

# Nutritional ecology of blowflies (Diptera, Calliphoridae): estimates of critical larval weight for pupation on two different diets

Cássio da Silva Ribeiro<sup>1</sup> & Cláudio José Von Zuben<sup>1</sup>

<sup>1</sup>Departamento de Zoologia/ Instituto de Biociências – Universidade Estadual Paulista – Campus de Rio Claro. Avenida 24A, 1515, Caixa Postal 199, Bairro Bela Vista, 13506-900 Rio Claro-SP, Brasil. cassiosr@gmail.com, vonzuben@rc.unesp.br

**ABSTRACT.** Nutritional ecology of blowflies (Diptera, Calliphoridae): estimates of critical larval weight for pupation on two different diets. Under natural environmental conditions, blowflies utilize discrete and ephemeral feeding resources such as decaying carcasses. Competition for food on such feeding substrates is usually very severe, and only the individuals that are capable of attaining the critical larval weight for pupation will be able to survive. This critical weight is hitherto unknown for several blowfly species; therefore, the current work is aimed at obtaining such a critical value for four blowfly species of the genera *Chrysomya* and *Lucilia*, deploying two types of feeding substrate, namely, artificial diet and macerated bovine meat. On the whole, the critical weights ranged from 30 to 35 mg. The lowest larval weight which permitted pupation was 30.0 mg for *Chrysomya megacephala* reared on macerated bovine meat. This species was also the best adapted to pupation at low larval weights in relation to the maximum larval weight for males. Regarding the pupation of females, the best-adapted individual was a *C. albiceps* specimen exhibiting a critical weight that was equal to 39.20 % of the maximum value obtained. Concerning all the species and diet types, the female individuals exhibited the lowest critical weights that produced viable pupae, probably representing an evolutionary strategy that favoured the survival of females, responsible for the egg formation, contributing to the establishment of future generations. Regarding the loss (in percentage) of adult biomass in relation to the third instar larvae, the females of *C. megacephala* lost less weight than males in both feeding substrates. On the other hand, such a loss of weight occurred in males of *C. albiceps* and *L. cuprina*.

**KEYWORDS.** Calliphorids; competition; food; larvae; minimum weight.

**RESUMO.** Ecologia nutricional de moscas-varejeiras (Diptera, Calliphoridae): estimativas de peso larval crítico para pupação em duas diferentes dietas. Em ambiente natural, as moscas-varejeiras utilizam como alimento, substratos discretos e efêmeros, como por exemplo, carcaças em decomposição. Normalmente, o nível de competição por alimento nesses substratos é bastante severo, e somente vão sobreviver os indivíduos que atingiram o peso larval crítico para pupação. Como esse valor não é conhecido para várias espécies de moscas-varejeiras, o objetivo do presente trabalho foi de obter esse peso crítico para quatro espécies de moscas-varejeiras dos gêneros *Chrysomya* e *Lucilia*, em dois tipos de substrato alimentar: dieta artificial e carne bovina moída. Os pesos críticos geralmente se situaram na faixa de 30 a 35 mg. O menor peso larval que permitiu a pupação foi de 30,0 mg em *C. megacephala* criada em carne bovina moída. Esta espécie também foi a mais adaptada a empupar com pesos larvais baixos em relação ao peso larval máximo para machos. Já a fêmea melhor adaptada a empupar foi uma *C. albiceps* com peso crítico representando 39,20 % do máximo obtido. Os menores pesos críticos que deram origem a pupas foram observados em fêmeas, para todas as espécies e dietas, representando provavelmente uma estratégia evolutiva que procura privilegiar a sobrevivência das fêmeas, responsáveis pela formação dos ovos, contribuindo para a formação das futuras gerações. Já com relação à perda em porcentagem, de biomassa do adulto em relação às respectivas larvas de terceiro instar, em *C. megacephala* as fêmeas perderam menos peso que os machos em ambas as dietas, sendo que para *C. albiceps* e *L. cuprina*, isto ocorre com os machos.

**PALAVRAS-CHAVE.** Alimento; califorídeos; competição; larvas; peso mínimo.

Food sources like feces, carcasses, fruits, fungi and decaying plants, are separate units and provide food for many animal species. These substrates are small and ephemeral and provide a limited amount of food (Hanski 1987).

The organisms that use these resources often face severe levels of competition and, for those that present competition of the exploitative type, each one search to eat food as quickly as possible before the complete exhaustion of resources (De Jong 1976, Lomnicki 1988). The results of this competition reflected in population parameters such as survival, fecundity and size of adults (Reis *et al.* 1994).

Examples of animals showing this type of competition are the larvae of blowfly species of the genera *Chrysomya* (Robineau-Desvoidy) and *Lucilia* (Robineau-Desvoidy), which have considerable importance to public health and

may be mechanical vectors of pathogens for humans, cause facultative myiasis in animals and humans, as well as being used in studies of legal medicine as forensic indicators (Zumt 1965; Furlanetto *et al.* 1984; Greenberg 1991; Guimarães & Papavero 1999). Moreover, the species *Chrysomya megacephala* (F.) is responsible for economic losses in Southeast Asian countries, where the fish exposed to the sun is constantly attacked by males and females of this species in search of food and oviposition sites, causing severe losses (Esser 1990, 1991).

The survival of competitors for ephemeral resources will also depend on the time of development (Slansky & Rodriguez 1987). Thus, the larvae that reach a minimum larval weight or the critical weight in a shorter time than others, have greater chances of survival. This critical weight is defined as the

minimum larval mass in which additional growth would not be necessary for the normal course of pupation (Ochieng' Odero-1992; Davidowitz *et al.* 2003), this being different for each species (Levot *et al.* 1979).

Artificial diets that have a more pleasant odor and a good cost benefit relationship have been used for the maintenance of species of flies in the laboratory. These diets present number and size of individuals equivalent to those created with meat (Leal *et al.* 1982; Constable & Griffith 1986; Taylor & Mangan 1987; Schmidt & Harris 1989; Chaudhury & Alvarez 1999; Tachibana & Numata 2001).

Data on minimum larval weight for pupation obtained experimentally with the use of diets based on meat and artificial diets are rare in the literature. Levot *et al.* (1979) studied the larval growth of some species of Calliphoridae and Sarcophagidae in beef liver, and estimated their critical larval weights for pupation. Von Zuben (1998) studied the minimum larval weight for pupation in *Chrysomya megacephala* (F.) using an artificial diet.

Therefore, more detailed studies of larval critical weight for pupation for different blowfly species, growing in different conditions, are necessary. The objective of this study is to observe what is the critical larval weight for pupation in four blowfly species of the genera *Chrysomya* and *Lucilia*, developing on two different feeding substrates, under laboratory conditions. The weight loss of the emerging adults in relation to the respective third instar larvae was also calculated.

## MATERIAL AND METHODS

Blowflies were collected in the Biosciences Institute of UNESP in Rio Claro, SP, Brazil, with the aid of a hand net and baits made with beef rotting. Once identified, specimens of *Chrysomya albiceps* (Wied), *C. megacephala*, *L. eximia* (Wied) and *L. cuprina* (Wied) were separated, each species in a cage at  $25 \pm 1^\circ\text{C}$  and a 12 hours photoperiod. Subsequently, the  $F_1$  generation of each species was obtained, whose larvae were kept in bottles with ground beef in climate chambers at  $25 \pm 1^\circ\text{C}$  and a 12 hours photoperiod.

From the oviposition of the  $F_1$  generation 100 larvae of each species were collected, forming the  $F_2$  generation. These larvae were separated and placed in vials containing macerated bovine meat (50 larvae) and artificial diet according to Leal *et al.* (1982) (other 50 larvae). Third instar larvae were removed from the flasks, washed, dried and weighed on an analytical balance. These larvae were isolated in small plastic bottles numbered, with a substrate of wood shavings, covered with organza and kept under the same conditions as before. From the viable pupae, the sex of the adults that emerged was determined, to obtain the minimum larval weight for pupation.

The maximum larval weights were determined by separating 50 larvae of the  $F_2$  generation and placing them in separate bottles for each species under the same conditions of the substrates used to obtain the minimum larval weight. The third instar larvae, when they began to leave the food substrate for pupation, were removed, washed, dried, weighed

and individualized, as before. The emerging adults were sexed and the maximum larval weight for each species and sex was considered as the largest larval weight giving rise to a viable adult.

The ability to pupation (percentage in relation to the maximum larval weight for each species) was calculated using the formula  $\% = \frac{A}{B} \times 100$ , where *A* corresponds to the minimum larval weight and *B*, the maximum larval weight for each species.

After the emerging adults have completed their cycle, we waited until the complete relaxation of its wings and then they were weighed and sexed. The calculation of the loss of biomass was done using the following formula:

The average weight loss of the larva until the adult stage  $= \left\{ \frac{Pl - Pa}{Pl} \right\} \times 100$ , where *Pl* is the larval weight, and *Pa* is the adult weight.

## RESULTS

Table I shows all the values of critical larval weight for pupation found for the four species studied. The lowest larval weight obtained was 30 mg, resulting in a female of *C. megacephala* created in ground beef. For the same species, the lowest larval weight necessary for pupation on artificial diet was 32.8 mg, also resulting in a female.

For *C. albiceps*, the lowest larval weight necessary for pupation was 31.4 mg in female bred in macerated bovine meat.

The species of the genus *Lucilia* presented minimum larval weights of 32.6 mg and 33.2 mg, respectively, for *L. eximia* and *L. cuprina* treated in macerated bovine meat and resulted in females.

After the larvae stop the feeding, due to food shortages or satiety, those who achieve the minimum larval weight for pupation tried to pupate. The results shown in Table II demonstrate the loss of biomass, in percentage, of the adults in relation to the respective third instar larvae, and indicated that the greatest losses occurred in females and males of *L. cuprina*, with 22.86 and 19.61% loss of biomass in artificial diet. In ground beef, the largest losses occurred in females of *L. cuprina* and males of *C. megacephala*, with 21.27 and 17.13% loss, respectively.

With respect to maximum larval weight, the specimen with

Table I. Critical larval weights (mg) necessary to pupation, separated by sexes and diets for the different species studied.

Species	Diets	Minimum weight (mg)	
		females	males
<i>Chrysomya albiceps</i>	artificial	31.5	34.8
<i>Chrysomya albiceps</i>	beef	31.4	32.6
<i>Chrysomya megacephala</i>	artificial	32.8	35.1
<i>Chrysomya megacephala</i>	beef	30	33.4
<i>Lucilia eximia</i>	artificial	34.1	34.4
<i>Lucilia eximia</i>	beef	32.6	34.1
<i>Lucilia cuprina</i>	artificial	33.4	37.3
<i>Lucilia cuprina</i>	beef	33.2	34.3

Table II. Mean loss weight (in percentage) between adults and third instar larvae for the different species, separated by diets and sexes.

Species	Artificial diet		Ground beef	
	Females	Males	Females	Males
	adult-larva	adult-larva	adult-larva	adult-larva
<i>Chrysomya albiceps</i>	18.31	15.37	15.85	14.05
<i>Chrysomya megacephala</i>	18.03	18.43	15.57	17.13
<i>Lucilia eximia</i>	17.98	14.93	15.54	16.46
<i>Lucilia cuprina</i>	22.86	19.61	21.27	15.76

the highest value was a male of *C. megacephala*, created in an artificial diet with a weight of 100.4 mg. The largest female was also obtained in *C. megacephala*, with 81.4 mg, obtained in diet of ground beef. The results are shown in Table III.

Table IV shows that the species best suited to pupate with low larval weight in relation to the maximum larval weight was *C. megacephala* with 34.96% of the maximum larval weight for males, in artificial diet. The better adapted female was of *C. albiceps*, with 39.20% of the highest weight obtained, in ground beef.

## DISCUSSION

In this study, some aspects of nutritional ecology of four blowfly species were studied, with emphasis on larval competition for food and minimum larval weight for pupation. It has been shown that for many insects, it is necessary to reach a critical larval weight for the occurrence of pupation (minimum weight for pupation) in its last instar (Ochieng' Odero-1992; Davidowitz *et al.* 2003).

The pupation and the subsequent adult emergence are possible only if the third instar larvae reach the minimum weight before the complete lack of its food source (Von Zuben 1998). In studies of weevils, it was observed that hunger can cause the complete desiccation of specimens in the cocoons, and is considered the major cause of natural deaths (Reardon & Spurgeon 2002).

Competition for food in blowflies is regarded as exploitative, where each larva attempts, independently, eat as much as possible until the complete food exhaustion (Levot *et al.* 1979, Lomnicki 1988, Godoy *et al.* 1996).

The result of competition for food is related to the degree of food assimilation by the competitors. In studies

Table IV. Percentage (%) of the maximum larval weight.

Species	Diets	Percentage (%)	
		females	males
<i>Chrysomya albiceps</i>	artificial	40.75	42.49
<i>Chrysomya albiceps</i>	beef	39.2	43.29
<i>Chrysomya megacephala</i>	artificial	42.43	34.96
<i>Chrysomya megacephala</i>	beef	41.84	45.19
<i>Lucilia eximia</i>	artificial	44.29	43.27
<i>Lucilia eximia</i>	beef	40.6	41.79
<i>Lucilia cuprina</i>	artificial	44.47	51.17
<i>Lucilia cuprina</i>	beef	43.34	46.23

with insects, Slansky & Rodriguez (1987) determined that the performance of the immature individual is genetically determined. Thus, insects that can assimilate resources more efficiently are those with a higher fitness, and not necessarily those who ate a greater quantity of food (Putman 1977; Slansky & Rodriguez 1987; Von Zuben 1993).

One of the adaptive advantages for this type of competition is that the species which have a low critical weight for pupation can get down in the process of pupation in a shorter time than others, and thus are more likely to give rise to adults (Lomnicki 1988, Von Zuben 1998).

In this study, the minimum larval weights necessary to pupation were ranging between 30 to 35 mg, with the exception of two cases with weights a little higher than 35 mg, and the lighter weight that allowed larval pupation was 30, 0 mg in *C. megacephala* created in ground beef. Analyzing the two diets used, it was not possible to determine which of the diets that provided a better condition for the development of the larvae. The best results on the ability of pupation with larval weight below the optimum were obtained in the two diets, but for different sexes.

The species most adapted to pupate with lighter larval weight in relation to the maximum was *C. megacephala* with 34.96% of the maximum larval weight, for males in artificial diet. This result confirms that obtained by Levot *et al.* (1979), who observed that *C. megacephala* is the species best adapted to pupate with low larval weights. The female better adapted to pupate was a *C. albiceps* with 39.20% of the maximum weight, in macerated bovine meat.

Another interesting aspect of this study was the fact that the lowest minimum weights that gave rise to pupae were observed in females for all species and diets, representing probably an evolutionary strategy that seeks to favor the survival of females responsible for the formation of eggs, contributing to the formation of future generations.

In relation to biomass loss in adults, in percentage, related to their respective third instar larvae in *C. megacephala*, females lost less weight than males in both diets, and for *C. albiceps* and *L. cuprina*, this occurs with males.

The results of the present study suggest that the species that achieves pupation with the lowest weight in relation to its maximum larval weight, and that less lose weight at adult emergence in relation to its respective third instar, should take advantage over competitors in situations of limited food resources. Thus, further studies are needed to further

Table III. Maximum larval weights (mg), separated by sexex and diets in the different species.

Species	Diets	Maximum weight (mg)	
		females	males
<i>Chrysomya albiceps</i>	artificial	77.3	81.9
<i>Chrysomya albiceps</i>	beef	80.1	75.3
<i>Chrysomya megacephala</i>	artificial	77.3	100.4
<i>Chrysomya megacephala</i>	beef	81.4	73.9
<i>Lucilia eximia</i>	artificial	77	79.5
<i>Lucilia eximia</i>	beef	80.3	81.6
<i>Lucilia cuprina</i>	artificial	75.1	72.9
<i>Lucilia cuprina</i>	beef	76.6	74.2

elucidate the differences in competitive ability among species of blowflies using the food substrate.

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