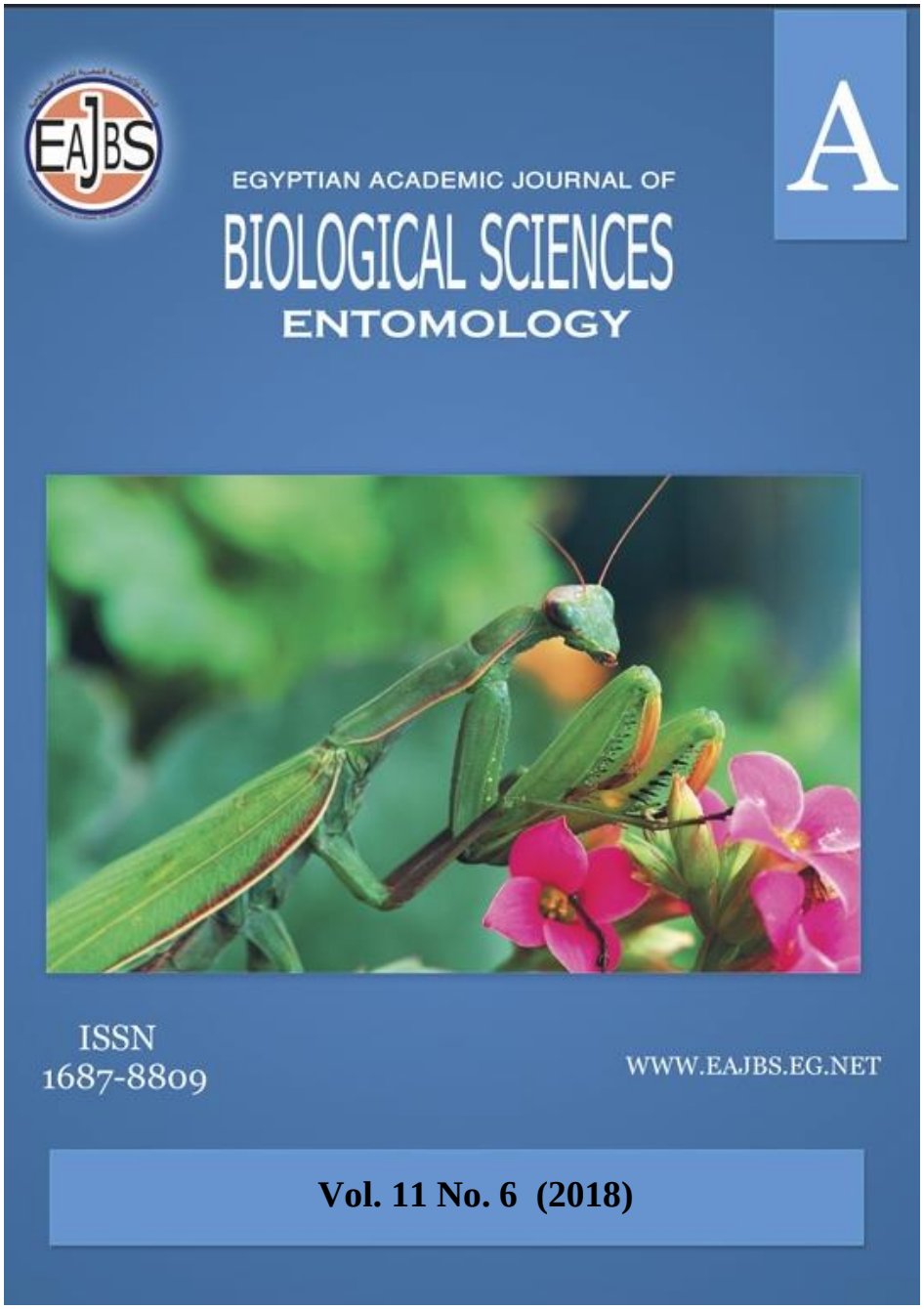

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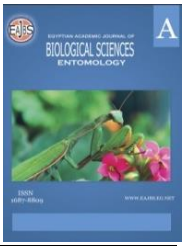

The Effect of Temperature Regimes and Tissue Types on The Development of *Chrysomya megacephala* Larvae (Diptera: callophoridae).

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ABSTRACT

This study investigated the effects of three temperature regimes representing several specific development conditions of this blowfly: (25.7, 28.4and 30.6ºC) and tissue types. Furthermore, *Chrysomya megacephala* colonies were reared on three different food sources (all rabbit carcasses, Liver and muscles) on the development of *C.megacephala* as important forensic fly, and their possible implications to calculate the postmortem interval (PMI). The results showed that the mean durations period from egg to adult eclosion of *C. megacephala* when reared on control rabbit at 25.7, 28.4 and 30.6 ºC were 303.5, 217 and194.5 hrs, respectively. In addition, the mean duration period from egg to adult eclosion when the larvae reared on liver tissue at 25.7, 28.4and 30.6oC were 275.5, 205.5 and 188.5 hrs, respectively. Moreover, The Larvae of *C.megacephala* reared on muscle tissues from rabbit carcasses, the mean duration from egg to adult eclosion at 25.7ºC 28.4ºC, and 30.6ºC were 293, 214.5 and 192.5 hrs, respectively.

From the previous results, it is obvious that the mean duration in larvae reared on control rabbit carcasses was longer than those fed on muscle which longer than those reared on the liver at all temperature regimes

INTRODUCTION

Knowledge of the distribution, biology, and behavior of insects found at a crime scene can provide information on when, where and how the crime was committed (Hall, 2008). The type and composition of fauna found on a corpse are indicative of its stage of decomposition (Anderson, 2009). Blowfly larvae play an important role in ecological function in the decomposition of animal remains. 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). Using growth parameter and larval length as a 'biological clock' (Chen *et al*. 2008). The use of

immature insects, in particular, the larvae of calliphorid flies, may not only contribute to the calculation of the PMI but can also provide evidence for the detection of the presence of drugs or other substances in the cadaver (Lima *et al*.2014). Some death that occurs by poisoning remains undiscovered until the body is wholly or partially skeletonised. In such cases, the analysis of toxicology using body fluids and tissues is almost impossible. Recently forensic entomologist has introduced a procedure using insects as a silent witness interpreting information concerning death. Temperature is a well-recognized abiotic factor that affects blowfly development. In most cases, warmer temperatures accelerate development while cooler temperatures have an inverse impact. This relationship has been documented in past growth studies on blowflies at varying temperatures Byrd and Allen (2001). The purpose of this study was to determine the effect of temperature regimes and tissue types on the development and growth of *C. megacephala larvae* as well to verify the time of emergence of adults that can affect the estimation of postmortem interval.

MATERIALS AND METHODS

Blowfly Source:

The colony of C. *megacephala* was reared in the research laboratory of the Department of Entomology, Faculty of Science, Ain-Shams University, and Cairo, Egypt. Several generations of these flies were reared prior to use. Similar to Sukontason *et al*. (2004) and Gabre *et al*. (2005**)**, these fly colonies were maintained at ambient temperature, (25.5 $\pm$ 2.5ºC), and natural light/dark photoperiod in a wooden box in the rearing room with fluctuating outdoor conditions. Temperatures were measured at each sampling time and the average temperature of rearing was calculated from these data (annual mean of 25.7ºC). Adults have reared on two kinds of food: **1)** a mixture of sugar & powdered milk. **2)** Fresh rabbit liver (used as both a food source and oviposition site). Water supply was a piece of paper as a long thread in a bottle filled with water. Small pieces of fresh rabbit liver and water supply were changed every 2 days. Subsequently, the oviposition sites were observed daily for the presence of eggs; if present, the eggs were transferred into a (12 x 15 x 6 cm) transparent plastic box, and 40 g of fresh rabbit liver was provided 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 adhesive rubber cord to prevent the larvae from crawling out. Immediately after the third larval instars observed, larvae were transferred into the rearing cage (40 x 40 x 56 cm). At the post-feeding stage, dry autoclaved sawdust was added as a medium for pupation.

Three domestic rabbits (1.25 – 1.3 kg in weight), *Oryctolagus cubiculum* (L.), were used in each experiment and killed by chloroform or ethyl either immediately following death the rabbits were dissected. A sterile scalpel made an incision made in the abdomen of each rabbit. The sample's liver and muscles were taken. 1.0 g of each solid tissue was dissected out over a stainless steel plate cooled with ice. After weighting, the solid tissues were wrapped in a piece of aluminum foil, put in a plastic bag and immediately frozen at-20°C until analysis. Three samples per tissue per carcass were used. After sampling, rabbits were sutured to reconstitute their initial anatomy**.**

Muscle and liver tissues were chosen to be rearing substrates for *Chrysomya megacephala* beside the whole rabbit.

Blowfly Experiment:

After oviposition of gravid females on rabbit liver, eggs were collected within 30 minutes. Each rabbit carcass was seeded in the natural openings by approximately 800 eggs of C.megacephala. About 200 newly emerged larvae were obtained and placed into each muscle and liver tissues to initiate the test colonies. Colonies established were maintained in the laboratory at 25.5 $\pm$ 2.5ºC with 12 h light and 12 hrs. dark.

Rabbit carcasses were placed into plastic boxes with wire netting to prevent contamination by other insects. When most of the larvae were mature third instars, dry sawdust was added on the top of each rabbit cadaver for post-feeding larvae to pupariate (Abd El- Samad2006).

Statistical Analysis:

SAS (1997) statistical analysis software was used to analyze all experimental data. Analysis of variance one- way (ANOVA) was used between the mean time spent in the developmental stages (Larvae, Pupae and Total) between all tissues(rabbit carcass, liver and muscle)that *C. megacephala* reared on, under 3 temperature regimes.

RESULTS

The Effect of Different Temperature Regimes on the Development Rate of *Chrysomya megacephala* Larvae:

1-Larvae Reared on Control Rabbit Carcasses:

The data in Table: (1) showed that the mean durations form the egg to adult eclosion of *C. megacephala* reared on control rabbit carcasses at 25.7ºC, 28.4ºC and 30.6 ºC, respectively were 303.5, 217 and 194.5 hrs. ANOVA analysis showed that there were strong significant differences for all developmental stages and the total periods at different temperatures (in all cases $p < 0.001$).

Regression analysis revealed that there were strong significant correlations (in all cases $p < 0.01$) for all developmental stages and total periods. The correlation coefficient (r) possessed a minus value, this indicated to the reduction of the total period of development with the increase of temperature (Table: 2).

Table 1: Mean durations period of the developmental stages of *C. megacephala* from rabbit colonies with different temperature regimes

Stage	Temperature regimes			p
	25.7	28.4	30.6	
Egg	23a	10b	8c	0.0001
Larvae	148a	115.5b	107.5c	0.0006
Pupae	132.5a	91.5b	79c	0.0005
Total duration /hrs	303.5a	217b	194.5c	0.0003

$P > 0.05$ = No significant difference
$P \leq 0.05$ = Significant difference
The same letters are significantly different
Different letters are not significantly different
(In the same row)

Table 2: Correlation between different stages of development of *C. megacephala* reared on rabbit colonies and different temperatures

Stage	Linear regression equation	r	p
Egg	Y = 101.9 - 3.13 X	-0.942	0.0049
Larvae	Y = 361 - 8.41 X	-0.958	0.0026
Pupae	Y = 413.8 - 11.08 X	-0.967	0.0015
Total	Y = 876.7 - 22.6 X	-0.962	0.0021

r: correlation coefficient between each stage and different temperatures (25.7, 28.4 and 30.6 ºC)

2-Larvae Reared on Liver Tissues From Control Rabbit Carcasses:

The data in (Table: 3) showed the mean period from egg to adult eclosion at 25.7ºC, 28.4oC and 30.6oC were 275.5, 205.5 and 188.5 hrs, respectively. There were strong significant differences for all developmental stages and the total periods (in all cases $p < 0.01$) at 25.7 ºC but were not found at 28.4ºC or

The data analysis in Table (4) showed that there were strong significant correlations (in all cases $p < 0.01$) for all developmental stages and total periods. The correlation coefficient (r) was by a minus value, this indicated to the reduction of the total period of development with the increase of temperature (Table 4).

Table 3: Mean durations period of the developmental stages of *C. megacephala* reared on liver with different temperature regimes

Stage	Temperature regimes			p
	25.7	28.4	30.6	
Egg	23a	10b	8c	0.0001
Larvae	131.5a	108b	105b	0.0042
Pupae	121a	87.5b	75.5b	0.0037
Total	275.5a	205.5b	188.5b	0.0021

$P > 0.05$ = No significant difference
$P \leq 0.05$ = Significant difference
The same letters are significantly different
Different letters are not significantly different
(Inthe same row)

Table 4: Correlation between different stages of development of *C. megacephala* reared on liver and different temperatures

Stage	Linear regression equation	r	p
Egg	Y = 101.9 - 3.13 X	-0.942	0.0049
Larvae	Y = 271 - 5.5 X	-0.923	0.0086
Pupae	Y = 360.13 - 9.4 X	-0.967	0.0016
Total	Y = 733.07 - 18.06 X	-0.953	0.0032

r: correlation coefficient between each stage and different temperatures (25.7, 28.4 and 30.6 ºC)

3-Larvae Reared On Muscle Tissues From Control Rabbit Carcasses:

The data in Table (5) showed the mean duration from egg to adult eclosion at 25.7°C, 28.4°C and 30.6oC were 293, 214.5 and 192.5 hrs, respectively. There were strong significant differences for all developmental stages and the total periods (in all cases $p < 0.01$) at different temperatures.

The data analysis in Table (6) showed that there were strong significant correlations (in all cases $p < 0.01$) for all developmental stages and total periods. The correlation coefficient (r) was by a minus value, this indicated to the reduction of the total period of development with the increase of temperature.

Table 5: Mean durations of the developmental stages of *C. megacephala* reared on muscle with different temperature regimes**.**

Stage	Temperature regimes			p
	25.7	28.4	30.6	
Egg	23a	10b	8c	0.0001
Larvae	140.5a	115b	107c	0.0006
Pupae	129.5a	89.5b	77.5b	0.0021
Total	293a	214.5b	192.5c	0.0008

P > 0.05 = No significant difference
P ≤ 0.05 = Significant difference
The same letters are significantly different
Different letters are not significantly different
(In the same row)

Table 6: Correlation between different stages of development of *C. megacephala* reared on muscle and different temperatures

Stage	Linear regression equation	r	p
Egg	Y = 101.9 - 3.13 X	-0.942	0.0049
Larvae	Y = 316.7 - 6.9 X	-0.969	0.0014
Pupae	Y = 402.9 - 10.77 X	-0.963	0.0021
Total	Y = 821.4 - 20.8 X	-0.963	0.002

r: correlation coefficient between each stage and different temperatures (25.7, 28.4 and 30.6 °C)

4-Differences in the Mean Durations in All Colonies:

Results in (Table 7,and Fig.1) showed that the mean duration in larvae reared on control rabbit carcasses was longer than those fed on muscle which longer than those reared on the liver at all temperature regimes (25.7, 28.4 and 30.6°C). The durations for rabbit, muscle, liver colonies at 25.7°C were 303.5 hrs, 275.5 hrs and 293 hrs, respectively. While at 28.4°C, durations were 217 hrs, 205.5 hrs and 214.5 hrs, respectively. Meanwhile, at 30.6°C, the mean total periods were 194.5 hrs, 188.5 hrs and 192.5 hrs, respectively. This indicated that the longest duration of larval and pupal stages from rabbit colonies followed by those from muscle colonies than the shortest periods were recorded for larval and pupal stages from liver colonies.

In all temperature regimes (25.7, 28.4 and 30.6°C), the mean development periods of *C. megacephala* had a linear relationship with temperature (R^2 range: 0.85–0.94).At 25.7°C, there was a significant difference between larval period on

liver tissues and both of rabbit carcass and muscle tissues but was not found between rabbit carcass and muscle tissues (p = 0.024). While there was no significant difference between pupal and total periods for all colonies (p >0.05At 28.4°C and 30.6°C there was no significant difference between Larval, pupal and total periods for all colonies (p > 0.05).

Table 7: P value from analysis of variance (ANOVA), two factor, of the mean time spent in the developmental stages (Larvae, Pupae and Total) between all tissues (rabbit carcass, liver and muscle)that *C. megacephala*reared on, under 3 temperature regimes.

Stage	At 25.7°C	At 28.4°C	At 30.6°C
	All tissues	All tissues	All tissues
Larvae	0.024	> 0.05	> 0.05
Pupae	> 0.05	> 0.05	> 0.05
Total	> 0.05	> 0.05	> 0.05

P > 0.05 = No significant difference
P ≤ 0.05 = Significant difference

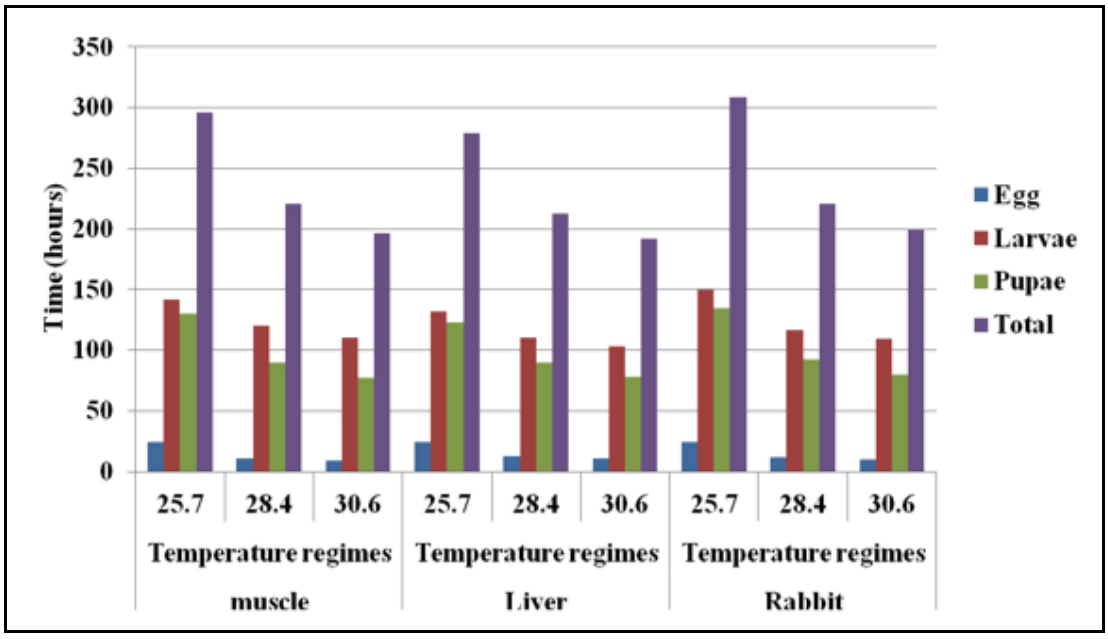

Fig. 1: Different mean durations of the developmental stages of *C. megacephala* with different temperature regimes (25.7, 28.4, 30.6 °C).

Based on these results, significant differences related to the life cycle of the oriental latrine fly (*C. megacephala)* that fed on different diets, (rabbit carcass, liver and muscle tissues), decreased gradually with rising temperature degrees. However, it was a significant variance at 25.7°Cat the larval period, it diminished at 28.4°C and 30.6°C. It means that the effect of tissue type at higher temperature degrees was not significant.

DISCUSSION

Temperature is probably the most influential environmental factor in the life history of populations, and controlling the insects' activity, oviposition rate and as

well as their overall development Marinho *et al*. (2006). Anderson (2000) who studied the development rate of some forensically important Calliphoridae (Diptera). The development rate was speeded up by higher temperatures, and the complete egg-adult developmental period between 1.4 to 2.3 times longer than the lower temperatures, Kamel *et al., (*2016) reported that the duration of each larval 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.

It is a well-recognized fact that insect development is temperature-dependent; that is, the normal metabolic rate is increased with increasing temperature, which results in a faster rate of development so that the duration of development decreases in a linear manner with increasing temperature within optimum ranges (Chapman, 1982).

Similar to Mohd *et al*. (2007), who reared *C. megacephala* larvae at different temperature degrees, the mean period from the larvae to reach adult hood at 27^{o}Cwas 8.5 days. Raising the temperature by 3 degrees reduced this period by 2days. A further increase of 3 degrees shortens the period by about another 2days to 5 days. The lowest temperature used was 27^{o}C, at which, development of the insect was the slowest compared to other temperatures. Smith, (1986), noted similar results.

The current results are comparable to Gosselin *et al.* (2011), who studied development times (days) of *Lucilia sericata*t that decreased with higher temperatures. In a temperature range (12.5-30°C), the median development times of *Lucilia sericatahave* a linear relationship with temperature (R^2 = 0.99).In addition, the current results are inconsistent with Boatright *et al*. (2010), who reared the secondary screwworm, *Cochliomyia macellaria* (Fabricius), on either equine gluteus muscle or porcine loin muscle at 20.8^{o}C, 24.3^{o}C, and 28.2^{o}C and found that *C. macellaria* needed35%more time to complete development when reared at 20.8 than 28.2^{o}C. Bambaradeniya *et al.*, (2019) reared the *Chrysomya megacephala* on four temperature regimes (20, 25, 27 and 38^{o}C) representing several specific development conditions of this blowfly and found that temperature significantly affected larval length and width over time. However, the fastest development was recorded at 38^{o}C for immature feeding on bovine muscles followed by those fed swine liver and swine muscles.

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ARABIC SUMMARY

تأثير أنظمة درجات الحرارة وأنواع الأنسجة على تطور يرقات Chrysomya megacephala (Diptera: calliphoridae)

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3-قسم علم الحشرات – كلية العلوم – جامعة عين شمس

في هذة الدراسة تم فحص ثلاثة أنظمة درجة حرارة تمثل العديد من شروط التطوير المحددة لهذا الذبابةكريزوما ميجاسيفالا وهم: (25.7 ، 28.4 و 30.6 درجة مئوية) وعلاوة على ذلك ، تربية ذبابة الجيفة باعتبارها ذبابة شرعية مهمة على ثلاثة مصادر غذائية مختلفة وهي كل جثة الأرنب والكبد والعضلات) وأثارهما المحتملة لحساب الفاصل الزمني بعد الوفاة وأظهرت النتائج عند تربيتها علي جثة الارنب من البيضة للطور البالغ عند درجة حرارة (25.7 ، 28.4 و 30.6 درجة مئوية)

كانت فترة النمو 303.5 و 217 و 194.5 ساعة على التوالي. بالإضافة إلى ذلك ، كان متوسط مدة الفترة من البيض إلى الطور البالغ عندما تربى اليرقات على أنسجة الكبد عند درجة ، 188.5، 205.5, 275.5,حرارة (25.7 ، 28.4 و 30.6 درجة مئوية) على التوالي. ، فإن اليرقات التي تم تربيتها و على أنسجة العضلات من جثث الأرانب ، وكان متوسط المدة من البيضة ل البالغين عند 25.7 درجة مئوية 28.4 درجة مئوية ، و 30.6 درجة مئوية ، 293 ، 214.5 و 192.5 ساعة ، على التوالي.

من النتائج السابقة ، يتضح أن متوسط المدة في اليرقات التي تم تربيتها على جثث الأرانب كانت أطول من تلك التي تغذت على العضلات و أطول من تلك التي تربى على الكبد في جميع

.