Ain Shams Journal of Forensic Medicine and Clinical Toxicology
Jan 2017, 28: 133-143

Role of House Fly in Determination of Post-Mortem Interval: An Experimental Study in Albino Rats

Ahmed K Elden Elfeky[1], Ismail M. Moharm and Bahaa El Deen W. El Aswad[2]

[1] Department of Forensic Medicine and Clinical Toxicology.
[2] Department of Medical Parasitology.

Faculty of Medicine, Menoufia University, Menoufia, Egypt.

Abstract

The potential for contributions of entomology to legal investigations has been known for at least 700 years, but only within the last two decades or so has entomology been defined as a discrete field of forensic science .There are many ways that insects can be used to help in solving a crime, but the primary purpose of forensic entomology is estimating time passed since death. Because blow flies arrive earlier in the decomposition process, they provide the most accurate estimation of time since death. House flies (Musca domestica Linnaeus) are medically and forensically important flies. **The aim** of this study was to investigate time passed since death according to different stages of house fly life cycle with time variations over months of the year. **Materials and methods**: 120 mature male albino rats were used, 2 rats were scarified every 6 days and left exposed to houseflies, the different stages of the fly life cycle in relation to different postmortem intervals and represented time were recorded, photographed and statistically analyzed.

Results: There is a highly significant statistical difference between the duration of the presence of the different stages housefly life cycle (eggs, 1st, 2nd, 3rd larval instars light colored (1^{st} stage) and dark colored pupae(2^{nd} stage) through different months of the year which was shorten in hot months of the year (August (9.6±1.57 h), July (1.5±0.52d), July (3.3±0.4d), August (7.6±0.69d), August (10.4±0.69d) and August (12.3±1.15d) respectively) and much prolonged in cold months of the year (January (27.2±2.85h) , January (3.6±0.51d), January (8.2±1.61d), Feb (14.1±1.52d), January (16.0±1.49d) , and January (21.2±1.22d) respectively).

The results showed that time after death could be estimated from certain forms of housefly stage and every stage denote the time with consideration to seasonal temperature state. There is statistically difference between the duration of the presence (p.m. interval) of the different stages of housefly life cycle through different months of the year which was shorten in hot months and much prolonged in cold months.**Conclusion:** postmortem interval could be determined through the presented forms of the housefly developmental stage in and around the dead body with consideration of presented date of observation.

Introduction

Forensic entomology can be defined as the use of insects, and their arthropod relatives, that inhabit decomposing remains to aid legal investigations (Byrd 2010). It is considered the most accurate method in determining the time elapsed since death, particularly when more than 3days have elapsed (Kashyab and Pillai 1989).The house fly is the most common of all domestic flies, accounting for about 91% of all flies in human habitations, and indeed one of the most widely distributed insects, found all over the world from the hot to cold regions near humans or their activities (Wiegmann et al., 2003).

As soon as death occurs, autolysis starts to occur with lysis of cells by digestive enzymes. The body starts decomposing. Bacteria present in the gastrointestinal tract start destroying and fermentation the soft tissue producing liquids and gases like hydrogen sulphide, carbon dioxide, ammonia, methane, sulfur dioxide and hydrogen. The volatile molecules escaping from the decomposing body attract insects (LeBlanc and Logan 2010). Also putrative sulfur-based compounds were found to be responsible for the initial attraction of the flies to the decomposing carcass (Ashworth and Wall 1994) but ammonium-rich compounds present on the carrion induce the flies to put their eggs or larvae (Joseph et al., 2011).

The adult house fly length range from 6 to 9 mm (Mandal 2015), with the female usually larger than the male. Adults usually live 15 to 25 days, but may live up to two months. Female flies need access to suitable food (protein) to allow them to produce eggs. (Brown et al., 2009). The female housefly usually mates once and stores the sperm for later use, lays batches of about 100 eggs on decayed, fermenting or rotting organic material. (Fasulo 2002)

Egg, larva, and pupa are the immature stages of house fly which can be used as entomological evidence for post-mortem interval estimation (Anderson et al., 2004)

Aim of the study

The aim of this study was to investigate time passed since death in experimental albino rats according to different stages of house fly life and correlate this with different with time variations over months along one year from the first of January 2016 to the end of December 2016.

Materials and methods

120 mature albino rat, 2 rats were scarified every 6 days (10 rat per month) by cervical dislocation under inhalation anesthesia and left exposed to houseflies, each dead rat was placed on a layer of soil in a small (30x20x 20 cm) box with wooden floor covered by clear glass plates and the sides were made of perforated nets with pores not allow passage of flies through it. The box was left uncovered until sufficient number of house flies attracted on dead rat then covered. Any other types of flies were discharged.

After appearance of eggs, adult flies were removed from the box to facilitate observation of the different stages of fly life cycle.

The different stages of house fly life cycle were closely observed at different days of different months through the study period and were recorded, photographed and statistically analyzed.

Every larva breeding experiment was photographed every day, starting with their occurrence as the first instar larvae until pupation. Samples of different instars larvae were collected and placed in boiling water to stop their development and allow their measurement. In this way we documented morphological changes during their development. Key developmental stages of each larva with the accurate date and time could be distinguished based on those photographs and by measuring their length.

Statistical Analysis

Data were tabulated and statistically analyzed using Statistical Package for the Social Sciences (SPSS) for windows version 16.0. Data were expressed as mean and standard deviation and Anova test was used, P-value of 0.05 or less is considered significant

Results

Eggs

The most common sites where eggs were deposited were among natural orifices like eyes and nostrils, anus, or any small wounds in rat. The time of hatching is vital and so eggs were monitored every few hours. Eggs were white in color about 1.2 mm in length, was laid and piled in small groups (fig7).

There is a statistical significant relation between durations of the presence of housefly eggs over the year; the longest period was in January (27.2±2.85h) followed by February (25.0±3.55 h) and the shortest period was in August (9.6±1.57 h) (Table1, and fig. 1).

Larvae

The first larvae emerged from the eggs after about early instar larvae are 2 to 5 millimeter (mm) legless, typical creamy whitish in color, cylindrical but tapering toward the head. (fig8), second larvae 5 -10mm long (fig9) and third instar larvae are more than 10mm (fig10).

The present study revealed that statistical significanct difference between durations of the presence of 1stinstar larva in days through different months of the year; the longest period was in January (3.6±0.51d) followed by (3.5±0.52d) in February and the shortest period was in July (1.5±0.52d) (Table 2 and Figure 2).

There was a statistical significant difference between the durations of the presence of the 2nd instar larvae through different months of the year; the longest period was in January (8.2±1.61d) followed by February (8.1±0.87d) and the shortest period was in July (3.3±0.4d) (Table 3 and Figure 3).

There was a statistical significant difference between the durations of the presence of the 3rd instar larvae through different months of the year; the longest period was in February (14.1±1.52d) followed by January (13.9±0.73d) and the shortest period was in August (7.6±0.69d). (Table 4 and Figure 4).

Pupae

Pupae were formed when the last larval skin form a capsule-like case, which varies in color from yellow, red , brown, to black as the pupa ages, complete their development till emerging flies escapes from the pupal cases through a hole on the front of its head (fig11).

Our results revealed that there was a statistical significant difference between the durations of the presence of the light colored stage pupae (from yellow, red to light brown) over the year; the longest period was in January (16.0±1.49d) followed by February (15.7±0.94d) in and the shortest period was in August (10.4±0.69d) (Table 5 and Figure 5).

There is a statistical difference between the durations of development of the dark colored pupae (dark brown and black) over the year; the longest period was in January (21.2±1.22d) followed by February (21.1±1.28d) in and the shortest period was in August (12.3±1.15d) (Table 6 and Figure 6).

Table (1): ANOVA one way statistical analysis of the duration of the presence of housefly eggs in hours over different months of the year

Months	N. of dead rats/month	Duration (Hr) Mean±SD	FT	P.value
January	10	27.20±2.85	65.6	0.000
February	10	25.00±3.55		
March	10	24.20±1.13		
April	10	22.00±2.66		
May	10	16.00±1.63		
June	10	15.60±0.69		
July	10	10.20±1.75		
August	10	9.60±1.57		
September	10	12.30±1.76		
October	10	17.20±1.93		
November	10	23.00±.299		
December	10	24.60±3.77		

FT: Anova test significant at P < 0.05.

Table (2): ANOVA one way statistical analysis of life span of 1stinstar Larvae in days over different months of the year

Months	N of dead rats/month	life span (days) Mean±SD	FT	P.value
January	10	3.60±0.51	14.78	0.000
February	10	3.50±0.52		
March	10	2.90±0.31		
April	10	2.70±0.67		
May	10	2.10±0.73		
June	10	2.00±0.23		
July	10	1.50±0.52		
August	10	1.90±0.31		
September	10	2.90±0.56		
October	10	3.00±0.81		
November	10	3.10±0.56		
December	10	3.40±0.51		

FT: Anova test significant at P < 0.05.

Table (3): ANOVA one way statistical analysis of life span of 2nd instar Larva in days over different months of the year

Months	N. of dead rats/month	life span (days) Mean±SD	FT	P.value
January	10	8.20±1.61	39.3	0.000
February	10	8.10±0.87		
March	10	6.20±0.63		
April	10	4.80±0.78		
May	10	4.00±0.66		
June	10	3.60±0.51		
July	10	3.30±0.48		
August	10	3.50±0.70		
September	10	5.80±0.63		
October	10	5.00±0.47		
November	10	7.30±0.67		
December	10	7.90±0.56		

FT: Anova test significant at P < 0.05

Table (4): ANOVA one way statistical analysis of life span of 3rd instar Larva per days over different months the year

Months	N. of dead rats/month	life span (days) Mean ±SD	FT	P.value
January	10	13.90±0.73	39.9	0.000
February	10	14.10±1.52		
March	10	12.30±1.15		
April	10	12.00±1.82		
May	10	10.20±1.22		
June	10	10.50±1.08		
July	10	8.90±0.56		
August	10	7.60±0.69		
September	10	11.30±0.82		
October	10	12.50±0.97		
November	10	12.70±0.67		
December	10	12.70±0.94		

FT: Anova test significant at P < 0.05.

Table (5): ANOVA one way statistical analysis of life span of light colored pupae per days over different months of the year

Months	N. of dead rats/month	Life span(days) Mean ±S.D	FT	P.value
January	10	16.00±1.49	30.3	0.00
February	10	15.70±0.94		
March	10	14.60±0.84		
April	10	13.80±1.61		
May	10	12.30±1.15		
June	10	12.20±1.47		
July	10	11.00±0.81		
August	10	10.40±0.69		
September	10	14.20±1.03		
October	10	14.40±0.96		
November	10	14.60±0.69		
December	10	14.80±0.91		

FT: Anova test significant at P < 0.05.

Table (6): ANOVA one way statistical analysis of life span of the dark colored pupae per days over different months of the year

Months	N. of dead rats/month	Life span(days) Mean±S.D	FT	P.value
January	10	21.20±1.22	68.008	0.000
February	10	21.10±1.28		
March	10	18.40±1.42		
April	10	18.50±1.71		
May	10	13.80±1.13		
June	10	13.90±1.66		
July	10	12.40±0.96		
August	10	12.30±1.15		
September	10	17.50±1.26		
October	10	19.10±0.73		
November	10	19.90±0.87		
December	10	20.90±0.99		

FT: Anova test significant at P < 0.05.

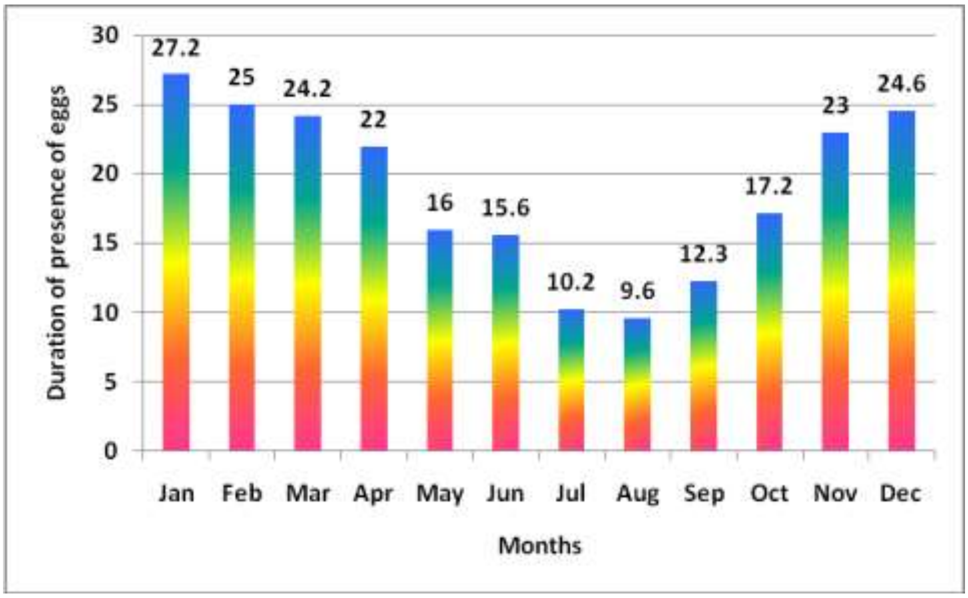

fig (1) life span of the housefly eggs in hours over different months of the year

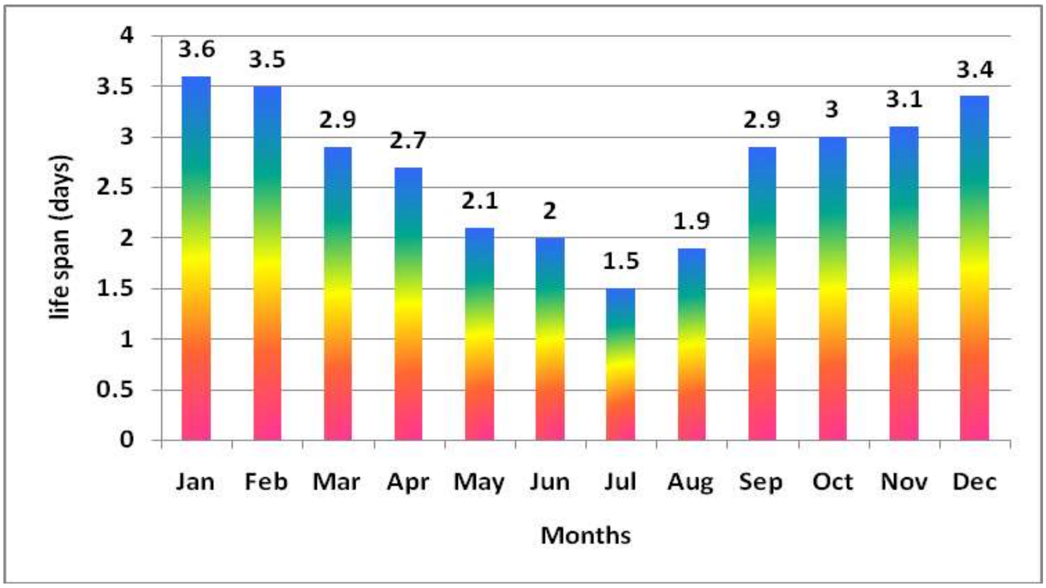

Fig. (2): The life span of the 1st instar Larva over different months the year

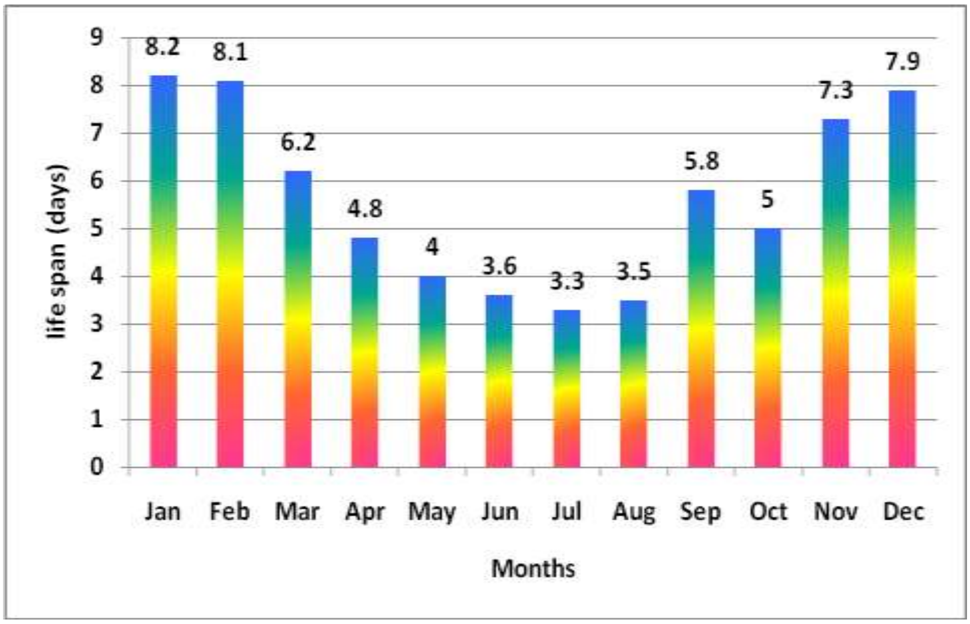

Fig. (3): The life span of the 2nd instar Larva over different months of the year

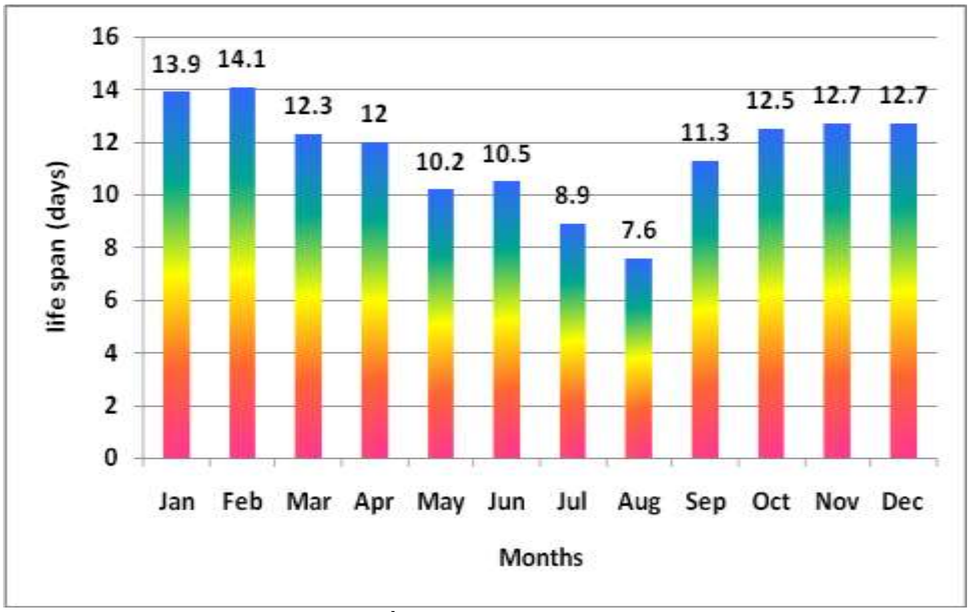

Fig. (4): The life span of the 3rd instar Larva over different months of the year

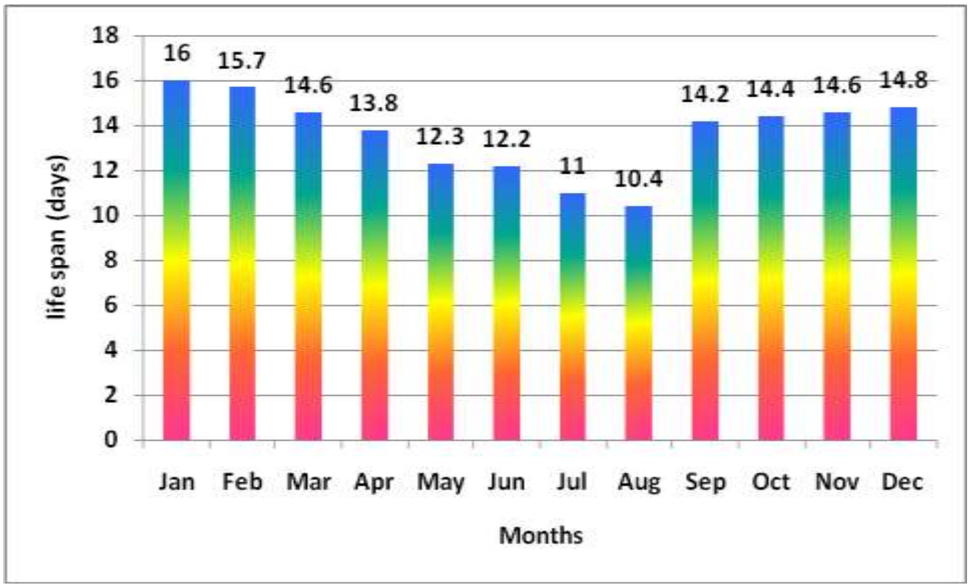

Fig. (5): The life span of the light colored pupae over different months of the year

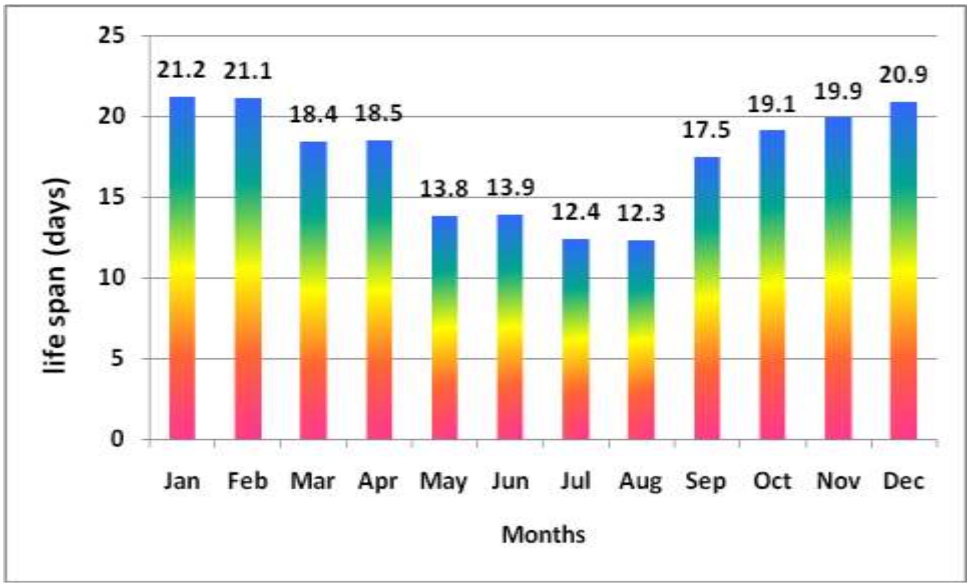

Fig. (6): The life span of the dark colored pupae over different months of the year

A B

Fig. (7) Patches of white eggs of house fly in the mouth of dead rat.

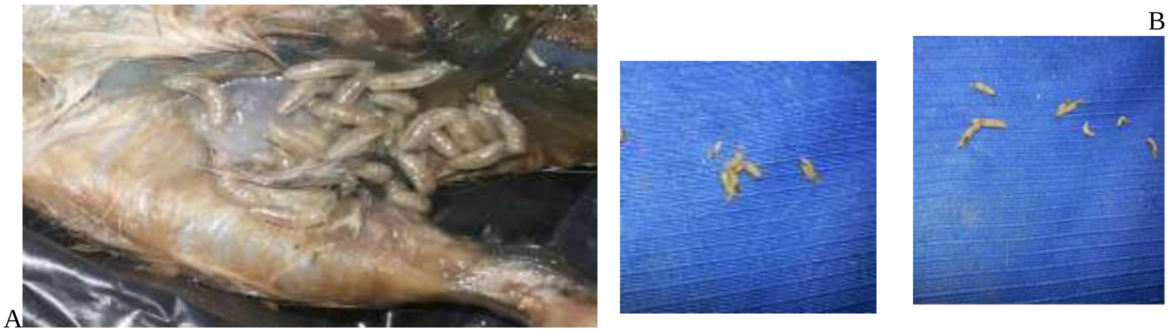

Fig. (8) number of 1st instr larvae(A,B)

Fig. (9) Number of 2nd instr larvae in relation to the dead rat (A,B,C and D)

Fig. (10): Number of 3rd instr larvae (A,B,C and D)

Fig. (11): Number of pupae of different colors and ages (A,B,C and D).

Discussion

Arthropods are found in a decomposing vertebrate corpse and these colonizers can be used to estimate time interval between death and corpse discovery at the death scene (Amendt et al., 2004).

Insect life cycles act as precise clocks, which begin within minutes of death. They can be used to closely determine the time of death when other methods are useless. They can also show if a body has been moved after death (Catts and Haskell 1990).

In the current study we searched for eggs in most common sites where eggs were deposited usually in natural orifices like eyes and nostrils, anus, or any small wounds in rat. Eggs were white eggs about 1.2 mm in length, was laid and piled in small groups (Hedges, 2004). The time of hatching is vital and so eggs were monitored every few hours. The findings showed that the duration required for hatching of housefly eggs was dependent on atmospheric temperature in different months of the year. This relation was statistically significant where this duration was much prolonged in January(27.2±2.85h) followed by February and December (cold months of the year) while much shortened in August, July and September (hot months of the year) with least time required for hatching in August (9.60±1.57 h).

Barnard and Geden in (1993) noted that legless maggot emerges from the egg in warm weather within eight to 20 hours.

The present study revealed that there was a statistical significant difference between the durations of the presence of the 1st instar, 2nd instar and 3rd instar larvae through different months of the year; where the longest period of the 1st instar larvae was in January (3.6±0.51d) followed by February (3.5±0.52d) and the shortest period was in July (1.5±0.52d), 2nd instar where longest duration was during January (8.2±1.61d) followed by February (8.1±0.87d) and the shortest period was in July (3.3±0.4d). As regards 3rd instar larvae, the longest period was in February (14.1±1.52d) followed by January (13.9±0.73d) and the shortest period was in August (7.6±0.69d), revealed that larval development was rapid in high temperature during summer months of the year.

This is in agreement with (Barnard and Geden 1993) who stated that the larva eat for four days in order to store nutrients for metamorphosis, goes through three instars and a full-grown maggot, 7 to 12 mm long, has a greasy, cream-colored appearance and the optimal temperature for larval development is 35 to 38°C (hot months), though larval survival is greatest at 17 to 32°C. Larvae complete their development in four to 13 days at optimal temperatures, but require 14 to 30 days at temperatures of 12 to 17°C (cold months).Also Sukontason et al. (2008) noted that during the larval stage, the fly passes through three different instars by molting and development of larvae is temperature dependent.

Tullis and Goff (1987) mentioned that by measuring the length or dry weight of the oldest larvae and comparing it with the reference data, age of the larvae can be estimated.

Following the larval phase, they transform into pupae, about 8 mm long, formed from the last larval skin form a capsule-like case, which varies in color from yellow, red, brown, to black as the pupa ages.

Results of the present study revealed that, there was a statistical significant difference between the durations of the development of the 1st stage pupae (light colored) over the year; the longest period from time of death was in January (16.0±1.49d) followed by February (15.7±0.94d) and the shortest period was in August (10.4±0.69d). As regarding the 2nd stage pupae (dark colored), the longest period was in January (21.2±1.22d) followed by February (21.1±1.28d) and the shortest period was in August (12.3±1.15d). This concluded that pupal development was rapid in high temperature during summer and decreased during low temperature in winter season months of the year.

Hogsette and Jacobs (2003) also noticed that Pupae complete their development in two to six days at 32 to 37°C, but require 17 to 27 days at about 14°C. The emerging fly escapes from the pupal case through the use of an alternatively swelling and shrinking sac, called the ptilinum, on the front of its head which it uses like a pneumatic hammer to break through the case and transformation from larva to adult usually takes 2–10 days.

Krafsur et al. (1985) showed that adult flies emerge from the pupae in warm climates after only 3–4 days of pupae formation. It can complete its life cycle in seven to ten days ranges from 14 to 90 days under suboptimal temperature.

So knowing the stage of insect inhabitation of corpse and the duration of stages in the insect´s life cycle can lead to an estimate of time since colonization. Estimating the time for insects to find the corpse and start laying eggs allowing the investigator to calculate the post mortem interval.

References

Amendt J, Krettek R, Zehner R (2004): Forensic entomology. Naturwissenschaften;91:51–65.

Anderson J.G., Dykema J.A, Goody R.M. (2004): Absolute, spectrally-resolved, thermal radiance: a benchmark for climate monitoring from space ,Journal of Quantitative Spectroscopy & Radiative Transfer 85 367–383

Ashworth JR, Wall R. (1994): Responses of the sheep blowflies Lucilia sericata and Lxuprina to odor and the development of semiochemical baits in Med Vet Entomology 8:303–9.

Barnard DR, Geden CJ. (1993): Influence of larval density and temperature in poultry manure on development of the house fly (Diptera:Muscidae). Environmental Entomology 22: 971-977.

Brown, B.V., Borkent, A., Cumming, J.M et al., (2009): Manual of Central American Diptera. Vol. 1. 714 pp. NRC Press, Ottawa.

Byrd JH and Castner JL. (2000): The Utility of Arthropods in Legal Investigations. Forensic Entomology, 1st ed.; p. 1-225. CRC Press, Florida.

Catts EP and Haskell NH (1990): Entomology and Death, a Procedural Guide, 1st ed.; p. 1-182. Joyce’s Print Shop, South Carolina.

Fasulo TR. (2002): Bloodsucking Insects and Filth-breeding Flies. Bug Tutorials. University of Florida/IFAS. CD-ROM. SW 156.

Hedges SA. (2004): The Mallis Handbook of Pest Control. 9th ed. pp.1396 .GIE Media, Cleveland.

Hogsette JA, Jacobs RD. (2003). The black dump fly: a larval predator of house flies. Journal of Economic Entomology 8: 54-71.

Joseph I, Mathew DG, Sathyan P, and et al., (2011):The use of insects in forensic investigations; An overview on the scope of forensic entomology , J Forensic Dent Sci. Jul-Dec; 3(2): 89–91.

Kapil Verma, Reject Paul MP (2013): Assessment of Post Mortem Interval (PMI) from Forensic Entomotoxicological Studies of Larvae and Flies Entomology, Ornithology& Herpetology j. 2:104.

Kashyab VK, Pillai VV. (1989): Efficacy of entomological methods in estimation of postmortem interval: a comparative analysis. *Forensic SciInt*; 40:245-50.

Krafsur ES, Black IV WC, Church CJ et al., (1985): Age structure and reproductive biology of a natural house fly (Diptera: Muscidae) population. Environmental Entomology 14: 159-164.

LeBlanc HN, Logan JG (2010): Exploiting Insect Olfaction in Forensic Entomology, Amendt J, Goff ML, Campobasso CP, Grassberger M, (eds). Current Concepts in Forensic Entomology. Netherlands: Springer; pp. 205–21.

Mandal, F B (2015): Human Parasitology, Book *2015*. Textbook of animal behavior, Chapter 2. pp. 235- PHI Learning Pvt. Ltd..ISBN via Google Books.

Sukontason K, Piangjai S, Siriwattanarungsee S et al., (2008): Morphology and developmental rate of blowflies s in Thailand: Morphology of second and third instars, application in forensic entomology. May; 102(*6*):1207-16. Parasitol Res 102:1207–1216.

Tullis K, Goff M L (1987): Arthropod succession in exposed carrion in a tropical rainforest on O’ahu Island, Hawai. J. Med. Entomol.; 24:332–9.

Wiegmann BM , Yeates, DK, Thorne JL et al.,(2003): Time flies, a new molecular time scale for brachyceran fly evolution without a clock, Systematic Biology, j;52 (6):745–56 December.

الملخص العربي

دور الذبابه المنزليه في تحديد زمن ما بعد الوفاه, دراسه تجريبيه في فئران التجارب البيضاء

أحمد كمال الدين الفقي١ و اسماعيل محمود محرم و بهاء الدين وديع الاسود٢

مقدمه : ان امكانية مساهمة علم الحشرات في التحقيقات القانونية والفحص الطبي الشرعي كان معروفا علي الاقل منذ سبعمائة سنة الا انه في العقدين الاخيرين أو نحوهما عرف علم الحشرات كحقل منفصل عن علم الطب الشرعي وهناك العديد من الطرق التي يمكن بها استخدام الحشرات للمساعدة في حل لغز الجريمه بيد ان الهدف المبدئي لاستخدام علم الحشرات الجنائي هو تحديد الوقت المنقضي بعد الوفاه ولان الحشرات الطائرة تصل للجسم في الاوقات الاولي لعملية التحلل الرمي فهي تساعد بدقه عاليه في تحديد الوقت المنقضي بعد الوفاه وتعد الذبابة المنزليه (ذبابة لينيوس المستأنسة) من الحشرات الهامة من الناحيتين الطبية والطبية الشرعية .

الهدف من هذه الدراسة : الهدف الرئيسي لهذه الدراسة هو التحقق من زمن ما بعد الوفاه وفقا لمختلف اطوار حياة الذبابة المنزليه علي مدارشهور العام.

طرق و مواد البحث : أجريت هذه الدراسة على مائة وعشرين من ذكور فئران التجارب البيضاء البالغة ، يتم الفصل العنقي لاثنين من الفئران كل ستة أيام تحت استنشاق مخدر حتي الوفاة ويتركا عرضه للذبابه المنزليه حيث تسجل المراحل المختلفة لدورة حياة الذبابة مع الاوقات المختلفة لما بعد الوفاة المختلفة ويتم تصويرها وتحليلها إحصائيا.

النتائج: أظهرت النتائج وجود اختلاف احصائي ذو دلالة عالية في اوقات الاطوار المختلفة (البويضات,الثلاثة اطوار اليرقية وطوري العذراء (الشرنقة)) في مختلف شهور السنه والتي كان اقصرها في شهور السنة الحاره (اغسطس ويوليو)

وكانت كالتالي:اقصرها في الاشهر أغسطس (٩,٦±١,٥٧) يوليه (١,٥±٠,٥٢)، يوليه (٣,٣±٠,٤)، أغسطس (٧,٦±٠,٦٩)، أغسطس (١٠,٤±٠,٦٩) وأغسطس (١٢,٣±١,١٥) يوما على التوالي وكانت اكثرها طولا في الأشهر الباردة من السنة يناير(٢٧,٢±٢,٨٥)، يناير (٣,٦±٠,٥١)، يناير (٨,٢±١,٦١)، فبراير (١٤,١ ±١,٥٢) (١٦,٠ ±١,٤٩), يناير(٢١,٢ ± ١,٢٢) يوما علي التوالي.

الاستنتاج و التوصيات: اوضحت النتائج انه يمكن تحديد الوقت الذي مر بعد الوفاة من خلال المراحل المختلفة لدورة حياة الذبابة حيث ان كل مرحلة تدل على الوقت بالنظر إلى درجة الحرارة والتغيرات في الموسمية. وجد ان هناك فرقا احصائيا بين زمن مختلف مراحل دورة حياة الذبابة المنزلية وفقا لاطوار النمو للذبابه ويكون اطولها وقتا في الشهور الباردة واقصرها في الشهورالحارة. وبالتالي فانه يمكن تحديد الوقت الذي مر بعد الوفاة من خلال المراحل المختلفة لدورة حياة الذبابة في وحول الجثة مع الاخذ في الاعتبار درجة حرارة الغلاف الجو.

[١] قسم الطب الشرعي والسموم – كلية الطب البشري – جامعة المنوفية

[٢] قسم الطفيليات الطبية – كلية الطب البشري – جامعة المنوفية