

**Effects of larval crowding on size and fecundity of the blow fly,
Calliphora vicina (Diptera: Calliphoridae)**

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***Calliphora vicina*, larval overcrowding, body size, development, fecundity, critical weights,
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Abstract. Increased crowding of larvae of the blow fly, *Calliphora vicina*, within a standard quantity of meat led to reduced larval and puparial weight, shortened development, reduced adult size, and lowered fecundity. Apparent “critical weights” for pupariation in overcrowded cultures were lower than those for larvae manually extracted from under-crowded cultures, suggesting that weight per se is not the decisive factor but – within a rather wide size range – a period of feeding after the moult to the third instar is necessary for onward development to the next instar.

INTRODUCTION

In many insect groups, inadequate larval nutrition or overcrowding results in reduced body size (e.g. Hanes & Ciborowski, 1992; Gu & Danthanarayana, 1992; Lyimo et al., 1992). Although such a relationship may seem self-evident, in groups such as the Diptera Cyclorrhapha, the consequences of larval crowding are far from trivial and may become important factors in survival and population growth. The larvae of blow flies (Calliphoridae) and flesh flies (Sarcophagidae), for example, often feed in carrion. However, since animal remains constitute a limited resource, intense larval competition for food may result if too many eggs or larvae are deposited in a carcass, leading, inter alia, to reduced body size (Baxter et al., 1973; Fraenkel, 1976; Kenny et al., 1992), lowered fecundity (Spradbery & Schweitzer, 1981), curtailed development time (So & Dudgeon, 1990; Goodbrod & Goff, 1990) and reduced adult survival (Nicholson, 1957; So & Dudgeon, 1990). Small body size and limited metabolic reserves may also compromise survival in those flies that overwinter in diapause (e.g. Denlinger, 1971a,b; Saunders, 1971, 1987).

In the present paper we examine the effects of larval crowding on the blow fly *Calliphora vicina*, measuring body size, fecundity and rate of growth, with particular reference to the so-called “critical weights” which may be necessary for successful further development.

MATERIAL AND METHODS

Culture methods

The strain of *Calliphora vicina* Robineau-Desvoidy used in this investigation was isolated from flies caught in Musselburgh, near Edinburgh, in August 1984. Since then it has been maintained in the laboratory under continuous illumination (LL) at 25°C (Saunders, 1987). Adult flies were provided with sugar and water ad libitum, and with meat (beef muscle) from about day 4 post eclosion. Larvae hatching from

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eggs laid on this meat were reared to maturity on meat supplemented by an artificial diet made from dried milk, yeast and agar. Mature larvae were allowed to disperse into dry sawdust to form puparia. All experimental cultures of flies and larvae were maintained under LL at 20°C.

Larval growth curve

Larval growth at 20°C was measured using 6 to 10 larvae extracted from a large culture every 12 h over a 10 day period. Each larva was cleaned with a paper tissue, weighed, and its instar determined according to the characters of the posterior respiratory spiracles. After weighing, each larva was placed in a separate glass vial containing about 1 cm of dry sawdust. Times of pupariation and eclosion, and the weights or sizes of resulting puparia and flies (see below), were recorded. These manually extracted larvae were also used to establish "critical weights" for successful pupariation and eclosion.

Crowding of larvae within their food

Cultures with different degrees of larval crowding were established by transferring 1, 5, 10, 20, 50, 100, 150, 200 or 300 newly hatched larvae to a standard "hamburger" made from 50 g of minced beef muscle. Several replicates of each condition were made, particularly at the lower densities. Larvae were collected as they wandered from their food; they were then weighed and maintained as above, checking at intervals for pupariation and eclosion success, and for weights and sizes of the resulting puparia and adult flies.

Measurements of size of adult flies

Adult females emerging from the manually extracted and crowded cultures were allowed 24 h to fully expand and were then killed by freezing. Body size was estimated by taking three measurements: maximum head width, a wing vein (length of wing vein R_{4+5} measured from its junction with R_{2+3} to the wing apex), and the length of the mesothoracic tibia.

Number of primary follicles

As an estimate of the potential fecundity of the flies reared in these experiments, emerging females were given access to sugar and water from the time of their eclosion, and then to meat on days 3 and 6 post-eclosion; this allowed some ovarian development to occur, facilitating identification of egg follicles upon dissection. Flies were killed by freezing on day 7 or 8 and the number of primary follicles, now large enough to identify, were counted for one ovary in each fly.

RESULTS

Larval growth rate

Figure 1 shows a larval growth curve for *C. vicina* at 20°C; it is similar to other such curves published for "higher" Diptera (e.g. Wentworth et al., 1981; Richard et al., 1987). Newly hatched larvae weighed about 0.3 mg, and grew rapidly to an average maximum weight of about 81 mg by day 5, although some larvae in excess of 100 mg were recorded. After day 5, fully-fed third stage larvae ceased gaining weight and by day 7.5 they were wandering from the culture. Wandering larvae showed a drop in weight due to crop emptying and gut purge.

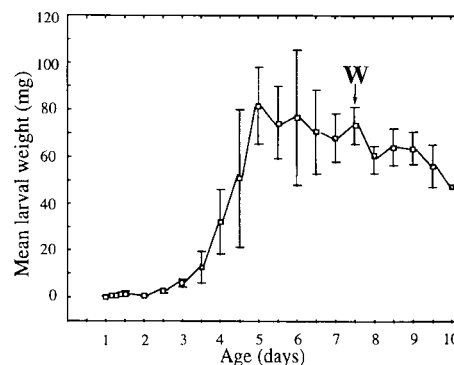


Fig. 1. Larval growth of *Calliphora vicina* at 20°C in an uncrowded culture. Error bars = $2 \times$ SEM. The majority of the larvae wandered from the meat by day 7.5 (W).

Pupariation and eclosion success in manually extracted larvae, and in larvae from overcrowded cultures

In the cultures subjected to manual extraction, none of the larvae below 30 mg pupariated. Figure 2a shows a bimodal distribution, larvae below 30 mg, some still in their first and second instar, persisting as larvae, whereas most of those above 30 mg, all third instars, successfully forming puparia. Since more than 50 per cent of the larvae in the 30 to 34.9 mg weight class formed puparia, this weight was identified as a “critical weight” for pupariation.

The distributions of larval weights and the proportions successfully forming puparia in cultures inoculated with 100, 150, 200 and 300 larvae are shown in Figs 2b–e. With 100 larvae per 50 g of food, most larvae attained 65 to 85 mg, but with 150, 200 and 300 larvae per culture, larvae left the meat at a lower weight. In these crowded cultures, successful pupariation was recorded in the 15 to 19.9 mg weight class, suggesting a much lower “critical weight” for pupariation than in the manually extracted cultures.

Figures 2f–j (right hand columns) show the proportion of each puparial weight class that successfully emerged as adults. Failure to complete development was more frequent for lighter puparia; this was characterised by early death within the puparium, through to fully formed flies that failed to emerge completely. Determination of a “critical weight” for successful eclosion was more difficult than for the larval-puparial transformation, but was estimated to lie between 20 and 30 mg.

Crowding and larval and puparial weight

The effects of larval rearing density (1 to 300 larvae per 50 g of food) on larval weight are shown in Fig. 3A. The data were subjected to an unrelated one-way analysis of variance (ANOVA). For larval weights, the effects of density were found to be significant ($F_{8,585} = 99.8$, $p < 0.001$); for puparial weight, $F_{8,537} = 146.2$, $p < 0.001$. Judging by the 95% confidence limits ($2 \times \text{SEM}$) for these data, initial densities of up to 50 larvae per culture had little effect on size. The greatest effects were seen with densities between 50 and 150. With larval weights, the mean values for 150, 200 and 300 larvae were also significantly different ($t = 2.77$, $df = 232$, $p < 0.01$) and with puparial weights, mean values over a density of 50 were all significantly different.

Crowding and developmental rate

Increasing density of larvae within the food led to more rapid development whether measured from inoculation of the first instar larvae to wandering (Fig. 3B), or to pupariation or adult eclosion (data not shown). The effects of density within each data set were significant (wandering, $F_{8,589} = 5.49$, $p < 0.01$; pupariation, $F_{8,538} = 128.5$, $p < 0.001$; eclosion, $F_{8,303} = 12.5$, $p < 0.001$), but in each case the greatest changes were found to occur between 50 and 150 larvae per 50 g of meat.

Crowding and adult size

Using the three measures of adult size (head width, wing vein length and mesothoracic tibia), increasing larval density was found to cause smaller female flies (head, $F_{8,155} = 23.7$, $p < 0.001$; wing, $F_{8,417} = 29.8$, $p < 0.001$; tibia, $F_{8,283} = 47.5$, $p < 0.001$) although, again, the greatest rate of change was observed to occur between densities of 50 and 150 larvae (Fig. 4). For head width, for example, mean measurements of size were not significantly

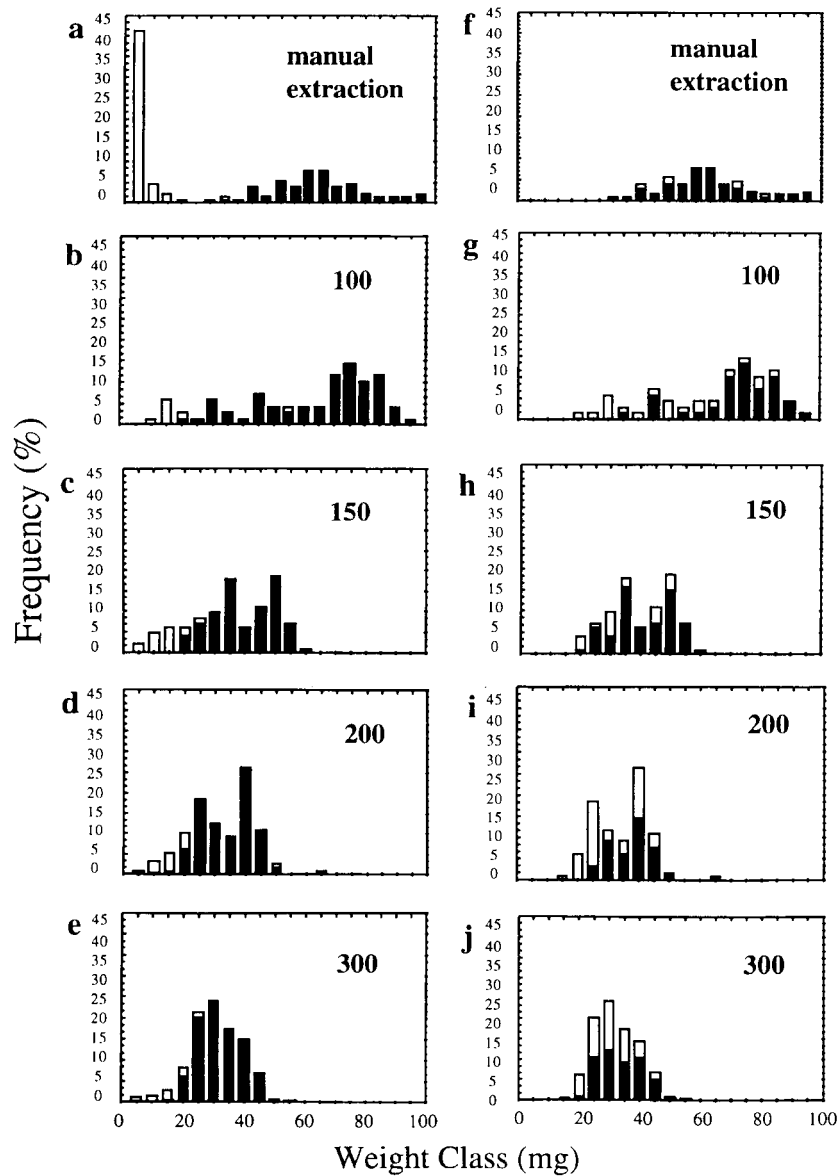


Fig. 2. Frequency percent of larvae of *C. vicina* in each weight class to form puparia (2a–e), or puparia to emerge as adults (2f–j). Open bars represent the proportion that failed to pupariate or eclo respectively. Numbers (100–300) show the density of larvae within the cultures.

different for culture densities of less than 50 or more than 150, but between 50 and significant drop in size occurred ($t = 3.06$, $df = 42$, $p < 0.001$). By pooling all the data emerging flies (Fig. 5) each of the measurements of size was shown to be positively correlated with larval weight.

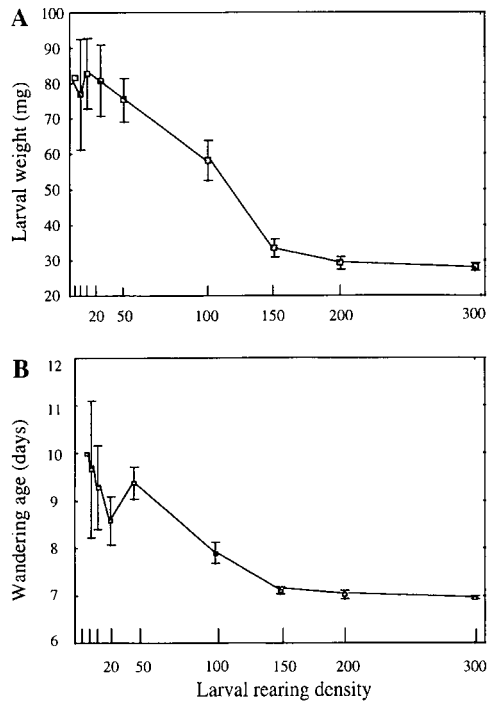


Fig. 3. The effect of larval rearing density on (A) the weight of wandering larvae of *C. vicina*, and (B) the age of the larvae at wandering from the meat. Error bars = $2 \times \text{SEM}$.

puparia, a shortened developmental time, reduced adult size and a lowered fecundity. For all but fecundity, the greatest changes in the effects of crowding are seen between 50 and 150 larvae per 50 g of food, suggesting that about 1 g of minced beef muscle is adequate and sufficient for full development of a larva of *C. vicina*. Fecundity, measured by the number of primary follicles developing in the ovary, also fell with crowding and body size (Fig. 6), but when compared with the data shown in Figs 3 and 4, the steep fall in mean values between 50 and 150 larvae per culture was not observed. This may suggest that a high capacity to produce eggs is preserved at the expense of body size.

These data on the effects of crowding are comparable to those obtained for other carrion-infesting Cyclorrhapha. For example, Baxter et al. (1973) (*Sarcophaga bullata*), So & Dudgeon (1990) (*Hemipyrellia ligurriens* and *Boettcherisca formosensis*) and Goodbrod & Goff (1990) (*Chrysomya megacephala* and *C. rufifacies*) all reported crowding-related reductions in body size, and Baxter et al. (1973), Kenny et al. (1992) (*Sarcophaga argyrostoma*) and Spradbery & Schweizer (1981) (*Chrysomya bezziana*) found a positive correlation between primary follicle number and size. In *C. vicina*, all females need exogenous protein for egg production (i.e. they are anautogenous), regardless of size, but in *Sarcophaga bullata* (Baxter et al., 1973) and *S. argyrostoma* (Denlinger, 1971c; Kenny et al., 1992) the largest flies from the least crowded cultures were able to produce mature

Adult size and fecundity

For each larval density the mean number of primary follicles (in one ovary) was calculated (Fig. 6). Between groups, the differences were significant ($F_{7,96} = 9.79$, $p < 0.001$), showing a reduction in mean follicle number with crowding. However the steep fall between densities of 50 and 150 larvae per culture, as seen for larval weight, developmental time and adult size (Figs 3 and 4), was not observed for follicle number. Nevertheless, pooling all of the data for flies from different density groups showed that the number of primary follicles was positively correlated with each measurement of size (wing vein, head width and tibial length) (Fig. 7), suggesting a strong relationship between adult size, and hence the amount of food available to the larvae, and fecundity.

DISCUSSION

The present results for *C. vicina* show that increases in larval crowding lead to a reduced body weight in mature larvae and

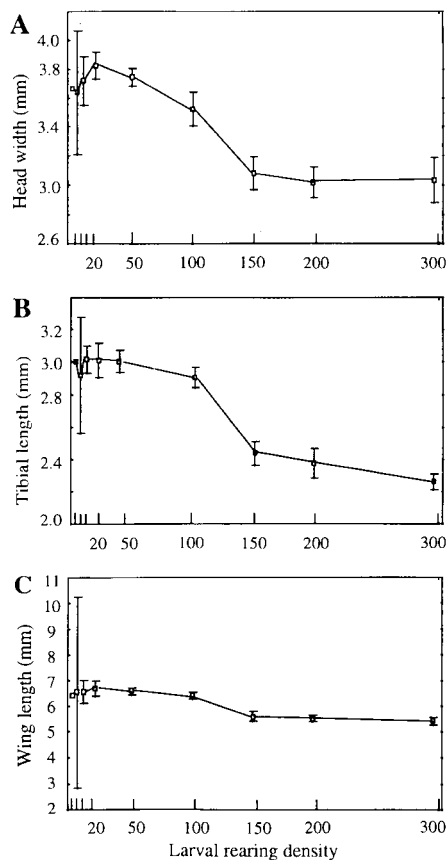


Fig. 4. The effect of larval rearing density on adult body size of *C. vicina*, as measured by (A) head width, (B) length of mesothoracic tibia, and (C) the length of a wing vein. Error bars = $2 \times$ SEM.

In cyclorrhaphous larvae there is no head capsule and few hard parts, but these larvae are also reported to need a certain body weight before they can pupariate (e.g. Ulyett, 1950). In this study, for example, larvae of *C. vicina* manually extracted from their food could only proceed to the puparium if they were greater than about 30 mg.

Žďárek & Sláma (1972) showed that 3rd instar larvae of *Sarcophaga argyrostoma* and *Calliphora vomitoria* need to feed for 3 to 10 h after the moult before they can develop into small puparia and diminutive adults. Larvae starved from the beginning of the third instar were found to survive for many days with no further development, although upon further feeding development could resume to full size. Similar results were reported for *S. bullata* (Žďárek, 1981) and the phenomenon in *S. argyrostoma* has been confirmed (Saunders & Bradley, 1984). These studies all suggest that 3rd stage larvae require a

eggs using food reserves obtained during larval life (i.e. they were autogenous). So and Dudgeon (1990) and Goodbrod & Goff (1990) also found accelerated development in crowded cultures of Calliphoridae and Sarcophagidae. In *Drosophila melanogaster* (Miller, 1964) and *Musca domestica* (Sullivan & Sokal, 1963), however, higher larval densities resulted in protracted development.

Working with the tobacco hornworm moth, *Manduca sexta*, Nijhout & Williams (1974) showed that 5th instar larvae emptied their guts on day 4 or 5, at which time they normally weighed about 8 to 10 g, and then proceeded to metamorphosis. Underfed larvae required a period of feeding (the phagophase) and could not initiate metamorphosis before they reached a weight of about 5 g. Larvae below about 5 g showed delayed PTTH release and sometimes underwent supernumerary larval moults; above 5 g an unidentified process was initiated which required 24 h to be completed before normal metamorphosis to a small sized pupa. In a subsequent paper, Nijhout (1975) demonstrated a threshold size for metamorphosis: 5th stage larvae with a head capsule width of less than 5.1 mm did not pupate and sometimes underwent supernumerary larval moults, whereas those above 5.1 mm proceeded to pupation. In some way, therefore, larvae are able to "measure" their own size or weight.

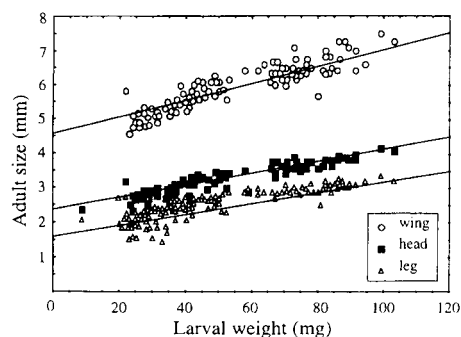


Fig. 5. The relationship between larval weight and adult body size in *C. vicina* as measured by wing length ($y = 4.54 + 0.026x$, $r = 0.815$, $p < 0.001$), head width ($y = 2.41 + 0.017x$, $r = 0.704$, $p < 0.001$) and tibial length ($y = 1.79 + 0.016x$, $r = 0.748$, $p < 0.001$).

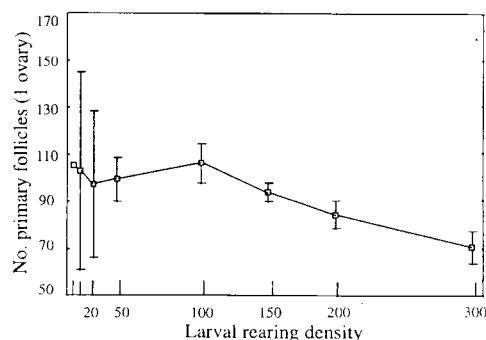


Fig. 6. The effect of larval rearing density on the fecundity of adult *C. vicina* (the number of primary egg follicles in one ovary). Error bars = $2 \times \text{SEM}$.

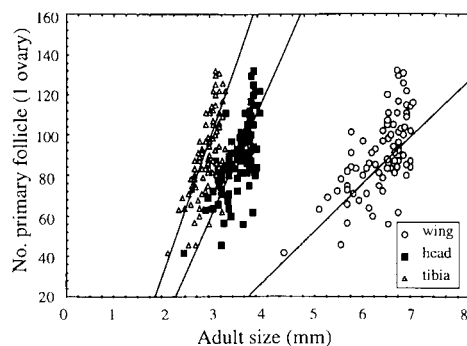


Fig. 7. The relationship between adult body size and fecundity (number of primary egg follicles in one ovary) of *C. vicina*. Wing length, $y = -80.54 + 25.486x$, $r = 0.472$, $p < 0.001$; head width, $y = -70.73 + 44.526x$, $r = 0.495$, $p < 0.001$; tibial length, $y = -76.99 + 56.765x$, $r = 0.474$, $p < 0.001$.

period of feeding before they are competent to form puparia.

The present results with *C. vicina* show that manually extracted larvae need to achieve a weight of more than 30 mg before they can pupariate, whereas those from grossly overcrowded cultures leave the meat earlier and may pupariate at weights as low as 15 to 20 mg. In the latter case the effects of overcrowding, such as the physical presence of other larvae, competition for food, or tainting of the meat with larval excreta, probably come into force quite early in larval development, so that overcrowded larvae may already be quite small when they moult to the 3rd instar (see also Fraenkel, 1976, for *S. bullata*). The results indicate therefore that a

“critical weight” for pupariation has little significance for these larvae, the important factor for onward progression, within a fairly wide size range, being the obligatory period of feeding following the moult to the third instar (Žďárek & Sláma, 1972).

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