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The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*

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Abstract

The use of entomological evidence in the estimation of the post mortem interval (PMI) often depends on the size and developmental stage of blowfly larvae collected from a corpse. Therefore, factors which can have an effect on the larval size and growth rate can have implications for reliable PMI determinations. This study explores the competitive effects of larval overcrowding on *Calliphora vomitoria* reared on three different pig tissues – liver, brain and muscle. The competitive feeding environment within the more crowded larval cultures resulted in increased development rates and the production of undersized larvae and adults. Variation in the extent of these effects was observed on each of the three body tissues, highlighting the importance of documenting the positions from which entomological evidence is recovered from a corpse.

Keywords: Competition; Density; Larval mass; Adult size; Survival; Forensic entomology; PMI

1. Introduction

The major use of insect evidence in forensic science is in the estimation of the post mortem interval (PMI), the time between death and discovery of a corpse. Estimates are most commonly determined by examining the developmental stage of a particular insect species found on a corpse. Knowledge of the time typically taken to reach this stage can then give an indication of the PMI.

In the UK, the Calliphoridae (Diptera), are of particular importance as these are the usual species forming the first wave of the faunal succession to arrive and lay eggs on a corpse. Those routinely encountered by forensic entomologists are the common bluebottle species – *Calliphora vomitoria* L. and *Calliphora vicina* Robinseau-Desvoidy and the greenbottles (*Lucilia* spp.).

There are two methods available for PMI estimation using blowfly development. One approach involves killing the larvae collected from a crime scene and comparing the measured larval length with reference data on larval size, related to temperature and time in a species-specific isomegalendiagram [1]. A second, more widely adopted approach involves calculating the accumulated degree hours or days (ADH/D) required for a larva to reach a particular stage of development. Both methods enable the age of a larva to be estimated and thus provide an indication of the PMI.

As the size and developmental stage of the larvae collected from a corpse provide an important indication of the PMI, factors which can affect the size and growth rate of blowfly larvae must be considered by forensic entomologists. Factors which have been most extensively studied include the effects of temperature variation [1–4] and the presence of drugs in body tissue [4]. Larval crowding and the

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resulting competition for food resources is also an important issue since previous studies have shown that it results in undersized individuals, which has potential implications for reliable PMI determinations [5–8].

Carcasses are often scarce in nature and female blowflies tend to lay many eggs on a corpse, resulting in high larval densities once these eggs hatch. The presence of other individuals increases oviposition by females, probably due to contact and chemical stimulation [9]. Larval aggregation is beneficial as feeding larvae pre-digest food resources by secreting salivary and proteolytic enzymes and, by aggregating in masses, an ample supply of these secretions is ensured [10]. In addition larval masses experience elevated temperatures, due to their metabolic activity, which can speed their development [11].

Group oviposition may also have detrimental effects if too many eggs are laid on a corpse. Larvae can become overcrowded, resulting in increased competition for limited resources. Previously published work indicates that intraspecific competition for food between larvae does not tend to result in high mortality, as one might expect. Rather, mortality is generally low – about 15% [12] – and smaller-sized individuals are produced, in larval, pupal and adult stages [5]. These undersized adults are still capable of laying full-sized and viable eggs, although usually in smaller numbers. This is indicative of exploitative competition, where a limited resource (in this case, food) is shared between all individuals [13] and survival is maintained at the expense of size. However, the production of undersized individuals has implications for the PMI estimation. If it is not accounted for, an underestimate of the PMI is possible.

This study reports on a series of laboratory experiments which explore the effects of varying degrees of larval crowding on the size and development of *Calliphora vomitoria* as there has been very little published to date on the competitive effects of this species. These effects have been investigated with *C. vicina* larvae feeding on different body tissue [14], indicating that *Calliphora vicina* larvae grow at different rates on different body tissues. A similar effect has also recently been observed in *Lucilia sericata* [15].

2. Materials and methods

Populations of *C. vomitoria* were raised and maintained in gauze covered cages (37 cm × 22 cm × 20 cm) at room temperature (∼20 °C) and flies were supplied with granulated sugar and water ad libitum. The cages contained a maximum of 200 flies in approximately a 1:1 ratio of males and females.

Flies were provided with liquid liver exudate for 4 or 5 days on a daily basis as a protein source to promote ovarian development of females. When eggs were required, fresh pigs' liver (∼5 g) placed inside a weighing boat (8 cm × 5 cm × 1.5 cm) was introduced to a cage as an oviposition stimulus and removed once sufficient eggs had been laid. The eggs were then left to hatch which occurred within 24 h of oviposition.

Cultures with different degrees of larval crowding were established by transferring 1, 5, 10, 20, 50, 75 or 100 newly hatched larvae to a 10 g (±0.1 g) piece of fresh food inside a weighing boat. The food source was either pigs' liver, purchased from a local supermarket, or pigs' brain or cheek muscle, purchased from a local abattoir. Each weighing boat was placed inside a plastic container (12 cm × 9.5 cm × 6.5 cm) filled with 50 g (±2 g) of multipurpose compost, required as a medium in which post-feeding larvae could pupate. This was sealed with a plastic lid pierced with six small air holes and maintained at 20 °C (Cooled Incubator, LMS, Sevenoaks, Kent, UK). All cultures were kept in constant darkness, except when incubators were opened to check on experimental progress, and three replicates of each condition were carried out.

Once larvae had pupated, the weighing boat containing any remaining food was removed from the plastic container. The first day of adult eclosion was noted for each culture and, after a minimum of 7 days had elapsed, all flies were killed by freezing (−20 °C, Lec freezer, UK). Personal observation indicated that 7 days after first adult emergence was ample time for all remaining flies to emerge.

After freezing, all adults from each culture were removed from the container and separated according to gender. The size of each adult was then determined by measuring the length of the posterior cross vein (dm-cu) on the left wing (Fig. 1).

The measurement of the posterior cross vein as an indication of adult body size is a well-documented approach [5–7,15]. The measurement of the full wing length is another technique adopted [8,16]. However, in this study measurement of the cross vein was preferred as it is usually still measurable even if the wing experiences considerable damage.

The left wing of each adult was removed and affixed to 90 gsm white paper using clear nail polish. The paper was then oven dried at 40–50 °C (Incubator: IH-180, Sanyo-Gallenkamp, UK) for a minimum of 24 h. Once dry, the wings were protected from damage by covering with a layer of clear adhesive tape.

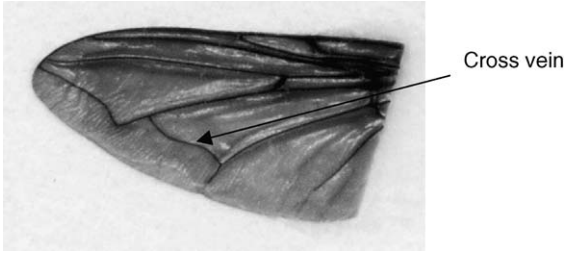

Fig. 1. Left wing of *C. vomitoria* adult, with the posterior cross vein (dm-cu) used in measurements indicated.

The wings were scanned as digital images using an Epson Stylus Colour CX5200 scanner, adopting the settings of 300 dpi resolution and 400% magnification suggested by Hwang [17]. The length of the cross vein was then measured using the computer software 'ImageJ v.1.26t', obtained via the Internet [18]. As suggested by Hwang [17], the program was calibrated to 47 pixels = 1 mm. The length of the cross vein was determined by recording the straight line distance between the points where the ends of the cross vein touch the longitudinal veins (Fig. 1). This measurement was recorded three times and a mean length calculated.

Statistical analyses (one way ANOVA and Fisher's protected least significant difference post hoc tests) were carried out using the SAS package Statview v.5.

3. Results and discussion

3.1. Effect of larval crowding on rate of development

An approximate indication of the speed at which *C. vomitoria* grow on different body tissues was obtained by recording the length of time taken for adults to emerge from puparia. Each culture was inspected on a daily basis after most larvae had pupated, and the first day of adult eclosion was recorded (Table 1).

Analysis of the data show that the effect of food type is significant over the range of larval densities included in this experiment ($F = 13.41$, $p < 0.0001$). However, there is little difference between the times taken to reach the adult stage of development at *low* larval densities. Therefore, when food is in plentiful supply differences between the three types of body tissue on the speed of *C. vomitoria* development are less evident. This appears inconsistent with Kaneshrajah and Turner's observations of *C. vicina* development [14], although that study only explored development during the larval stages and a concerted effort was made to ensure that food was always in excess so that competitive effects could be ruled out. Thus, direct comparisons with results shown here cannot be drawn without further investigation.

Faster development is observed in the more crowded larval cultures reared on liver and muscle (Table 1), which is consistent with studies undertaken with other blowfly species, such as *C. vicina* [5]. Increased temperatures, which can reach up to 40 °C [11] and the more efficient feeding within larval aggregations [10] are thought to be the primary factors contributing toward this trend.

Larvae reared on brain in crowded cultures display the opposite effect – development slows down (Table 1). Observations of the remaining food levels throughout this experiment showed that brain was consumed more quickly than either liver or muscle. In the severely overcrowded cultures, containing 75 and 100 larvae, the brain food source was entirely depleted by the time larvae had reached the third instar. In contrast, the liver and muscle used in the corresponding crowded cultures was never completely consumed by the larvae.

Research undertaken in the early 1970s [19] showed that third instar larvae of *C. vomitoria* and *Sarcophaga argyrostoma* (a fleshfly species) must feed for 3–10 h after the moult between second and third instar before they can pupate and become adults. Development halts at the third instar if larvae are unable to feed from this stage, although they can remain alive for several days. If a new food supply is discovered during these few days, development can resume as normal [19]. This behaviour among *C. vomitoria* larvae may provide an explanation for the delayed emergence of adults in crowded cultures reared on brain.

The survival of *C. vomitoria*, determined as the percentage number of individuals reaching adulthood, is very low in the crowded larval cultures reared on brain (Fig. 2).

Development of those individuals not reaching adult eclosion was found to primarily cease during the larval stages. It is therefore possible that the few individuals which reached the adult stage in these crowded cultures may have ceased development for several days during the third larval instar, and then utilised dead *C. vomitoria* larvae within the culture as a new food source in order to resume development, thus providing an explanation for the delayed adult eclosion observed. This rationalisation is

Table 1
Development times for *C. vomitoria* to reach adult eclosion on alternative body tissues, at different larval densities reared at 20 °C

Larval density[a]	Liver		Brain		Muscle	
	Mean no. days to adult eclosion	S.D.	Mean no. days to adult eclosion	S.D.	Mean no. days to adult eclosion	S.D.
1	22.3	0.577	24.0	0.000	24.0	2.000
5	22.0	0.000	22.7	0.577	22.0	0.000
10	21.7	1.155	21.7	0.577	22.0	0.000
20	21.3	0.577	21.0	0.000	21.0	0.000
50	21.3	1.155	21.0	0.000	21.3	0.577
75	20.7	1.155	24.3	2.517	21.0	0.000
100	20.0	0.000	27.5	4.950	21.0	0.000

[a] Given as numbers of larvae per 10 g wet weight food.

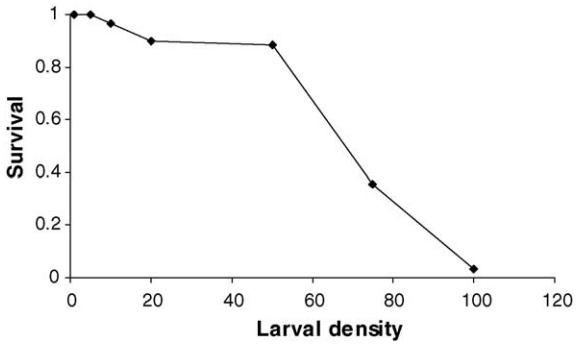

Fig. 2. Survival rate of *C. vomitoria* reared on pig brain as a function of increasing larval density, expressed as the percentage number of individuals reaching adult eclosion.

supported by personal observations in which third instar *C. vomitoria* larvae were seen attacking a dead larva of the same species when contained inside a petri dish with no other food source.

Very little is known about cannibalism among blowflies. Research into the cannibalistic behaviour of two blowfly species, *Chrysomya rufifacies* and *Chrysomya albiceps*, which are known predators of other dipteran larvae, has been conducted [20,21]. Both exhibit cannibalism, but at different stages of larval development. *C. rufifacies* have sufficiently developed mouth parts to kill prey by the second larval instar, but *C. albiceps* do not attack others until the third instar.

Cannibalism appears to be secondary to the attack of other species. In the study based on *C. albiceps* [21], it is only observed in cases when prey or other food sources, such as carrion, are absent. Although these studies involve blowfly species already known to attack other larvae, hunger is unmistakably a driving force inducing cannibalism. Therefore, the possibility of *C. vomitoria* larvae consuming others of the same species when alternative food sources have been depleted is a plausible consideration.

3.2. Effect of larval crowding on adult survival rate

The survival rate of *C. vomitoria* reared on each tissue type is expressed as a percentage by comparing the number of adults produced in each culture with the initial larval density (Table 2).

Both larval density ($F = 21.71$, $p < 0.0001$) and food type ($F = 4.54$, $p = 0.017$) have a significant effect on the survival of *C. vomitoria*. The effect of larval density is to be expected, and is more pronounced. Increased competition during feeding is known to reduce survival rates [6,10,22]. Differences between the alternative food sources are only significant between brain and liver ($p = 0.006$) and brain and muscle ($p = 0.039$). This is consistent with the experimental observation that brain was consumed more rapidly than either liver or muscle, running out entirely before larval feeding was complete in the more crowded cultures.

The detrimental effects of larval *undercrowding* on survival, documented by dos Reis et al. [10] after studies conducted with *Chrysomya putoria* and *Cochliomyia macellaria*, are not evident with *C. vomitoria* (Table 2). By aggregating in masses, larvae are able to increase the efficiency of feeding by ensuring a plentiful supply of digestive fluid is produced for the pre-digestion of food. Mackerras and Freney [23, cited in 10] noted that single larvae are unable to produce enough of these digestive secretions to obtain adequate amounts of food for themselves. This was not seen in this study: in all cultures containing a single larva, full development to the adult occurred.

3.3. Effect of larval crowding on adult size

Increasing larval density produces smaller adults (Figs. 3–5) and this effect is significant on all three body tissue types (liver: $F = 65.98$, $p < 0.0001$; brain: $F = 129.83$, $p < 0.0001$; muscle: $F = 42.62$, $p < 0.0001$). This is consistent with previous studies on other blowfly species [5–8].

Table 2
Average survival rates of adult *C. vomitoria* reared on alternative body tissue at different larval densities reared at 20 °C

Larval density[a]	Liver		Brain		Muscle	
	Mean no. of adults per replicate	Percentage survival	Mean no. of adults per replicate	Percentage survival	Mean no. of adults per replicate	Percentage survival
1	1	100	1	100	1	100
5	5	100	5	100	5	100
10	8	80	10	100	9	90
20	15	75	18	90	17	85
50	42	84	44	88	47	94
75	47	63	27	36	59	79
100	64	64	3	3	24	24

[a] Given as numbers of larvae per 10 g wet weight food.

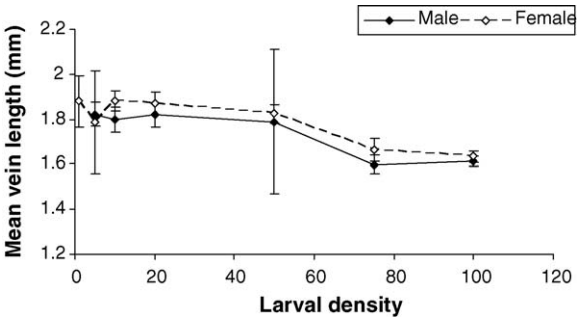

Fig. 3. The effect of larval density on adult size, after larval rearing on pig liver (error bars indicate ±2S.E.).

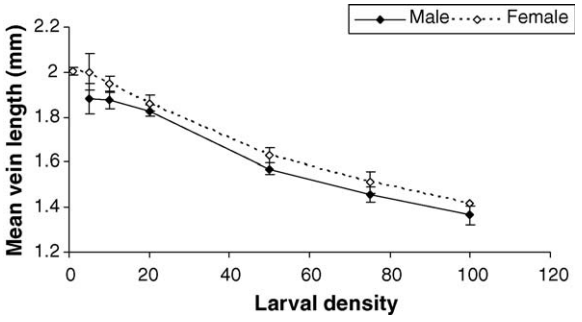

Fig. 4. The effect of larval density on adult size, after larval rearing on pig brain (error bars indicate ±2S.E.).

In full-sized blowflies the adult female is usually noticeably larger than the adult male, and this trend is consistently adhered to throughout the range of larval rearing densities ($p < 0.0001$ for liver, brain and muscle, Figs. 3–5). Studies with *C. vicina* suggest that this sex-dependent element into adult size becomes less important when extreme levels of larval competition for food are experienced [8]. This consequence is not observed with the results of this experiment. Larval density does not affect the size of male and female *C. vomitoria* adults in a significantly different manner on any of the three food types (liver: $F = 0.10$, $p = 0.981$; brain: $F = 0.42$, $p = 0.839$; muscle: $F = 1.65$, $p = 0.145$).

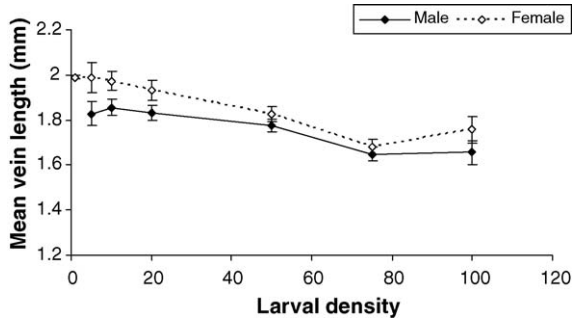

Fig. 5. The effect of larval density on adult size, after larval rearing on pig muscle (error bars indicate ±2S.E.).

Table 3
Composition of brain, muscle and liver

	Human [24]		Pig [25]	
	Brain (%)	Muscle (%)	Muscle (%)	Liver (%)
Water	78	75	72	72
Lipids	10–12	5	5	3.4
Proteins	8	18–20	21.5	21.1
Carbohydrates	1	1	0	3

No information on pig brain is available and human data are used for comparison [24,25].

In 1995, Saunders and Bee published work [5] on the effects of larval overcrowding on the size of *C. vicina*. They observed that the greatest changes in the effects of larval crowding occur between 50 and 150 larvae per 50 g of minced beef muscle. Therefore they concluded that approximately 1 g of minced beef muscle is adequate for the full development of a larva of *C. vicina*.

In this study, the greatest changes in the effects of larval crowding on the size of *C. vomitoria* are observed at different intervals on each of the three body tissues. Table 3 shows the differing compositions of brain, muscle and liver. Human brain data are provided in the absence of pig brain information. There are considerable differences in the fat/protein ratios and carbohydrate levels of the different tissues.

C. vomitoria development on liver is the most efficient of the three body tissues investigated. The greatest changes in the effects of larval crowding are seen between larval densities of 50 and 75 larvae per 10 g of food, suggesting that about 0.2 g of pig liver is sufficient for full larval development.

Brain appears to be the least nourishing of the three tissue types utilised. Lower protein levels, a softer consistency and slightly higher water content appear to promote its greater and more rapid consumption compared to the other tissues offered. The greatest changes in the effects of crowding are observed when the initial larval density is above 10 larvae per 10 g of food, suggesting that approximately 1 g of pig brain is required for full development of a larva of *C. vomitoria*.

For the most direct comparison with the results obtained by Saunders and Bee [5] the effects of larval growth on pig muscle are considered. Analysis of the results obtained indicates that the greatest changes are seen when the larval density is above 20 larvae per 10 g of food. This suggests that approximately 0.5 g of pig muscle is a sufficient amount to ensure full development of a larva of *C. vomitoria*, half the quantity of minced beef muscle required for complete larval development of *C. vicina*.

Although comparisons between this study and the work conducted by Saunders and Bee [5] can be made, conclusions are difficult to draw, primarily due to the different origin of the muscle used in each experiment. This is likely to cause variation in the results, as illustrated by studies with

Lucilia sericata [15]. Also the beef muscle offered to *C. vicina* was minced prior to use, unlike the pig muscle provided to *C. vomitoria*. It was initially thought that the difference in the food surface area might affect the rate of digestion by larvae. However, Clark et al. [15] have shown this not to be the case.

Other factors influencing the competitive interactions between larvae also require consideration. The space available to larvae within a culture may affect the levels of mutual interference experienced by individuals, and may therefore influence their feeding behaviour. Obviously the experimental conditions implemented in this study were identical throughout so that comparisons between food sources can reliably be made. However, the exact conditions adopted by Saunders and Bee [5] are unknown, thereby making any differences between the experiments arising due to these factors more difficult to verify.

4. Conclusions

Overcrowding during the larval stages of development results in a competitive feeding environment, and as such, size, development rate and also survival, are all affected.

In general, increased larval crowding results in faster development of *C. vomitoria*, most likely due to a combination of the higher temperatures attained and the more efficient feeding which is possible within large larval aggregations. An increased development rate can mislead the forensic entomologist during casework and may result in an *overestimate* of the PMI if not taken into account.

The development rate at different larval densities was considered on three body tissues; liver, brain and muscle. Variation between the food sources was observed, especially in the highly overcrowded cultures. This highlights the importance of documenting the positions from which entomological evidence is recovered from a corpse, as this can indicate the most likely tissues to have been consumed by larvae [14,15].

Larval crowding also affects the size of *C. vomitoria*. The general tendency to produce undersized individuals is observed on liver, brain and muscle, although the greatest changes are noticed at different degrees of crowding on each tissue type, re-emphasising the importance of knowing the likely feeding positions of larvae on a corpse. The production of undersized individuals also has implications for the PMI estimation. If not accounted for, an *underestimate* of the PMI is possible.

In this study, size measurements of *C. vomitoria* were conducted using adult flies. Determination of the PMI in criminal investigations is usually conducted by size measurements of blowflies in the larval stages. Some larval size measurements were recorded during secondary experiments not reported here, which indicate that larval crowding also influences the size of *C. vomitoria* larvae, an observation supported by studies on other blowfly species, such as *C. vicina* [5,22].

This study highlights the importance of considering the effects of larval overcrowding during casework as PMI errors of up to 2 days are evident from the results obtained here. However, interpretation of these findings in terms of larval growth requires further investigation.

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