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Influence of Fluctuated Room Conditions on the Development of the Forensically Important *Chrysomya albiceps* (Wiedemann) (*Diptera: Calliphoridae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SSR and ASY designed the study and wrote the protocol. Author SSR managed analysis of the study and wrote the manuscript. Author ASY wrote part of the manuscript. Author EEZ managed the experimental process, collected all data and did the literature search. Author ZMEB performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Aims: To describe the influence of the seasonal variations on the development of blow fly *Ch. albiceps* (Wiedemann) stages as regards to the fluctuated temperature,
Study Design: Eggs and larvae of the *Ch. albiceps* were collected from rabbit carcasses, the development period for immature and adult stages under naturally circulating room conditions were observed.
Place and Duration of Study: Observations were carried out for a year (June, 2012 – May, 2013) at Zoology Dept., Zagazig University, Zagazig, Egypt.
Methodology: Groups of newly formed pupae were kept in rearing jars for adult emergence. The development time (in days) from emergence to egg laying and immature growth was determined.

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Results: The overall development time of the *Ch. albiceps* exhibited significant seasonal variations corresponding to temperature changes. High temperatures accelerate the overall development, whereas low temperatures slow it down. The average period between emerging of *Ch. albiceps* adults, mating, spawning, larval and pupal development at low temperatures in winter was sustained the record average of 25-51 days when reared at 11℃. This period, was decreased gradually to reach average of 19-27 days at 15℃ in autumn, 16-23 days at 18℃ in spring and 17-19 days, in summer months when temperature average was 23℃. Adult emergence of the *Ch. albiceps* fly was positively affected by temperature, it ranged from 96.12±0.24%, in summer (23℃), to 81.79±0.77 in winter (11℃). Generally female emergence was in high rates more than males and the higher female ratio was during winter (1.62). Also changes in temperatures due seasonal variations showed significant effect on the pre-oviposition and incubation periods, female fecundity, percentage of egg hatching and time of larval development.
Conclusion: Changes in temperature according to seasonal variation considered an important factor for the development time for the forensically important *Ch. albiceps*

Keywords: Chrysomya albiceps (Wiedemann); forensic entomology; development time; seasonal variation.

1. INTRODUCTION

Flies, especially Calliphoridae, are the first insects to arrive on a corpse after death, and, for forensic entomologists, they play a privileged role in defining the post mortem interval [1]. *Chrysomya albiceps* (Wiedemann 1819) is a common Calliphorid species found in southern Europe, Afrotropical, Oriental (from India to China) and Neotropical regions (Central and South America) [2]. *Ch. albiceps* is normally a carrion breeder and is frequently involved in primary and secondary cutaneous myiasis in mammals [1,2,3], the first stage larvae feed on exudations of the decomposing flesh, the second- stage and third-stage larvae are predaceous, feeding on other blowfly larvae [4]. This predatory behavior may possibly lead to decline in the population numbers of native species [2]. Therefore, *Ch. albiceps* demonstrate greater survival ability [5] and definitely contributes to successful dissemination in nature, as pointed out by [6,7]. The life history of *Ch. albiceps* has been summarized by many authors [2,8,9,10,11].

Ch. albiceps is one of the most forensically important insects found in Egypt. Its rapid and consistent time of arrival at a body following death makes it a potentially quite accurate tool for the calculation of post mortem interval. The estimation of post mortem interval using insect evidence depends largely upon the accurate estimation of life cycle duration. Although some workers believe that lab studies under room temperature cannot depict larval development accurately [12], it can be applicable to indoor forensic situations where environmental factors are fairly constant [13]. Therefore, the present study was conducted to study the developmental time of *Ch. albiceps* immature and adult stages for a year under naturally circulating room conditions.

2. MATERIALS AND METHODS

2.1 Stock Colony

The stock colony of *Ch. albiceps* eggs and larvae were collected from a rabbit carcasses weighed (1300-1500g), maintained in front of the laboratory 's window at Zoology Dept., Zagazig University, Zagazig, Egypt from June, 2012 – May, 2013. Larvae were reared in a plastic jar (10.5 cm diameter × 7 cm height) contained approximately 50 gm fresh beef under laboratory condition. About 50 larvae were kept in each jar to avoid the intraspecific competition that may stunt growth, and the effects of maggot-generated heat that may stimulate growth [11]. The rearing jars were thoroughly cleaned every day by washing them with soap and 70% alcohol. The newly formed pupae, each were kept in clean rearing jars containing dry autoclave sieved sawdust (3-4 cm depth) as a medium for pupating and covered with muslin secured tightly with a rubber band. The pupae were sieved from the sawdust and transferred to rearing cages (30×30×30 cm) and observed daily till adult emergence. Each cage was made of a wooden floor and roof and three sides of wire gauze. The front side was provided with a wooden part holding a circular hole fitted with a piece of cloth sleeve to facilitate inserting of the pupae, daily supply food, cleaning of the cage and removal of deposited eggs.

2.2 Development Time Determination

2.2.1 Adult stage

To determine the adult emergence and Sex ratio of the *Ch. albiceps* fly**,** three groups of 100 newly formed pupae were used and the number of emerged males and females were recorded. The pre-oviposition periods were determined by using five pairs of adults, each consisting of one newly emerged virgin female and one day-old virgin male. Each pair was kept into small cage (20×15×15 cm) and supplied with 10% sucrose solution, sucrose granules and fresh beef meat. The cages were observed twice daily for oviposition and the time at which eggs were laid were recorded and the experiment was repeated five times. The adult longevity was investigated by using four cages (30×30×30 cm) each contained 40 pairs of newly emerged adults, 20 males and 20 females. They supplied with 10% sugar solution and sucrose granules. Daily mortality of adults from each cage was recorded. The experiment was repeated four times. Five pairs of adults, each consisting of one virgin male and one virgin female were used to determine the female fecundity and each pair was kept in a small cage and supplied with 10% sugar solution, sucrose and fresh beef meat. The number of eggs laid by a single female was recorded. The experiment was repeated five times.

2.2.2 Egg stage

To calculate the incubation period and percentage of egg hatching, ten batches of eggs were obtained from 10 different gravid females. The number of eggs in each patch was recorded and then transferred into rearing jars provided with a small piece of fresh beef meat, then covered by muslin secured tightly with a rubber band. They were checked twice a day till hatching. Incubation period and egg hatchability were recorded. The experiment was repeated five times.

2.2.3 Larval stage

Duration of the whole larval stage under laboratory conditions was carried out by using ten groups of 50 newly hatched 1st instar larvae. Each group was kept in a rearing jar supplied with approximately 50g fresh beef meat. The jars were observed for pupation. The experiment was repeated three times.

2.2.4 Pupal stage

Five groups of 100 newly formed pupae, each were kept in clean rearing jars covered by muslin secured tightly with a rubber band. These jars were observed daily till adult emergence for determining pupal duration. Experiment was repeated three times.

2.3 Statistical Analysis

Obtained data sorted by season were analyzed by one-way Analysis of Variance (ANOVA) and multiple pair-wise comparison of the means were then compared by least significant difference (LSD) test to examine the seasonal variations in adult and immature developmental rates. The SPSS software (Version 11 for windows, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. RESULTS

During the present study, seasonal variations on some developmental parameters of *Ch. albiceps* were carried out under circulating room conditions. Data illustrated in Fig. (1) cleared that the percentage of adult emergence showed significantly seasonal variations, the highest percentage of emergence (96.12±0.24%) was observed at high temperatures (23±1℃) in summer season (P<.001), that decreased gradually with the decrease of temperature to reach the lowest percentage (81.79±0.77%) at low temperature (11±1℃) in winter season (P<.001). Sex ratio of the emerged flies was recorded as the percentage of females to males. It was (1.19: 1), (1.56: 1), (1.47: 1) and (1.62: 1) during spring, summer, autumn and winter, respectively as illustrated in Fig. (2). There was no significant difference among ratios of summer, autumn and winter, whereas there was a highly significant decrease during spring. Data illustrated in Fig. (3) showed that the mean pre-oviposition period of *Ch. albiceps* was also significantly varied among seasons. The shortest period (4.06±0.09 days) was observed at high temperature (23±1℃) in summer. This period increased in spring (5.82±0.09) and autumn (7.18±0.12) and reached the highest significant increase (13.28±0.13 days) at low (11±1℃) temperature in winter. The mean values of female fecundity showed the highest significant rates (287.68±3.64 egg/female, P<0.001) at high temperature in summer and the lowest significant rates (194.12±3.59 egg/female, P<0.001) at low temperature in winter as shown

in Fig. (4). The mean adult longevity of both male and female flies of *Ch. albiceps* showed great significant variations among seasons, Fig. (5). At high temperature in summer male and female longevity recorded 9.81±0.12 and 10.06±0.11 days, respectively. Gradual increase of fly longevity was recorded in a positive correlation with temperature to reach its maximum increase in winter as 23.73±0.23 and 24.05±0.22 days for male and female flies, respectively.

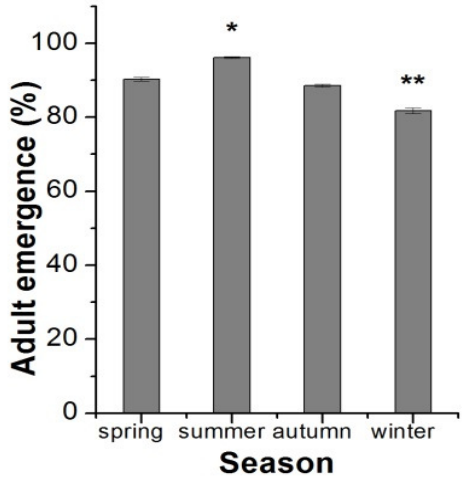

Fig. 1. Mean % of adult emergence of *Ch. albiceps* during the four seasons of the year

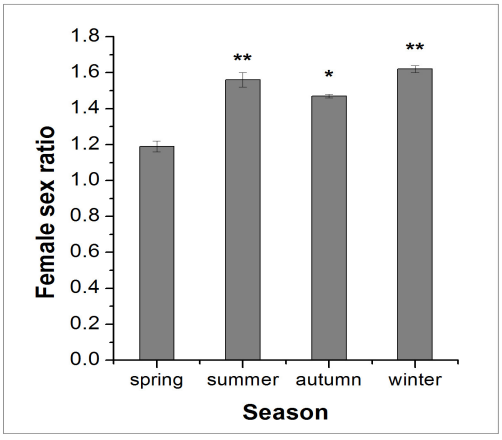

Fig. 2. Female sex ratio of *Ch. albiceps* during the four seasons of the year

Data illustrated in Figs. (6 & 7) showed results of the egg development as represented by the mean incubation period and percentage of egg hatching among seasons. Period of incubation was significantly higher in winter (2.64±0.03 days) compared with the insignificantly different in the other seasons (0.98±0.01, 0.90±0.01and 1.11±0.02 days, in spring, summer and autumn, respectively). Percentage of egg hatching was significantly high (93.84±0.93) at high temperatures in summer, significantly lower, 89.30±1.06 and 86.11±1.25 days, at lower temperatures in spring and autumn, respectively and significantly low (73.23±1.02) at low temperature in winter.

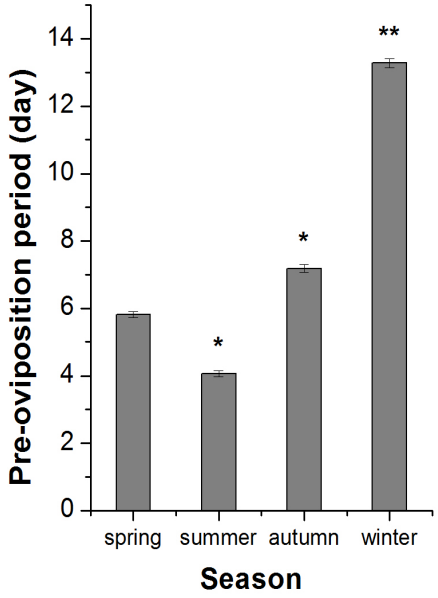

Fig. 3. Mean preoviposition period of *Ch. albiceps during the four season*

The mean larval and pupal duration was illustrated in Figs. (8 & 9). Values of larval duration in spring and summer were fairly close, 6.96±0.03 and 6.34±0.02 days, respectively, but showed significant increase in autumn and winter to reach 9.94±0.02, 15.68±0.08 days, respectively. The mean pupal duration of both males and females showed also significant difference among seasons. High temperatures in summer showed highly significant reduction in pupal duration for both males and females (6.86±0.02 and 7.23±0.01 days, respectively). Lower temperatures in spring and autumn showed significant increase for both sexes: for males (10.85±0.03 - 11.06±0.04), and for females (11.06±0.04 - 11.97±0.04), respectively. The considerably low temperatures in winter showed highly significant elongation of pupal duration, 18.69±0.11 and 19.27±0.12 for male and female, respectively.

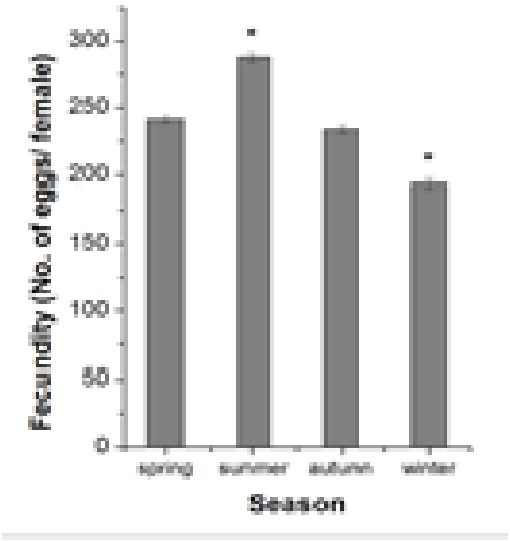

Fig. 4. Fecundity (No. of eggs/ female) of *Ch. albiceps* in days during the four seasons

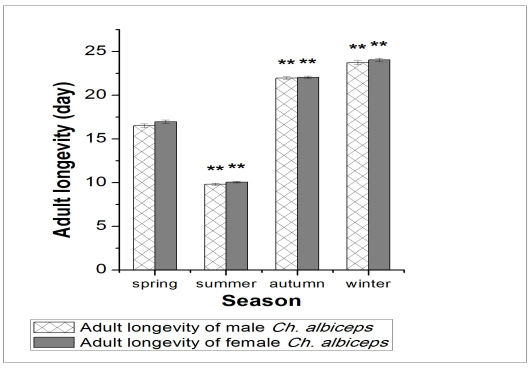

Fig. 5. Mean adult longevity of male and female *Ch. albiceps* during the four seasons

4. DISCUSSION

The species *Ch. albiceps* considered of great potential for forensic use due the great number of immature individuals developing on the carcasses and on the number of visiting adults, a fact that has been observed in this study and also on most studies conducted in urban areas [14,15,16,17,18,19]. Results of this study revealed that the overall development time of the forensically important blow fly *Ch. albiceps* exhibited seasonal variations corresponding to temperature changes. High temperatures accelerate the overall development, whereas low temperatures slow it down. The average period between emerging of *Ch. albiceps* adults, mating, oviposition, larval and pupal development at low temperatures in winter was sustained the record average of 25-51 days when reared at 11℃. This period, was decreased gradually to reach average of 19-27 days by the increase of temperature to 15℃ in autumn and average of 16-23 days at 18℃ in spring. The shortest developmental period was 14-17 days in summer months when temperature average was 23±1℃. The short period during high temperatures, was recorded by [9] (8-12 days when reared at 28℃) and [2,10,11] who recorded 9.5 - 10.5 days when *Ch. albiceps* reared at 30℃. The duration of the life cycle described in this study from egg to adult stage was more or less shorter than data provided by [20] (26 days under natural environmental conditions, 28±2℃), [21] (23–28 days under field conditions), and [22] (32 days at 16℃ and 20 days at 21℃), and somehow similar to the information by [23] (12–15 days at 22℃), [22] (14 days at 27℃) and [24] (14 days at 22±1℃). This disparity between our results and others can be mainly explained by two factors; room temperature at which the fly was reared and the characterizations of the local populations that had been studied. It is proved that the development larvae as well as its post-feeding dispersal [25,26] are temperature dependent and variation has been observed in development time for geographically distinct populations [27].

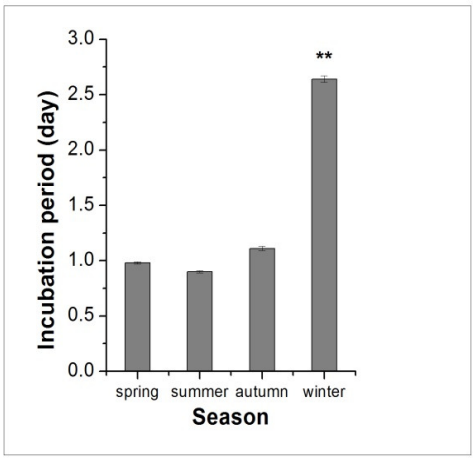

Fig. 6. Mean incubation period of *Ch. albiceps* during in days the four seasons

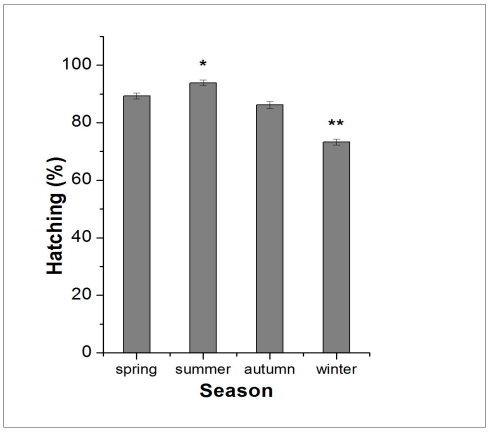

Fig. 7. Mean percentage of egg hatching of *Ch. albiceps* during the four seasons

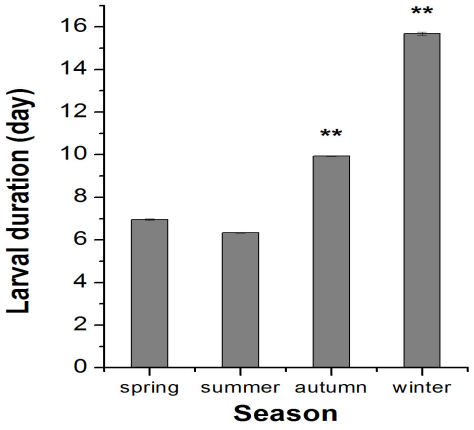

Fig. 8. Mean larval duration of *Ch. albiceps* in days during the four seasons

Percentage of adult emergence of the *Ch. albiceps* fly was positively affected by variations of seasonal temperature, it ranged from 96.12±0.24% in summer 23℃, to 81.79±0.77% in winter at 11℃. This percentage was somewhat higher than reported by others [28] that found the percentage of adult emergence of *Ch. albiceps* is 84.44% at 30 C°. On the contrary there was an inverse relationship between temperature and the pre-oviposition period. The highest values were recorded during winter (13.28 days) at the lowest temperature and the lowest values were recorded at the highest temperature during summer (4.06 days). Our data assured by other data [29] that found the preoviposition period of *Ch. albiceps* took about 4.25 days at high temperatures.

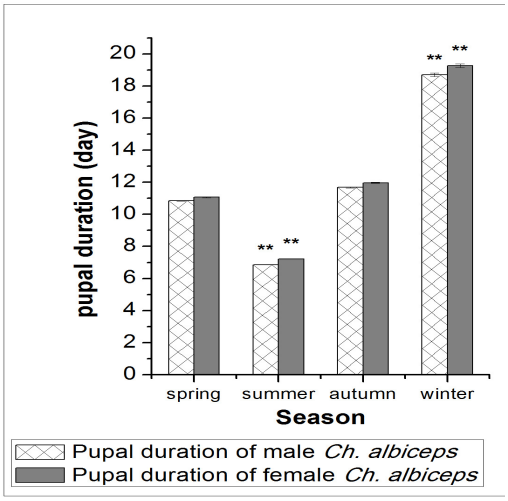

Fig. 9. Mean pupal duration of male and female *Ch. albiceps* in days during the four seasons

Significant effect of temperature on female fecundity, 287.68±3.64 egg/ female at 23±1℃ while 194.12±3.59 egg/female at 11±1℃ was in agreement with other authors, for *L. sericata* [30,31,32] and for *L. cuprina* [33] and some other dipterans that display discrete ovarian cycles [34]. Since female *L. cuprina* showed a limited tendency to mate more than once, the first mating is the essential one for inseminating the eggs all-over the life span [35]. Results showed that temperature affect Incubation period and egg hatchability (Figs. 6 & 7). Incubation period ranged from 0.90 to 2.64 days and egg hatching ranged from 73 to 93% according to temperature. These result assured by other reports that found the egg hatching of calliphorid flies was temperature dependent and ranged in 24-36 hours [1[and the incubation period (16 h.) at 27℃ [36]. Our observations on egg hatchability of *Ch. albiceps* were somewhat, close to that reported by [37] that reached 87.2±3.5% at 30℃. On the other hand, the present value was higher in comparison with those observed by [38] who found that at 25±1℃ and the percentage of egg hatching of *Ch. albiceps* was only 70%.

The minimum larval and pupal duration of *Ch. albiceps* flies recorded at high temperatures was in agreement with other reports. The larval and pupal viability were found to be higher at 27 and 32℃ than at 22℃, larval and prepupal survival rates were low at 18℃, mean duration and viability of the pupal stage at 22, 27 and 32℃ were 9.36, 4.7 and 3.0 days and 93.8, 100 and

100%, respectively [37]. These results and ours supported data reported in Brazil [39] stating that *Ch. albiceps* populations significantly increase during warmer periods of the year.

There was no significant difference in the development time of pupal stage and adult longevity between both sexes when reared at different temperatures. This result was on the contrary to other reports that found adult longevity of females (20.56 days) is significantly (*P*= .05) longer than that of males (12.76 days) of *L. cuprina* [33] and that of male and female of *Ch. albiceps* was 12.06 and 18.07 days, respectively [29]. However, female *L. sericata* gave a field estimate of 46.2 – 53.3 days for the complete life span [40] and all emerged *Ch. megacephala* adults survived 20 days and the mean longevity of male and female was 25.3 and 25.8 days, respectively [41].

5. CONCLUSION

Changes in temperature according to seasonal variations considered an important factor for the developmental times for the forensically important *Ch. albiceps*. High temperature accelerates the growth and development, whereas low temperatures slow it down. The conclusion of this study has major implications in forensic science. First, by using this data for larva rearing in forensic entomology cases to determine the post mortem interval (time since death). Second, slight variation in temperature will influence growth and indirectly influence estimation of time of death. Thus to ensure a more accurate estimation of time of death, history of surrounding temperature in the location where a body was found must be taken into consideration.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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