Journal of Insect Physiology 95 (2016) 98–104

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Heat accumulation and development rate of massed maggots of the sheep blowfly, *Lucilia cuprina* (Diptera: Calliphoridae)

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ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form 14 September 2016
Accepted 15 September 2016
Available online 17 September 2016

Keywords:
Thermogenesis
Development rate
Post-mortem interval
Forensic entomology
Lucilia cuprina

ABSTRACT

Blowfly larvae aggregate on exposed carcasses and corpses and pass through three instars before wandering from the carcass and pupating. The developmental landmarks in this process can be used by forensic entomologists to estimate the time since the insects colonised the carcass, which sets a minimum post mortem interval. Large aggregations of feeding larvae generate a microclimate with temperatures up to 15 °C above ambient conditions, which may accelerate larval development and affect forensic estimates of post-mortem intervals. This study investigated the effects of heat accumulated by maggot masses of *Lucilia cuprina* at aggregations of 20, 50 and 100 larvae, each at incubation temperatures of 18 °C, 24 °C and 30 °C, using body length and life stage as developmental indicators. Aggregation temperatures reached up to 18.7 °C above ambient temperature, with significant effects of both size and temperature of the aggregation on the development time of its larvae. Survivorship was highest for all life stages at 24 °C, which is near the developmental optimum of *L. cuprina*. The results of this study provide a broadly applicable method of quantifying heat accumulation by aggregations of a wide range of species of forensic importance, and the results obtained from such studies will demonstrate that ambient temperature cannot be considered the only source of heat that blowfly larvae experience when they develop on a carcass. Neglect of temperatures within larval aggregations will result in an overestimation of post-mortem intervals and thus have far-reaching medicolegal consequences.

1. Introduction

Blowflies (Diptera: Calliphoridae) are useful indicators of the time that has elapsed since a carcass or corpse was exposed to colonization by insects (Higley and Haskell, 2010; Villet et al., 2010; Rivers and Dahlem, 2014). Gravid females may arrive at carrion within minutes of death, and will lay eggs promptly under optimal conditions (Matuszewski and Madra, 2015). Larvae can hatch from 6 h onwards, depending on ambient temperatures and precocial fertilisation (Erzinçlioğlu, 1990; Wells and King, 2001), and then feed on the carcass until they reach the post-feeding stage in preparation for pupation (Villet et al., 2010; Rivers and Dahlem, 2014). The rate of larval development depends largely on larval body temperature (commonly approximated by using ambient temperature as a surrogate), and this relationship can be used to estimate when the carcass was initially colonised by arthropods based on larval size or developmental stage (Dourel et al., 2010; Higley and Haskell, 2010; Johnson et al., 2012) within certain limitations (Dourel et al., 2010; Villet et al., 2010).

Blowfly larvae are commonly found developing in large aggregations (Richards et al., 2008; Rivers et al., 2011; Charabidze et al., 2013; Johnson and Wallman, 2014), a characteristic shared with larvae of some other insect taxa (e.g., Klok and Chown, 1999; Reader and Hochuli, 2003; Durieux et al., 2012). Gregarious feeding allows larvae to optimise the consumption of a resource, which may be available to them for a limited period only (Tsubaki and Shiotsu, 1982; Charabidze et al., 2013). Co-operative feeding within a blowfly larval mass releases enzymes and ammonia, allowing the larvae to efficiently macerate food externally (Fitzgerald and Peterson, 1988). In blowflies, the formation of maggot masses may be beneficial to the development of larvae, but aggregation may also lead to intraspecific competition and increased predation and disease transmission among larvae (Reader and Hochuli, 2003; Rivers et al., 2011).

Aggregation also leads to the development of a microclimate in the mass that is up to 32 °C warmer than ambient air temperature (Johnson and Wallman, 2014). High temperatures may kill potentially harmful bacteria on carrion, but may also lead to thermal

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http://dx.doi.org/10.1016/j.jinsphys.2016.09.009
0022-1910/

stress (Higley and Haskell, 2010; Rivers et al., 2011), with negative consequences for larval growth and mortality, and thus for forensic estimates. Most species have a thermal optimum around which they can successfully develop to adulthood, with development and even survival increasingly compromising at temperatures deviating further from this optimum (Richards and Villet, 2008; Higley and Haskell, 2010; Rivers et al., 2010). It has been suggested that blowfly larvae employ several cooling mechanisms to counteract the elevated temperatures generated in aggregations and avoid compromised development. These mechanisms include physiological thermoregulation by convection, conduction and evaporation (Rivers et al., 2010, 2011) and discontinuous feeding that allows for behavioural thermoregulation by periodical retreat to cooler microclimates (Charabidze et al., 2013). Larvae may also temporarily break into smaller groups with larger effective surface-to-volume ratios, or leave the mass completely, to dissipate excess heat (Heaton et al., 2014; Johnson et al., 2014).

The sheep blowfly, *Lucilia cuprina* (Wiedemann), is widespread and common in Africa, Australia and parts of Asia and North America (Zumpt, 1965; Williams and Villet, 2013), and is attracted to carrion and rotting flesh, *inter alia* (Richards et al., 2009b). This species also causes sheep strike and wound myiasis (Ulyett, 1950; Zumpt, 1965; Tantawi et al., 2010; Kingu et al., 2012; Kuria et al., 2015). As such, this blowfly is of forensic significance and can be used to estimate the time since carrion were exposed to colonisation by insects. The species develops fastest at temperatures of 32–45 °C (Williams and Richardson, 1984), and optimally (in terms of mature size and survivorship) at ~24 °C (Kotzé et al., 2015).

The aim of this study was to investigate the effect of the size of larval aggregations on the development rate and survival of larvae of *L. cuprina* at different ambient temperatures. Raised temperatures within the maggot masses should increase mortality among larvae and compromise survival of post-feeding stages, as noted by Kotzé et al. (2015). By quantifying the effect of heat accumulation on the development of this blowfly, it is hoped that more accurate estimation of the PMI can be made in medicolegal investigations where it is present.

2. Materials and methods

2.1. Study species

Blowfly eggs were obtained from adults of *L. cuprina* housed in a culture at the Department of Zoology and Entomology at the University of Pretoria. This culture had been reared at 24 °C for over 20 generations on a diet of sugar, milk powder and water, with chicken liver as an oviposition substrate (Kotzé et al., 2015).

2.2. Protocol

Large numbers (>850) of eggs were harvested within an hour of their oviposition on chicken livers. Fifteen feeding cups were prepared, consisting of 50 g catering grade chicken liver in a sealed 125 ml polystyrene cup with holes pierced along the upper rim. Each cup was placed in a larger container with 3.0–3.5 cm-deep air-dried sand for pupation, and sealed with fine mesh to prevent larvae escaping and parasitoids entering.

A cluster of 20, 50 or 100 eggs was placed into each cup (n = 5). Eggs were isolated and counted individually using a pair of soft-tipped forceps, but were placed in the replicate cups in clusters to prevent desiccation before hatching. Replicates were then placed in a calibrated incubator (model 0102A: AFH Devers and Co. (Pty) Ltd., Johannesburg) set at 24 °C (±1 °C) (Kotzé et al., 2015). Relatively small aggregations were investigated in this study due to limited rearing and incubator space. While it is acknowledged that the number of larvae in aggregations on carcasses in the field may exceed thousands (Richards et al., 2008), such aggregations vary far more in surface area than in thickness, so this does not limit the results of this study; aggregations of 50–100 individuals, which may be found on small carcasses or where a single female has laid on a carcass, are only slightly less thick than substantially larger masses (Rivers et al., 2011; Charabidze et al., 2013). In addition, the smallest aggregation size was run to verify the assumption made by Kotzé et al. (2015) that heat accumulation would be minimal in a group of 20 larvae (*cf.* Goodbrod and Goff, 1990).

Sampling took place every 6 h until pupation (Richards and Villet, 2008). At each sampling event, one larva was removed from each cup, killed by immersion in boiling water for 30 s, and transferred to 70% ethanol for 1–2 h, which did not cause them to contract or swell (Adams and Hall, 2003). Larval length was measured using a pair of high-accuracy digital callipers (Series 1101, Insize Co., China), and life stage was determined by counting the posterior spiracular slits under a dissecting microscope (Smith, 1986).

At each sampling event, a calibrated type T thermocouple attached to a digital thermometer (MT630, MajorTech, China) was placed into the largest portion of the maggot mass, or the larger of the two secondary masses if the primary mass had split, and the temperature of the mass recorded (±0.1 °C). Even though larvae were removed using the protocol described above, by the onset of wandering there were still sufficiently large numbers of maggots present in each replicate to accumulate excess heat (Supplementary Table 1). This protocol, using length of developing larvae, rather than their mass as was done in similar studies (Wells and Kurahashi, 1994; Johnson and Wallman, 2014), was selected because it is more closely aligned with real-life forensic entomological practice. In medicolegal entomological cases, maggot-mass temperatures and ambient temperatures are measured; larvae are removed from the crime scene and killed, preserved, and measured in a laboratory; and the age of each larva is determined from the information about temperature, length and developmental stage. This age is then used to estimate the time since exposure of the corpse to insects (Amendt et al., 2007, 2015; Villet et al., 2010; Ridgeway et al., 2014).

During the wandering period, sand was sifted every 6 h to recover prepupae. Once larvae had pupariated successfully, individuals were transferred to microcentrifuge tubes held at the same incubation temperature to record the time that they spent in pupation and the rate of adult eclosion.

The above procedure was replicated three times at 24 °C, and again in the same incubator three times each at temperatures of 18 °C and 30 °C, based on thermophysiological data presented by Kotzé et al. (2015). However, at 18 °C, eight (rather than five) replicates were prepared containing clusters of 20 eggs because the longer development time at such a low temperature required a larger number of larvae to provide all of the necessary dead samples and still have enough live larvae remaining to estimate the survivorship of post-feeding and adult stages with satisfactory precision. All treatments were conducted in a random order, involving eggs drawn from various mothers from approximately six generations of the laboratory culture. Treatments were thus well replicated and randomised, and any incubator and genetic effects were strictly controlled by this procedure.

2.3. Data analysis

The median time taken to reach each developmental landmark after hatching (Richards and Villet, 2008) and the survivorship of each replicate at each sampling event were calculated. Survivorship of larvae was expressed as a percentage of larvae remaining after each sampling event per treatment, based on known numbers