

ORIGINAL ARTICLE

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Temperature requirements for the growth of immature stages of blowflies species, *Chrysomya albiceps* and *Calliphora vicina*, (Diptera:Calliphoridae) under laboratory conditions

Mojtaba Salimi¹, Yavar Rassi^{1*}, Mohamadali Oshaghi¹, Omid Chatrabgoun², Mojtaba Limoei³ and Sayena Rafizadeh⁴

Abstract

Background: In forensic entomology, a way to estimate Post Mortem Interval (PMI) is to use development data of the first colonizing insects on a dead body. *Chrysomya albiceps* and *Calliphora vicina*, two members of the family Calliphoridae, are the first waves of necrophagous insects that are the most entomologically important fly species because of its consistent time of arrival and colonization of the body following death. Regarding the bioclimatic, environmental conditions and difference in geographic population, it seems necessary to collect data about the forensically important species in each region. In this research, the growth rate of immature stages of *C. albiceps* and *C. vicina* was studied in controlled laboratory conditions.

Results: The results of this study showed that with increasing temperature, the duration of development was reduced. The highest period of development, for both species, is related to pupae stage and involves about half of the entire development period. The amount of Accumulated Degree Days (ADD) for the egg stage to mature of the species *C. vicina*, at two temperatures of 15°C and 20°C, are calculated as 416 and 396 DD, respectively.

Conclusion: This study showed, temperature had significant effects on rate of the development on *Chrysomya albiceps* and *Calliphora vicina* and the amount of heat energy needed to development for these species is specific. Actually, the relationship between the development rate and temperature was significant and dependent to species and geographical regions.

Keywords: Forensic, Calliphoridae, Larva, Temperature, Iran

1. Background

When a dead body is discovered, the main question is to find the time spent on death. This question can be answered in two ways using entomology data: first, using the succession data of insect's taxa that have time and food preferences and are changed based on the stage corpse putrefaction; and second, using the development data of the oldest and largest maggots of the first

colonizing species. Succession data are mostly used for the cases in which a long time has been spent on death while development data are mostly used for the cases in which a short time has been spent on death. Studies have shown that insect development is directly related to environmental data such as temperature and humidity. Degree day model using development data of necrophagous insect on the carcass particularly first colonizers and environmental data such as temperature can be estimate the time of death (Kumara et al., 2017; Bansode et al., 2016; Chua, 2013). As a microhabitat, the corpse is used differently by the insects that colonize the

* Correspondence: rassiy@tums.ac.ir

¹Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences(TUMS), Tehran, Iran
Full list of author information is available at the end of the article

body. Some insects use body as food source and reproduction, some others as a place for prey and parasitization of larvae and others as a habitat. In terms of forensic entomology, all these arthropods provide some information and evidence on the crime scene. The most important insects in the forensic entomology that are attracted to the corpse immediately after death are two families of flies known as Calliphoridae and Sarcophagidae (Al-Mesbah et al., 2011; Al-Shareef & Al-Qurashi, 2016). The number of different species of this family is variable based on the geoclimatic and environmental conditions of the region. In each region, the number of one species of this family may be more than the other ones based on environmental conditions. Thus, PMI is calculated based on the development stages of dominant species (Al-Shareef & Al-Qurashi, 2016; Smith, 1986). *Chrysomya albiceps* is mostly found in temperate and subtropical regions during the hot months of the year and temperature plays a key role in determining the variation of its dispersion (Fig. 1) (Al-Shareef & Al-Qurashi, 2016; Smith, 1986; Tabor et al., 2004; Grassberger et al., 2003). This species is reported to be seen in afro-tropical, oriental, south central America and southern Europe and the areas located at altitudes of 1300–3100 m above sea level (Queiroz, 1996; Szpila et al., 2008). In most of these researches, this species is known as *primary species* and is considered as the first colonizer that is responsible for using dead bodies (Al-Mesbah et al., 2011; Bharti & Singh, 2003). Facultative predation, Competition and cannibalism are different nutrition strategies that are adopted in the second and third larvae stages of this species facing various conditions (Szpila et al., 2008; Faria & Godoy, 2001). *Calliphora vicina* is a member of the family Calliphoridae which is considered as a eusynanthropic species with endophilic tendencies and it is mostly seen during the cold months (Fig. 1) (Faucherre et al., 1999). Temperature is one of the critical factors that is effective in laying activities and development of larvae of this species. It can be active within a range of 10–12°C to 28–30°C (Faucherre et al., 1999; Erzinclioglu, 1996; Williams, 1984). In different studies, this species is considered as the first

and only colonizer on the corpse, especially during the cold months of the year (Bharti, 2009).

Generally, there is a bilateral relationship between temperature and development of insects. The insect growth is a linear function of temperature variation (Defilippo et al., 2013). The rate of development and growth of insect larvae can be obtained using different methods, the most common of which is to calculate ADD/ADH (Grassberger & Reiter, 2001; Marchenko, 1989; Anderson, 2000; Oshaghi et al., 2009). The difference in geographic populations of insects makes them tolerate a range of temperature, exclusive to each species, during different stages of growth. Therefore, ADD/ADH is different for the insect population of each geographic region (Davies & Ratcliffe, 1994; Donovan et al., 2006). The goal of this research is studying the growth parameters of pre-mature stages of forensic dominant species, i.e. *C.albiceps* and *C.vicina*, under constant laboratory temperature conditions and calculating ADD for them as well as comparing the development data of these species with populations living in other geographical areas.

2. Materials and methods

Sampling location and period

This research was done under both field and laboratory conditions. The field of research was conducted in the Bisetoon protected area, west of Iran, 13 kilometers far of the city of Kermanshah, 34° 24' 37" N and 47° 14' 42" E, comprising a land area of 54,950 km² and elevation about 1320 m above sea level. The average precipitation here is 400–450 mm per year and the average maximum and minimum temperatures are 30°–35°C and -2°C, respectively. The flora of this region is mostly meadow and it has four distinct seasons in terms of temperature conditions.

Rearing in field conditions

In this research, inbreeding rabbits, about 2.5 kg in weight, were used. The rabbits were first killed by chloroform and then, weighed and immediately transferred to the field. The carcasses were put in cages (150 × 90 × 90) with 1.25 cm meshes with their floors

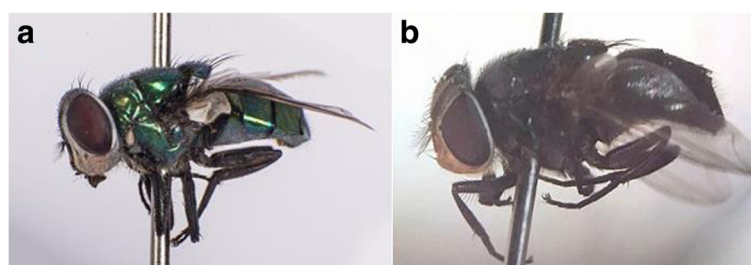


Fig. 1 Pictures of *Chrysomya albiceps* (a) and *Calliphora vicina* (b)

being directly in contact with the ground. The sampling was done from mature flies using insect net through sweeping from the corpse in immature stages with forceps. The corpse had degeneration in four stages, i.e. decay, bloated, fresh and dry in the field and the duration for each stage depends on the season during which it happens. Eggs and larvae clusters of the stage one are collected from the corpse using forceps and put in transparent plastic containers (15×10×10) cm that have lace fabric lids for air conditioning. Each container is supplied with a 3 cm layer of sawdust, a piece of rabbit meat covered by an aluminum sheet and daily humidity. The goal was to detect the mature species as well as to compare the adults out of pupa with the sampled ones in terms of number. The adult samples were transferred to cages (50×50×50) cm with fabric laces containing rabbit meat substrate with humidity and a few hours later, the egg and larvae samples were put into other breeding containers and then transferred immediately to the laboratory of Kermanshah School of Public Health. In this study, larval and adult of blowflies were identified based on descriptive and pictorial keys (Szpilla, 2010a; Szpilla, 2010b).

Rearing in laboratory conditions

The identified samples were bred and evolved under constant-temperature conditions of the laboratory. In order to avoid making mistake during all breeding stages, both in the field and laboratory, rabbit meat substrate was used to feed the maggots. In the laboratory, the samples were put into incubator with constant temperature, humidity and photoperiod similar to the field conditions. Then, the growth stages of the samples were investigated regularly.

Development zero (Dz) temperature

The development of different growth stages of insects proceeds between two ranges of lower and upper development threshold temperature outside of which the growth stops. Development zero temperature or lower development threshold temperature is different based on the species, different stages of development of each species and geographic conditions. By reviewing previous studies, i.e. Marchenko, (Marchenko, 1989) Queiroz, (Queiroz, 1996) Grassberger and Reiter, (Grassberger & Reiter, 2001) Richard et al., (Richards et al., 2008) and Al-Shareef and Al-Qurashi, (Al-Shareef & Al-Qurashi, 2016) conducted in different geographic populations, some numbers were calculated for the Dz temperature of the species *C.albiceps* and regarding all of which an average temperature was determined for each stage as follows: egg stage to first larvae (9.72 °C); larvae stage one to second stage (11. 14°C); second stage to third stage (13°C); larvae stage three to pupal (14.48 °C) and

for pupal stage to adult (14. 32°C). Overall, 10. 11°C and 15. 38°C were determined for the egg to adult and first larval to adult, respectively. Referring the studies done before, different temperatures were also suggested for the Dz temperature of the species *C.vicina*: Kamal (Kamal, 1958) (Dz= 1°C); Vingradova (Vinogradova & Reznik, 2015) and Marchenko (Marchenko, 1989) (Dz= 2°C); Davies and Ratcliffe (Davies & Ratcliffe, 1994) (Dz= 3. 5°C); Haskell (Haskell et al., 2000) (Dz= 6°C); Ames and Tuner (Ames & Turner, 2003) (Dz= 1°C), Donovan et al., (Donovan et al., 2006) based on linear approximation estimation method (Dz= 1°C) and Defilippo et al. (Defilippo et al., 2013) (Dz= 1. 6°C) were considered. In this study, we considered 2°C for all the growth stages of the species *C.vicina*. ADD was calculated in the laboratory based on the formula suggested by Wilson and Barnett (Gennard, 2007):

$$ADD = \text{Days } (T_{\text{constant}} - T_{\text{basement}}).$$

3. Results

Rearing in field conditions

The results obtained from breeding eggs and larvae clusters that were collected from the rabbit body during spring and summer with average temperatures 17. 5°C and 27°C, respectively, showed that the *C.albiceps* was dominant compared with other Calliphoridae species. In autumn, the results obtained from breeding eggs and larvae clusters showed that the abundance of *C.vicina* was 70% more than other species. In the winter, the results confirmed that *C.vicina* was only active species.

Development of *C.vicina* and *C.albiceps* immature stages in laboratory

The development period for the species *C.albiceps* was measured in incubator in two constant temperatures of 25°C and 30°C, and 40% humidity. This period for the species *C.vicina* was measured in two constant temperatures of 15°C and 20°C, and 70% humidity. In the case of *C.albiceps*, as the temperatures increases from 25°C to 30°C, the brooding time as well as the time spent for the next development stages reduce to half. Furthermore, it was found the same pattern for the first larval instar to second instar as well as the second to third instar. The pupa duration at the temperatures of 30°C and 25°C were 3 and 5 days, respectively. The total development from egg to adult for *C.albiceps* at 25°C and 30°C were 11 and 8. 5 days, respectively (Table 1). For the species *C.vicina*, the difference in the growth time of first and second larval instar, at temperature 20°C, was half the time when the temperature was 15°C. The development of third larval instar include feeding and post feeding stages, took 8 and 5 days at the temperatures 15°C and 20°C, respectively. The maximum difference is associated with the pupal period since it took 18 and 13 days

Table 1 Development duration and thermal requirements (ADD) of *Chrysomya albiceps* (second generation) at constant temperatures (RH = 50%, Photoperiod = 14: 10)

Life stages	Development duration (Day+/-SD)		ADD (°C)		Dz(°C)
	25 °C	30 °C	25 °C	30 °C	
Egg hatching	1+/- 0.0	0.5-1+/- 0.0	15.28	10.1-20.3	9.72
1st larval instar (L1)	1+/- 0.0	1+/- 0.0	13.86	18.86	11.14
2nd larval instar (L2)	2+/- 0.0	1+/- 0.0	24	17	13
3rd larval instar (L3)	3+/- 0.0	2+/- 0.0	31.56	31.04	14.48
Pupal stage	5+/- 0.0	3.5+/- 0.0	53.41	54.9	14.317
First larval instar to adult eclosion	11	7.5	105.82	109.65	15.38
Egg to Adult eclosion	12	8-8.5	178.68	159.12	10.11

n= 2

at temperatures 15°C and 20°C, respectively. Overall, the whole period of development from first larval instar to adult for *C.vicina* at temperatures 15°C and 20°C lasted 32 and 21 days, respectively (Table 2).

Temperature requirement in laboratory condition

The results showed that the development period was in inverse proportion to temperature. In other words, as the temperature increases to upper threshold, growth is accelerated and the length of the development period is reduced. During the growth process, the base threshold temperature for any stage was increasing in such a way that the minimum threshold of growth temperatures for the egg to first larval instar, first to second instar, second to third instar, third larval to pupal and pupal to adult were considered as 9.72 °C, 11.14°C, 13°C, 14.48 °C and 14.317 °C, respectively. In *C.albiceps*, the length of the development of each stage relative to the total development period at the temperatures 25°C and 30°C were as follows: egg to first larval (9%, 6.25%), first to second larvae (9%, 12.5%), second to third larvae (18%, 12.5%), third larva to pupa (27%, 25%), pupal to adult stage (45.5%, 44%) and first larvae to adult was (90%, 94%). For *C.vicina*, the greatest contribution of the development period, in temperatures 15 and 20°C, is assigned to the pupal to adult period that accounted for 62 and 56 percentage of the total development period, respectively. The

development time of third instar larvae to pupa stage were calculated 24 and 25 percentage of the total pre-mature time at 15°C and 20 °C respectively. Also, the development time from egg to the end of third instar larvae were observed about 14.24 and 18.57 percentage of the total time at 15°C and 20°C respectively. Similar to *C.albiceps*, the greatest contribution of the development period of *C.vicina* is assigned to the pupal period. It was quite evident that temperature had a key role, especially in low temperatures, in making significant differences in temperature requirement of growth of ADD in *C.vicina* at 15°C and 20°C. For *C.albiceps*, the majority of required ADD was related to the immature period, and the same applied to *C.vicina*. As the larvae development, the amount of ADD increased too such a way that, at temperatures 15°C and 25°C, the amount of ADD for the third larvae were determined as 104°C and 90°C respectively, and for the pupal stage, it was similar and equal to 234°C (Table 2).

4. Discussion

In this study, as other researches (Al-Shareef & Al-Qurashi, 2016; Grassberger et al., 2003; Queiroz, 1996; Grassberger & Reiter, 2001; Augul, 2009) we found that as the temperature increases, the length of the development is shortened. In *C.albiceps*, the length of larvae stage (first larval to Pupal instar) in 25°C and 30°C were 6 and 4 days,

Table 2 Development duration and thermal requirements (ADD) of *Calliphora vicina* (second generation) at constant temperatures (RH = 70%, Photoperiod= 10: 14)

Life stages	Development duration (Day+/-SD)		ADD (°C)		Dz(°C)
	15 °C	20 °C	15 °C	20 °C	
Egg hatching	2+/- 0.0	1+/- 0.0	26	18	2
1st larval instar (L1)	2+/- 0.0	1+/- 0.0	26	18	2
2nd larval instar (L2)	2+/- 0.0	1+/- 0.0	26	18	2
3rd larval instar (L3)	8+/- 0.0	5+/- 0.0	104	90	2
Pupal stage	18+/- 0.0	13+/- 0.0	234	234	2
Egg to adult eclosion	32	21	416	396	2

n= 2

respectively. Different researchers determined different values for the length of this stage: Velez and Wolff (Velez & Wolff, 2008) (8–10 days at 25°C); Grassberger (Grassberger et al., 2003) (8–10 days); Al-Shareef and Al-Qurashi (Al-Shareef & Al-Qurashi, 2016) (6 days); Queiroz (Queiroz, 1996) (5 days at 27°C); Augul and Jassim (Augul, 2009) (3 days at 30°C); AL-Shareef and Al-Qurashi (Al-Shareef & Al-Qurashi, 2016) (5 days); Richard et al. (Richards et al., 2008) (5.5 days); Shiravi et al. (Shiravi et al., 2011) (2–3 days at 28°C). In our research, the length of the pupal period for *C.albiceps* at 25°C and 30°C were determined as 5 and 3.5 days, respectively. Again, other researchers determined different numbers: Grassberger (Grassberger et al., 2003). 1 and 5.9 days at 25°C and 30°C, respectively; Al-Shareef and Al-Qurashi, (Al-Shareef & Al-Qurashi, 2016) 5.5 and 4 days at 25°C and 30°C, respectively; Queiroz, (Queiroz, 1996) 4.7 and 3 days at 27°C and 32°C respectively and Shiravi (Shiravi et al., 2011) (4–6 days at 28°C). In addition, in our study, the length of egg to adult eclosion, at 25°C and 30°C were determined as 12 and 8–8.5 days, respectively. Other researchers got the followings: Grassberger (Grassberger et al., 2003) 13 and 9.9 days at 25°C and 30°C; Velez and Wolff (Augul, 2009) (14 days at 25°C); Al-Shareef and Al-Qurashi, (Al-Shareef & Al-Qurashi, 2016) 11.5 and 9 days at 25°C and 30°C respectively; Shiravi et al. (Shiravi et al., 2011) (8–12 days at 28°C); and Queiroz (Queiroz, 1996) (7 days at 32°C). The figures calculated for the life cycle of the species *C.albiceps* were more similar to the ones obtained in Al-shareef (Al-Shareef & Al-Qurashi, 2016) and Shiravi (Shiravi et al., 2011) studies and were different from others. The reason seems to be natural. As noted in various studies, inherent biogeographically variation between population (Marchenko, 1989; Donovan et al., 2006; Szpilla, 2010a; Greenberg & Kunich, 2002) food substrate (Greenberg & Kunich, 2002; Kounouz & Kamel, 2017) humidity, population density and other intrinsic factors are considered as the reason of such differences (Faria & Godoy, 2001; Rosa et al., 2006). It seems that histolysis and histogenesis of the adult organs in immature stage were the reason of the highest thermal requirement of ADD in both 25°C and 30°C, during the pupal period, and the length of this development stage has been mentioned in most researches (Al-Shareef & Al-Qurashi, 2016; Queiroz, 1996; Bharti, 2009; Defilippo et al., 2013; Donovan et al., 2006; Richards et al., 2008; Augul, 2009; Velez & Wolff, 2008). Our findings about the life cycle of *C.vicina* showed that this species will also have shorter life cycle as the temperature increases such a way that at 15°C, its life cycle, from the egg stage to adult, it took 32 days while at 20°C, it took 21 days. For this species, other researchers obtained similar results: Reibe et al. (Reibe et al., 2009) at 13°C and 20°C, 45 and 23 days, respectively; Defilippo et al. (Defilippo et al., 2013) at 15°C and 20°C, 30.5 and 20

days, respectively; Bharti (Bharti, 2009) at 20°C and 25°C, 19 and 15.29 days. The results obtained from our study were more near to the ones obtained from the study done by Defilippo et al. (Defilippo et al., 2013). In terms of rate of development in *C.vicina* and *C.albiceps*, the results of this study were compatible with the finding of the studies of Greenberg, (Greenberg, 1991) (Lowne 1890), Defillipo et al (Defilippo et al., 2013) Such a way that the length of post-feeding and pupal periods were determined one third and half the total immature development stage, respectively. The amount of ADD was calculated for *C.albiceps* at 25°C and 30°C respectively, as 179 and 159 which correspond to the results obtained from the study done by Shiravi et al. (Shiravi et al., 2011) the amount of ADD, at 28°C, was calculated between 130 and 195 DD. Moreover, the amount of ADD for the first larvae instar to adult of this species at 25°C and 30°C was calculated as 96°C and 110°C respectively. In the study done by AL-Shareef, (Al-Shareef & Al-Qurashi, 2016) the length of this period, at the same temperature, has been determined as 110 and 129. The life cycle of these species depends on some other factors, such as the geographic differences of population and genetics. Actually, the rate of development in necrophagous insects is the response of organisms to their thermal environment. Determining the rate of development of forensically important insects such as *C.albiceps* and *C.vicina* can give us valuable information concerning the circumstance of the death including the postmortem interval.

5. Conclusion

This study emphasizes the importance of determining of developmental data for these species and other first colonizers on the carcass in each geographical region. The results of this study will be usable to estimate of the PMI in the western of Iran and similar regions.

Acknowledgments

This work was supported by Tehran University of Medical Sciences, Tehran, Iran project No.34233.

Funding

This work was supported by Tehran University of Medical Sciences, Tehran, Iran project No.34233.

Availability of data and materials

They are available.

Authors' contributions

MS Sampling of insects on carcass and samples breeding. YR Managing of Project and writing of Project and Manuscript. MO Help in writing the project. OC Assistance in data analysis. ML Assistance in field and Lab. SR Assistance in Lab and editing of paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Necessary ethical approval was obtained from School of Public Health, Tehran University of Medical Sciences Ethics Committee, code No.IR.TUMS.SPH.REC.1395.1634.

Consent for publication

All authors have complete consent to print article information.

Competing interests

The authors declare that they have no competing interests.

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Author details

¹Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences(TUMS), Tehran, Iran.

²Department of Mathematical, Statistics and Probability, Malayer University, Malayer, Iran. ³Departments of Public Health and Health Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran. ⁴National Institute for Medical Research Development(NIMAD), Tehran, Iran.

Received: 7 October 2017 Accepted: 14 March 2018

Published online: 20 March 2018

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