Thermoregulation in Larval Aggregations of Carrion-Feeding Blow Flies (Diptera: Calliphoridae)

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ABSTRACT The growth and development of carrion-feeding calliphorid (Diptera: Calliphoridae) larvae, or maggots, is of great interest to forensic sciences, especially for estimation of a postmortem interval (PMI). The development rate of calliphorid larvae is influenced by the temperature of their immediate environment. Heat generation in larval feeding aggregations (=maggot masses) is a well-known phenomenon, but it has not been quantitatively described. Calculated development rates that do not include internally generated temperatures will result in overestimation of PMI. Over a period of 2.5 yr, 80 pig, *Sus scrofa* L., carcasses were placed out at study sites in north central Florida and northwestern Indiana. Once larval aggregations started to form, multiple internal and external temperatures, and weather observations were taken daily or every few days between 1400 and 1800 hours until pupation of the larvae. Volume of each aggregation was determined by measuring surface area and average depth. Live and preserved samples of larvae were taken for species identification. The four most common species collected were *Lucilia coeruleiviridis* (=*Phaenicia*) (Macquart) (77%), *Cochliomyia macellaria* (F.) (8.3%), *Chrysomya rufifaces* (Macquart) (7.7%), and *Phormia regina* (Meigen) (5.5%). Statistical analyses showed that 1) volume of a larval mass had a strong influence on its temperature, 2) internal temperatures of masses on the ground were influenced by soil temperature and mass volume, 3) internal temperatures of masses smaller than 20 cm^3 were influenced by ambient air temperature and mass volume, and 4) masses larger than 20 cm^3 on the carcass had strongly regulated internal temperatures determined only by the volume of the mass, with larger volumes associated with higher temperatures. Nonsignificant factors included presence of rain or clouds, shape of the aggregation, weight of the carcass, species composition of the aggregation, time since death, or season.

KEY WORDS forensic entomology, homeostasis, maggot mass, thermal ecology, crime scene

The larval development of blow flies (Diptera: Calliphoridae) on a deceased individual is of great interest to forensic science, especially for estimation of time since death (=postmortem interval or PMI) (Catts and Goff 1992, Keh 1985). Female calliphorid flies can find and deposit eggs on a body within minutes after death (Anderson and VanLaerhoven 1996), except in situations where physical barriers or environmental conditions prevent access to the body. The age of the oldest larvae found on a body can be used for estimation of the PMI, but for this estimation to be valid, the age of the larvae must be accurately determined based on species development rate characteristics, modified by environmental conditions.

As poikilotherms, calliphorid larvae experience a wide range of temperatures that determine their rate of development (Sharpe and DeMichele 1977). Estimation of the temperature regime under which the larvae developed is not as simple as determining the prevalent ambient temperatures during its development period. Calliphorid larvae usually form dense aggregations, or maggot masses, while feeding, and it has been documented for at least 65 yr (Deonier 1940) that these aggregations generate internal heat, presumably through exothermic digestive processes. Temperatures within larval aggregations have been recorded at >50°C when the ambient temperature was below 30°C (Anderson and VanLaerhoven 1996). Others also have noted high temperatures in larval aggregations (e.g., Cianci and Sheldon 1990, Campobasso et al. 2001, Joy et al. 2002). Goodbrod and Goff (1990) measured the effects of calliphorid larval population density (defined as the number of larvae per gram of substrate) on development rate in a laboratory study. Marchenko (2001) postulated that the number of larvae influences the magnitude of increased temperatures in larval aggregations relative to ambient. Turner and Howard (1992) noted that site differences affected the temperatures of larval aggregations, and they postulated that the size of the aggregation also may influence its temperature. We know of no systematic field study that focuses on the effect of the characteristics of a larval aggregation on its internal temperature.

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Accurate development rates of calliphorid larvae are required for determination of postmortem intervals based on their age. Because aggregation temperatures may differ from ambient temperature, larvae within an aggregation can have development rates different from the rate predicted by ambient temperature. We therefore analyzed the internal temperature of calliphorid larval aggregations based upon characteristics such as mass location, mass size, and environmental factors.

Materials and Methods

Use of human cadavers for the study of calliphorid development is not practical for large numbers of replicates. The rate of decomposition and the succession of fly colonization on pigs, *Sus scrofa* L., with a body weight ≈23 kg may be similar to that of humans (Anderson and VanLaerhoven 1996, Campobasso et al. 2001, Schoenly and Hall 2002); therefore, pig carcasses of approximately that weight were selected as animal models for this study.

All pig carcasses were purchased from a commercial livestock market near the study sites. Before purchase, the pigs were killed by the market operator with a shot into the top of the head from a 0.22 caliber rifle. Each carcass was immediately double-bagged in a heavy-duty plastic trash bag, weighed to the nearest 2.3 kg, and transported to the study site.

Study Sites and Data Collection. Two study sites, one site in north central Florida and the other in northwestern Indiana, were used in this study. The Florida study site was located in a wooded, 50-ha parcel near Earleton, FL. The Indiana study site was located in a wooded section of a 400-ha farm near Rensselaer, IN. All pig carcasses were placed in part-shade under the tree canopy.

Trials were begun approximately monthly from 15 November 2001 to 5 March 2004 in Florida and monthly from 18 June to 22 September 2003 in Indiana. Each trial consisted of three pigs placed at least 18.3 m apart. The pig carcasses were removed from the plastic bags and placed directly on undisturbed ground, and then wire cages (90 cm in length by 60 cm in width by 50 cm in height) were placed over the pigs to protect them from scavengers. The cages, which were lifted off during sampling times, were constructed of heavy wire mesh (5 by 5 cm), and they were secured with at least four bungee cords attached to tent stakes driven into the ground. After placement, the carcasses were not disturbed or moved during the study. All data and specimens were collected between 1400 and 1800 hours. After the pigs were in position, temperatures collected included ambient air, ground–pig interface, ground at 5 cm under the pig, and ground at 5-cm depth 3 m from the pig, all with a Taylor 9841 digital thermometer (Forestry Suppliers, Jackson, MS). Other daily measurements were of prevailing weather conditions, and wind velocity (in meters per second). Additionally, ground surface temperatures were recorded 1–2 m from each carcass every 30 min with Hobo (Onset Computer Corporation, Onset, MA) data loggers.

Once aggregations started to form, the aforementioned measurements were taken daily during warmer months, and every second or third day for cooler months, until the larvae dispersed for pupation. Depth and temperature measurements were obtained by gently inserting the warmed stainless steel part of the digital thermometer into five to 50 locations in the aggregation, depending on size (approximately every 2 cm, at depths of 1, 3, and 5 cm, or the maximum depth of the aggregation). These temperatures and the dimensions of each aggregation were marked on pig-shaped outlines, and digital photographs were taken of each mass accompanied by a linear scale for size reference. External temperatures were taken with a Raytek hand-held infrared thermometer (Forestry Suppliers, Jackson, MS). Only one temperature reading at the approximate center of each aggregation was taken in Indiana.

Samples of ≈100–600 larvae from each aggregation were collected on each visit. Reference specimens are available at the University of Florida. The location on the body or ground from where each sample was taken, and the sample number was noted on the data collection sheet. Half of the collected larvae were preserved by boiling them in water for 2 min and then they were placed into vials of isopropyl alcohol. The other half of the collected larvae were reared to adults. Preserved larvae were counted, sorted by instar, and then third instars were identified to species. Reared adult specimens also were identified to species (Gruner 2004).

Additional volume and temperature data were obtained from published values in Goodbrod and Goff (1990). In that study, 25, 50, 100, 200, and 250 larvae of *Chrysomya rufifaces* (Macquart) larvae were reared on 25 g of beef liver (plus 500 larvae on 12.5 g of liver) to have ratios ("larval density") of 1, 2, 4, 8, 10, and 40 larvae per gram of substrate. We calculated the approximate maximum volume of the *C. rufifaces* aggregations in that study. Maximum lengths of larvae were estimated from their Fig. 3, and then we compared live *C. rufifaces* specimens in our possession with the lengths obtained from the figures to calculate the volume that individual larva occupied (D.H.S., personal observation). Aggregation volumes from the Goodbrod and Goff (1990) study were then estimated as volume occupied per larva multiplied by the number of larvae in the aggregation. Finally, we estimated the maximum aggregation temperatures from fig. 4 of the same study.

Data Analysis. The dimensions of the larval aggregations were determined with a combination of field notes, field sketches, and photographs. The surface area of each aggregation was estimated by a geometric approximation method. We drew an irregular polygon that closely followed the perimeter of the aggregation on a scale drawing or measured photograph, then broke the irregular shape into a combination of simpler geometric shapes (e.g., squares, triangles, trapezoids), Finally, we calculated the combined area of the

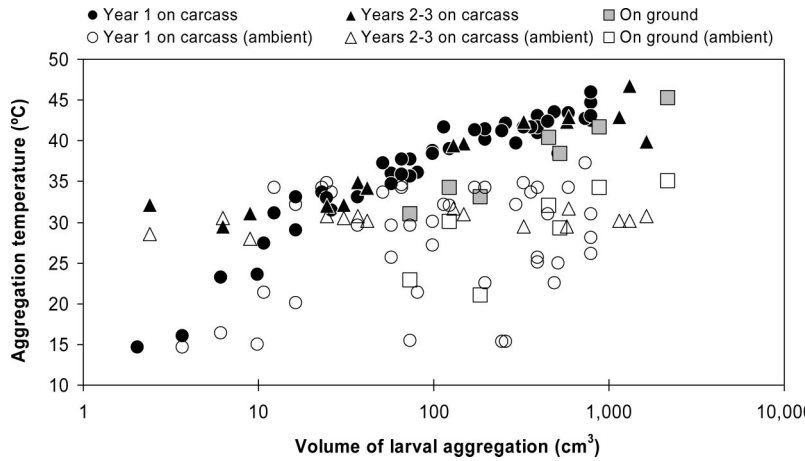

Fig. 1. Temperatures of larval aggregations from the Florida study compared with volume. Ambient temperatures are shown in white, and the corresponding aggregation temperatures are shown in black or gray. Close correspondence in the volume–aggregation temperature relationship can be seen between years in the larger volumes regardless of ambient temperature, but the smaller volume aggregations show little similarity because ambient temperature significantly affects these smaller masses. On ground, larval aggregations on the ground; on carcass, larval aggregations on the carcass. Ambient, temperature readings taken in the air near the carcass at the same time as the corresponding aggregation temperature.

simpler shapes. To obtain the volume of the aggregation, we multiplied the area of the aggregation by the mean of the five to 50 depth measurements recorded on the data sheet. Any mass that had indefinable borders, had a depth that could not be accurately established or was not tightly packed was not used in any analysis.

This estimation procedure was developed because it was simple to implement and allowed adjustments for parts of the mass that could not be photographed. It also allowed in situ measurement of volume without disturbing the larvae. Most importantly, it can be used by investigators in the field who may lack ready access to more sophisticated apparatus.

After all useable masses were measured, duplicate measurements were identified (defined as masses on the same pig, similar location of mass, but on a different date), and one of the duplicate measurements was randomly selected to represent that mass. The other duplicates were removed from the database.

We used the larger data set from Florida for statistical analysis and model parameterization, and the Indiana data for validation. We tested the effect of volume, ambient and soil temperatures, visible surface area of the mass, area/depth ratio, species composition, carcass size (Hewadikaram and Goff 1991) and larval density (Goodbrod and Goff 1990) on the maximum temperature of each larval aggregation. Effects of rain and clouds also were examined. Multiple linear regression was used to analyze the effect of all variables (Hintze 2004), and transformations were applied when necessary to normalize variance (see Results). Mindful of the ≈10 repeated tests that were necessary to analyze all of the factors, we adopted a comparisonwise α level of $1 - (1 - 0.05)^{1/10} = 0.0051$ (Jones 1984). Significant regression results were combined into a custom linear model, and the model fit was calculated using the coefficient of determination, r^2, directly from the model results (Neter and Wasserman 1974).

Large aggregations have been reported as having internal temperatures that are greater than ambient (Turner and Howard 1992, Marchenko 2001), so we first looked at the effect of volume on temperature.

Results

In total, 68 pigs in Florida and 12 pigs in Indiana were used, with an average weight of 24.3 ± 5.3 kg (mean ± SD). Several larval aggregations formed on each carcass during decomposition, with 67 aggregations from 29 carcasses in Florida and 17 aggregations from 12 carcasses in Indiana meeting our standards for volumetric analysis. All months were represented in Florida except December, whereas months represented in Indiana were June through November. The ambient temperatures found during the afternoon sampling periods ranged from 13.5 to 37.2°C, and the overall ambient temperatures during the field trials ranged from −3.0 to 37.2°C.

The volume of an aggregation had a strong influence on its afternoon internal temperature [$F_{(2,\ 64)} = 197.53$; $P < 0.0001$], and both ln(volume) and ln(volume)^2 were highly significant contributors to the model [$F_{(1,\ 64)} = 106.37$ and 45.24, respectively; $P < 0.0001$) (Fig. 1, equations 1–3), and the residuals were normal and consistent. In contrast, volume was a poor predictor of the difference between mass temperature and ambient temperature, and the model variance varied between small and large volumes. These are indications that the difference between ambient and aggregation temperature was not explained well by aggregation volume.

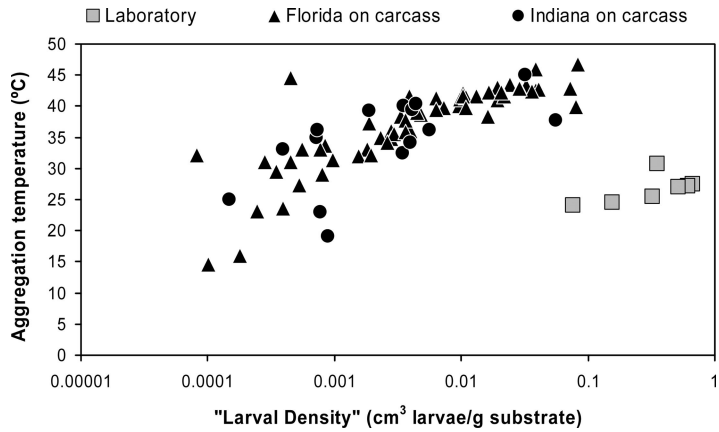

Fig. 2. Temperatures of larval aggregations from the Florida study compared with larval density. The three data sets (Florida, Indiana, and laboratory from Goodbrod and Goff 1990) show the wide range of densities found. Larval aggregations found on the ground were excluded from this figure. Note the wide separation between the laboratory data and the two field trials using pig carcasses, indicating the poor power of larval density to predict aggregation temperature.

The average depth of most aggregations was 2–5 cm, although some were >10 cm in depth. After the volume of the mass was taken into account, no relationship was found between the residual aggregation temperature and the log-transformed ratio of the depth of the mass to its surface area [$F_{(1, 65)} = 3.61$; $P = 0.06$ and $F_{(1, 64)} = 0.79$; $P = 0.38$ with one outlier removed], which indicates that the shape of the larval aggregation had little influence on its temperature.

There was no effect of postmortem interval on the residual aggregation temperature [$F_{(1, 65)} = 0.79$; $P = 0.38$] after the effect of volume was removed, which implied that larval age or instar had no effect on aggregation temperature. Aggregations with first instars had temperatures equivalent to similarly sized aggregations of second or third instars.

Pig weight, which ranged from 15.9 to 31.8 kg, had no effect on aggregation volume [$F_{(1, 65)} = 0.05$; $P = 0.82$] or the residual aggregation temperature [$F_{(1, 65)} = 0.02$; $P = 0.89$]. If larval density was a predictor of larval aggregation temperature, we would expect substrate weight to be independently significant, because substrate weight (pig carcass) and larval volume are the two independent factors in larval density. The previous test was not powerful, because the weights of the pig carcasses were all similar, whereas the volume of the larval masses varied widely. To increase the range of substrate weight and the power of the larval density test, we included data from Goodbrod and Goff (1990), who used a much smaller substrate weight. After removing the highly significant ln(volume) variable, substrate weight was still insignificant as a predictor of aggregation temperature [$F_{(1, 72)} = 1.85$; $P = 0.18$]. Nonsignificance of substrate weight indicates that larval density was immaterial for predicting aggregation temperature (Fig. 2).

Ambient temperature had a significant effect on the temperatures of small masses (<20 cm^3) on the carcass [$F_{(1, 6)} = 18.32$; $P = 0.005$; equation 2]. For masses of all sizes on the ground, surface soil temperature was somewhat significant [$F_{(1, 5)} = 16.96$; $P = 0.009$; equation 3]. In both cases, higher ambient temperatures were associated with higher aggregation temperatures. By comparison, ambient temperature did not influence the temperature of masses larger than 20 cm^3 that were on the carcass [$F_{(1, 50)} = 0.74$; $P = 0.39$], which indicated that these larger larval masses regulated their temperatures over a wide range of ambient temperatures (14.6–37.2°C at the time of measurement, −3.0–37.2°C overall). Seasonality, expressed as the month the sample was taken, was also not significant after the effect of volume was removed [$F_{(8, 49)} = 0.78$; $P = 0.62$].

In total, 5,039 calliphorid larvae were reared and identified from the Florida samples. Seven species were found: *Lucilia coeruleiviridis* (=*Phaenicia*) (Macquart) (3,876 specimens, 76.9%), *Cochliomyia macellaria* (F.) (418, 8.3%), *Chrysomya rufifaces* (Macquart) (386, 7.7%), *Phormia regina* (Meigen) (275, 5.5%), *Chrysomya megacephala* (F.) (78, 1.5%), *Calliphora livida* Hall (4, 0.1%), and *Calliphora vicina* Robineau-Desvoidy (=*Calliphora erythrocephala* Meigen) (1, 0.0%). There were 22 larval aggregations found in which a single species made up at least 95% of each mass (19 *L. coeruleiviridis*, one *C. macellaria*, and two *C. rufifaces*), six masses where two species made up at least 20% each of the total calliphorid larvae (all containing *L. coeruleiviridis* with one other species), and one mass where three species made up at least 20% each of the total larvae. Once volume and ambient temperature were accounted for, the residuals for *L. coeruleiviridis* aggregations averaged 0.12 ± 0.29 (mean ± SE), the other single species aggregations averaged 0.06 ± 0.85, and the multiple-species aggregations averaged −0.56 ± 0.72. These data were too sparse for a robust statistical analysis, but all of the standard errors overlapped zero, and it seemed that there was no difference in internal aggregation temperature among the species assemblages. The species composition of the aggregations in Indiana was not analyzed, but aggregations

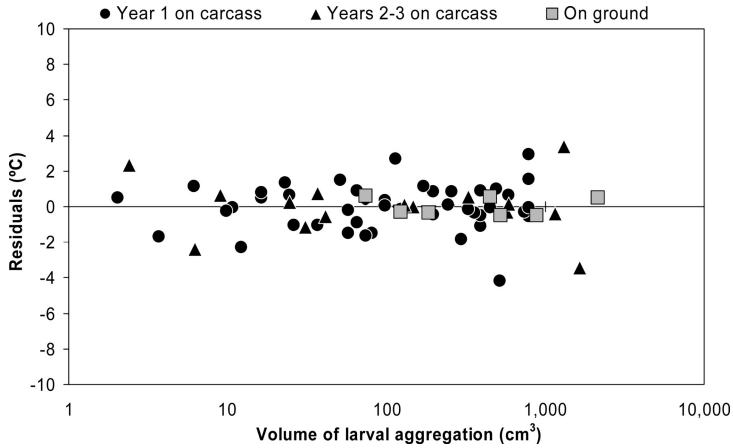

Fig. 3. Residuals of temperature from larval aggregations in Florida after application of equations 1–3. On ground, larval aggregations on the ground; on carcass, larval aggregations on the carcass.

there were dominated by *L. coeruleiviridis*, similar to the Florida study.

After sorting all preserved larval samples in Florida by instar, we found that there were seven aggregations containing only first instars, and four aggregations containing only third instars, but none with only second instars. The rest of the aggregations ($n = 56$) contained a mix (1–2, 1–2-3, or 2–3 instar) of larval stages. After accounting for volume and ambient temperature, the residual temperatures for first instar aggregations averaged -0.16 ± 0.83 (mean $\pm$ SE), mixed aggregations averaged 0.10 ± 0.18, and third instar aggregations averaged -0.40 ± 0.59. Again, these data were too sparse for a robust statistical analysis, but all of the standard errors overlapped zero, and there seemed to be no residual influence of instar on the internal aggregation temperature.

Weather may affect the thermal properties of larval aggregations, so we separated from the Florida data sampling days that were entirely cloudy ($n = 18$ aggregations), and those that were entirely sunny ($n = 14$ aggregations). We found no difference in the internal temperature of aggregations on sunny days versus cloudy days after removing the effect of volume [$F_{(1, 30)} = 0.91$; $P = 0.35$], but all the pig carcasses were in locations where they generally did not receive full sun. Similarly, we separated sampling days that had >0.1 in of rainfall occur while we were actively sampling ($n = 9$ aggregations) and compared them to entirely dry days ($n = 50$ aggregations), and we found no difference in the internal temperature of aggregations after removing the effect of volume [$F_{(1, 57)} = 0.09$; $P = 0.77$]. In our wooded location, the weather during the sampling day had no effect on internal temperatures during the afternoon sampling period.

After analyzing likely contributors to the temperature of a larval aggregation, final models were determined using the Florida data, based on the significant contributions made by each factor. The volume at which ambient temperature became insignificant for the determination of temperature was between 16 and 25 cm^3; we used 20 cm^3 in the models.

For masses larger than 20 cm^3 on the carcass, the final predictive model of temperature was dependent only on aggregation volume:

$$T_{max} = 7.9578 + 9.6294 \cdot \ln(v) - 0.6546 \cdot \ln(v)^2 \quad [1]$$

where v is aggregation volume (cubic centimeters), and T_{max} is the highest temperature (Celcius) in the aggregation.

For masses smaller than 20 cm^3 on the carcass, a negative interaction between ambient temperature and volume in the model indicated that the influence of ambient temperature decreased as aggregation volume increased:

$$T_{max} = -10.8651 + 1.4133 \cdot T_a + 11.8232 \cdot \ln(v) - 0.3918 \cdot T_a \ln(v) \quad [2]$$

where T_a is the ambient air temperature (Celsius).

For masses of any size found on the soil, there was strong dependence on volume and a lesser effect from soil temperature:

$$T_{max} = 13.5624 + 0.2633 \cdot T_s + 3.0272 \cdot \ln(v) \quad [3]$$

where T_s is the soil surface temperature (Celsius).

Combining the three models explained almost all of the variability in the Florida data, with no measurable bias ($n = 67$, $r^2 = 0.960$, residual average $\pm$ SE = -0.015 ± 0.16) (Fig. 3). The combined model also explained the majority of the variability in the validation data from Indiana but with a possible bias of <1° ($n = 17$, $r^2 = 0.666$, residual average $\pm$ SE = -0.84 ± 0.99 (Fig. 4). Finally, the fit of the small-mass model to the laboratory data (Goodbrod and Goff 1990) was good, but it had a low r^2 value because of the few data points and relatively flat slope ($n = 7$, $r^2 = 0.328$, residual average $\pm$ SE = 0.32 ± 0.69) (Fig. 4).

The parameter in the model that was most difficult to measure precisely was the volume of the larval

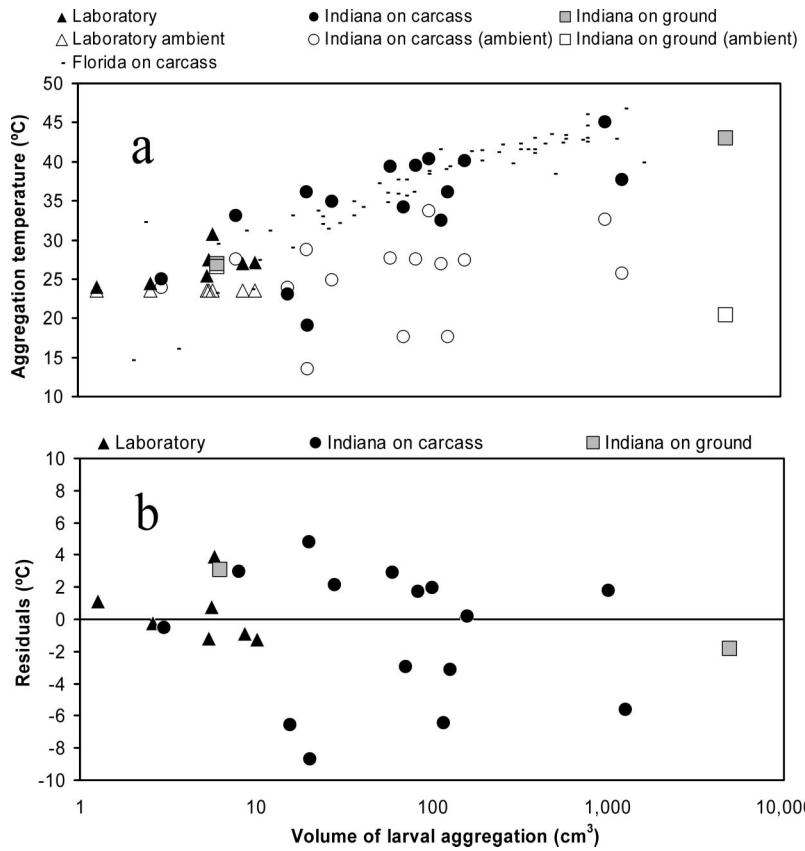

Fig. 4. (a) Temperatures of larval aggregations compared with volume. (b) Residuals of temperature from larval aggregations after application of the models, equations 1–3. Ambient temperatures are shown in white, and the corresponding larval aggregation temperatures are shown in black or gray. Laboratory, data from Goodbrod and Goff (1990); on ground, larval aggregations on the ground; on carcass, larval aggregations on the carcass. Ambient, temperature readings taken in the air near the carcass at the same time as the corresponding aggregation temperature.

aggregation. We tested for the sensitivity of the final model to errors in volume estimation, and we found that the model was not sensitive to volume estimation errors, probably because the volume parameter is log transformed. For example, a large 30% error in volume measurement introduced a maximum model error of only 0.68°C over the 4 orders of magnitude of aggregation volumes that we measured. Such a small model error indicates that the model is robust with realistic field error rates in volume estimation.

Discussion

Heat production from larval aggregations has been acknowledged in the literature (e.g., Cianci and Sheldon 1990, Turner and Howard 1992, Campobasso et al. 2001), but the strong relationship found here between aggregation volume and internal temperature has not been reported previously. In this study, larval aggregations found on the carcasses that had a volume >16–25 cm^3 (smaller than a table-tennis ball) had afternoon temperatures that were fully independent of ambient temperature.

Aggregations with a volume of 20–50 cm^3 had internal temperatures of ≈30–35°C, the optimum temperature range for calliphorid larval growth (Davidson 1944, Byrd and Butler 1996). Larvae within these aggregations would become resistant to effects of low temperature such as diapause (Vinogradova and Zinovjeva 1972) or low temperature inactivation (Sharpe and DeMichele 1977). Aggregations larger than 50 cm^3 had core temperatures higher than optimum, even lethal (Wigglesworth 1967), but larvae from the centers of large masses move deliberately to the cooler periphery (Anderson and VanLaerhoven 1996; S.V.G., personal observations), where in addition to escaping from the hot center of the mass, evaporative cooling of the wet larvae would occur, so the average temperature experienced by a larva would be less than the hottest temperatures in the aggregation.

Far from being a wasted by-product of decay and digestion, metabolic heat production in larval aggregations seems to be coupled with cooling mechanisms to optimize the larval thermal environment. The mechanism of thermal regulation is beyond the scope

of this article, but it may be related to the alteration of digestive enzymes, or to bacterial levels, or by variation in transit times between feeding and cooling off. Evidence of active heating and cooling include aggregation temperatures that were cooler than ambient (Fig. 1; also see Toolson 1987), and aggregation temperatures that were within a degree or two of other aggregations of similar volume, even when ambient temperatures differed by as much as 20°C. Even with cooling mechanisms, apparently lifeless maggots were seen several times around the periphery of large masses, perhaps the result of overheating. It seems that at small volumes (<20 cm^3), there is not enough metabolic output to provide regulation, and at large volumes (perhaps $>1{,}000$ cm^3), there is too much metabolic output for cooling mechanisms to overcome.

Although not included in any calculations, we noted that groups of loosely associated larvae had lower temperatures than tight aggregations of comparable size. Therefore, the relationship found here between volume and temperature applies only to tightly packed masses that can retain their metabolic heat. In contrast, masses deep in the interior of the pig had temperatures as high as 50.7°C, and although we were unable to measure the volume of these interior masses with any accuracy, these temperatures were 5–7°C hotter than the largest and hottest masses found on the surface of the pig. It may be that masses deep inside wound channels are better insulated against convective and radiative heat loss than are masses exposed on the surface; therefore, they maintain higher overall temperatures. Larval aggregation temperatures of >50°C are extreme, but they have been reported previously (Anderson and VanLaerhoven 1996, Deonier 1940). Small masses, and masses found on the soil, were significantly affected by ambient temperatures, possibly because those masses shed heat too rapidly to the environment for the larvae to regulate the mass temperature.

In the larger Florida study, we found that the hottest temperature in each aggregation was not always exactly in the center of the mass, so we took multiple temperature readings throughout each aggregation. Aggregation temperatures in Indiana were ≈0.84°C lower compared with similarly sized aggregations in Florida, possibly because the hottest temperature in each mass was not found with the single temperature reading that was recorded in each aggregation. The otherwise good fit of the model to data from a vastly different climatic zone suggests that it may be universal for the four species commonly encountered in this study.

Our study was not designed to experimentally test for effects from sunlight versus shade. Other investigations have found no effect (Joy et al. 2002) or possibly some effect (Reed 1958, Shean et al. 1993). All of our pig carcasses were in partial shade, and although we found no difference in aggregation temperatures between cloudy days and sunny days, it is possible that aggregations that develop in full sun may experience hotter temperatures due to extra solar heating (Cianci and Sheldon 1990). We also did not experimentally test for the effects of precipitation on the thermal regulation of the larval aggregations, but by comparing the rainy days with the sunny days, no difference was found in aggregation temperatures. There were only nine aggregations in the final database that were sampled during rain, so we cannot say that any amount of rain would not disrupt thermoregulating larvae. During one notably violent storm, some larvae were seen moving to form new aggregations on the underside of the carcass. Higher aggregation temperatures have been associated with later instars (Cianci and Sheldon 1990), but in our sampling of aggregations made up of only first instars, mixed instars, or only third instars, we found that the instar in an aggregation had no effect on aggregation temperature.

Linking the volume of an aggregation with afternoon temperatures was the first step in characterizing the thermal environment of larvae found within an aggregation. Other factors need to be better understood before a complete picture of the thermal environment of a larva can be established. These factors include both diurnal temperature cycling and changes in aggregation volume over time through mechanisms of oviposition, immigration, emigration, and mortality. Also, the aggregations found in this study were dominated by *L. coeruleiviridis*, with *C. macellaria*, *C. rufifaces*, and *P. regina* also present in large numbers, but it is likely that some other forensically important calliphorids do not share the same aggregating or thermal properties. More information is required to build a model of the thermal environment within an aggregation through space and time and to more accurately estimate the growth rates of larvae found on a body.

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