The movement of fly larvae within a feeding aggregation

Dr Vivienne Heaton, PhD

Keele University, United Kingdom

(+44) 1782 733115

v.g.heaton@keele.ac.uk

Dr Colin Moffatt, PhD

De Montfort University, United Kingdom

(+44) 7876 644941

colin.moffatt@dmu.ac.uk

Dr Tal Simmons, PhD

Virginia Commonwealth University, United States

(804) 828 3295

tlsimmons@vcu.edu

Abstract

Dipteran larvae from a number of families feed in aggregations. Rotation of blow fly (Diptera: Calliphoridae) larvae within an aggregation has been reported anecdotally many times. However, there is a lack of quantitative data on such larval movement, which is necessary to better understand the advantage of this gregarious behaviour. A recent development in tagging methods provided an opportunity to address this gap in knowledge. In fifteen aggregations of 500 *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) larvae, the location of four tagged individuals was recorded at 10 minute intervals. All larvae were seen to rotate, alternating between the periphery and within. There was much variation in the relative proportions that larvae were seen in these two locations among aggregations (χ^2 = 78.4, df = 58, p = 0.038), perhaps as a result of differences in mass shape and, therefore, surface area: volume ratio. There were also differences between larvae within aggregations (χ^2 = 25.6, df = 14, p = 0.029), which may give rise to differences in development rate, perhaps as a result of intra-specific competition. Further work would be required to verify this competition, and to establish whether the limited resource is temperature, food, oxygen, or some other requirement.

Introduction

Aggregation of insect larvae is a common phenomenon which confers advantages on the constituent individuals (Denno and Benrey 1997; Parrish and Edelstein-Keshet 1999) and so can be termed an *Allee effect* (i.e., individuals of many species may benefit from the presence of conspecifics, Stephens et al. 1999). Fly larvae from a number of families form such aggregations in a range of food substrates including mushrooms (Jaenike and James 1991; Heard 1998), fallen fruit (Atkinson 1985), and in aquatic situations (Wotton 1992). Perhaps most interest in dense, feeding cohorts has been focussed upon those associated with carrion, in particular larvae of necrophagous blow flies (Calliphoridae) (Charabidze et al. 2011; Heaton et al. 2014; Johnson and Wallman, 2014). Perhaps gregarious behaviour observed in aggregations benefits individuals in a cohort by ensuring sufficient proteolytic enzymes are secreted over a large surface area, facilitating liquefaction of the food substrate and maximising larval feeding efficiency (Goodbrod and Goff 1990; Green et al. 2003; Rivers et al. 2011).

While the knowledge of larval aggregations is far from recent (Deonier 1940), basic questions about 'maggot masses' have not been sufficiently addressed. We do not know, for example, whether all individuals within a mass exhibit the same behaviour. Is consequence of larval aggregation is a faster rate of development

as a result of an increased temperature (Johnson and Wallman 2014)? Research on blow flies has shown that larval aggregations generate heat significantly higher than ambient (Deonier 1940; Richards and Goff 1997; Slone and Gruner 2007; Richards et al. 2009) and that the rise in temperature is proportional to the size of the aggregation (Charabidze et al. 2011; Heaton et al. 2014, Johnson and Wallman 2014). However, in some cases, the resulting temperatures in large aggregations can exceed what is thought to be the lethal limit, causing the occasional larva to perish (Slone and Gruner 2007; Kelly et al. 2009). Because most individuals survive and go on to complete development at such high temperatures they must somehow regulate their temperature and thus avoid overheating. Thermal imaging reveals that aggregations are not a uniform temperature and thermal gradients are known to occur in sufficiently large aggregations (Heaton et al. 2014; Johnson et al. 2014).

Many anecdotal reports describe blow fly larvae appearing to circulate within an aggregation between the hot centre and the cooler periphery, and that larvae will select an optimum position for development when presented with a temperature gradient (Catts 1992; Byrd and Butler 1996; Ames and Turner 2003; Slone and Gruner 2007; Charabidze et al. 2011; Hückesfeld et al. 2011; Rivers et al. 2011). It seems plausible that circulating larvae are regulating their temperature by alternating between locations where temperatures are above

and below optimum, that is, at the centre and the periphery of a maggot mass. However, it is almost impossible to keep track of a larva in a mass, since they are practically identical, and move continuously, so anecdotal reports are unreliable.

The literature does not contain any quantitative investigation of larval circulatory movement in flies save for that of Johnson *et al.* (2014) which reported that larvae of *Chrysomya rufifacies* (Macquart) and *Calliphora vicina* (Robineau-Desvoidy) spent 60% of their time within 1 ^{O}C of the aggregation's maximum temperature. However, this figure was based upon monitoring only one individual in each maggot mass, and importantly, no indication of the variation around this figure is given. The authors acknowledged issues with their experimental design, which limited the quality and quantity of data they were able to collect. Most notable was the fact that aggregations were continually divided by cling film, and were repeatedly separated to enable visualization of the tagged larvae, so causing repeated disturbance. Moreover, the tagged (by food dye) individual had spent more than a day away from its parent mass before it was reintroduced, prior to data collection. Time spent away from the aggregation may have altered the foraging behaviour of the larva or negatively impacted on its feeding and development. Further quantitative data are called for, by a less disruptive, more realistic approach, though this presents its own challenges as demonstrated by the shortage of such studies in the literature.

Recent advances in the technology of tagging animals have presented further possibilities for investigating larval movement more quantitatively. Boulay and colleagues (2016) investigated collective decision-making in two species of forensically important blow fly, *Lucilia sericata* (Meigen) and *Calliphora vomitoria* (Linnaeus). In their paper they describe the use of a novel tagging method for blow fly larvae, which allowed them to differentiate between species in heterospecific experiments. Using a cyanoacrylate glue which fluoresces under ultraviolet light, they were able to apply a visible mark externally to the anterior region of the dorsal surface. It is possible that such a marking might have impeded larval movement. However, the authors reported that this was not the case and tagged larvae were observed to behave as normal. It should also be noted that to limit heat generation within the mass, aggregations were composed of just 40 individuals (Boulay et al. 2016). Whilst contributing to our understanding of larval movement, their research focused on the movement of individuals across a surface, rather than the more realistic case of an aggregation with an identifiable centre and periphery. Another technique for tagging larvae was proposed by Rosati and colleagues (2015) who describe marking larvae with fluorescent fingerprint powders, either by ingestion or applying topically. Whilst their results show potential, it should be noted that larvae were not monitored whilst in an aggregation, which could have consequences for the topically applied powders given the nature of the mass.

The aim of the study presented herein was to quantify the movement of individual larvae within aggregations of the blow fly *Lucilia sericata* using a relatively new tagging technique, and therefore, generate scientifically-robust, replicated data. Visible implant elastomer (VIE) has been successfully used to tag blow fly larvae such that individuals could be tracked within an aggregation in real time (Moffatt 2013). VIE is a brightly coloured, bio-compatible elastomer material, which when injected into translucent animal tissues, cures to form a gelatinous internal tag. Using this technique, blow fly larvae were tagged without impediment to subsequent development in the 80% of larvae which survived the process (Moffatt 2013). For this research several VIE-tagged individuals were observed for each mass, after having spent only a short time away from it. Thus, it was intended that the data collected would give a more complete picture of the actual situation than had been published previously.

Materials and Methods

Adult *Lucilia sericata* were housed in cages that were kept in a walk-in incubator. Conditions inside the incubator were maintained at a constant 22 °C with a relative humidity of around 60% and a 16:8 hour (light:dark) light regime. Adults were provided with a constant supply of water and sugar, augmented by pork liver to provide the necessary nutrients for gonad development.

Prior to setting up each replicate, further pork liver was introduced into fly cages as an oviposition medium for two to three hours, during which time a sufficient quantity of eggs had been laid. Eggs remained in the same conditions for approximately 24 hours until first instar larvae began hatching. Of these recently eclosed larvae, 500 were transferred to 200 g of pork muscle, which was cut to dimensions of 100 x 90 x 25 mm. Care was taken to ensure the feeding substrate contained no bone and minimal fatty tissue. Earlier trials had shown this larva:meat ratio was most suitable, being dense enough to promote the formation of a mass without being so large that tagged larvae would be easily lost within the aggregation. For five aggregations, observations were recorded regarding the shape, size and position of the mass in relation to the feeding substrate. Measurements were taken for maximum and minimum mass diameter, as well as depth, using a Mitutoyo Absolute Digimatic Calliper 0-200 mm (accuracy ±0.02 mm). All aggregations were circular or slightly oval in shape with diameters ranging from 55-70 mm and a depth of approximately 15–20 mm, or 2-3 larvae deep. Aggregations were often observed to position themselves on two faces of the substrate at any one time, with part of the mass feeding on a vertical face and spreading upwards onto the horizontal, or top, surface of the meat. However, it needs to be stressed that these are generalised measurements for aggregations composed of early third instar larvae. Constant larval movement resulted in slight variations in mass shape and position during

the course of the experiment, whilst dimensions such as diameter and depth were expected to increase over time as larvae continued to feed and develop.

The meat and the larvae were held in a plastic container, which measured 270 x 270 x 160 mm. The bottom of the container was lined with paper towel to absorb any excess moisture that might result from decomposition and liquefaction of the meat. To ensure the container was well ventilated a panel (50 x 100 mm) was removed from the lid and fine netting was secured in its place to prevent larval escape. The lid was removed during data collection so the mass could be properly viewed. The process was repeated to produce 15 replicates.

During the trails, three randomly selected aggregations had their surface temperatures recorded at regular five minute intervals using a FLIR T425 thermal imaging camera (FLIR Systems Ltd (UK), 2 Kings Hill Avenue, West Malling, Kent, ME19 4AQ UK). Data collection commenced at the start of second larval instar, which permitted time for the larvae to amass, and ceased once larvae began to disperse away from the aggregation. Surface temperatures were recorded instead of internal temperatures since the repeated insertion of a thermometer or temperature probe caused to the larvae to be disturbed and the aggregation to temporarily disperse. Temperature data showed that a typical mass of this size reared under these conditions generated mean surface temperatures of 23.3

°C (SD=0.94), peaking during 3rd instar at approximately 26 °C (Fig. 1). This was deemed an appropriate temperature range for this experiment since it falls several degrees below the temperatures suspected of triggering stress-induced behaviours, meaning that any circulatory movement observed in the aggregation is not solely attributed to thermoregulation.

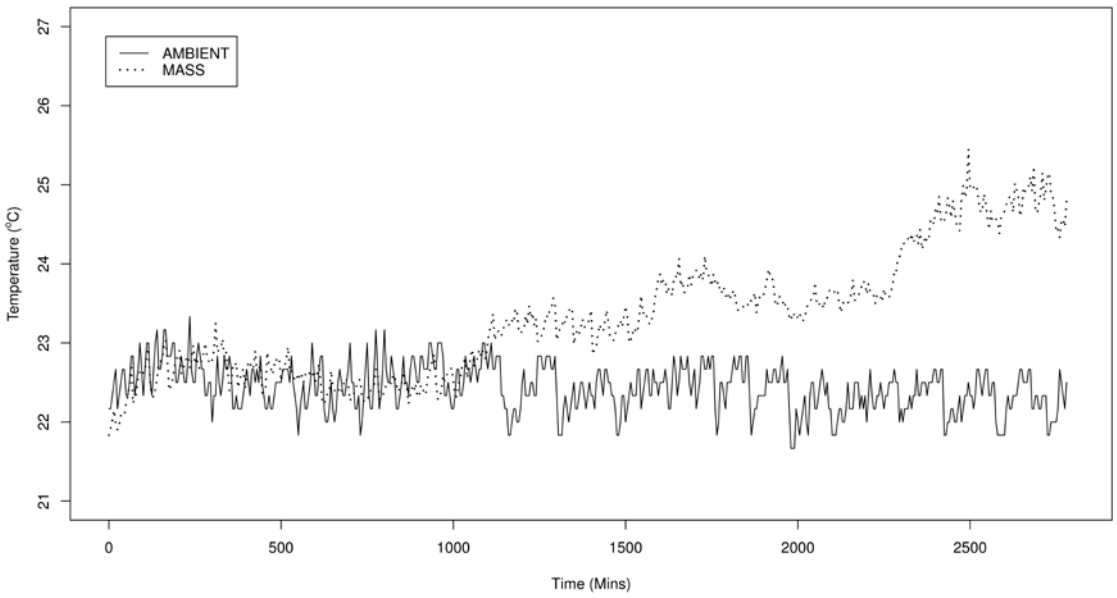


Fig. 1 - Mean surface temperature of the mass (°C) versus time (mins) for aggregations containing 500 larvae and reared at a constant ambient temperature of 22 °C. Temperatures recorded from the start of 2nd larval instar.

Tagging larvae

Through regular observation of the developing larvae, the time when more than half of an aggregation had reached 3rd instar was identified, whereupon four individuals were randomly selected and removed. These larvae were injected with visible implant elastomer (Northwest Marine Technology Inc., Washington, USA) in the 11th segment, dorsally in the midline between two tissue masses (Moffatt 2013). Each of the four was injected with a different fluorescent colour, which under ultra-violet (black) light makes it easier to see the tag. Once tagged, the four larvae were then placed on a separate piece of pork muscle for around 30 minutes to verify they had survived the tagging procedure unaffected, before being returned to their original mass. From an hour after this, aggregations were observed every 10 minutes for four hours, and each of the four-tagged larvae recorded as being visible and therefore at the periphery, or not visible and so within the mass. Thus data comprised counts of a maximum of 25, which were converted to proportions of observations at the periphery.

Statistical Analysis

A generalized linear mixed-effects model (GLMM) using a logit link for binary data, took into account that observations were repeated on the same larvae in different masses, and was used to establish whether there were differences between masses, and differences between larvae in the same masses in terms of time spent at the periphery. The model captured the structure of the data;

repeated observations on the same individuals clustered within masses, the significance of each being tested by deletion and comparison using the chi-squared statistic. The intraclass correlation coefficient allowed a simple comparison between mass and maggot in terms of the variation they explained. A new variable indicating whether a larva's position had changed (from visible to not visible or *vice versa*) between observations also constituted binary data, so was analysed in the same way. While 10 minutes was an arbitrary interval, and speed of circulation cannot be deduced from these observations, this new variable does reflect something of how active the larvae were. Data were analysed using the statistical package R (R Core Development Team, 2015) using the lme4 package (Bates et al. 2015). It should also be noted that whilst the term 'centre' is used throughout the results section for ease of interpretation, the exact location of tagged larvae not visible at the periphery cannot be confirmed. Whilst some larvae would indeed have been feeding at the centre, others may have been moving through the aggregation but out of sight.

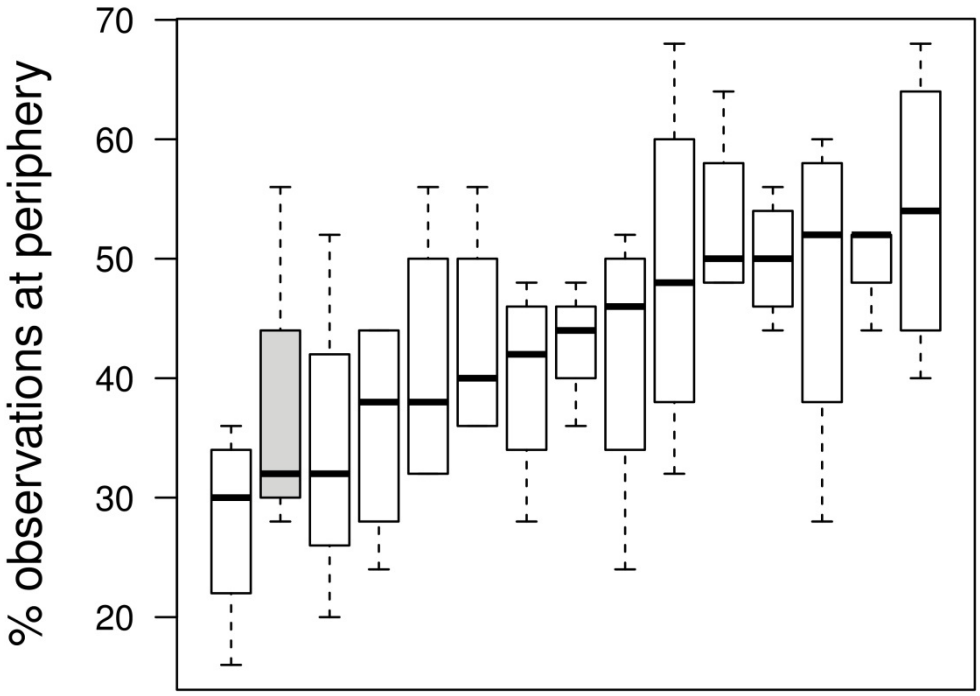
Results

Larvae tagged with VIE were quickly and easily identified within the aggregation, facilitated by the momentary use of black light. The proportions of time spent at the periphery of the aggregation had an approximately normal distribution for all tagged larvae *en masse* and Grubb's test identified an outlier ($G = 3.37$, $n = 60$, p

224 = 0.012), which was removed from analyses. This atypical larva was observed
225 moving particularly slowly, and spent considerably longer at the periphery than
226 all others. It is possible that this individual was injured during the tagging
227 process though had appeared unaffected immediately afterwards.

228

229 The proportions of time that larvae spent at the periphery are shown as a box
230 and whisker plot for each aggregation in Figure 2, where the wide variation
231 between larvae and aggregations can be clearly seen. Mixed-effects models
232 showed that differences among larvae within aggregations were significant (χ^2 =
233 78.4, df = 58, p = 0.038), but that differences among aggregations were also
234 significant (χ^2 = 25.6, df = 14, p = 0.029); an aggregation influenced the larvae
235 within it, but individual larval variation was still evident. The percentage of time
236 individuals spent at the periphery ranged from 16 to 68% (mean = 43%), and
237 within the same aggregation the largest difference between larvae was 32% and
238 68%. The lowest variation was only 8% with a median of 52%. The intraclass
239 correlation coefficients for aggregation and maggot were 0.040 (61%) and 0.026
240 (39%) respectively; differences amongst aggregations explained more of the
241 variation than differences amongst maggots.



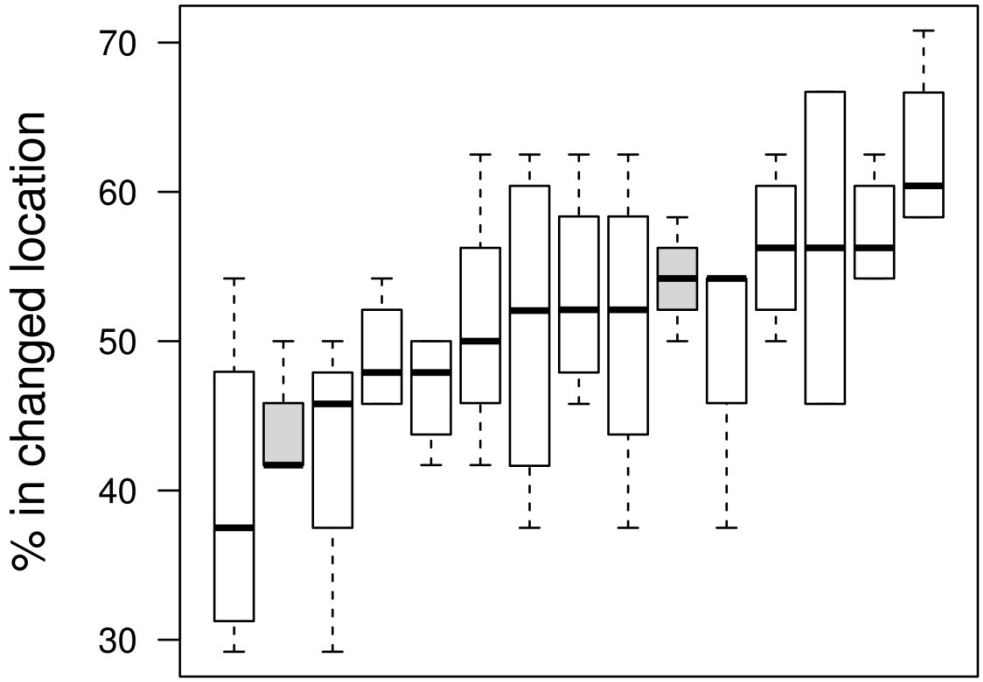
242

243 **Fig. 2** – Box and whisker plot showing the percentage of observations tagged
244 larvae spent at the periphery in each of the 15 experimental aggregations,
245 arranged in order of median values for each mass to facilitate interpretation.
246 Each aggregation had 500 larvae, four of which were tagged, but the one shaded
247 is based upon three of these (aggregation containing the outlier).

248

249 All larvae appeared to be in a state of continuous movement in all aggregations.
250 In fact, 49% of all observations showed a different location on subsequent
251 observations. The 95% confidence interval for the location of the population
252 mean, produced from a GLMM model, was between 47.6% and 50.5%
253 (asymmetry is expected). This is a long way from 0%; the value at which no

254 rotation occurs; clear evidence for the rotation of larvae. There were no
255 differences either among larvae within aggregations (X^2 = 44.1, df = 57, p = 0.89)
256 or among aggregations (X^2 = 17.9, df = 14, p = 0.21) for this 'change metric',
257 although the actual percentage varied from 29% to 71% as illustrated in Figure 3.
258 The intraclass correlation coefficients showed that practically all variation was
259 explained by the aggregation relative to maggots within aggregations; the
260 aggregation influenced the likelihood of change between observations. A single
261 additional outlier (Grubb's Test: G = 3.81, n = 59, p = 0.001) was identified and
262 removed from this analysis, as it rotated atypically quickly.



263

264 **Fig. 3** – Box and whisker plot for the 15 experimental aggregations (*N* = 500)
265 showing the percentage of observations where tagged larvae (*n* = 4) were

observed in a different location (periphery or centre) to the previous observation recorded 10 minutes earlier. Data are arranged in order of median values for each mass to facilitate interpretation. Each aggregation had 500 larvae, four of which were tagged, but the ones shaded are based upon three following the removal of an outlier.

Discussion

Blow fly larvae continually rotate between the periphery and the centre of a larval aggregation at a rate influenced by the collective more than the individual. This continual motion may be in response to their immediate environment (Charabidze et al. 2008; Boulay et al. 2015) for which there are a number of possible explanations including thermoregulation, foraging behaviour and avoidance of hypoxic conditions.

It has been suggested on numerous occasions that larvae feeding in aggregations are capable of regulating their temperatures to avoid overheating (Catts 1992; Ames and Turner 2003; Slone and Gruner 2007; Sharanowski et al. 2008; Kelly et al. 2009; Hückesfeld et al. 2011; Amendt et al. 2011; Charabidze et al. 2011). When confronted with a temperature step, blow fly larvae exhibit reflex-like evasive behaviour, retracting their anterior segments and moving away from unfavourable temperatures (Hückesfeld et al. 2011). This thermophobic

behaviour may also manifest inside an aggregation, directing larvae away from the hot centre and out towards the cooler periphery where they experience evaporative cooling (Catts 1992; Ames and Turner 2003; Slone and Gruner 2007; Hückesfeld et al. 2011; Charabidze et al. 2011; Rivers et al. 2011). However, since the aggregations studied in this experiment contained only 500 larvae, it is questionable that any circulation observed was a result of individuals regulating their temperature to avoid potentially harmful overheating. Aggregations of this size are not expected to generate temperatures exceeding 26 – 27 °C (Heaton et al. 2014), several degrees cooler than the proposed stress-inducing temperatures of 50 °C recorded in large aggregations (Richards and Goff 1997; Slone and Gruner 2007). In smaller aggregations, it may be more reasonable to assume that circulatory behaviour is a result of larvae re-positioning themselves along a thermal gradient for optimal development and not stress relief (Catts 1992; Byrd and Butler 1996; Ames and Turner 2003; Slone and Gruner 2007; Hückesfeld et al. 2011; Charabidze et al. 2011; Rivers et al. 2011), or an innate behaviour better suited to larger aggregations which are more common in real situations (Vasconcelos et al. 2014; Moffatt et al. 2015).

Blow fly larvae do not feed continually but regulate their foraging behaviour (Charabidze et al. 2011; Charabidze et al. 2013), where individuals move out to the periphery to search for new feeding sites. It is this which creates the familiar

rolling turnover. Larvae feeding in an aggregation might also experience periods of little (hypoxia) or no (anoxia) oxygen, especially if the aggregation is dense or partially submerged in decompositional fluids. Carrion is a hypoxic microhabitat (Hoback and Stanley, 2001), where larvae and bacteria remove oxygen from the surrounding air. Larval hypoxia could therefore contribute to mass rotation, with individuals moving away from the hypoxic centre to areas at the periphery where oxygen is at greater concentrations (Hoback and Stanley, 2001). However, whilst this might influence the behaviour of larvae in large masses composed of thousands of individuals, it is unlikely to be an issue in smaller aggregations, similar to those used in this research. It seems likely that circulation of larvae is influenced by all of thermoregulation, foraging behaviour and oxygen requirements, and their relative importance may be related to the size and shape of an aggregation.

The large variation seen in the proportion of time spent at the periphery may be a consequence of the shape of an aggregation. In an aggregation which is relatively flat, the surface area: volume ratio (SA:V) is large and the distance to the periphery is always short, causing the aggregation to lose heat faster (Contreras 1984). In such an aggregation, when a larva is not feeding, it is likely to be visible at the periphery. In an aggregation which is closer to spherical, the SA:V ratio is relatively low and a larva is less likely to be visible at the periphery if

not feeding. A better quantification of larval activity would take into account the SA:V, but its measurement is extremely difficult, not least because the aggregation is continually moving and changing shape. Whilst all the aggregations monitored in this research were circular in shape, they did vary slightly in diameter and depth. However, it seems unlikely that a difference in depth of up to 5 mm would account for the significant differences observed between larvae and the proportion of time they spent at the periphery.

If differences between aggregations can be explained, at least in part, by the shape of the aggregation, differences between larvae in the same aggregation must be accounted for in different ways. Whilst some larvae appear to divide their time evenly between the centre and the periphery, significant deviation from this was also evident. If it can be inferred that this variation also extends to the time spent feeding, then it seems likely that the movement in an aggregation is an explanation for differences in development rate of the insects therein. It seems plausible that this is the result, at least in part, of intra-specific competition (Ullyett 1950; Smith and Wall 1997). Further work needs to be done to establish whether intra-specific competition does indeed drive this phenomenon, and further to establish whether the limited resource is temperature, food, oxygen, or some other requirement.

The results of this study imply that all larvae circulate between the periphery and the centre of the aggregation at a similar rate. However, there are a number of factors related to the tagging procedure and sampling techniques used that could have influenced these findings and should be taken into consideration. It is plausible that some larvae may have been injured during the tagging operation, though did not appear so in the period immediately afterwards. Tagged insects exude a liquid from the syringe needle's entry point, which makes all appear damaged initially (Moffatt 2013). Injury may have reduced the larvae's ability to locomote to some degree, and so modified their movements within the aggregation. The timing of observations might also have influenced the results. If larval rotation is indeed cyclic, as the results of this study suggest, then there is a slight risk that the observations made at regular intervals may have been synchronized with the rate of rotation of some larvae. Further research using similar methods may benefit from continuous observation of at least some aggregations.

Conclusion

This research has provided quantitative, scientifically-robust evidence for an often-stated assertion that has so far lacked evidence. Larvae are in a constant state of motion as they circulate between the centre of the aggregation and its periphery. The proportion of time larvae spend at the periphery varies

371 significantly between individuals as well as aggregations, and whilst some larvae
372 appeared to rotate between the two locations faster than others, no significant
373 differences were recorded in this respect. With the development of new tagging
374 techniques, such a VIE, there is now potential to collect quantifiable data which
375 will contribute to further understanding the phenomenon of larval aggregations.

376

377 **References**

378 Amendt, J., Richards, C.S., Campobasso, C.P., Zehner, R., and Hall, M.J.R. 2011.
379 Forensic entomology: applications and limitations. Forensic Science, Medicine
380 and Pathology, **7**: 379–392.

381

382 Ames, C. and Turner, B. 2003. Low temperature episodes in development of
383 blowflies: implications for postmortem interval estimation. Medical and
384 Veterinary Entomology, **17**: 178–186.

385

386 Atkinson, W.D. 1985. Coexistence of Australian rainforest Diptera breeding in
387 fallen fruit. Journal of Animal Ecology, **54**: 507–518.

388

389 Bates, D., Maechler, M., Bolker, B., and Walker, S. 2015. Fitting Linear Mixed-
390 Effects Models Using lme4. Journal of Statistical Software, **67**: 1-48.

391

392 Boulay, J., Betremieux, C., Hédouin, V., and Charabidzé, D. 2015. A first insight
393 into the scanning behaviour of the presocial blow fly larvae: Scanning behaviour
394 of presocial blow fly larvae. Physiological Entomology, **40**: 317–324.

395

396 Boulay, J., Deneubourg, J.L., Hédouin, V., and Charabidzé, D. 2016. Interspecific
397 shared decision-making in two forensically important species. Proceedings of the
398 Royal Society B, **283**.

399

400 Byrd, J.H. and Butler, J.F. 1996. Effects of Temperature on *Cochliomyia macellaria*
401 (Diptera: Calliphoridae) Development. Journal of Medical Entomology, **33**: 901–
402 905.

403

404 Catts, E.P. 1992. Problems in estimating the postmortem interval in death
405 investigations. Journal of Agricultural Entomology, **9**: 245–255.

406

Charabidze, D., Bourel, B., and Gosset, D. 2011. Larval-mass effect: characterisation of heat emission by necrophageous blowflies (Diptera: Calliphoridae) larval aggregates. Forensic Science International, **211**: 61–66.

Charabidze, D., Bourel, B., Leblanc, H., Hedouin, V., and Gosset, D. 2008. Effect of body length and temperature on the crawling speed of *Protophormia terraenovae* larvae (Robineau-Desvoidy) (Diptera Calliphoridae). Journal of Insect Physiology, **54**: 529–533.

Charabidze, D., Hedouin, V., and Gosset, D. 2013. Discontinuous foraging behaviour of necrophagous *Lucilia sericata* (Meigen 1826) (Diptera: Calliphoridae) larvae. Journal of Insect Physiology, **59**: 325-331.

Contreras, L.C. 1984. Bioenergetics of huddling: test of a psycho-physiological hypothesis. Journal of Mammalogy, **65**: 256–262.

Denno, R. and Benrey, B. 1997. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. Ecological Entomology, **22**: 133–141.

Deonier, C.C. 1940. Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest. Journal of Ecological Entomology, **33**: 166–170.

Green, P.W.C., Simmonds, M.S.J., and Blaney, W.M. 2003. Diet nutriment and rearing density affect the growth of black blowfly larvae, *Phormia regina* (Diptera: Calliphoridae). European Journal of Entomology, **100**: 39-42.

Goodbrod, J.R. and Goff, M.L. 1990. Effects of larval population density on rates of development and interactions between two species of Chrysomya (Diptera: Calliphoridae) in laboratory culture. Journal of Medical Entomology, **27**: 338-343.

Heard, S.B. 1998. Resource patch density and larval aggregation in mushroom-breeding flies. Oikos, **81**: 187–195.

443 Heaton, V., Moffatt, C., and Simmons, T. 2014. Quantifying the temperature of
444 maggot masses and its relationship to decomposition. Journal of Forensic
445 Science, **59**: 676–682.

446

447 Hoback, W.W. and Stanley, D.W. 2001. Insects in hypoxia. Journal of Insect
448 Physiology, **47**: 533–542.

449

450 Hückesfeld, S., Niederegger, S., Schlegel, P., Heinzel, H.E., and Spiess, R. 2011.
451 Feel the heat: The effect of temperature on development, behavior and central
452 pattern generation in 3rd instar *Calliphora vicina* larvae. Journal of Insect
453 Physiology, **57**: 136–146.

454

455 Jaenike, J. and James, A.C. 1991. Aggregation and the coexistence of
456 mycophagous Drosophila. Journal of Animal Ecology, **60**: 913–928.

457

458 Johnson, A.P. and Wallman, J.F. 2014. Effect of massing on larval growth rate.
459 Forensic Science International, **241**: 141–149.

460

461 Johnson, A.P., Wighton, S.J., and Wallman, J.F. 2014. Tracking movement and
462 temperature selection of larvae of two forensically important blow fly species
463 within a “maggot mass.” Journal of Forensic Sciences, **59**: 1586–1591.

464

465 Kelly, J.A., van der Linde, T.C., and Anderson, G.S. 2009. The Influence of
466 Clothing and Wrapping on Carcass Decomposition and Arthropod Succession
467 During the Warmer Seasons in Central South Africa. Journal of Forensic Sciences,
468 **54**: 1105–1112.

469

470 Moffatt, C. 2013. Using visible implant elastomer to tag insects across life stages:
471 a preliminary investigation with blow flies (Diptera: Calliphoridae). The Canadian
472 Entomologist, **145**: 466–470.

473

474 Moffatt, C., Heaton, V., and de Haan, D. 2015. The distribution of blow fly
475 (Diptera: Calliphoridae) larval lengths and its implications for estimating post
476 mortem intervals. International Journal of Legal Medicine, **130**: 287–297.

477

478 Parrish, J.K. and Edelstein-Keshet, L. 1999. Complexity, pattern, and evolutionary
479 trade-offs in animal aggregation. Science, **284**: 99–101.

R Core Development Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richards, C.S., Price, B.W., and Villet, M.H. 2009. Thermal ecophysiology of seven carrion-feeding blowflies in Southern Africa. Entomologia Experimentalis et Applicata, **131**: 11–19.

Richards, E.N. and Goff, M.L. 1997. Arthropod succession on exposed carrion in three contrasting tropical habitats on Hawaii Island, Hawaii. Journal of Medical Entomology, **34**: 328–339.

Rivers, D.B., Thompson, C., and Brogan, R. 2011. Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion. Bulletin of Entomological Research, **101**: 599–611.

Rosati, J.Y., Robinson, S.D. and Devine, R. 2015. Investigating the potential of fluorescent fingerprint powders as a marker for blow fly larvae (Diptera: Calliphoridae). Journal of Forensic Sciences, **30**: 653-658.

Sharanowski, B.J., Walker, E.G., and Anderson, G.S. 2008. Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. Forensic Science International, **179**: 219–240.

Slone, D.H. and Gruner, S.V. 2007. Thermoregulation in larval aggregations of carrion-feeding blow flies (Diptera: Calliphoridae). Journal of Medical Entomology, **44**: 516–523.

Smith, K.E. and Wall, R. 1997. Asymmetric competition between larvae of the blowflies *Calliphora vicina* and *Lucilia sericata* in carrion. Ecological Entomology, **22**: 468–474.

Stephens, P.A., Sutherland, W.J., and Freckleton, R.P. 1999. What Is the Allee Effect? Oikos, **87**: 185.

Ullyett, C.G. 1950. Competition for food and allied phenomena in sheep-blowfly populations. Philosophical Transactions of the Royal Society, **234**: 77–174.

518 Vasconcelos, S.D., Soares, T.F., and Costa, D.L. 2014. Multiple colonization of a
519 cadaver by insects in an indoor environment: first record of Fannia trimaculata
520 (Diptera: Fanniidae) and Peckia (Peckia) chrysostoma (Sarcophagidae) as
521 colonizers of a human corpse. International Journal of Legal Medicine, **128**: 229–
522 233.

523

524 Wotton, R.S. 1992. Feeding by blackfly larvae (Diptera: Simuliidae) forming
525 dense aggregations at lake outlets. Freshwater Biology, **27**: 139–149.