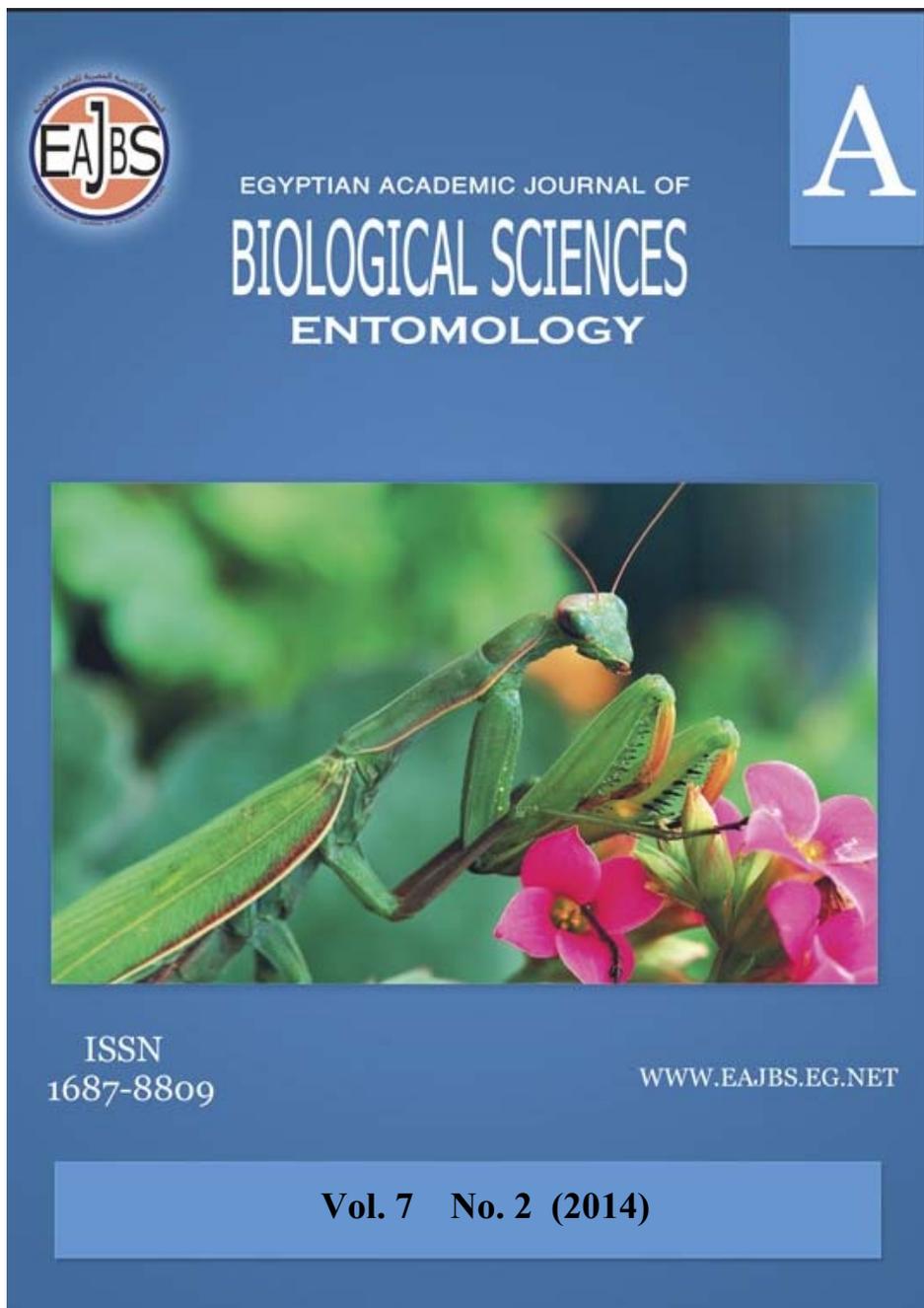


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Influence of larval density on some biological and demographic aspects of *Chrysomya megacephala* (Diptera: Calliphoridae).

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ABSTRACT

The objective of this work was to study the influence of larval density on some aspects of the population ecology & survival of *Chrysomya megacephala*, analyzing demographic aspects of adults kept under experimental conditions. A positive effect of density on survival and pre-oviposition period was observed, while no simultaneous influence of density in sex ratio. The results showed that the demographic parameter for life expectancy at intrinsic growth rate (e_0), total fecundity (TF), net fecundity (NF) and mean reproductive age (T) increased with increasing the density of population larvae. Other population parameters such as the intrinsic growth rate (r), the finite growth rate (λ) and the time to double population (DT) were also calculated.

Keywords: *Chrysomya megacephala*, Demographic aspects, Reproductive parameter and rearing density.

INTRODUCTION

Calliphoridae adults (*Diptera: Oestroidea*) or blowflies are important in regard to sanitary biology, medical, veterinary, and forensic Entomology, Setyaningrum & Al Dhafer, (2014). Blowflies are useful indicators in legal investigations. The age of larvae can be used to estimate how long a corpse has been exposed to the environment Byrd & Castner, (2010). Blowflies are distributed worldwide and cause medical problems by transmitting various diseases to human as well as animals and losses to the animal industry Ghandour, (1988). In nature, larvae of blowflies are found in ephemeral substrates for feeding. These substrates consist of separate and small units, such as feces, carcasses, fruits, fungi and decaying plants, usually large number of individuals exploring the same source of food Kneidel, (1984). Substrate in which immature stages of blowflies develop discrete and ephemeral are saturated with insects of one or more species Akinson & Schorroks, (1981). There is often an intense competition for resources with influence the larval behavior Hanski, (1987). This situation can produce competitive interaction as a function of density, external and internal temperature of the substrate, which frequently influences the population dynamics, may cause impact of demographic of the species Brewere, (1994). The blowflies face severe levels of competition. The result of this competition is seen on populational parameters as survival, fecundity and size of the resulting adults, with direct effects on the populational dynamics of the species involved Von Zuben *et al.*, (2001). Among blowflies, the oriental latrine fly *Chrysomya megacephala* (Fabricius) is one of the most common blowflies in Egypt (Gabre,

1994). *C. megacephala* has medical, veterinary importance and it has geographic distribution expansion. Tomberlin *et al.*, (2001). This species is among the most dangerous dipteran vectors of enteric pathogens Greenberg, (1973). It is the dominant vector of helminthes parasite eggs In Malaysia Sulaiman *et al.*, (1989), and it is an important pollinator of mango in Australia Anderson *et al.*, (1982). In populational ecology studies, it is necessary to investigate, among other factors, the demographic aspects of adults, with direct influence on the populational dynamics of the species involved. Many demographic studies in insects analyze the mortality pattern of the cohort as a function of time. However, dynamic behavior usually depend on factors associated with demography, such as growth rate and life history characteristic Uchmanski, (1999). Life history and population growth are subject to high variation among different species and population Gotelli, (1995).

Fecundity data may be totaled throughout the life time of a cohort and considered together with the survival data Carey, (1993). Because of *C. megacephala's* medical and veterinary important and its expanding geographic distribution, the ecology of *C. megacephala* deserves more attention than it has received so far. More precise estimates of reproductive rates and mean reproductive age, as those obtained in this study could be very useful in understanding the population dynamics of insects in general and of these blowflies in particular.

This study aimed to analyze the effect of larval density on certain demographic aspects of *C. megacephala* under experimental conditions and its effects on survival, fecundity, sex ratio and rate of reproduction.

MATERIAL AND METHODS

Insect rearing

A colony of *Chrysomya megacephala* was initially established in the Department of Entomology, Faculty of Science, Benha University. Adults from the stock colony of *C. megacephala* were kept in cages (38 × 38 × 56 cm) at 31.2±1.47°C, 14 h. photoperiod and 43.61±9.3 % R.H. The cages were made with a wooden floor and wire gauze on three of the sides. The fourth side was wooden with a circular hole fitted with a cloth sleeve to facilitate daily feeding, cleaning of the cage, and removal of eggs. Adults were supplied daily with granular sucrose, water, and beef meat. Water was supplied by dipping a piece of cotton as a wick in a bottle filled with water, and 50gm. of ground beef was provided in 400 ml a Plastic cup. Egg batches were removed daily and transferred to a fresh piece of beef placed in a rearing enamel bowl (35 cm in diameter) covered with muslin secured with a rubber band. At the pre-pupal stage, dry autoclaved sawdust was added to the bowl as a medium for pupation. Pupae were sieved from the sawdust and transferred to adult cages described above for adult emergence.

Three cages were made for each density with the adults of the F2 generation of *C. megacephala*. Data of female mortality and fecundity were recorded daily. Regarding the fecundity, four identical flasks with the same amount of rotting ground beef were placed on the cages daily at regular time intervals. The presence or absence of eggs and the number of eggs laid were then recorded daily.

Estimate of demographic parameters for *C. megacephala*

The necessary demographic parameters to make a life table followed the traditional calculation methodology, as described by Papadopoulos *et al.*, (2002).

Estimate of reproductive rates of *C. megacephala*

From the daily mortality data and number of eggs laid, it was possible to estimate reproductive parameters (total fecundity (TF), net fecundity (NF), reproductive rates (net reproductive rate (R_0) and average daily egg production (ADP), intrinsic growth rate (r) and

finite growth rate (λ), mean reproduction age of the cohort (T) and population duplication time (DT).

Statically analysis

Data was statistically analyzed using System Analysis Statistics Program (SAS), version 6.12,1998. Data were presented as mean \pm standard deviations. The effect of different densities on population dynamics were studied using one- way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results in table (1) declared a significant effect of density on pre adult survival rate, where ($p \leq 0.001$, $df = 2$ and $f = 56, 29$). Survival of larvae and pupae were similar at each rearing density. This means that no mortalities were recorded during pupal stage. They were 74.66 ± 16.04 , 152.5 ± 14.84 and 208.3 ± 62.29 respectively for densities 100, 200 and 400, from this data we concluded there were direct relationship between larval density and survival.

These results agree with Reis *et al.*, (1994), Reigada and Gody, (2006) while Good brod *et al.*, (1990) reported that the larval mortality decreased with increasing density until and optimum density was reached 8 larvae /g liver for *C. megacephala* and 10 larvae /g liver for *C. rufifacies* Zuban *et al.*, (2000) concluded the larval competition may depend on both larval density and the amount of food in different situation with the same proportion of larvae per gram of food. Survival and fecundity have been analyzed in flies by Reis *et al.*, (1994), which focused on intra- and inter- specific competition and showed the competitive processes, which act on the species, may induce changes in population behavior, this agree with our results.

Table 1: Effect of larval density on the survival of *C. megacephala* pre- adults.

Larval density	No of larvae Survived Mean \pm SD	No of pupae Survived Mean \pm SD	Percentage Pre-adult Survival Mean \pm SD	df	F	P (Value)
100	74.66 \pm 16.04	74.66 \pm 16.04	74.66 \pm 16.04	2	56.29	0.001**
200	152.5 \pm 14.84	152.5 \pm 14.84	65.75 \pm 1.80			
400	208.3 \pm 62.29	208.3 \pm 62.29	50.75 \pm 2.00			

** Highly significant

While the larval competition for limited resources can be scramble or contest types Birch, (1957). In scramble, all individuals are equally affected. It occurs when some species decreases the environmental carrying capacity, stimulating the homogeneously partition of resources among individuals De Jong, (1976). *Chrysomya* species usually exhibit scramble competition Reis *et al.*, (1996), but depending on alternative mechanisms, such as intraguild predation, detected in *C. albiceps* Polis *et al.*, (1989). The increase of intraspecific competitor numbers influenced the survival in *C. megacephala*. However, larval density influenced fecundity of adults (Reis *et al.*, 1996). On other hand, intraspecific competition in *C. megacephala*, *C. putoria* and *Cochliomyia macellaria*, has been investigated, suggesting its negative influence on survival and fecundity, in different ranges of densities, (Reis *et al.*, 1994; 1996), these results disagree with our study.

Data in Table (2) showed that the larval density significantly influenced in pre-oviposition period and has not significantly influenced in sex ratio where ($p \leq 0.007$, $f=12.78$ and $df=2$). The pre-oviposition period for densities 100, 200 and 400 were 7.29 ± 1.11 , 20.00 ± 8.13 and 21.00 ± 8.12 days, respectively. The higher values were 21.00 ± 8.12 observed at density 400, followed by 20.00 ± 8.13 at density 200, while lower values were 7.

29± 1.11 days, observed at density 100. These results disagree with what have been found by Gabre *et al.*, (2005) in which the pre-oviposition period was 7 days. Zubydi, (2000) found that the longest pre-oviposition period was observed for *C. bezziana* & *C. megacephala* were (13.6, 40.8 days) respectively at 20° C. Sex ratio at the three densities 100, 200 and 400 were 1.07 : 1, 0.98 : 1 and 1.38 : 1 respectively. These results agree with those obtained by Gabre *et al.*, (2005), and Zubydi, (2000) for *C. albiceps*, and may indicate that the number of males increases at high larval density.

Table 2: Effect of larval density on sex ratio and pre-oviposition period of *C. megacephala*.

Larval density	Sex ratio(♂/♀)	pre-oviposition period (Mean±SD)	df	f	P (Value)
100	1.07 : 1	7.29± 1.11	2	12.78	0.007
200	0.98 : 1	20.00± 8.13			
400	1.38 : 1	21.00± 8.12			

A detailed sensitivity analysis of population dynamics has shown that the most sensitive demographic parameter for *C. megacephala* in terms of influence on dynamics is not survival Godoy *et al.*, (1996). Thus, these results are not in accordance with our results. Fecundity is the most sensitive parameter for *C. megacephala*, as it can cause significant changes in behavioral dynamics, leading the population from a two-point limit cycle to a one-point stable equilibrium Godoy *et al.*, (1996). In our study the fecundity affected by density where its values were high at density 400 followed by 200 and 100 respectively (Table 3) while in study done by Rosa *et al.*, (2004), focusing on intra- and inter specific competition among *C. albiceps*, *C. megacephala* and *C. macellaria* with different amounts of food, it was observed that *C. megacephala*, *C. macellaria* and *C. albiceps* exhibited similar survival at densities of both 200 and 1,000 with either 25 or 50g of food for third instar larvae. In our experimental design, 50g of food were used to test the effect of density. This condition, associated with density sets, could explain the presence of significant difference in survival. Competition for food affects the fitness of adults with implications for population dynamics and demography Hastings, (1997).

The demographic parameters for the life expectancy at initial age (e_0) estimate were calculated along with the reproductive rates and mean reproduction age estimates for *C. megacephala*. The results of our study showed that total fecundity (TF) and net fecundity (NF) were directly proportional to density, the greatest values 2645.33±814.29 obtained in the greatest density 400, followed by 1946.66±672.67 obtained in density 200 and 1601.00±163.38, obtained in density 100, respectively. The net fecundity was 1391.90 ±595.47, 1864.13±734.08 and 2651.00± 859.89 for densities 100, 200 and 400 respectively. This showed substantial differences in terms of female fecundity among larval densities (100, 200 and 400) feeding on the same amount of food. The previous results disagree with Carvalho and Zuben, (2006) while density was inversely proportional to average daily egg production (ADP). The greatest values were 38.93±1.79 eggs produced in the smallest density 100 and the smallest values were 37.012± 14.02 eggs produced in the greatest density 400. The mean reproductive age (T) was directly proportionally to density, the greatest values 41.67 ±12.09 obtained at density 400 and the lowest value 31.96±1.51 obtained at density 100. The data in Table (3) illustrated that the initial life expectancy values (e_0) obtained for *C. megacephala* females in our study, varied from 35.83 ± 1.46 days for density 100 to 51.49 ± 10.30 days for the density 400 larvae and 45.53 ± 13.26 days for density 200. These values were higher than those obtained by Von Zuben *et al.*, (1996) for the same species, which was between 33 and 40 days and another study done by Gabre *et al.*, (2005) the life expectancy of a new egg is 32 days. A possible explanation for the difference observed between our results

and these two studies may be due to, the males and females were developed on an artificial diet, and the oviposition substrate for the females was not provided.

Table 3: Reproductive parameter rates and mean reproduction age for different densities of *C. megacephala*

	Formula	Larval density 100 (Mean±SD)	Larval density 200 (Mean±SD)	Larval density 400 (Mean±SD)
e0	$\sum Lx$ □□□=x	35.83 ± 1.46	45.53 ± 13.26	51.49 ± 10.30
TF	$\sum Mx$ □□□=x	1601.00±163.38	1946.66±672.67	2645.33±814.29
NF	$\sum lxMx$ □□□=x	1391.90 ±595.47	1864.13±734.08	2651.00± 859.89
R0	$\sum lmx$ □□□=x	695.98± 12.21	937.04 ± 369.07	817.16 ± 321.73
ADP	$\sum lxMx \div e0$ □□□=x	38.93±1.79	38.03 ±13.28	37.012± 14.02
T	$\frac{\sum lmx}{\sum xlmx}$ □□□=x	31.96±1.51	35.94±11.3 9	41.67 ±12.09
r	$\ln(R0)/ T$	0.203±0.046	0.18738±0.057	0.155±.057
(λ)	<i>re</i>	1.226±0.0012	1.17± 0.39	1.17±0.366
DT	$\ln(2)/r$	3.38 ± 0.11	3.566 ± 1.11	4.28 ± 1.2 2

e0 , individual life expectancy at birth; *x* , initial age; β, age of last individual; *Lx*, live *per capita* ratio between the ages *x* and *x+1*; *TF*, total fecundity; *Mx*, number of eggs per female at age *x*; *NF*, net fecundity; *R0*, net reproductive rate; *ADP*, average daily egg production; *T*, mean reproduction age of cohort in days; *lx*, ratio of surviving individuals at age *x*; *mx* = *Mx*/2, number of female progeny produced per female at age *x*; *DT*, time, in days, to double the population.

The net fecundity rate values (*R0*) obtained for *C. megacephala* were proportionately smaller than those obtained for the fecundity Parameters, since the survival probability values are used to calculate *R0*, that is, the individuals which have already died in a certain time interval are disregarded. Considering the average daily egg production (*ADP*), the greatest values were observed on the cages of density 100 and the smallest values were observed for density 400. These results are similar to those of Carvalho and Zuben (2006) and could be explained by the fact that a greater quantity of food resources in the case of the smaller density, would result in bigger adults with greater fecundity. Concerning the mean reproduction age (*T*), the greatest value obtained was from the cages created from concentrations starting at 400 larvae, while the density 100 had the lowest reproduction age. This could be explained by the fact that the initial life expectancy for females was higher for cages originated from densities of 200 larvae or more. Thus, ovipositions by females with greater longevity seemed to have influenced this result, increasing the mean reproduction age in this case. Analyzing the populational growth rate (*r*) and the population doubling time (*DT*), it was seen that the growth rate was greater for the population with density 100, which presented the smallest *DT* value in days, indicating that a smaller individual density and a greater availability of food for larval development had an effect on viability and fecundity of the resulting adults, allowing a greater and faster population growth in the population of the

species studied where ($p \leq 0.014$, $df=2$ and $f= 9.323$). The population doubling time were 3.38 ± 0.11 , 3.566 ± 1.11 and 4.28 ± 1.22 for densities 100, 200 and 400, respectively. According to Southwood, (1981) and Huffaker *et al.*, (1984) r-strategists are characterized by a high r, a large fecundity (large R₀) and short generation time (T) this results disagree with our results which recorded high (r) with small fecundity R₀ and short generation time (T). The short generation time was 0.203 ± 0.046 , 0.18738 ± 0.057 and 0.155 ± 0.057 for densities 100, 200 and 400, respectively. Esser, (1990) reported that for understanding of the population dynamics of *C. megacephala*, data suitable for life table analysis must be collected on major diets under both laboratory and field conditions. For example, nutrients and food preference play important roles on the developmental rate and fecundity of organisms. *C. megacephala* preferentially oviposited on fish spiked with recently laid eggs, and D'Almeida *et al.*, (1996) found that meat was a less suitable diet than fish for *C. megacephala*. The results of Von Zuben *et al.*, (1993) and Reis *et al.*, (1994), which noted that the fecundity values of *C. megacephala* decreased as larval competition for limited food resources increased, as in the case of *C. albiceps* Queiroz and Milward-de-Azevedo, (1991). Von Zuben *et al.*, (2000) studied the effect of different levels of larval aggregation on fecundity and reproductive investment in *C. megacephala*, and they observed that the results of larval competition for food were different in situations where their aggregation level (number of competitors per amount of available food) was the same, but with proportionately different competitor densities and amounts of food. These results indicated that larval competition could depend on the larval aggregation level on the feeding substrate. Goodbrod and Goff (1990) demonstrated that the larval aggregation of blowflies, with consequent production of proteolytic and salivary enzyme secretions would increase the efficiency of the feeding process, and consequently, accelerated the larval development rate. The flies might have fed on the ground beef which was made available daily. This way, the females were able to lay eggs throughout their lives, and it was possible that the last eggs came from a second or even third gonotrophic development in a single female Linhares, (1988). In natural environment, it is very difficult to obtain precise data on reproductive rates.

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ARABIC SUMMARY

تأثير كثافة اليرقات على بعض الجوانب البيولوجية من *Chrysomya megacephala* (ذوات الجناحين : كاليفوريدى).

عبد الوهاب عبدالمقصود ابراهيم ، عايدة سعيد كامل ، ناهد محمد حلمي ، دينا عادل مروان
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يهدف هذا العمل الى دراسة تأثير كثافة اليرقات على بعض الجوانب البيئية والسكانية والبقاء لحشرة *Chrysomya megacephala* ، وتحليل الجوانب الديموغرافية للاطوار البالغة في ظل ظروف تجريبية. واستخدمت ثلاثة كثافات لليرقات مختلفة ١٠٠ و ٢٠٠ و ٤٠٠ على التوالي لتحديد البقاء، وفترة ما قبل وضع البيض، والنسبة الجنسية و البيئة السكانية. واثبتت النتائج وجود اختلاف كبير بين بقاء الحشرة و فترة ما قبل وضع البيض مع كثافة اليرقات. أظهرت النتائج أن المعلمة الديموغرافية لمتوسط العمر المتوقع عند معدل نمو جوهري (e0) ، الخصوبة الكلية (TF) ، صافي الخصوبة (NF) والاطوار البالغة في سن وضع البيض (T) زادت مع زيادة كثافة اليرقات. وحسبت المعلمات السكانية مثل معدل النمو الجوهري (e0) و معدل نمو محدود (λ) والوقت الازم لمضاعفة عدد السكان (DT). أظهرت النتائج أن الخصوبة الكلية (TF) وصافي الخصوبة (NF) كانت تتناسب تناسباً طردياً مع الكثافة، والقيم العظمى التي تم الحصول عليها في أكبر كثافة ٤٠٠ تليها ٢٠٠ و ١٠٠ على التوالي. أظهرت هذه الاختلافات جوهرياً من حيث الخصوبة بين الإناث و كثافة اليرقات التي تتغذى على نفس الكمية من المواد الغذائية. كانت الكثافة تتناسب عكسياً مع معدل إنتاج البيض اليومي (ADP). لوحظت القيم العظمى في أصغر كثافة ١٠٠ و القيم الصغرى في أكبر كثافة ٤٠٠. و متوسط سن الإنجاب (T) يتناسب تناسباً عكسياً مع الكثافة ، كانت القيم العظمى التي تم الحصول عليها في كثافة ٤٠٠ و ١٠٠.