IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 3 Ver. III (May- Jun.2016), PP 05-11
www.iosrjournals.org

Influence of Temperature and Larval Food oncertain Biological Aspectsof *Chrysomyamegacephala*(Diptera: Calliphoridae)

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Abstract: The influence ofthreerearing temperatures; 20, 25 and 30°C on the duration,percentage survival, fecundityand wing formation in*Chrysomyamegacephala*(Diptera: Calliphoridae) were investigated. The duration ofall stages significantly decreased with the increase in rearing temperature. The highest survival of adults and the lowest malformationwere recorded at 25°C.Fecundity was not significantly affected by the change in temperatures. Larvae feed on beef meat had the highest fecundity and survival,followed by those feed on chicken meat andbeef liver. Larvae reared on artificial media produced adults with low survival and failed to lay any eggs.We hypothesize that time for each stage of development will be affected by the larval rearing substrate (media type) as well as the temperatures to which the flies were exposed to throughout their life cycles.
Keywords: Biology, *Chrysomyamegacephala*, Temperature, Forensic entomology.

I. Introduction

Forensic entomologists estimate the minimum time between death and discovery of a corpse (PMI) mainly in terms of the parameters of body size and developmental stages of blowflies which are found in or on a corpse (Li et al., 2014).Understanding of factors influencing larval development is incomplete, and the applicability of laboratory data is somewhat restricted to ideal or artificial conditions, not frequently encountered in case work of each country. For these reasons, the availability of accurate development data for commonly encountered blowfly species in different ecological niches is of utmost importance.

Current standard development rate curves of blowflies are produced by extrapolation from analysis of developmental rate at controlled environmental conditions (Li et al., 2014). Studies on the effect of temperature and nutrient composition of diet on larval growth of the blowfly are sparse in the forensic entomology literature. *Chrysomyamegacephala*, is a forensically important blowfly species in many parts of the world including Egypt (Lee et al., 2004, Sukontusonet.al., 2008 and Ibrahim et al., 2014). As a result, their larvae are usually collected to be reared to adults and some to be preserved in order to determine the PMI of the victim (Chen et al., 2007).Previous experiments on the development of blowfly larvae showed that temperature and the components of the diet play a key role in larval development (Flores et al., 2014 and Li et al., 2014).

The aim of this study, therefore, was to investigate the relation betweentemperature and diet type on certain biological parameters of *Chrysomyamegacephala*(Fabricius) (Diptera: Calliphoridae). The blowfly *C. megacephala*was selected for thiswork because our preliminary surveys revealed that it isone of the most dominant species that infests carcasses in the local area of Qaluobiya, Egypt. Moreover, it is also one of the first saprophagous organisms to arrive and lay eggs on a body after death (Ibrahim et al., 2013).

II. Materials And Methods

Fly Maintenance:

The strain of *C. megacephala* used in this experiment was an established culture originating from individuals trapped in Qaluobiya (29_590 N; 106_ 540 E), Egypt. These flies were inbred for three generations. Flies were reared in an incubator(1.5m×1m×.5m) for several generations prior to use. Flies were maintained at an ambient temperature, (25.5 $\pm$ 2.5^{o}C), and 12:12 light/dark photoperiod in a wooden boxes (40×40×56). Adults were provided with a mixture of sugar (50%) & powdered milk and fresh meat (used as a food source and oviposition site). Water was provided through along thread of paper immersed in bottle filled with sterile water.Small pieces of fresh meat were added and water supply was changed every 2 days. The oviposition site was provided with a layer of 10 Cm of sterile sawdust to keep moisture in the plastic cup. The oviposition sites were observed daily for the presence of eggs; if present, eggs were transferred into (12 x 15 x 6 cm) transparent plastic boxes, containing a layer of sawdust 10Cm and provided with 50 gm. of fresh meat as larval food. The lid of each box was rectangular, likes a fine mesh suitable for ventilation and prevention of other small insects entering the box to oviposit in it. The lid was sealed tightly with an adhesive rubber cord to prevent the larvae from crawling out. Immediately after the appearance of third instars, larvae were transferred into clean sterilized jars containing 5cm sterile sawdust as a medium for pupation. Emerged adults were transferred into the rearing cage (40 x 40 x 56 cm).

Effect of Temperature:

The influence of three rearing temperatures; 20, 25 and 30°C on the duration, percentage survival, fecundity and wing formation in *C. megacephala*were investigated.Different stages of the fly (eggs, larvae, pupae and adults) were separated from the colony, transferred to a plastic container (10X6 Cm.) and were provided with the diet. This container was placed insidelarger one (12 X 15 X 6 Cm.) containing vermiculite, and was covered with nylon netting. Insects were maintained in growth chambers at 27 ºCand 70 % RH and were daily observed.

Three cages were used for each temperature with the adults of the F2 generation of *C. megacephala*. Data on female mortality and fecundity were collected daily. Regarding the fecundity, four identical cups with the same amount of minced meat were placed on the cages daily at regular time intervals. The presence or absence of eggs and the number of eggs laid were recorded daily.

Effect of Diets:

Fresh liver, beef and chicken meat were obtained from the local market on the day of slaughtering. Fifty grams of each diet wereused in each treatment. Diets were stored at a refrigerator until used. The diets were thawed and equilibrated to room temperature before the transfer of egg masses.An artificial diet of fish food was purchased from the market and was soaked in 10 ml of distilled water for 24 hours for softening. The artificial diet used contained 40% crude protein, 14% crude ash, 0.6% zinc, 3.5 crude fibers, 3.5% calcium 3.5% salts and 10% moisture.

Eggs were separated from the meat and kept on Petri dishes with a portion of the tested diet.After hatching, 30 larvae were transferred to a plastic container (10X6) with the corresponding diet. This container was placed inside larger one (12X15X6) containing vermiculite and was covered with nylon netting.Insects were maintained in a growth chamberat 27 ºC and 70 % RH and were daily observed.

Data and Statistical Analysis of Data:

Data was statistically analyzed using System Analysis Statistics Program (SAS), version 6.12,1998. Significant levels were located at probability level ≤ 0.01.

III. Results

Effect of Temperature on the Duration of Different Stages of *C. megacephala*

The influence of three rearing temperatures; 20, 25 and 30°C on the duration, of*C. megacephala*is shown in table (1).

Table (1): Effect of temperature on the duration of different stages of *C. megacephala*under laboratory conditions

Temp.	Duration in hours (hrs)									
	Eggs	Larvae				Pupae			Adults	Totalduration
		L1	L2	L3	Total	Pre-pupae	pupae	Total		
20°C	18.0a ±0.8	18.8a ±2.5	45.1a ±6.4	65.4a ±8.5	129.2a ±23.3	107.6a ±10.6	236.2a ±72.0	343.8a ±118.3	31950 a ±37.5	810.5a ±179.6
25°C	15.0b ±3.8	17.0a ±0.3	31.9b ±0.4	75.7a ±0.4	124.6a ±30.9	141.0b ±0.8	108.3b ±4.1	249.3b ±23.1	239.20b ±0.7	628,0b ±31.5
30°C	12.0c ±3.6	17.8a ±0.4	30.3b ±0.5	71.0a ±1.0	119.1b ±27.9	0.00c	143.0c ±1.5	143.0c ±1.5	233.20c ±1.8	507.3c ±34.8

Means followed by the same letter in each column is not significantly different at P ≥ 0.001

Data in table (1) indicate that at 20 °C egg, larvae, pupae and adults durations were18**,** 129.23, 343.78and 319.5hrs, respectively.At 25 °C egg, larvae, pupae and adults durations were 15**,** 124.56, 249.28and 239.2hrs, respectively.At 30 °C egg, larvae, pupae and adults durationswere 12, 119.1, 143and 233.2hrs, respectively. The total durations of the insect at 20, 25 and 30 °C were 810.51, 628.04 and 507.3 hrs. respectively. The durationsof each stage and the total duration decreased with the increase in temperature.The most recognizable variation in duration was observed during the pre-pupae and pupae stages. Both stages appeared to be less predictable than other stages. This can be clearly seen in the pre-pupaestage which spent 107.6 and 141 hrs. to reach the pupae stage at 20 and 25 °C, respectively,whereas larvae transformed directly to pupaeat 30 °C. In case of pupae the shortest duration (108.3 hrs.) was recorded at 25 °C, followed by 143 and 236.2 hrs. at 30 and 20 °C, respectively.Because larval behavior is not always similar, the indicator used for pinpointing the onset of the post feeding stages was determined when the larvae began to travel around the circumference of the container.

Effect of Temperature on Adult Survival and Fecundity

The influence of three rearing temperatures; 20, 25 and 30°C on adult survival and fecundityof*C. megacephala*is shown in table (2).

Table (2): Effect of temperature on adult survival rate and fecundity of *C. megacephala* under laboratory conditions.

Temp.	Survival rate	f	P	Fecundity	f	p
20 ºC	43.42 ±2.30	30.792	0.001	236.34 ±10.54	0.453	0.659
25ºC	65.75 ±1.8			225.00 ±25.00		
30ºC	55.23 ±4.20			235.60 ±2.41		

A highly significant difference was found in the survival of *C.megacephala* reared at different temperatures.The highest survival rates were observed at25ºC and the lowest survival rates were observed at 20 ºC. The survival rates of adults were 43.42, 65.75 and55.23 at 20 25 ºC and 30 ºC, respectively.
No significant difference was found in the fecundity of adults reared at the three degrees of temperature tested. The recorded fecunditywas236.34±10.54, 225±25, and 235.6±2.41 at20 ºC, 25ºC and 30ºC, respectively.

Effect of Temperature on Wing Formation of *C.megacephala*

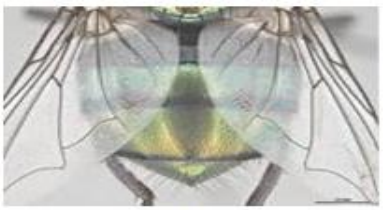
The data in table (3) summarize the results of inbreeding *C.megacephala* at different temperatures under controlled conditions.

Table: (3) Effect of temperatures on wing formation of *C. megacephala* under controlled conditions:

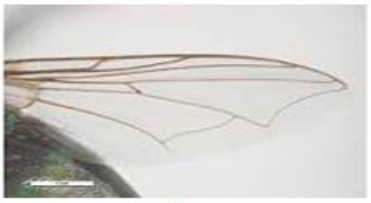
Temp.	Normal wings (%)		Malformed one wing (%)		Malformed two wings (%)	
	Male	Female	Male	Female	Male	Female
20ºc	76.39 ±4.88	76.39 ±1.84	2.43 ±3.44	2.3 ±1.80	18.33 ±7.87	_
25ºc	98.33 ±2.357	100	1.66 ±2.35	_	_	_
30ºc	74.19 ±8.97	94.58 ±2.43	14.13 ±6.66	3.7 ±2.6	11.64 ±2.3	_
F value	717	4.753	4.735	2.066	7.863	
P value	0.013	058	0.058	0.208	0.021	

Inbreeding *C. megacephala* under laboratory conditions resulted in many forms of wing malformations. Males were significantly more sensitive to changes in temperature than females.A highly significant difference was found in the percentages of normal wings at different temperatures. The highest percentage of normal wings was observed at 25ºC. Normal male wings represented 76.39, 98.33and 19% at 20, 25and30 ºC, respectively**.**Normal females'wingswere 97.66, 100 and 94.58%at 20, 25and 30 º C, respectively.

The malformation in one wing of males was 2.43, 1.66 and 14.13 % at 20º, 25 and 30 º C, respectivelyIn females**,** the malformation in one wing was 2.3 at 20ºC, 3.7 at 30 º C, and no wing malformation was recorded at 25 º C. The least malformationin wings was recorded in malesand females at 25 º C,andthe highest malformation was recorded at 30 º C.



A



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d

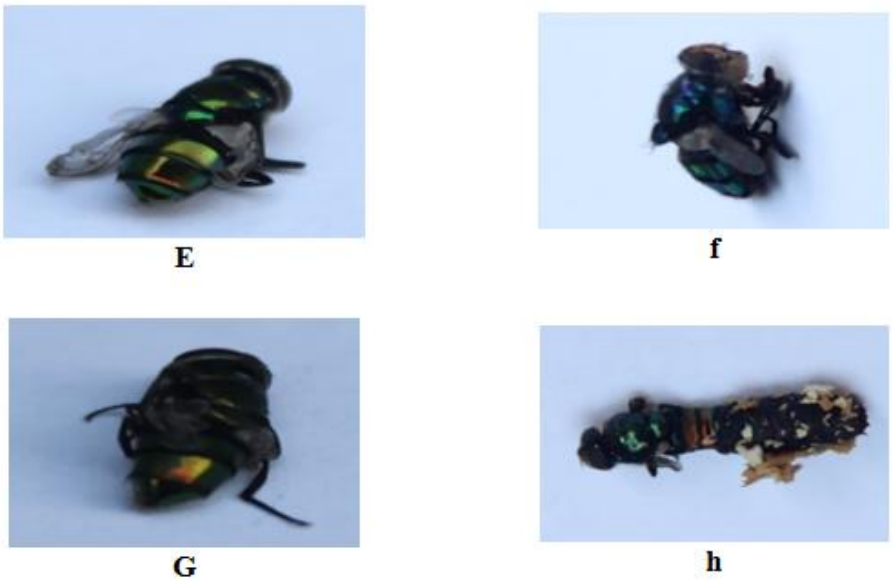

Fig. (1): Different forms of wing malformationin*C.megacephala* reared under laboratory conditions
a and b : Normal wings; c and d : Malformation in one wing; e, f , g and h : Malformation in both wings

Effect of Diettype on Adult Longevity
The effects of diet typeof larvae on adult longevity are shown in table (4).

Table: (4) Effect of different diets on adult longevity of *C.megacephala* reared in the laboratory at (27.00±1.74ºc) and RH (42.36±12.39%)

Diets	Male longevity (Mean ± SD)	t	p	Female longevity (Mean ± SD)	t	p
Beef meat	25.9±0.40	5.412	0.001	26.4±0.60	8.473	0.001
Chicken meat	19.9±0.374			18.2±0.734		
Liver beef	19.0±0.816			17.5±0.355		
Artificial media (fish food)	8.0±0.816			7.4±0.432		

A highly significant difference was found in both males and females longevitiesemerged from larvae reared on different types of diets.The longevitiesof males by using beef meat, chicken meat, liver beef and artificial media were25.9±0.4, 19.9±0.374, 19.0±0.816 and 8.00±0.816 days, respectively. The longevities offemales by using beef meat, chicken meat, liver beef and artificial media were 26.4±0.6, 18.2±0.734, and 17.5±0.355 and 7.4±0.432 days, respectively.These results clearly indicate that the longevities of males and females were greatly affected by the type of food provided for larvae.

Effect of Diet Type on Survival Rate and Fecundity
The data in table (5) show the effect of rearing media on survival rate and fecundity of*C.megacephala*.

Table (5) Effect of diet type on the survival rate and fecundity of *C. megacephala* reared under laboratory conditions (27.±1.74ºC) and 42.36±12.39% RH)

Diets	Survival rate Mean±SD	t	p	Fecundity Mean±SD	t	p
Beef meat	65.75±1.8	23.87	0.001	225.00±25.0	5.646	0.001
Chicken meat	53.33±6.23			200.33±18.37		
Liver beef	50±4.08			195.66±6.34		
Artificial media (fish food)	21.83±2.89			0.00		

Results of the present work indicate that the type of diets provided for larvae significantly affect($p \leq 0.001$) the survival rate and fecundity of adults of *C. megacephala* .By using the beef meat, chicken meat liver beef and artificial diet, the highest survival rate (65.75 ±1.8) was recorded in males emerged from larvae reared

on beef meet media followed by 53.33±6.23 when using chicken meat then 50±4.08 by using liver beef. The lowest Survival rate was 21.83±2.89 by using the artificial media.

The statistical analysis of data indicated that the variation in larvae diets had a significant effect on the fecundity of adults ($p \leq 0.001$). The highest fecundity was found by using beef meat, whereas the lowest fecundity was found by using liver beef. By using the artificial media the insects did not lay any eggs. The recorded fecundity of adults in case of beef meat media, chicken meat media, liver media and artificial media, were 225.0±25.0 200.33±18.37, 195.66±6.34 and 0.00,respectively.

IV. Discussion

Effect of Temperature

Temperature is probably the most influential environmental factor in the life history of populations, particularly in organisms with short life cycles such as insects (Levine and Levine, 1991). In most cases, warmer temperatures accelerate development of flies while cooler temperatures have an inverse impact.This relationship has been documented in past growth studies on blow flies at varying temperatures (Greenberg, 1991; Byrd, and Allen 2001; Byrd, and Butler, 1996; Byrd and Butler, 1997). Results of the present work demonstrateda direct relation between temperature and development. The durations of each stage and the total duration decreased with the increase in temperature. These findings are supported by findings of other investigators as Marinho*et al*., (2006) who concluded that temperature influencesand controls the insects' activity, oviposition rate and as well as their overall development. On the other hand, the recorded variations in duration of different stages of developmentin our results and results of other investigators, as, Smith, 1986, Wells &Kurahashi, 1994,Ismail *et al.,* 2007, Mohd*et al*., 2007 and Velez&Wolf, 2008, may be due to the differences in larval density, day length and type of food used. The most recognizable variation in duration was observed during the pre-pupae and pupae stages. Both stages appeared to be less predictable than other stages. This can be clearly seen in the pre-pupae stage which spent 107.6 and 141 hrs. to reach the pupae stage at 20 and 25 °C, respectively, whereas larvae transformed directly to pupae at 30 °C.This may be due to the fact that larvae cannot regulate their own body temperature, so, when temperature is above the tolerable threshold, as seen in our study; the larvae pupate sooner than normal to avoid being desiccated.

Our results cleared that survival of *C. megacephala* is significantly influenced by temperature, and the highest rate of survivalwas found at 25°c .These findings agree with Krebs, (2001) who found that the survival *C. megacephala* is reduced at high temperature. Meanwhile, our results disagree with what have been found by other investigators as Reigada& Godoy, (2006) who showed that temperature had no significant effect on survival of this insect and Godoy *et al.,* (1996) who stated that the most sensitive demographic Parameter for *C. megacephala*in terms of influence on dynamics is not survival.

Our study cleared that temperature had no significant effect on fecundity. Again, these results disagree with what have been found by Godoy *et al*., (1996) who stated that fecundity may be the most sensitive parameter for *C. megacephala*, as it could cause significant changes in behavioral dynamics, leading the population from a two-point limit cycle to a one-point stable equilibrium. Our results also disagree withTomberlin*et al*., (2001)who found that *C.megacephala* prefers warm climates and display a correlation between warmer temperature and higher fecundity

Temperature usually influences the biological variables which determine the body size(Tomberlin*et al.,* 2001). Our results showed that temperature had an effect on malformation of wings. Male were more sensitive to changes in temperature than females. No apparent explanation for this difference is available, but males may exhibit more sensitivity to thermal variations than females, since a decrease in terms of body size was detected only for them. This agree with what been reported by Tomberlin*et al.,* (2001) and Reigada& Godoy, (2006). Those authors found a correlation between wing size and temperature.Yu-Wei Hu andChaolangLe(2015)examined the effects of two temperatures (20 and 30 °C) on sexual size dimorphism (SSD) in six populations of the blowfly, *C. megacephala* and found that body size increased with temperature in all the populations studied, and the sexes differed in phenotypic plasticity of body size in response to rearing temperature. This created substantial temperature-induced variation in SSD (i.e. sex × temperature interaction). Males were often smaller than females and may be more sensitive. On other hand,further studies should address whether this variation can be produced by adaptive canalization of one sex against variation in temperature, or whether it may be a consequence of non-developmental differences between the sexes.

Effect of Diet Type

Our results declared that variation in diets had a significant effect on fecundity, survival and adult longevity. Decomposed beef proved to be the best diet for rearing *C. megacephala*, which was understandable since larvae of Calliphoridae develop in animal carcasses. Leal *et al*., (1982) reported the same results for *Calliphora. putoria*. The highest survival rate, adult's longevity and fecundity were recordedin insects reared on beef meat followed by chicken meat, then liver. This may be due to a higher protein content of meat than those

found in other diets.This agree with Barbosa *et al.*2004, who concluded that beef meat is an efficient diet for breeding larvae of microphagous dipterans, but it releases a disagreeable odor characteristic of decomposing organic matter and increases the possibility of secondary contamination of other insects that are attracted to it, in addition to being associated with high laboratory costs. On the other hand, D'Alemedia*etal.*, (1996) found that the meat was lesser suitable than fish for *C.megacephala.*

Insects reared on the artificial diet had short longevity, low survival rate and females failed to lay eggs. This may be due to low protein content in artificial diet. These results disagree with what have been found by Rabelo*et al.,* (2011) who reared *C. megacephala* on four substrates: minced beef and semi-synthetic diets with the addition of sardine, rumen or chicken eggs. No differences in total developmental times, adult's longevity and sex ratio were detected among larvae reared on different diets and the overall mortality was lower when beef was used as food. Also our resultsdisagree with Von Zuben*et al.,* (1993) and Reis *et al*., (1996) who find no significant difference between meat and liver diets on fecundity. Artificial diets proved unfavorable for rearing *C. megacephala*,

Many authors concluded that the type of tissue fed to immature blow flies can impacts their size and development rate (Kaneshrajah and Turner, 2004, & Clark *et al.*, 2004). The last author determined that *Luciliasericata* larvae reared on porcine tissue grew faster and larger than those on bovine tissue, while larvae fed lung and heart of both tissue types grew faster and larger than larvae fed liver. Clark *et al.*, 2004 recorded similar results for *Calliphoravicina* larvae with those fed pig lung, kidney, heart or brain growing faster and larger than those provided pig liver. Tarone and Foran(2006) showed that, even when fed only beef liver, *L. sericata* larvae possess the potential to develop at different rates depending on the experimental conditions (specifically factors affecting liver moisture and the condition of the pupation substrate.

Furthermore, these data, when compared to those available in the literature, indicate developmental differences that could be due to genetic differences in populations or possibly methods employed during the studies. Caution should be emphasized when applying development data for this species from one region to forensic investigations in other eco-regions as such differences in development based on temperature, tissue fed upon by larvae, population genetics, and methodologies used in the studies could represent error in estimating the time of colonization.

References

[1]. Barbosa, L. S.; Jesus, D. M. L. and Aguiar-Coelho, V. M. (2004): Longevidade e capacidadereprodutivadeecasaisagrupados de *Chrysomyamegacephala* (Fabricius, 1794) (Diptera: Calliphoridae) oriundos de larvascriadasemdieta natural e oligolítica.RevistaBrasileira de Zoociências, 6 : 207-217.

[2]. Byrd, J.H.and,J.F.Butler (1996): Butler, Effects of temperature on *Cochliomyiamacellaria* (Diptera: Calliphoridae) development, J. Med. Entomol. 33 (6) 901–905.

[3]. Byrd,J.H. and J.F. Butler,(1997): Effects of temperature on *Chrysomyarufifacies* (Diptera: Calliphoridae) development, J. Med. Entomol. 34 (3) 353–358.

[4]. Byrd, J.H., J.C. Allen,(2001): The development of the black blow fly, Phormiaregina (Meigen), Forensic Sci. Int. 120 79–88.

[5]. Chin, H.C.; Marwi, M.A.; MohdSalleh, A.F.; Jeffery, J. and Omar, B. (2007): A preliminary study of insect succession on pig carcass in a palm oil plantation in Malaysia. Tropical Biomedicine 24(2): 23-27.

[6]. Clark, K., L. Evans, and R. Wall, (2004): Growth rates of the blowfly, Luciliasericata, on different body tissues, Forensic Sci. Int. 156, 145–149.

[7]. D'Almeida, J.M.; José, R. and Salviano, B., (1996): Feeding preference of the larvae of *Chrysomyamegacephala*(Fabricius) (Diptera: Calliphoridae) and *Ravinia belforti*(Prado e Fonseca) (Diptera: Sarcophagidae) concerning different diets. Mem.Inst. Oswaldo Cruz, Rio de Janeiro. 91 (1): 137–138.

[8]. Flores M., Michael Longnecker B. , Jeffery K. Tomberlin A.(2014): Effects of temperature and tissue type on *Chrysomyarufifacies* (Diptera: Calliphoridae) (Macquart) development. Forensic Science International 245, 24–29

[9]. Godoy, W.A.C., C.J. Von Zuben, S.F. Reis & F.J. Von Zuben.(1996): Dynamics of experimental populations of native and introduced blowflies (Diptera: Calliphoridae): Mathematical modelling and the transition from asymptotic equilibrium to bounded oscillations. Mem. Inst. Oswaldo Cruz 97: 641-648.

[10]. Greenberg, B.(1991): Flies as forensic indicators, J. Med. Entomol. 28 565–577.

[11]. Kaneshrajah G., B. Turner, (2004): Calliphoravicina larvae grow at different rates on different body tissues, Int. J. Legal Med. 118, 242–244.

[12]. Kreps, C.J. (2001): Ecology: the experimental analysis of distribution and abundance. New York: Harper & Row, (p) 715.

[13]. Leal, T. T. S.; Prado, A. P. and Antunes, J. A. (1982): Rearing the larvae of the blowfly *Chrysomyachlopyga*(Wiedemann) (Diptera: Calliphoridae) on oligidic diets. RevistaBrasileira de Zoologia, 1: 41-44.

[14]. Lee, H.L.; Krishnasamy, M.; Abdullah, A.G. and Jeffery, J. (2004): Review of forensically important entomological specimens in the period of 1972-2002. Trop Biomed. 21(2): 69-75.

[15]. Levine, O.S.; Levine, M.(1991):. Houseflies (*Muscadomestica*) as mechanical vectors of Shigellosis. Infect. Immun., v.31, p.445-452,.

[16]. Li, X., Genping Li, Hongwei Li, Qingshan Wang, and Lihua Wan, J. (2014): The Effect of Dietary Fat Levels on the Size and Development of *Chrysomyamegacephala* (Diptera: Calliphoridae) J. Insect Sci. 14(174): DOI: 10.1093 pp. 1-5.

[17]. Marinho, C.R.; Barbosa, L.S.; Azevedo, A.C.; Queiroz, M.M.; Valgode, M.A. and Aguiar-Coelho, V.M. (2006): Diversity of Calliphoridae (Diptera) in Brazil's Tingua Biological Reserve. Braz J. Biol. 66(1A): 95-100.

[18]. Mohd I., I.; Khairul, O.; Ong, H. K.; Nurhaslina, H., Ezlan, E.; Kaswandi, M. A.; Ahmad Rohi, G.; Jamaludin, M. and Baharuddin, O. (2007): Accelerating *Chrysomyamegacephala*maggot growth for forensic entomological cases. JurnalSainsKesihatan, 5 (1): 17-26.

[19]. Rabêlo, K.C.N.; Thyssen,P.J.; Salgado,R.L.; Araújo,M.S.C.andVasconcelos,S.D.(2011): Bionomics of two forensically important blowfly species Chrysomyamegacephala and Chrysomyaputoria (Diptera: Calliphoridae) reared on four types of diet.Forensic Science International, 210 (1-3):257–262.
[20]. Reigada, C. and Godoy, W.A. (2006): Larval density, temperature and biological aspects of Chrysomyamegacephala (Diptera: Calliphoridae).Arq.Bras. Med. Vet. Zootec.58(4): 562-566.
[21]. Reis, S.F., M.A. Teixeira, F.J. Von Zuben, W.A.C. Godoy & C.J. Von Zuben. 1996. Theoretical dynamics in experimental populations of introduced and native blowflies (Diptera: Calliphoridae). J. Med. Entomol. 33: 537-544
[22]. Smith, K.G.V. (1986): A manual of forensic entomology. The trustees of British museum of natural history, London and Comstock Publishin Associates, New York, 1-205.
[23]. Sukontason, K.; Piangjai, S.; Siriwattanarungsee, S. and Sukontason, K.L. (2008): Morphology and developmental rate of blowflies Chrysomyamegacephala and Chrysomyarufifaciesin Thailand. Appl. Foren. Entomol.Parasitol. Res.,102(6): 1207-1216.
[24]. Tarone, A.M., and D.R. Foran, (2006): Components of developmental plasticity in a Michigan population of Luciliasericata (Diptera: Calliphoridae), J. Med. Entomol. 43 (5) 1023–1033.
[25]. Tomberlin JK, Reeves WK, Sheppard DC (2001). "First record of Chrysomyamegacephala,Calliphoridae: Diptera) in Georgia, USA". Florida Entomologist 84 (2): 300–1. doi:10.2307/3496184. JSTOR 3496184.
[26]. Velez Marcia, C. and Wolf, Martha. (2008): Rearing five species of Diptera Colombia in semi controlled field environments. Museu de Zoologia da UniversidadedeS .o Paulo. PapeisAvulsos de Zoologia ,48(6): 41-47.
[27]. Yu-WeiHuandChaolangLe(2015): Sexual size dimorphism decreases with temperature in a blowfly, Chrysomyamegacephala (Fabricius) (Diptera: Calliphoridae) Ecological Entomology Volume 36, Issue 1, 111–115.
[28]. ZubenV., Stangenhaus G, Godoy WAC. Competição larval em Chrysomyamegacephala (F.) (Diptera: Calliphoridae): efeitos de diferentesníveis de agregação larval sobreestimativas de peso, fecundidade e investimentoreprodutivo. RevistaBrasileira de Biologia. 2000;60:195–203.