered in plastic wrap (LPDE) perforated with pin holes throughout the 1m lanes to allow air flow. The plastic wrap was secured to the top of each lane using silicon to prevent gaps

149 which would allow transfer of larvae between lanes (see Fig. 1b & 1c). Multiple test runs were performed to
150 ensure wandering larvae could neither escape, nor transfer between lanes. The framework was contained
151 within a temperature controlled laboratory of 21°C with an even light distribution to prevent singular
152 negative phototaxis. Chemical trails have been shown to influence movement of larvae in replicates (Arnott
153 & Turner, 2008; Boulay *et al*, 2015), however it was not possible to exclude this from the study entirely as
154 not all conditions could be thoroughly cleaned or removed between replicates due to the nature of the
155 experimental set up. However, direction of travel was not a consideration within the current study.

156

157 *2.4 Measurements during study*

158 Larval behaviour and development were observed with the assistance of time lapse photography to monitor
159 movement along the experimental lanes and record both quantitative data and qualitative observations
160 (Persohn, 2015). A Nikon D7000 camera, whose electronic controls include an interval mode (Barnett *et al,*
161 2011) was mounted on a Benbo Classic No. 1 Tripod Kit 1m directly over the centre of the experimental
162 framework (Fig 1a). To avoid disturbance to the time lapse photography due to battery changes, the camera
163 was powered by a mains supply. A Nikon 12-24mm zoom lens was used, set at 12mm (equivalent to 18mm
164 with a full-frame 35mm camera) and lighting was provided by a Bowens Gemini Esprit 250 Studio Flash
165 (Bowens International Limited 2007) connected to the camera set at f/11 and ISO 100. This light was
166 sufficient to overcome the room lighting (LED daylight lighting) which caused a glare on the plastic used to
167 secure the larvae in the run. The lighting was angled to minimise glare to a small section of the experimental
168 area (Fig. 1b). Images were captured every 15 seconds over the time span of the experiment using Nikon
169 RAW format NEF and then converted to jpg at 4928 x 3264 pixels, see Fig. 1b. This resolution allowed for the
170 possible need to digitally zoom in to specific areas of the experiment, see Fig. 1c 1920 x 1200 pixels. The
171 series of photographs were then assembled into a video using iStopMotion by Boinx
172 https://www.boinx.com/istopmotion/mac/ and loaded into Screenflow
173 https://www.telestream.net/screenflow/overview.htm to mask out the top and bottom of the image area

(so that only the lanes were visible) then exported in .mov and .mp4 format. Note that for convenience, the resolution has been decreased for the .mp4 file available online (supplementary material). Recordings were made until pupariation of the final larvae (see table 1) at which point, all experimental samples were removed and placed into separate containers to allow development to adult stage. Unfortunately it was not possible to allow full development within the experimental lanes due to difficulty in removing adult flies at the end of testing. Puparia were placed into empty plastic 35ml hinged pots containing air holes in the lid with no added pupariation medium and kept within the same temperature and light controlled environment. All samples were kept in this secondary environment until development of the adult fly at which point samples were placed into a -20^{o}C freezer and measurements taken immediately. The weight and length of puparia were recorded on removal from the experimental set up and, additionally, as the length of dispersal has been shown to have an effect on emerging adult flies (thought to be due to the energy expenditure during the post-feeding stage (Mai & Amendt, 2012)), measurements were taken of the fly weight and length (anterior to posterior), wings (basicosta to wing tip), thorax (postsutural area from dorsal view), and the widest part of the abdomen to record any observable differences in adult flies. At the start of each replicate, 10 or 15 larvae (initial studies commenced with 10 larvae per lane which was later increased to 15 as no issue with crowding was observed) were placed at one end of the eight lanes (always the same starting location) and movement observed. Eight replicates were completed of which two monitored 10 larvae in each lane and six monitored 15, providing measurements from 110 larvae in each mock environment with mortality rates recorded. More detailed observations of the initial movement of larvae were made in a single trial over a one hour period from commencement of one of the n=15 larvae studies. Lanes were divided into 20cm sections with use of the fixed tape measures and the number of larvae within each 20cm location from the initial starting point were recorded every 5 minutes up to 1 hour. When pupariation was reached, the location from which each puparia was recovered in each replicate was also recorded.

3.0 Results

3.1 Behavioural observations

When placing the larvae onto each surface, there were marked differences in behaviour which were observable immediately dependent on surface type. Larvae placed onto the long pile (20mm) carpet began to bury almost immediately, resulting in 49% of puparia found within 10cm of the starting location. The remaining puparia were recovered at a distance no greater than 40cm with 12% in 11-20cm, 20% 21 – 30 cm and 18% 31- 40cm (with 1% of larvae not surviving). In comparison, larvae placed on all other surfaces dispersed throughout the full length of the lane, demonstrated in Fig. 2. The resulting distributions from the single one hour observation are visualised in Fig. 3 which demonstrates that larvae within the long pile carpet dispersed between the 0-40 cm ranges within this time; therefore it is likely that dispersal ceased after 1 hour due to the locations of puparia recovered.

For all surfaces except long pile carpet, short pile carpet, and ribbed matting, larvae were observed to have moved to the end of the 1m run within 10 minutes. Using observations taken from time lapse photography as an indication of orthokinesis, overall speed of travel was observed to be much greater in those conditions classed as 'exposed' (ribbed matting, smooth flooring, laminate flooring, laminate flooring containing obstacles, and burnt laminate flooring) than those in the 'enclosed' conditions (short pile (10mm) carpet and long pile (20mm) carpet). The greatest rate of movement appeared to be within the burnt laminate flooring lane; which can be observed in the time lapse video provided as supplementary material.

3.2 Developmental analysis

The measurements taken from all puparia and adult flies in all eight experimental conditions are summarised in Table 1. Due to non-normality, a Kruskal-Wallis test was performed using R (R Core Team, 2016) on each of these variables, with surface type as the independent variable. Where significant differences were indicated ($P \leq 0.05$), pairwise Wilcoxon Signed Rank tests were performed using Holm corrections to compensate for the increased risk of type I errors. Surface type did not have any significant effects on fly

length, wing length, abdomen width, or fly weight, with only thorax width producing a significant variation between the laminate floor and the long pile carpet ($P<0.01$). However, as no effect was seen between these conditions within other measurements of the emerging flies, no inference of the effect of substrate on the adult characters measured can be made from this observation. Puparia weight and length as well as day of pupariation and day of emergence did show some significant variations through pairwise comparison. A summary of this analysis can be seen in Fig. 4 which presents the resulting P values of the significant comparisons.

Puparia weight showed a significant difference between long pile carpet and ribbed matting ($P=0.04$) and puparia length was significantly different between the laminate containing sawdust and all other conditions except the two carpeted environments. However, a value of $P=0.05$ (equal to the confidence level) was observed between laminate containing sawdust and burnt laminate flooring so some caution must be taken with regard to this finding. Puparia length in the long pile carpet was also significantly different from the lengths in the smooth wood, ribbed matting, laminate floor, and laminate with obstacles surfaces. In addition, puparia length was also significantly different between the laminate floor and short pile carpet surfaces. Despite these findings at the puparia stage, no resulting effect was seen within the emerging fly.

Puparia measurements – key findings

- *Puparia weight was mostly unaffected by surface conditions.*
- *Puparia length in the condition of laminate containing sawdust was greater than all 'exposed' conditions (SW; RM; BL; LF; LO).*
- *Puparia length in the condition of long pile carpet was greater than all 'exposed' conditions except the burnt laminate.*

Day of pupariation showed significant variation between 'enclosed' and 'exposed' environments with the exception of the pairwise comparison between long pile carpet and ribbed matting surfaces. A significant difference in day of pupariation also occurred between the burnt laminate floor compared to all other

surfaces except the laminate floor. Additionally, the day of pupariation was significantly different between the ribbed matting and laminate floor surface. The day of emergence was generally consistent between conditions with the only significant difference (P=0.04) observed between the laminate containing sawdust and the burnt laminate flooring.

Day of pupariation – key findings

- *Pupariation occurred sooner in the 'enclosed' conditions except between the long pile carpet and ribbed matting.*
- *A delay to pupariation was observed within the burnt laminate floor environment compared to all other conditions except the laminate floor.*
- *Day of emergence was only statistically different between conditions of the burnt laminate floor and laminate containing sawdust, with burnt laminate emergence being slightly delayed.*

4.0 Discussion

4.1 Larval activity

The results of these investigations indicate that behaviour of wandering larvae is affected by the surface over which they are travelling with preliminary findings outlining two major groupings – a surface enabling burying behaviour ('enclosed') and smooth surfaces preventing burial ('exposed'). The current approach of utilising sawdust in laboratory settings, although not allowing burial within the substrate itself, appears to mimic the burial behaviour sufficiently to allow larvae to pupariate and is seen here to imitate some carpeted surfaces (with a minimum depth of 10mm) in an indoor setting with regards to subsequent intrapuparial development. This finding is despite the fact that through time lapse photography it was observed that larvae continued to wander for a considerable period of time through sawdust as opposed to larvae within carpet which remained static once buried. This may indicate that sawdust did not allow enough coverage from light to halt dispersal or perhaps that sufficient contact of the surrounding substrate with the

cuticle was not achieved. The long pile carpet provided favourable conditions for pupariation (Green, 1951) providing an area which promoted burrowing behaviour and appeared to be of a sufficient depth to halt further dispersal. This may be due to the depth of this carpet (20mm) providing sufficient coverage which was not achieved as efficiently in the short pile carpet in which larvae were more active. A previous factor discussed by Arnott & Turner (2008) was the immediate availability of a suitable pupariation site in laboratory conditions which is not realistic of indoor or outdoor environments. The current study indicates that while sawdust reflects surfaces that are 'exposed' in relation to larval activity (Fig. 3), sawdust also reflects surfaces that are 'enclosed' and facilitate burial in relation to intrapuparial development (Fig. 4c) despite being moved to a secondary environment upon pupariation. This may therefore indicate that surface environment in the dispersal and early puparial period continued to effect development throughout the intrapuparial period.

4.2 Distance travelled

The distance travelled within the post-feeding phase, and therefore the time taken to reach pupariation, is dependent on the environment encountered by larvae and is a factor which may need to be considered by the entomologist when investigating the path travelled to reach the pupariation site. Previous authors make recommendations on the distance which should be searched from a body to locate possible larvae (Amendt *et al*, 2007; Lewis & Benbow, 2011) and identify potential pupariation sites. It has been seen that these recommendations are transferable to indoor conditions containing smooth floors which may cover not only alternate rooms but possibly different levels within the property. Conversely, in a carpeted environment of at least 10mm pile, it may be unlikely that larvae have travelled a great distance from the body before pupariating and therefore entomological evidence may be located within a short distance of the feeding source (possibly no further than 1m). This may have some inference of speed travelled over certain outdoor conditions as Nuorteva (1977) noted that larvae travelled slower over a moss covered floor; the behaviour on laminate/hard soil and moss/carpet may therefore be comparable.

297 Although few studies on indoor dispersal have been conducted, behavioural observations are noted within
298 some cases for both human and non-human studies. Sandford (2015) discussed three cases of entomological
299 evidence present on pets which had died within a property after their owners. A unilateral movement of
300 larvae was noted within the smooth surfaced environment, with dispersal observed along a base board.
301 Additionally within a bathroom, puparia were discovered within a pile of clothes outside of the room
302 through the dispersal of larvae in search of a suitable pupariation medium. The flooring type should
303 therefore be the first consideration when contemplating possible locations of entomological evidence which
304 may have reached third instar/post-feeding stage.

305

306 *4.3 Developmental variation*

307 Despite differences in the levels of kinesis between all of the mock environmental conditions, the combined
308 data analysis showed little significant variation in the size and weight of emerging adult flies. Although
309 'exposed' conditions saw a greater level of activity, this apparent increase in energetic cost did not have the
310 same resulting effect on adults as has been observed in previous research. Pupariation did however occur at
311 a slightly delayed rate due to unfavourable conditions. Activity was seen to cease within the first hour for
312 those larvae on long pile carpet as opposed to those on smooth surfaces which were active up until the point
313 of pupariation, however puparia weight between conditions was fairly consistent. Significant variation in
314 puparia weight was only seen between ribbed matting compared to long pile carpet with those in the
315 carpeted surface significantly heavier ($P=0.04$) which may be due to a lack of activity in this environment.
316 Surprisingly, although little variation was seen in the weight of the puparia, puparial length was seen to vary
317 between some of the environmental conditions. Most notably, this was seen to occur in laminate containing
318 sawdust and all other conditions except the two carpeted environments; a variation between 'enclosed' and
319 'exposed' conditions (but with caution taken as to one finding of $P=0.05$). This would indicate that common
320 laboratory conditions using sawdust mimic that of an environment in which wandering larvae are able to
321 bury with respect to the resulting effect on puparia length. However, larval activity in sawdust is similar to

that seen in other 'exposed' conditions and therefore does not mimic carpet with regards to possible dispersal distance. Variation is however seen in those environments which are more unyielding and prevent the identification of a suitable pupariation site. Additionally, variation in length was seen to differ significantly between long pile carpet and the following conditions which presented puparia of a smaller size: smooth wood, ribbed matting, laminate floor, and laminate containing obstacles again indicating that increased motility increased energy expenditure and therefore produced smaller puparia. Unquestionably, the most dramatic observation in behaviour and observed speed of travel was seen within the burnt laminate floor condition but surprisingly, no significant variation on puparial or fly measurements was observed. This condition did however present significant findings in day of pupariation and demonstrated the only significance in day of emergence, which was seen between the laminate containing sawdust and burnt laminate (P=0.04). This may indicate that the amount of time spent travelling impacted on the development in the puparial stage and delayed emergence. There may be scope here for research to identify a chemical stimulus produced by the burnt wood which altered dispersal behaviour rather than the surface texture alone.

5.0 Conclusion

The study demonstrated that dispersal in sawdust induced a subsequent intrapuparial development similar to that caused by dispersal in carpet of at least 10mm pile depth. Previous assumptions of reduced dispersal have been demonstrated within carpeted conditions as no puparia were discovered beyond 40cm in long pile (20mm) carpet and only 24% of puparia discovered beyond this distance in short pile (10mm) carpet. This knowledge may potentially influence the search strategy employed at indoor scenes depending on the surface encountered. The immediate availability of a suitable substrate was shown through Kruskal-Wallis testing to produce a significant result with respect to day of pupariation, however the ranges observed here could be comparable to that which is naturally observed and may not be of a significant level that would impact upon current time of death estimations. The variations observed in the current study with regard to

development (especially in relation to puparia length) and larval activity are worth noting and greater research is required within this area to investigate any possible implications that surface type may have upon development. Due to puparia being moved to a secondary environment, further study is also required to examine if exposure to these surfaces for the full intrapuparial period would have any further effect upon development. Additionally, the transferability of these results to a more natural environment allowing radial dispersal is required as well as study of a greater range of carpeted surfaces to examine activity when pile depth is <10mm to identify at what point dispersal is affected. Possible advice and guidelines may therefore be able to be generated for the examination of indoor scenes in relation to the type of surface encountered.

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458 Table 1: Average developmental measurements of 110 larvae (adjusting for mortality rates) under the experimental conditions

Lane number and surface	Mortality rate of larvae (%)	Mortality rate of puparia (%)	Puparia length (mm)	Puparia weight (g)	Fly length (mm)	Fly weight (g)	Wing length (mm)	Thorax width (mm)	Abdomen width (mm)	Day of pupariation	Day of emergence
1. Smooth wood (SW)	0.0	3.6	7.8 (SD±0.59)	0.042 (SD±0.005)	8.6 (SD±0.65)	0.033 (SD±0.005)	7.2 (SD±0.53)	3.09 (SD±0.25)	3.63 (SD±0.31)	9.7 (SD±0.44)	20.1 (SD±1.45)
2. Short pile carpet (SP)	0.0	8.2	7.9 (SD±0.65)	0.045 (SD±0.005)	8.5 (SD±0.69)	0.033 (SD±0.004)	7.3 (SD±0.58)	3.09 (SD±0.29)	3.60 (SD±0.34)	9.4 (SD±0.51)	20.2 (SD±1.48)
3. Long pile carpet (LP)	0.9	6.4	8.1 (SD±0.51)	0.043 (SD±0.004)	8.6 (SD±0.60)	0.033 (SD±0.007)	7.3 (SD±0.64)	3.17 (SD±0.31)	3.61 (SD±0.30)	9.5 (SD±0.44)	20.0 (SD±1.92)
4. Ribbed matting (RM)	2.7	10.9	7.8 (SD±0.57)	0.041 (SD±0.005)	8.5 (SD±0.65)	0.032 (SD±0.006)	7.1 (SD±0.81)	3.06 (SD±0.26)	3.54 (SD±0.36)	9.6 (SD±0.50)	20.3 (SD±1.51)
5. Burnt laminate (BL)	0.0	6.0	7.9 (SD±0.49)	0.043 (SD±0.004)	8.5 (SD±0.68)	0.033 (SD±0.007)	7.3 (SD±0.58)	3.09 (SD±0.23)	3.60 (SD±0.31)	10.0 (SD±0.65)	20.6 (SD±1.46)
6. Laminate floor (LF)	0.9	3.6	7.7 (SD±0.63)	0.041 (SD±0.005)	8.5 (SD±0.67)	0.032 (SD±0.005)	7.2 (SD±0.59)	3.04 (SD±0.22)	3.53 (SD±0.31)	9.8 (SD±0.48)	20.4 (SD±1.54)
7. Laminate & obstacles (LO)	0.9	5.6	7.8 (SD±0.63)	0.042 (SD±0.005)	8.5 (SD±0.59)	0.032 (SD±0.005)	7.1 (SD±0.83)	3.06 (SD±0.27)	3.53 (SD±0.40)	9.7 (SD±0.54)	20.2 (SD±1.42)
8. Laminate & Sawdust (LS)	0.0	6.7	8.1 (SD±0.55)	0.043 (SD±0.005)	8.6 (SD±0.67)	0.033 (SD±0.006)	7.2 (SD±0.84)	3.11 (SD±0.30)	3.59 (SD±0.34)	9.4 (SD±0.39)	19.9 (SD±1.48)

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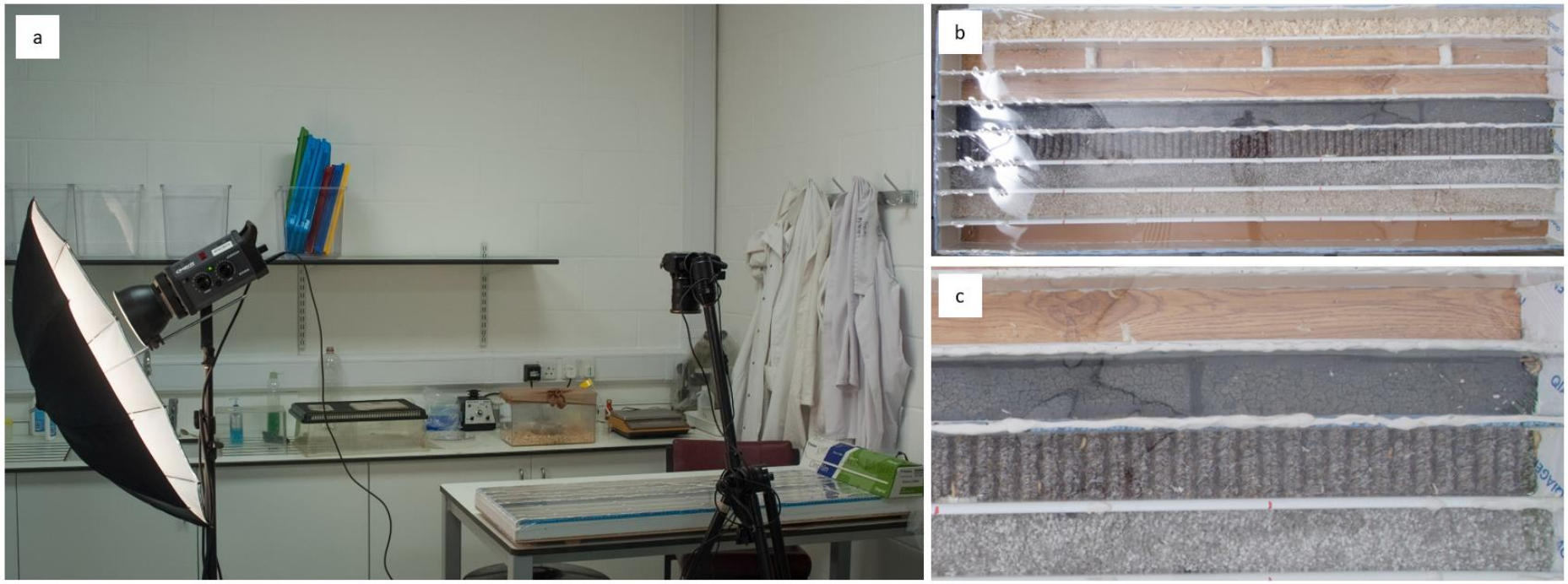

Fig. 1: a) Photographic time lapse apparatus in the temperature controlled laboratory. b) Photograph showing all lanes and plastic covering. From the top of the image the surfaces are laminate & sawdust (LS), laminate & obstacles (LO), laminate floor (LF), burnt laminate (BL), ribbed matting (RM), long pile carpet (LP), short pile carpet (SP) and smooth wooden surface (SW). c) Digital zoom, 1920 x 1200 pixels allowing parts of the experiment to be visualized without losing image quality.

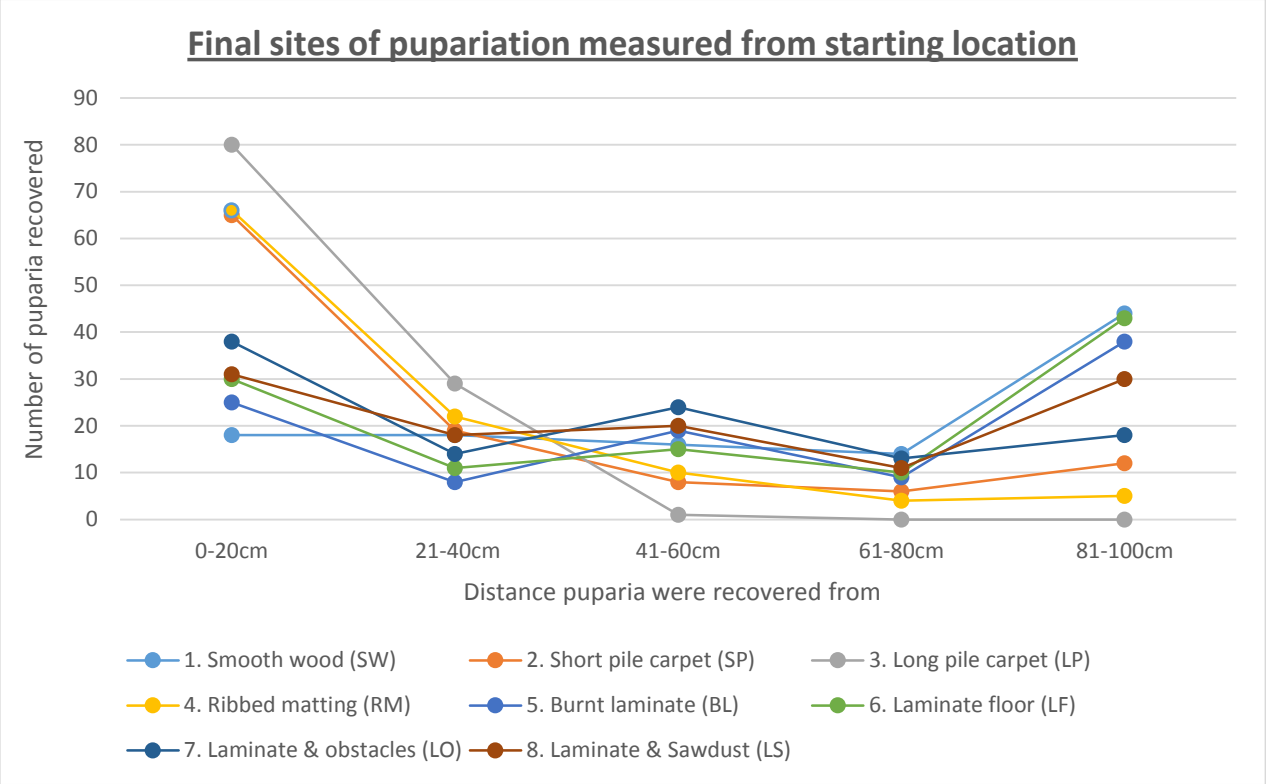

Fig. 2: Distance puparia were recovered within each experimental condition divided into 20cm sections.

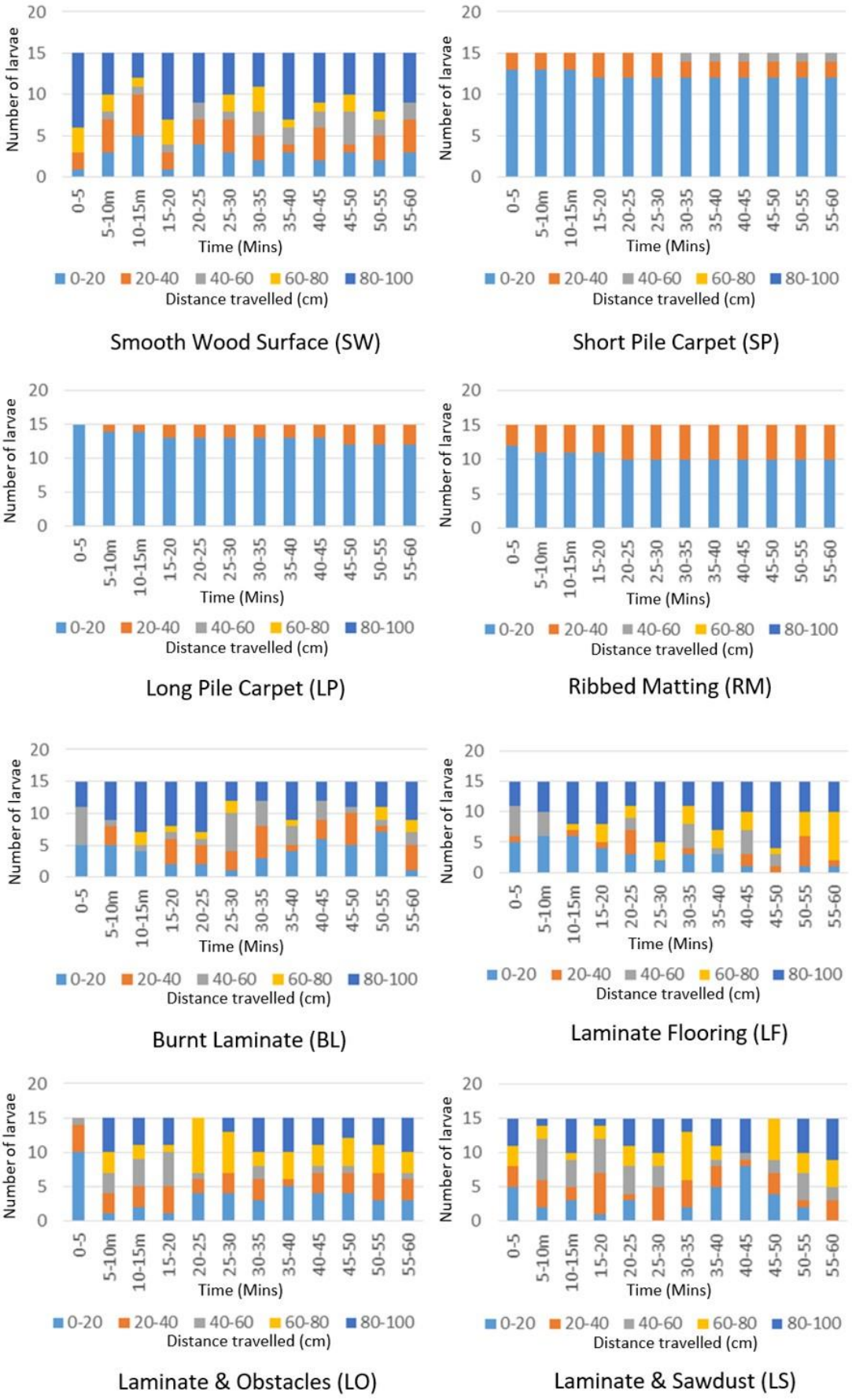

Fig. 3: Distribution of larvae during the first hour in a single experimental run showing number of larvae on the Y axis and time in minutes on the X axis. The number of larvae within each 20cm section of the experimental lane is displayed using colour.

	SW	SP	LP	RM	BL	LF	LO	LS
SW								
SP								
LP								
RM			0.04					
BL								
LF								
LO								
LS								

a. Puparia weight pairwise comparison

	SW	SP	LP	RM	BL	LF	LO	LS
SW								
SP								
LP	0.00							
RM			0.00					
BL								
LF		0.02	0.00					
LO			0.01					
LS	0.00			0.00	0.05	0.00	0.00	

b. Puparia length pairwise comparison

	SW	SP	LP	RM	BL	LF	LO	LS
SW								
SP	0.00							
LP	0.00							
RM		0.01						
BL	0.00	0.00	0.00	0.00				
LF		0.00	0.00	0.00				
LO		0.00	0.00		0.00			
LS	0.00			0.00	0.00	0.00	0.00	

c. Day of pupariation pairwise comparison

	SW	SP	LP	RM	BL	LF	LO	LS
SW								
SP								
LP								
RM								
BL								
LF								
LO								
LS					0.04			

d. Day of emergence pairwise comparison

Fig. 4: Pairwise comparisons of the eight experimental conditions (smooth wooden surface (SW), short pile carpet (SP), long pile carpet (LP), ribbed matting (RM), burnt laminate (BL), laminate floor (LF), laminate & obstacles (LO), laminate & sawdust (LS)). a) Significance values of pairwise comparisons for puparia weight. b) Significance values of pairwise comparisons for puparia length. c) Significance values of pairwise comparisons for day of pupariation. d) Significance values of pairwise comparisons for day of emergence.

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