Jahangirnagar University J. Biol. Sci. **9**(1 & 2): 49-58, 2020 (June & December)

Estimation of accumulated degree hours-based post-mortem intervals in mammalian and avian model

Md. Golam Mostafa*, Mst. Shahanaz Parvin Shobha, Anjuman Ara and Saadia Ahmad
Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

The post-mortem interval (PMI) of a jungle cat (*Felis chaus*) and a chicken (*Gallus gallus domesticus*) were estimated as a mammalian and an avian model, respectively. The estimations were performed adopting the Accumulated Degree Hours (ADH) method using blowfly, *Lucilia sericata.* The required developmental time from oviposition to the 3rd instar larvae of the blowfly species was considered for the determination of PMI in each of the study. The results revealed that the calculated PMI of jungle cat was 5.29 days. On the other hand, the estimated PMI of the chicken was 2.83 days. The difference of these two PMIs in two different models might be due to the variation of temperatures and other related factors in the development of the larvae of the blowfly species. This study might offer a new approach in the medico-legal investigations with a view to resolving homicide as well as other suspected death of animals including wildlife in Bangladesh.

Key words: Estimation, PMI, Mammalian, Avian, ADH method.

INTRODUCTION

Forensic entomology is a field of forensic sciences, which deals with insect-based information in the investigation of legal cases relating to unnatural deaths of humans or other animals including wildlife (Gennard, 2007). The most important application of forensic entomology is the estimation of Post-mortem interval (PMI), which means the time that has elapsed since the death of the victim. PMI possesses great potentials in the medico-legal investigations of homicides and other unnatural deaths of animals (Byrd & Castner, 2000). Since many insects are associated with the dead bodies, they are always a potential source of evidence in case of human murders and suspicious deaths of animals including poaching or illegal hunting of wildlife.

After the death of an animal, insects are usually the first organisms to appear on the dead body to colonize in a successive way (Catts & Goff, 1992). The cadaver undergoes a recognized sequence of decompositional stages from fresh to skeletal over time and inherently follows remarkable physical, biological as well as different chemical changes (Byrd & Castner, 2009). During the course of decomposition, different types of

* Corresponding author. Email: bdmostafa@yahoo.com

arthropods predominantly insects complete their life cycles on the dead body (Byrd & Castner, 2000).

The insect-based information can help in the precise estimation of PMIs in animals including humans. The immature stages of the insects particularly the sarcophagous insects found on the dead body can provide evidence in estimating the PMI ranging from one day up to a month or more depending on the insect species involved and the climatic conditions at the crime scene (Ahmed & Joseph, 2016). Among the sarcophagous insects, the most commonly used insects in the forensic investigations are the true flies of order Diptera and the predominant families are Calliphoridae (blow flies), Sarcophagidae (flesh flies) and Muscidae (house flies). Among them Calliphorids and Sarcophagids generally arrive within minutes of the death of animals (Bala & Sharma, 2016). However, without appropriate and professional approaches to the matter, particularly collection of insect evidence, presentation and accurate estimation of PMI, the judgments about the subject is affected in court (Amendt *et al*., 2011).

Many important homicide cases have been resolved in courts in the developed countries by using entomological data with effective and well-established techniques. Among them such cases in France, Belgium, England, Czech Republic, Russia, Canada and in the United States are exemplary (Benecke, 1998).

Khatun *et al*., (2019) carried out first experiment on the estimation of PMI in Bangladesh, but still the field remains unexplored and obscure in this country largely because of lack of awareness of the benefits. Although the experiment on the estimation of PMI in human models is utterly important, the present study was carried out using a jungle cat and chicken model because human dead bodies cannot be easily used due to legal complications.

MATERIALS AND METHODS

The study was conducted from February to April in 2019 in the Botanical Garden and at the Medical and Forensic Entomology laboratory of Department of Zoology in Jahangirnagar University. In this study netted forensic cage, insect rearing cages, glass vials, forceps, thermometer, hygrometer, incubator, alcohol, plastic cups, sweeping net, scales, needles, slides, dissecting and light microscopes, formalin, masks, gloves and polythene bags were used as to perform the experiments.

The experiment and the experimental animals were a dead jungle cat (*Felis chaus*) and a chicken (*Gallus gallus domesticus*). The dead body of the jungle cat was found near the Wazed Miah Science Research Center in Jahangirnagar University campus while the carcass of the chicken was collected from the dumping ground near the Jahangirnagar University campus area.

Blow fly larvae were collected from the cadaver and reared in the laboratory on rotten Tilapia fish as their natural diet. The development time of *L. sericata* (Meigen) from egg to pupae were observed at temperature of 21.3± 1°C and 31± 1°C respectively because of

the prevailing average temperature of that particular time of the study sites and the rearing was completed to obtain their adult stages for morphological identification of the insects. The adult insect samples were collected from the corpse and preserved in 80% ethanol for identification.

Maximum and minimum temperature data of the experimental period were recorded from Geography and Environment department of Jahangirnagar University. The developmental stages of blow fly and the instars were determined by their size and the number of slits present in the posterior spiracles (Youseffi *et al.*, 2012). The identification of the adult fly was done by studying their morphological characters under light microscope as described by Williams *et al.*, (2014). The ADH method was used to determine the PMIs in both model animals as described by Khatun *et al.*, (2019) and Suri Babu *et al.*, (2013). The ADH was calculated for *L. sericata* life cycle in both cases by using the following formula: **ADH=Time (Hrs.)×(Average temperature-Minimum development threshold temperature**) and the reference developmental durations for *L. sericata* used in the study were obtained from Cervantes *et al.*, (2017).

RESULTS AND DISCUSSION

Identification of adults and larvae: The adult flies were metallic green in color with a (Plate 1) ridge just above the squama. The rear wing was flap-like with tufts of hair on it and a yellow bicosta. There were four para-vertical setulae or occipital bristles and metallic blue to black fore femora. The life cycle of this blow fly included eggs, three larval instars, pupa and adults. The eggs were found usually ball like, white or pale yellow. The larvae were yellow or grayish in color and conical in shape and passed three instars (Plate 2). The 1st instar larvae were identified by one slit, 2nd instar larvae were identified by two slits and 3rd instar larvae were identified by three slits in the posterior spiracles (Plates 3 and 4).

Plate 1. Adult blow fly *(L. sericata*) on chicken carcass

Plate 2. The third instar larvae of *L. sericata*

Plate 3. Posterior spiracles of the 3rd instar larvae of *L. sericata*

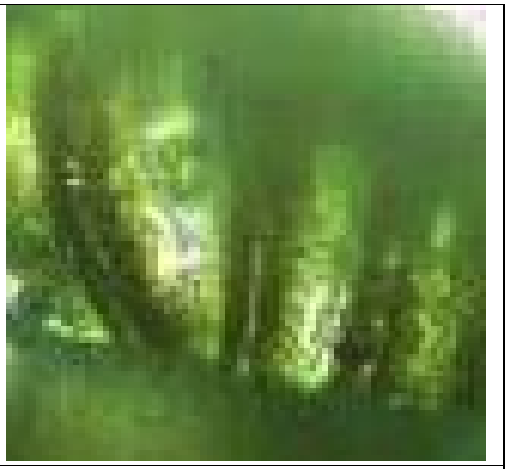

Plate 4. Three slits in the posterior spiracles of the 3rd instar larvae of *L. sericata*

Estimation of PMI: By observing different developmental stages of *L. sericata* and different decompositional stages of the dead bodies the PMI of a chicken (Plate 1) and of a jungle cat (Plate 5 and 6) and were estimated. The temperature of the study area was 21.3±1°C. The development time of *L. sericata* at 24°C was utilized to calculate ADH. Cumulative ADH required for the development of the 3rd instar larvae of the fly was calculated by using meteriological data of the study site as shown in Table 1. Finally, the time of death and PMI of the mammalian (Table 2) and avian model were estimated by using the ADH method (Table 3 and Table 4).

Estimation of PMI of the jungle cat

Table 1. Accumulated Degree Hours (ADH) for *L. sericata* from 1st February to 9th February 2019

Date	Temperature °C			Threshold Temp. °C	Degree Day (DD)	Accumulated Degree Hours (ADH)	Cumulative ADH
	Max.	Min.	Average				
1/2/2019	28.3	17	22.65	10	12.65	303.6	2318.6
2/2/2019	28.3	17.3	22.8	10	12.8	307.2	2015
3/2/2019	29.6	13.6	21.6	10	11.6	278.4	1707.8
4/2/2019	30.3	12.3	21.3	10	11.3	271.2	1429.4
5/2/2019	30.3	12.6	21.45	10	11.45	274.8	1158.2 ←
6/2/2019	30	12	21	10	11	264	883.4
7/2/2019	29.6	11.3	20.45	10	10.45	250.8	619.4
8/2/2019	29.3	12	20.65	10	10.65	255.6	368.6
9/2/2019	29.6	13	21.3	10	11.3	113	113

- The arrow sign indicates the position of required ADH in the table.
- Degree Days (DD)=(Average. Temp.-Threshold Temp.)°C
- Accumulated Degree Hours (ADH)=DD x Time in hours

Table 2. Accumulated Degree Hours (ADH) method for determining the post -mortem interval (PMI) of a dead jungle cat

Accumulated Degree Hours method	
Collection of 3rd instar larvae	at 10.00 hours on 9th February 2019
The reference ADH taken by *L. sericata* to reach the third instar stage at 24°C	(15.8 hrs. + 42.3 hrs.) × 24°C= 1394.4 ADH
Total ADH from 9th to 5thFebruary (Table 1)	ADH= Development time (hrs.)× Growing Degree Day value (DD) (°C) (10 hrs. × 11.3°C) +(24 hrs. × 10.65°C)+ (24 hrs. × 10.45°C) +(24 hrs. × 11°C)+ (24 hrs. × 11.45°C) =1158.2 ADH
Difference between ADH taken by *L. sericata* species to reach the third instar stage at 24°C - Total ADH of 5th,6th,7th,8th ,9thFebruary 2019	(1394.4 – 1158.2) ADH= 236.2 ADH
Dividing 236.2 ADH by the Growing Degree Day value(DD) of 4th February 2019 (11.3) (Table 1)	236.2 ADH/ 11.3°C =20.90 hrs.
Determination of oviposition and PMI	Backward counting of 20.90 hrs.from 4thFebruary 2019 revealed that the female *L. sericata* species might have laid its eggs on the dead body at 3.10 hrs.on 4th February. Total hours = (9th February: 10 hrs. + 8th February: 24 hrs. + 7th February: 24 hrs. + 6th February: 24 hrs. + 5thFebruary: 24 hrs. +4th February: 20.90 hrs.)= 126.9hrs= 5.29 days
Thus the death of the jungle cat might occur at 3.10 hrs.on 4th February 2019 and the calculated PMI after obtaining the third instar larvae was 5.29 days).	

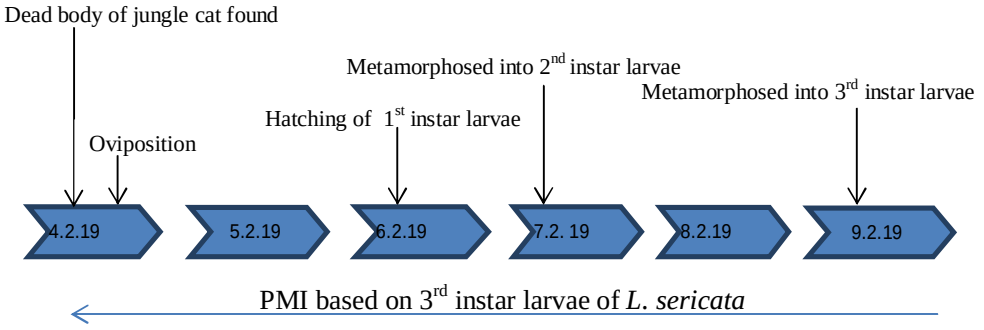

Fig. 1. The timeline indicating the main events in the dead body of the jungle cat

Plate 5. Blow flies on the wound of the jungle cat

Plate 6. 3rd instar larvae in the mouth of dead jungle cat

Estimation of PMI of the chicken

Table 3. Determination of Accumulated Degree Hours (ADH) for *L. sericata* from 29thMarch to 2ndApril 2019

Date	Temperature in °C			Threshold Temp. °C	Degree Day (DD)	Accumulated Degree Hours (ADH)	Cumulative ADH
	Max.	Min.	Average				
29/3/19	32	25.3	28.65	10	18.65	447.6	2031.9
30/3/19	31.6	25	28.3	10	18.3	439.2	1584.3
31/3/19	29.3	22	25.65	10	15.65	375.6	1145.1
1/4/19	31.6	26.6	29.1	10	19.1	458.4	769.5
2/4/19	31	25.6	28.3	10	18.3	311.1	311.1

- The arrow sign indicates the position of required ADH in the table.
- Degree Day (DD)=(Average.Temp. - Threshold Temp.)°C
- Accumulated Degree Hours (ADH)=DD x Time in hours

Table 4. Accumulated Degree Hours (ADH) method for determining the Post-Mortem Interval (PMI) of a dead chicken

Accumulated Degree Hours method	
Collection of 3rd instar larvae	at 17.00 hours on 2nd April 2019
The reference ADH taken by *L. sericata* to reach the third instar stage at 30°C.	(11.2 hrs + 28.8 hrs) × 30°C= 1200 ADH
Total ADH from 2nd April to 31th March (Table 3)	ADH= Development time (hrs) × Growing Degree Day value (DD) (°C) (17 hrs × 18.3°C) +(24 hrs × 19.1°C)+ (24 hrs × 15.65°C) =1145.1 ADH
Difference between ADH taken by *L. sericata*species to reach the third instar stage at 30°C - Total ADH of 1st April, 2nd April and 31th March 2019	(1200– 1145.1) ADH= 54.9 ADH
Dividing 54.9 ADH by the Growing Degree Day value(DD) of 30th March 2019 (18.3) (Table 3)	54.9 ADH/ 18.3°C =3.00 hrs
Determination of oviposition and PMI	Backward counting of 3.00 hrs from 30th March 2019 revealed that the female *L. sericata* species might have laid its eggs on the dead body at around 21.00 hrs on 30th March 2019. Total hours = (2nd April: 17hrs+ 1st April: 24 hrs+ 31th March: 24 hrs+ 30th March: 3hrs)= 68 hrs= 2.83 days
Thus the death of the chicken might occur at 21.00 hrs on 30th March 2019 and the calculated PMI after obtaining the third instar larvae was 2.83 days).	

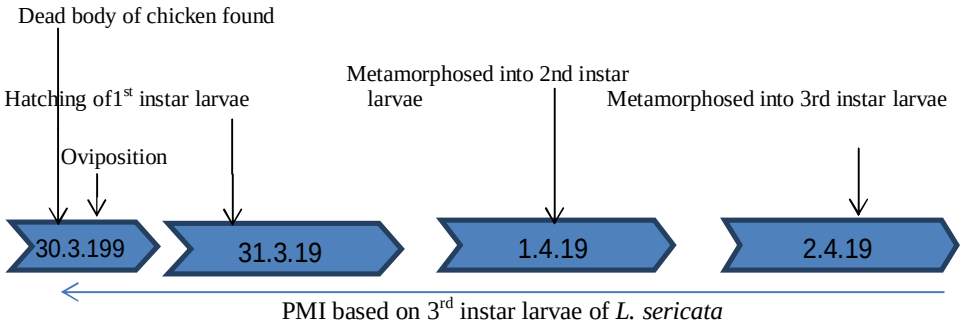

Fig. 2. The timeline indicating the main events in the dead body of the chicken

During the study, it was noticed that the rate of decomposition of chicken was faster than the jungle cat. The investigations were carried out on two fresh dead bodies of a mammalian and an avian model to understand the role the most predominating insect species on the ground. There was difference between the calculated PMIs in case of two animal models. This difference might be due to the variation of temperatures as the larvae

of the blowfly species developed at 21.3± 1°C in the carcass of jungle cat while the larvae of the same species developed at 31± 1°C in chicken. The study also revealed that the blowfly, *L. sericata* was found most predominant as well as suitable due to the size and rearing availability of this species. Islam *et al*., (2016) and Khatun *et al*., (2018) also reported the presence of blowfly in the carcasses at Jahangirnagar University, Bangladesh. Researchers around the world have used different animals as models for estimations of PMIs. Shean *et al.*, (1993) used pig carcasses, Patrician & Vaidyanathan (1995) used rats, Tantawi *et al*., (1996) used rabbit carcasses and Khatun *et al.*, (2019) used only mammalian models-mongoose and mouse in their experiments respectively for the estimation of PMIs.

Although various types of methods are used for estimation of PMI, the present study used ADH method to estimate post-mortem interval (PMI). Anderson (2000) used the newer concept of accumulated degree days (ADD). Ames and Turner (2003) also applied ADD for estimating post-mortem interval (PMI). The developmental stages of blow fly *(L. sericata)* larvae were used to calculate post-mortem interval (PMI) in our study because the adult flies were predominant and were found to be the first to reach the carcasses. Similar observations were made by Reed (1958), Tantawi *et al.,* (1996), Bharti & Singh (2003).

Kulshrestha & Satpathy (2002) used maggots of *Chrysomya rufifacies* from three corpses in three cases in the year 2001 from Bhopal, India for the estimation of post-mortem interval (PMI). Ramos-pastrana (2017) estimated the PMI based on the life cycle of *Chrysomya albiceps* in Colombia and the calculated PMI was of 229 hours (9.5 days). Anderson (1999) described the use of entomology to determine time of death in the illegal killing of two young bear cubs in Manitoba, Canada.

Greenberg & Wells (1998) used human dead bodies and reported that the phoridae fly may may be only insect evidence, especially in sealed apartments, which are inaccessible to large insects. Bala & Sharma (2016) calculated the PMI of a 23 years old female corpse found in the rice field of Punjab, India by using ADH method. The PMI of the female was calculated 9.6 days, whereas the autopsy surgeon estimated a PMI to 10 to 12 days. Khatun *et al.,* (2019) carried out an experiment in which the PMIs of two mammalian models – mongoose and mouse were estimated by using ADH method. In their studies they reported that the time of death of mongoose was at around 15.49 hours and the calculated PMI based on the 3rd instar larvae was 3.65 days. In the second case, the estimated PMI based on the 3rd instar larvae was 2.62 days.

However, forensic entomology is not only used to determine the time of death in human death investigations but also it can be equally applicable to wildlife. Since the numbers of poaching are increasing day by day and more animals are killed around the world, the present study can be used in homicide, suspected death and wildlife poaching cases in Bangladesh.

Conclusion: The 3rd instar larvae of blow fly *L. sericata* collected from the mammalian *(F. chaus)* and avian (*G. g. domesticus*) models were used to estimate the post- mortem intervals (PMIs). In the first case study, the obtained results showed that the death of the jungle cat occurred at 3.10 hrs and the calculated PMI based on the third instar larvae was 5.29 days. In the second case study, results revealed that the death of the chicken occurred at 21.00 hrs and the estimated PMI based on the third instar larvae was 2.83 days. There was no significant difference marked between the sequences of faunal succession on both carcasses. The use of forensic entomology in determining the post-mortem interval (PMI) has not been explored properly in our country. Therefore, this study offers necessary tools in the investigations of homicide, suspicious death of animals including wildlife poaching cases in Bangladesh.

Acknowledgement: The research work was carried out in the Laboratory of Medical and Forensic Entomology and at the garden of the Department of Zoology, Jahangirnagar University, Dhaka, Bangladesh. The authors are indebted to the Chairman of the department for extending necessary permissions and facilities to conduct the work.

Declaration: The authors declare that they have equal contributions to this research and have no conflict of interests.

REFERENCES

Ahmed, A.B. and Joseph, S.G. 2016. Estimation of postmortem interval using the blow fly *Phaenicia (Lucilia) sericata* (Diptera: Calliphoridae) in Kaduna, Northern Nigeria. *Int. J. Res. Med. Sci.* 4(8): 3417- 3420.

Ames, C. and Turner, B. 2003. Low temperature episodes in development of blowflies: implications for post-mortem interval estimation. *Med. Vet. Entomol*. 17(2): 178-86.

Amendt J, Richards C.S, Campobasso C.P, Zehner R, and Hall M.J.R. 2011. Forensic entomology: applications and limitations. *Forensic Sci Med Path*. doi:10.1007/s12024-010-9209-2.

Anderson, G. S. 1999. Wildlife forensic entomology: determining time of death in two illegally killed black bear cubs. *J. Foren. Sci*. 44(4): 856-859.

Anderson, G. S. 2000. Minimum and Maximum Development Rates Of Some Forensically Important Calliphoridae (Diptera).*J. Foren. Sci*. 45(4): 824-832.

Bala, M. and Sharma, A. 2016. Postmortem Interval Estimation of Mummified Body Using Accumulated Degree Hours (ADH) Method.A Case Study from Punjab (India). *J. Foren. Sci. & Criminal Inves*. 1(1): 555552.

Benecke, M. 1998. Six forensic entomology cases: description and commentary. *J Forensic Sci;* 43:797–805.

Bharti, M. and Singh, D. 2003. Insect Faunal Succession on Decaying Rabbit Carcasses in Punjab. *J. Foren. Sci. 48*: 1133-1143.

Byrd, J. H. and Castner, J. L. 2000. Forensic entomology: The Utility of Arthropods in Legal Investigations. Boca Raton: *CRC Press, pp.*440.

Byrd, J. H. and Castner, J. L. 2009. Forensic entomology: The Utility of Arthropods in Legal Investigations. 2nd ed. *CRC Press, pp.*681.

Catts, E.P. and Goff, M.L. 1992. Forensic entomology in criminal investigations. *Annu.Rev. Entomol*. 37:253-272.

Cervantes, L., Dourel, L., Gaudry, E., Pasquerault, T. and Vincet, B. 2017. Effect of low Temperature in the development cycle *of Lucilia sericata (Meigen)(Diptera, Calliphoridae):* implications for the minimum postmortem interval Estimation *Foren. Sci Res. 3(1): 52-59.*

Gennard, D. E. 2007. Forensic Entomology.Wiley, Chichester, UK.PP.224.

Greenberg, B. and Wells, J. D. 1998. Forensic Use of *Megaselia abdita* and *M. scalaris* (Phoridae; Diptera): Case Studies, Development Rates and Egg Structure. *J. Med. Entomol.* 35(3): 205-209.

Islam, M., Hossain, A. Mostafa, M. G., and Hossain, M. M. 2016. Forensically important insects associated with the decomposition of mice carrion in Bangladesh. *Jahangirnagar University J. Biol. Sci*. **5(1)**: 2016.

Khatun, R., Mostafa, M. G., Haque, M. E. Ahmad, S. and Akter, T. 2018. Formulation and evaluation of the efficacy of an artificial diet for two forensically important flies. *Asian Australas. J. Biosci. Biotechnol*. 3(2): 86-92.

Khatun, R., Mostafa, G. and Saadia, A. 2019. Application of forensic entomology to estimate the post-mortem interval (PMI) in mammalian models in Bangladesh. *EJBPS. Sci*, 6(5): 38-45.

Kulshreshtha, P. and Satpathy, D. K. 2002. *Ch. Rufifacies* development and forensic entomology-recent case studies. *JIAFM*. 24(1): 6-9.

Patrician, L. A. and Vaidyanathan, R. 1995. Arthropod Succession In Rats Euthanized With Carbon Dioxide and Sodium Pentobarbital.*J. New York Entomol. Soc.* 103(2): 197- 207.

Ramos-Pastrana, Y. and Wolff, M. 2017. Postmortem interval estimation based on *Chrysomya albiceps* (Diptera, Calliphoridae) in a forensic case in the Andean Amazon, Caqueta, Colombia. *Acta Amazonica*, 47: 369-74.

Reed, H. B. 1958. A Study of Dog Carcass Communities in Tennessee, With Special Reference to the Insects. *Am Midl. Nat*. 59(1): 213-245.

Shean, B. S., Messinger, L. and Papworth, M. 1993. Observations of Differential Decomposition on Sun Exposed Vs Shaded Pig Carrion. *Coastal Washington State. J. Foren. Sci.* 38(4): 938-949.

Suri Babu, B., Sharma, H. and Bharti, M. 2013. Estimation of Postmortem Interval by rearing *Chrysomya rufifacies* (Macquart, 1842) (Diptera : Calliphoridae): A case study from Central India. *J. Foren. Med. Toxicol.* 14(2): 1-12.

Tantawi, T. I., ElKady, E. M., Greenberg, B. and ElGhaffar, H. A. 1996. Arthropod Succession on Exposed Rabbit Carrion in Alexandria, Egypt. *J. Med. Entomol*. 33(4): 566-580.

Williams, K. A. and Villet, M. H. 2014. Morphological identification of *Lucilia sericata, Lucilia cuprina* and their hybrids (Diptera: Calliphoridae). *Zookeys*, 420: 69-85.

Youssefi, M. R., Rahimi, M. T. and Marhaba, Z. 2012. Occurrence of Nasal Nosocomial Myiasis by *Lucilia sericata* (Diptera: Calliphoridae) In North of Iran. *Iranian J. Parasitol.* 7(1): 104-108.