International Journal of Entomology and Nematology Research,.7, (1), 41-55, 2023
Print ISSN: 2399-1151(Print)
Online ISSN: 2399-116X (Online)
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development-UK

# Effect of Temperature and Type of Tissue on the Development of *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) in Sri Lanka

**Bambaradeniya Y.T.B**
Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.

**Karunaratne W.A.I.P**
Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.

**Tomberlin J.K**
Department of Entomology, Texas A&M University, College Station, TX.

**Magni P.A**
Discipline of Medical, Molecular and Forensic Sciences, Murdoch University, Murdoch, WA, Australia.
Murdoch University Singapore, King's Centre, 390 Havelock Road, Singapore 169662.

doi: https://doi.org/10.37745/ijenr.16 vol7n14155 Published September 20 2023

**Citation**: Bambaradeniya Y.T.B., Karunaratne W.A.I.P., Tomberlin J.K., Magni P.A., (2023) Effect of Temperature and Type of Tissue on the Development of Chrysomya rufifacies Macquart (Diptera: Calliphoridae) in Sri Lanka, International Journal of Entomology and Nematology Research,.7, (1), 41-55

**ABSTRACT:** *Chrysomya rufifacies (Macquart), is a species of biosecurity, medical and forensic importance. This fly is a pathogen-transmitting species, myiasis producer, and early colonizer of human and animal remains. Despite the commonality of Ch. rufifacies in Sri Lanka, developmental studies have never been performed in this region and the effects of an intraspecific diet have never been tested. In the current research, Ch. rufifacies immatures were reared on skeletal muscle, liver, and heart from domestic swine, with colonies maintained at 25°C and 28°C. The minimum time needed to complete each instar of life at 25°C was the fastest on liver (224.14 h), followed by skeletal muscle (249.33 h) and heart (251.64 h). Whereas at 28°C, fly development was fastest on heart muscle (178.27 h), followed by liver (178.50 h) and skeletal muscle (186.17 h). This study revealed that temperature has a significant impact on the total developmental time, as well as on the length and the width of the fly immatures.*

**KEY WORDS:** *Chrysomya rufifacies*, Intraspecific diet, Forensic Entomology, Growth rate, Larval length, Larval width

## INTRODUCTION

*Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), the hairy maggot blow fly, is one of the dominant carrion-feeders native to the Australian and Oriental regions (Baumgartner,

1993). However, this species has expanded its geographical territories to include Central America (recorded in 1978) and Mexico, as well as the United States (e.g. Texas, Arizona, California and Florida, recorded in 1982, 1986, 1988 and 1991, respectively) (Baumgartner, 1993; Holdaway, 1933). Generally, *Ch. rufifacies* is attracted to, feeds and oviposits on various substrates, ranging from carrion of vertebrates, wounds, human faeces, and refuse (Baumgartner, 1993).Therefore, this species is of biosecurity, medical and forensic importance as a pathogen-transmitting species, myiasis producer, and colonizer of human and animal remains. Understanding the time of colonization of *Ch. rufifacies* on a certain substrate is the base for a correct estimation of the minimum time since death (minPMI) and the colonization time (CI) connected with cases of negligence in medical and veterinary cases of forensic importance (Sukontason et al., 2005). Differently from other calliphorids typically found in forensic settings (e.g. *Lucilia* sp. and *Calliphora* sp., Diptera: Calliphoridae), when reaching the third instar of life, *Ch. rufifacies* larvae develop predatory and cannibalistic habits (Shiao and Yeh, 2008). As a consequence, this species can modify the assemblage of the necrophagous community feeding on a carcass and, in different ecological context, it can play a crucial role in reducing populations of nuisance fly species (e.g. *Musca* sp., Diptera: Muscidae) (Faria, 2004; Byrd and Castner, 2009). When present on decomposing remains, *Ch. rufifacies* larvae can be easily distinguished from the larvae of other calliphorides by their unique appearance; each of their body segment is covered by fleshy protrusionsending with sclerotized spines (Byrd and Castner, 2009). However, differentiation with the carrion-feeder, *Chrysoma albiceps* (Wiedemann) (Diptera: Calliphoridae),is difficult due to great morphological similarities, especially in their larval characters (Silva et al., 2012). Literature distinguish the larvae of these two species only on the basis of the location of the collection.*Ch. albiceps* is present from the Palaeartic Region throughout Africa and established in Brazil and Canary island, while *Ch. rufifacies* established in the Oriental and Australasian regions as well as in the in the southeastern, central, and southwestern portion of the United States (Smith, 1986; Baumgartner, 1986). In these countries, *Ch. rufifacies* is often the principal cause of myiasis (Sukontason, 2005) and the most abundant fly colonizing human and animal remains during in the first wave of decomposition (Smith, 1986), hence of primary forensic importance (Brundage et al., 2014).

Several development studies on *Ch. rufifacies* have been already conducted, but the vast majority were performed in temperate regions, e.g. USA and Australia (Flores et al., 2014; Byrd and Butler, 1997; Goodbord and Goff, 1990, O'flynn, 1983). To date, no research has been conducted in Sri Lanka, therefore in this country, the estimation of the CI and the minPMI using *Ch. rufifacies* can only be based on the limited studies conducted in India (Subramanian and Mohan, 1980), Thailand (Sukontason et al., 2008), and China (Hu et al., 2019). In these three research, local populations of *Ch. rufifacies* were reared in laboratory on media of different nature (e.g. bovine, equine, and canine). Such media were used as an alternative to swine (*Sus scrofa* L.) carcasses or flesh despite the latter is considered to be more relevant to the field of forensic entomology and as it has been proved to be the best surrogate for human remains (Bambaradeniya et al., 2017; Dekeirsschieter et al, 2013). The aim of the present research is to provide the first data on the development of *Ch. rufifacies* in Sri Lanka on swine medium of different types (hearth, muscle, liver). Results of this research will be useful in future case work associated with decomposing/colonized human remains in this particular

region. Overall, this study provides future insights into the importance of fly development data to the investigation of crime scenes.

## MATERIALS AND METHODS

### Initiation of a *Ch. rufifacies* fly colony

*Ch.rufifacies* larvae were collected from a carcass of a decomposing Indian crested porcupine (*Hystrix indica* Kerr) found in a partially vegetated area near the Department of Zoology of the University of Peradeniya (Sri Lanka) (7°15'9.00" N 80°35'28.79" E). Larvae were collected according to standard practice and guidelines for forensic entomology (Amendt et al., 2007), and divided in two subsamples. The first subsample was preserved to confirm the identification of the species and the instar of life of the larvae (James, 1947), the second subsample was used to develop the colony (Byrd and Castner, 2009). The colony was hosted in the entomology laboratory at the University of Peradeniya for the entire duration of the study. *Ch.rufifacies* larvae were reared on swine liver placed on a plastic plate in a plastic box (25x25x25 cm) containing a layer of saw dust (2 cm) and covered with a fine-meshed cloth to allow airflow. When the majority of the larvae reached the post-feeding instar, the plate with the remaining liver was removed to facilitate the larvae to use the sand as pupation site. After four days following the beginning of the pupation, pupae were removed from the sand and placed in an insect-rearing cage (30x30x30cm) equipped with a solution of sugar and milk in a hydroponic system. As the adults emerged from the pupae, the feeding source was replaced with a fresh one every three days. One week after the eclosion of the first adult from the pupae, adults were provided with a tissue paper blotted with pig blood and a 50g piece of tilapia fish (*Oreochromis niloticus* (L.)), to stimulate the development of eggs (Bambaradeniya et al., 2018). The same material was provided after five days, to be used as an oviposition site. The colony was observed every six hours, and the fly eggs were removed with a fine paint brush (size 04). Eggs were obtained from the first three generations of the flies of the colony, to maintain the genetic consistency among the individuals used in the experiment.

### Preparation of the rearing media and developmental study

For the purpose of this research, three different types of rearing media were used: swine heart tissue, swine skeletal muscle and swine liver tissue. The rearing media were prepared using 2 kg of each tissue type, purchased from the animal farm facility at Department of Agriculture of the University of Peradeniya. Tissues were finely chopped, divided in 100g aliquots and stored in a refrigerator ($4^{\circ}$C) in labelled polythene bags.Experimental treatments were prepared placing 50 to 60 *Ch. rufifacies* eggs collected from the colony on 100g of each tissue type. Eggs and rearing medium where then placed on a plastic plate in a plastic box (25x25x25cm) containing a 2cm layer of saw dust and covered with a fine-meshed cloth. For each treatment, three rearing boxes (i.e. three replicates) were placed inside an incubator (POL-EKO-APARATURA sp.j., Wodzislaw Slaski, Poland:Model- KK 240). This study was performed at 25°C and 28°C , in both cases with 70% relative humidity (RH) and photoperiod of 12:12 L:D. Observations were made hourly to note the time of hatching and completion of each additional development instar.

The three largest larvae present in each of the treatments were sampled every eight hours until the beginning of pupation. Larvae were parboiled and preserved in 70% alcohol to determine the length and the width (Amendt et al., 2007; Day and Wallman, 2006). For each of the larvae, the length was measured with the aid of a digital microscope (Microview USB digital microscope 650X, China), while the cross width of the last posterior segment were measured with a verniercaliper (Baker DC20, reading- 0.02mm, Measuring range- 0- 200mm, USA).

**Data analysis**

Data of larval length and width were analysed with the analysis of covariance test (ANCOVA) using Minitab 14. The aim was to determine the effect of the type of tissue and the temperature (independent variables) on length and cross width variation (dependent variables) of larvae over time (covariate) ($P< 0.05$). Effect of the type of tissue and temperature on the total development time was also determined through an analysis of variance (ANOVA) using Minitab 14. Tukey multiple range tests were used to compare all the significant differences between tissue types under total time, length and cross-width change ($P< 0.05$). Length and cross width overtime were graphically illustrated using sigma plot version 10 software. The mean length and cross width changes of larvae was illustrated with 95% confidence intervals.

## RESULTS

**Effects of tissue type and temperature on the development of *Ch. rufifacies***

The time needed by *Ch.rufifacies* to complete the development from egg to adult decreased with the increase of the temperature from 25°C to 28°C. At 25°C, the minimum time needed to complete the life cycle was recorded for specimens reared on liver (224.14 h), followed by those reared on skeletal muscle (249.33 h) and heart tissue (251.64 h). At 28°C, development occurred faster on heart tissue (178.27 h) followed by liver tissue (178.50 h), and skeletal muscle (186.17 h) (Table 1 and Figure 1).The total development time versus the rearing temperature was found to be significantly different (df= 1, $P \leq 0.0001$, F = 129.26); however, the type of tissue did not have a significant effect on the development when assessed in conjunction with temperature (df= 2, $P = 0.054$, F = 3.61).

Table 1: Time (mean hours ± SE,N=3) needed by Ch. rufifacies to complete each developmental instar when reared on different swine tissues and exposed to different laboratory temperatures.

| Instar of life | Skeletal Muscle | | Liver Tissue | | Heart Tissue | |
|---|---|---|---|---|---|---|
| | 25°C | 28°C | 25°C | 28°C | 25°C | 28°C |
| **1st instar** | 25.1±0.51 | 21.27±0.72 | 25.20±0.97 | 20.00±0.57 | 24.90±0.67 | 20.43±0.71 |
| **2ndinstar** | 25.45±1.92 | 24.53±5.27 | 24.59±1.29 | 15.26±1.26 | 24.34±0.55 | 16.53±1.28 |
| **3rd instar** | 80.30±6.63 | 73.10±0.49 | 60.14±4.14 | 70.21±1.06 | 80.12±7.14 | 69.43±2.43 |
| **Pupa** | 118.47±3.10 | 68.07±2.18 | 114.2±2.90 | 72.43±1.10 | 123.06±1.20 | 72.27±1.28 |
| **Total Developmental Time** | **249.33±6.59** | **186.17±3.58** | **224.14±9.31** | **178.50±0.55** | **251.64±7.55** | **178.27±0.84** |

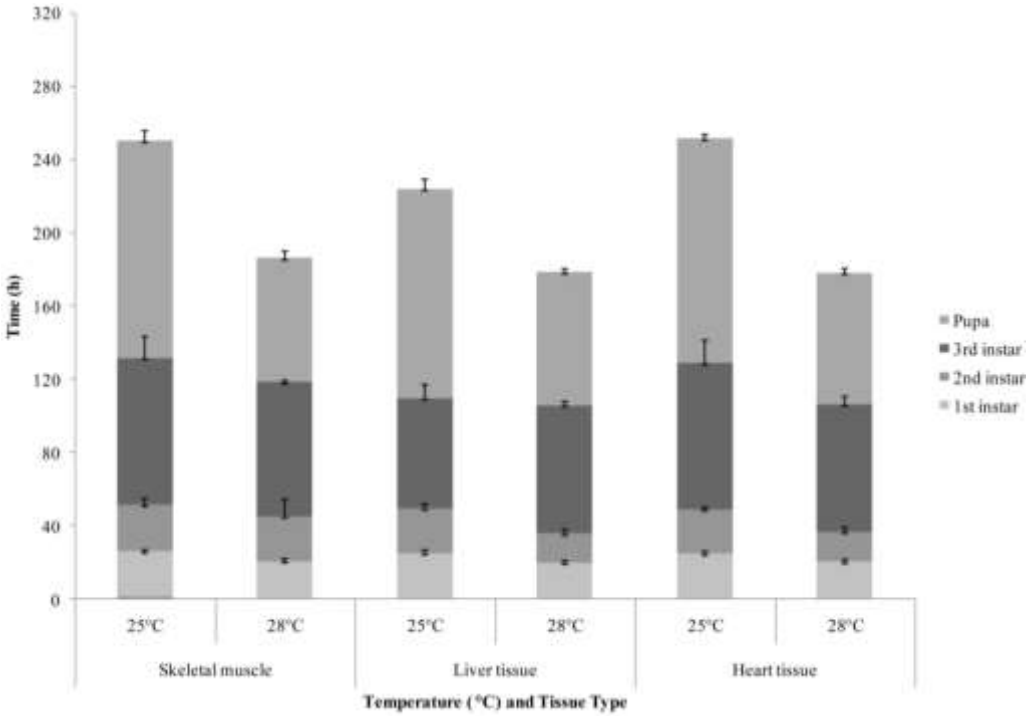



**Figure 1**: Development time (mean hours + SE) needed by *Ch. rufifacies* to complete each developmental instar when reared on different swine tissues and exposed to different laboratory temperatures.

**Effects of tissue type and temperature on the length of *Ch. rufifacies* larvae**

As expected, larval length increased from the 1st to the 3rd instar of life, then began decreasing during the post-feeding period, until reaching the pupa instar. When *Ch. rufifacies* were reared at 25°C, the length of the larvae started to decrease 50 hours after the eclosion from the egg, while larvae reared at 28°C started to decrease their length after 70 hours (Fig 3).
Overall, larvae reared on skeletal muscle at 28°C were the shortest in length (the mean length for any of the instars was the smallest of all the treatments), while larvae reared on heart tissue at 25°C were the largest in length (Fig. 2). The effect of the temperature on the larval length was found to be statistically significant (df= 1, $P \leq 0.0001$, F= 23.85), while the type of tissue on which the larvae were reared on didn't produce any statistical differences on their length (df= 2, $P$= 0.167, F= 1.84).

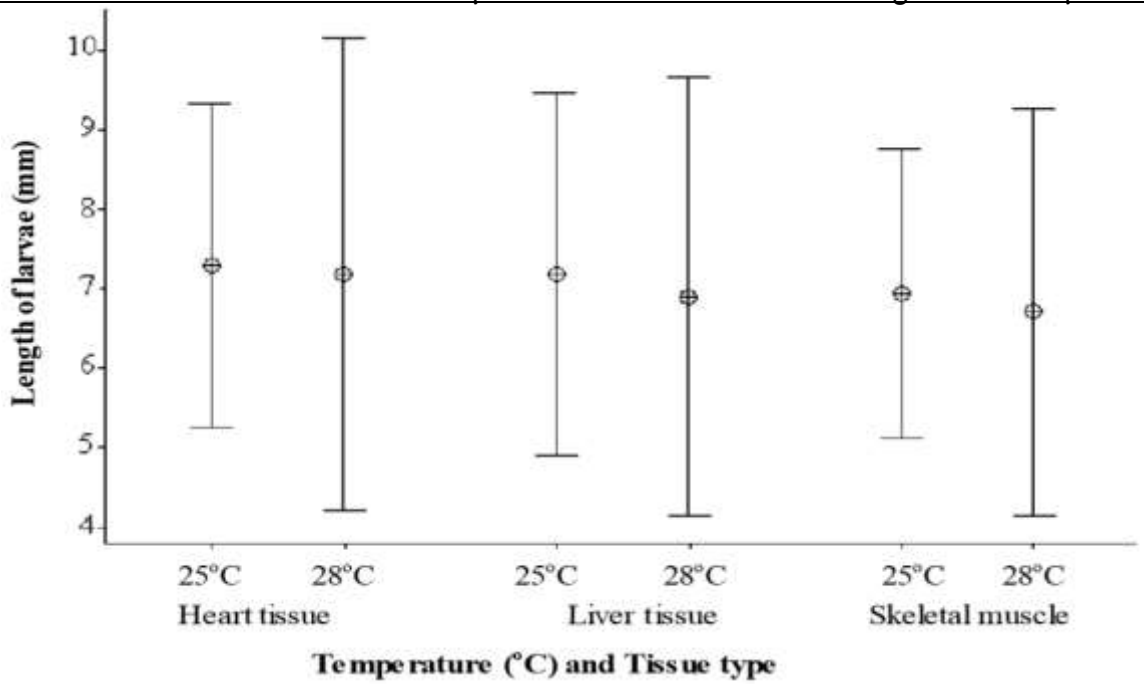
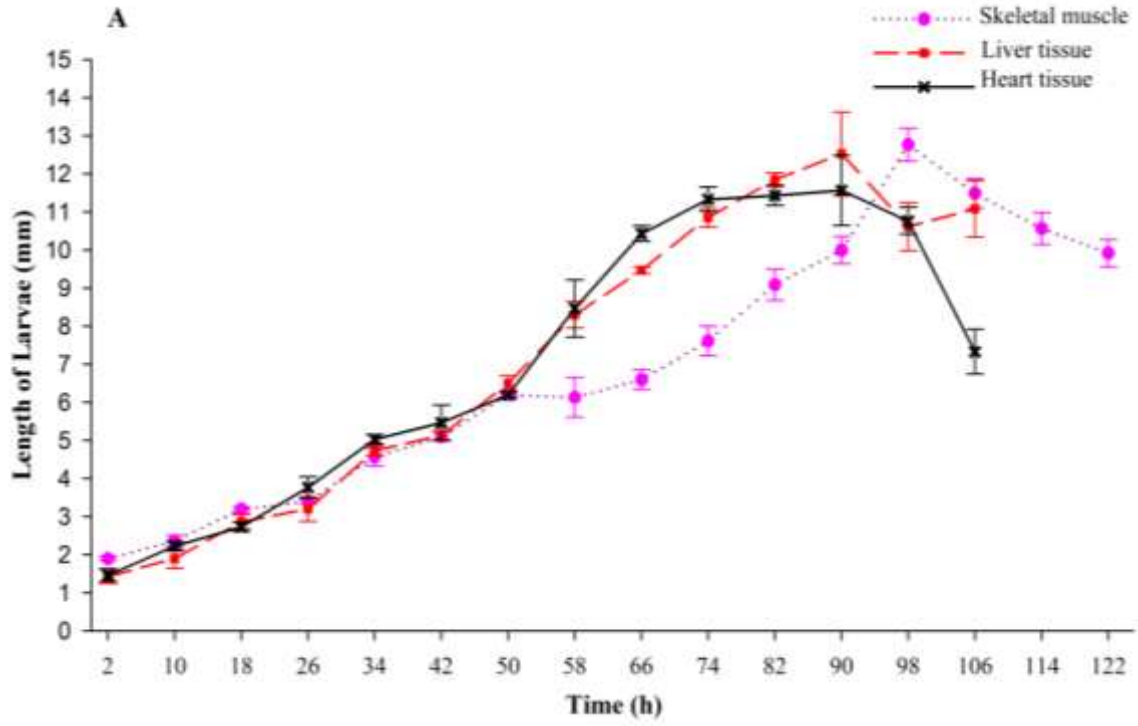



**Figure 2:** Mean length (mm) (±95% confidence intervals) of *Ch. rufifacies* larvae reared on heart tissue, liver tissue and skeletal muscle at 25°C and 28°C.



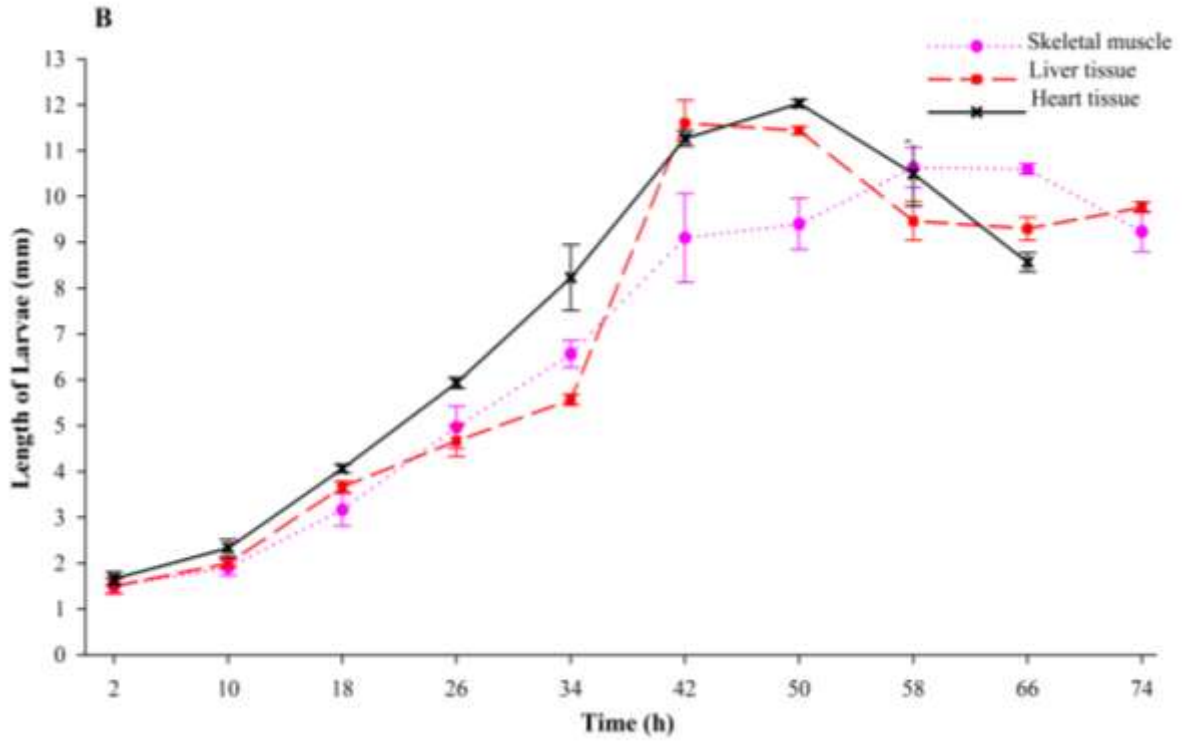



**Figure3:** Modification of the length of *Ch.rufifacies* larvae during their development from egg to pupa, when reared on skeletal muscle, liver tissue, and heart tissue at (A) 25°C and (B) 28°C, 70% RH, and 12:12 (L:D).

**Effects of tissue type and temperature on the cross width of *Ch. rufifacies* larvae**

Larval cross width gradually increased with time for all the treatments at both temperatures (Fig. 5).Larvae showed a sudden increase in their cross width after 34h from the hatching of the egg, regardless of the type of rearing medium (Figure 5 B). *Ch. rufifacies* recorded the lowest mean cross width when reared at 25°C on skeletal muscle, while the largest mean cross width was recorded when larvae were reared at 28°C on liver tissue (Fig 4). Both temperature and type of tissue were found to have significant effects of the larval cross width (df= 1, $P \leq$ 0.0001, F= 101.590 and df= 2, $P$= 0.043, F=3.29, respectively).

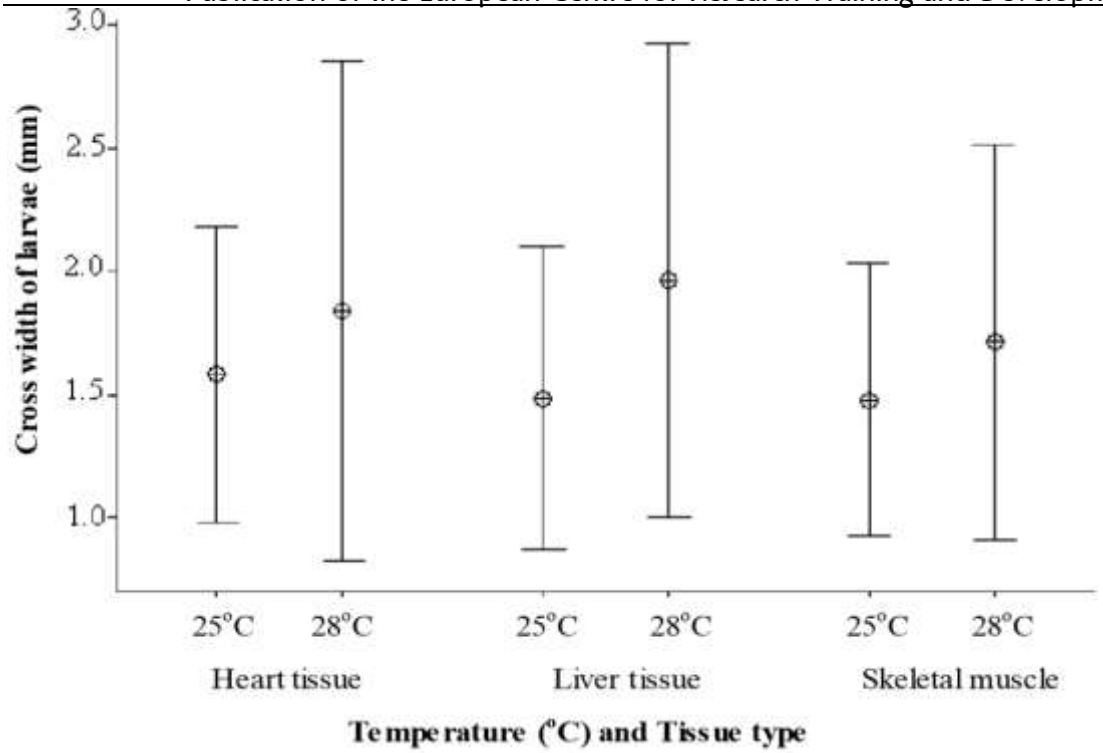
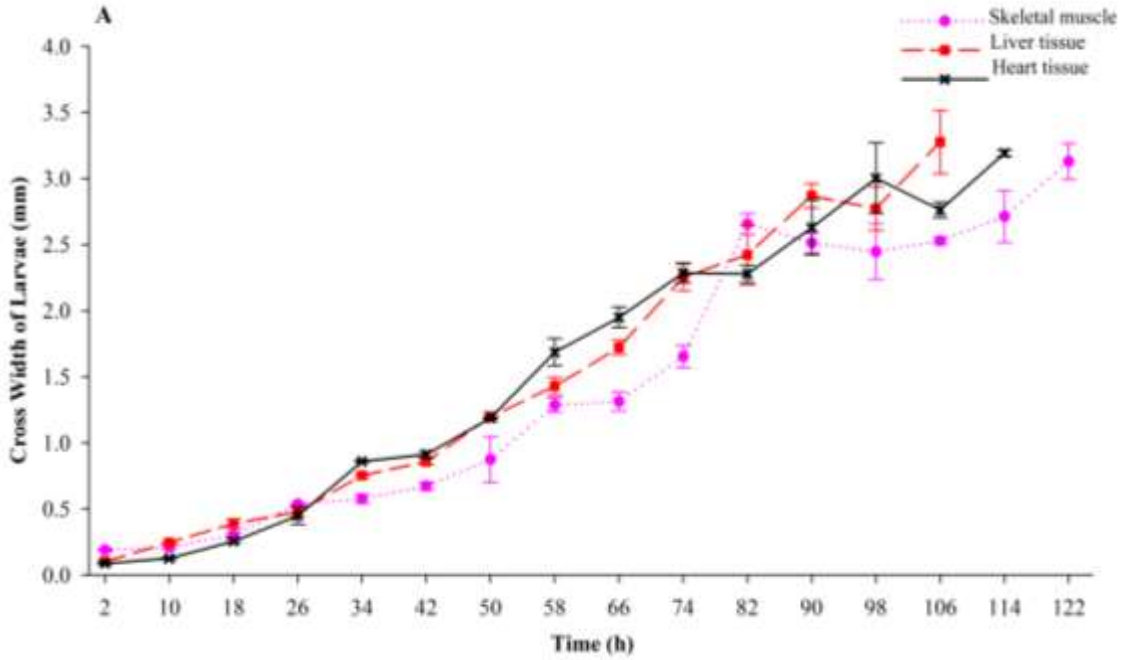



**Figure 4:** Mean cross-width (mm) (±95% confidence intervals) of *Ch. rufifacies* larvae reared on heart tissue, liver tissue and skeletal muscle at 25°C and 28°C.



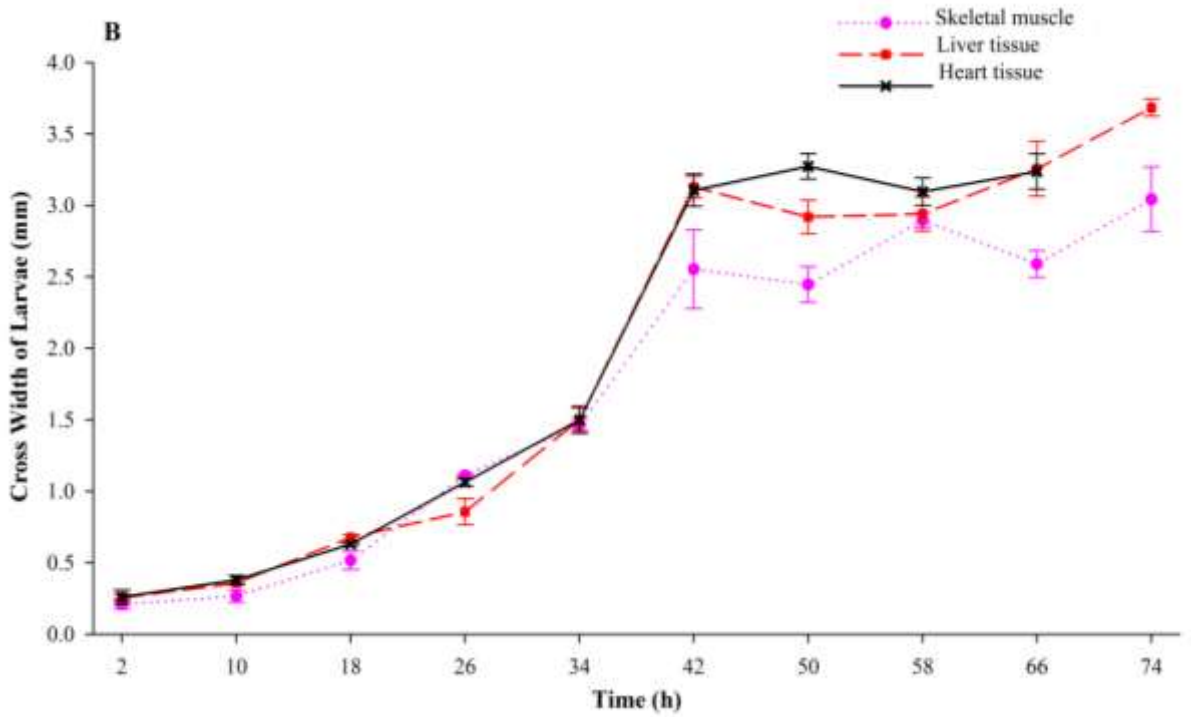



**Figure 5:**Modification of the cross width of *Ch.rufifacies* larvae during their development from egg to pupa, when reared on skeletal muscle, liver tissue, and heart tissue at (A) 25°C and (B) 28°C, 70% RH, and 12:12 (L:D).

## DISCUSSION

The present research is the first to develop growth tables for a Sri Lankan population of the blowfly *Ch. rufifacies*, considering different temperatures (25°C and 28°C) and rearing media (swine skeletal muscle, liver tissue, and heart tissue). Data obtained by this research will be of importance to aid in forensic investigations involving humans and animals found dead under suspicious circumstances and in cases of myiasis caused by suspected negligence in this region of the world.

*Ch. rufifacies* is a common blowfly in many regions of the world, however, developmental studies are limited to certain areas, opening questions regarding the possible geographical and ecological adaptations of this fly to tropical regions such as Sri Lanka (Liyanage et al., 1995). Furthermore, such studies lack in consistency with regards to the flies' rearing medium. Flores et al. (2014) studied the development of *Ch. rufifacies* feeding on porcine, equine, and canine muscle at 20.8°C, 24.8°C, and 28.3°C in Texas, USA. Byrd and Butler (1997) studied the development of this species feeding on swine muscle in Florida (USA) at mean cyclic temperatures of 15.6°C, 21.1°C, 26.7°C, and 35.0°C and a constant temperature of 25.0°C. In addition, Goodbrod and Goff (1990) examined the impact of larval density of *Ch. rufifacies* on their development. This experiment was performed in Hawaii (USA) using bovine liver as the food source for the larvae. Swiger et al. (2014) conducted a study using *Ch. rufifacies* along with *Ch. megacephala* on chicken, a medium which had been rarely used for fly development studies. A study conducted in Queensland (Australia) showed the variation in the development

of *Ch. rufifacies* in decomposing carrions placed in natural environments as well as at four manipulated constant temperature regimes (20°C, 25°C, 28°C and 34°C) (O'flynn, 1983). A comprehensive study conducted by Hu et al. (2019) in China was instead focused on the development of *Ch. rufifacies* on swine muscle at seven temperature regimes (16°C, 19°C, 22°C, 25°C, 28°C, 31°C, and 34°C). The closest regional studies to Sri Lanka on *Ch. rufifacies* were conducted in Thailand and India. In Thailand, Yanmanee et al. (2016) recorded the development of this species on bovine muscle, at temperatures ranging from 15°C to 39°C. In addition, Sukontason et al. (2008) conducted a study in Thailand at 27,4 and 27,7 °C in swine liver. In India, three separate studies were conducted using again bovine muscle, but considering the development at 18-28°C (Verma, 2013) and 24-32°C (Ziddiki and Zambare, 2017). In addition, Subramanian and Mohan (1980) conducted a study at 25.6°C. A summary of the outcomes of these studies is provided in Table 2. It is possible to notice that none of the aforementioned studies on this species have examined the effect of a diet based on different tissues from the same animal (*intraspecific variation*) on the fly development. Such studies are important for determining life history variation of different fly life instars in relation to varying internal and external factors of the tissue (Bambaradeniya et al., 2017).

Intraspecific variation has been tested for sister species, with interesting outcomes. Beuter and Mendes (2013) conducted a development study on *Ch. albiceps* reared on four types of swine tissue (e.g., liver, abdominal fat, muscle, and brain). In this study, the greatest rate of development was observed when larvae were reared on brain tissues, followed by muscle and fat, whereas larvae were not able to complete the development on liver. In addition, Tyssen et al. (2014) carried out a study using four types of bovine tissue (liver, muscle, tongue, and stomach) to determine the impact on the development of *Ch.megacephala* Fabricius (Diptera: Calliphoridae), *Ch. albiceps*, and *Ch.putoria* Wiedemann (Diptera: Calliphoridae). In this study, the lowest rate of development was observed for specimens reared on bovine liver. This study is the very first development study examining the development variation of *Ch. rufifacies* at 25°C and 28°C, when reared on different tissue types of the same source animal.

Results of the present research show that the total time needed by *Ch. rufifacies* to complete their life cycle decreases with the increase of temperature from 25°C to 28°C, for all the tissues tested. Previous studies aligned with the current finding, confirming that *Ch. rufifacies* develop at an optimum at very high temperatures (Yanmanee et al., 2016). In the present study, at 25°C the life cycle of *Ch. rufifacies* reared on any of the tissue types was completed within approximately 10 days; for the treatments at 28°C, the life cycle was completed in 7.5 days. Generally, the type of tissue provided to the fly immatures as rearing medium didn't statistically affect the time needed to complete the cycle of life. Results on sister species, however, show the opposite (Beuter and Mendes, 2013; Thyssen et al., 2014). Tyssen et al. (2014) described *Chrysomya* species (*Ch. albiceps*) having a low rate of development if reared on bovine liver. The authors justified these results describing this medium as the one with the highest tendency to dry up when compared to other tissues. Considering the variation of the larval length in relation to the type of food source, Beuter and Mendes (2013) indicated minimum values in *Ch. albiceps'* length when reared on liver, whereas highest records when reared on muscle and brain tissues. Accordingly with the previous studies, the present research confirmed that when *Ch. rufifacies* larvae were reared on heart tissue, their length was larger – even if not statistically significant, when compared to the liver tissue. Overall, the variations in

growth rates of larvae may be associated to the larvae rearing medium, due to factors such as texture and moisture level of the tissue and the feeding preference of larvae (Thyssen et al., 2014; Bambaradeniya et al., 2019).

The geographical region of the fly population used for the study can also impact the developmental patterns. For example, in determining the temperature of the lower developmental threshold (LDT) for *Ch. rufifacies* in South-East Asia, Yanmaneeet al. (2016) deduced this as11.04±0.58°C (by a linear regression method); whilst for O' Flynn (1983)in Australia, Marchenko (2001) in Russia and Warren (2006) in Canada the LDT temperature was 9°C. Due to the available number of data sets, an appreciation for the broad variability in development for this species globally can be made. Such variation should be taken into consideration when estimating a minPMI for specimens collected from human remains. However, the selection of the most suited development data set and the minimum threshold temperature for the calculation of the minimum PMI should be based on the regional similarities of the considering fly populations as to minimize the bias associated with the genetic distance of geographically apart populations (Yanmanee et al., 2016).

The present study highlighted that the pupation periods of *Ch. rufifacies* required more time when reared at lower temperature. However, for all the tissue tested, the difference in the time required for the pupation between 25°C and 28°C was 50.8 h. A similar observation was recorded by Flores et al. (2014) within the temperature range of 24.4-28.3°C, with a 44 h difference in development. In addition, Hu et al. (2019) recorded a 29.80 h gap for specimens reared at temperatures separated by 3°C.This acceleration of pupation activity could be due to increased metabolism.

Future studies should be directed towards gathering development data of forensically significant fly species specifically reared on different types of swine tissues to increase the relevance of the data to the actual PMI calculations in conducted human remains. Furthermore, studies should be conducted to validate and assess the applicability of development data obtained in laboratory-based settings to the estimation of colonisation times in field situations within various forensic cases.

**Table 2: Summary of development studies carried out on Ch. rufifacies species. N/A not applicable as the specimens didn't complete the cycle of life.**

| Author | Country and year of publication | Rearing medium | Temperature (°C) | Total developmental time (days) |
|---|---|---|---|---|
| Subramanian and Mohan | India (1980) | N.A | 25.6 | 15.5-16 |
| O'Flynn | Australia (1983) | Mixture of muscles | 28 | 11 |
| | | | 34 | 8 |
| Goodbrod and Goff | Hawaii, USA (1990) | Bovine liver | 23.5 | 7.25 |
| Byrd and Butler | Florida, USA (1997) | Lean Swine muscle | 15.6 | 25.5 |
| | | | 21.1 | 13.08 |
| | | | 25 | 12.04 |
| | | | 26.7 | 9.25 |
| | | | 32.2 | 7.5 |
| Sukontason et al. | Thailand (2008) | Swine liver | 27.2 | 14.5 |
| | | | 27.4 | 10 |
| Verma | India (2013) | Bovine muscle | 18-22 | 7 |
| | | | 20-24 | 6 |
| | | | 24-28 | 5 |
| | | | 26-30 | 5 |
| Flores et al. | Texas, USA (2014) | swine | 20.8 | 17 |
| | | | 24.4 | 12.5 |
| | | | 28.3 | 10.1 |
| | | Equine | 20.8 | 15.1 |
| | | | 24.4 | 11.6 |
| | | | 28.3 | 10.1 |
| | | Canine | 20.8 | 13.7 |
| | | | 24.4 | 12 |
| | | | 28.3 | 13.7 |
| Swiger et al. | Florida, USA (2014) | Chicken | 28 | 35.3 |
| Yanmanee et al. | Thailand (2016) | Bovine muscle | 15 | 25.77 |
| | | | 18 | 22.81 |
| | | | 21 | 14.1 |
| | | | 24 | 11.08 |
| | | | 27 | 9.04 |
| | | | 30 | 7.79 |
| | | | 33 | 7 |
| | | | 36 | 7.19 |
| | | | 39 | N/A |
| Siddiki and Zambare | India (2017) | Bovine liver | 24 | 8 |
| | | | 32 | 8.2 |
| Hu et al. | China (2019) | Lean swine muscle | 16 | 36.25 |
| | | | 19 | 23.59 |
| | | | 22 | 16.93 |
| | | | 25 | 12.13 |
| | | | 28 | 9.69 |
| | | | 31 | 8.01 |
| | | | 34 | 6.68 |

**Acknowledgements**
The authors would like to thank Shivani Radia and Veronica Mitchell for their useful suggestions.

**REFERENCES**

1. Amendt, J., campobasso, C. P., gaudry, E., Reiter, C., Leblanc, H. N., Hall, M. J. (2007). Best practice in forensic entomology—standards and guidelines. -*International journal of legal medicine*, 121(2): 90-104.
2. Bambaradeniya, Y. T. B., Karunaratne, W. A., Tomberlin, J. K., Goonerathne, I., Kotakadeniya, R. B., &Magni, P. A. (2019). Effect of Temperature and Tissue Type on the Development of the Forensic Fly *Chrysomya megacephala* (Diptera: Calliphoridae). *Journal of Medical Entomology*, 56, 1571- 1581.
3. Bambaradeniya,Y.T.B., Karunarathne, W.A.I.P., Goonerathne,I., Kotakadeniya, R.B. and Tomberlin, J.K. (2018). Effect of Temperature and Tissue Type on the Development of Myiasis Causing fly; *Chrysomya bezziana* (Diptera: Calliphoridae). *Journal of Medical Entomology*, 52, 625- 631.
4. Bambaradeniya,Y.T.B., Karunarathne, W.A.I.P., Tomberlin, J.K., Goonerathne, I., and Kotakadeniya, R.B. (2017). Temperature and Tissue Type Impact Development of *Lucilia cuprina* (Diptera: Calliphoridae) in Sri Lanka. *Journal of Medical Entomology*, 55, 285- 291.
5. Baumgartner, D. L. (1986). The hairy maggot blow fly *Chrysomya rufifacies* (Macquart) confirmed in Arizona. *Journal of Entomological Science*, 21: 130-132
6. Baumgartner, D. L. (1993). Review of *Chrysomya rufifacies* (Diptera: Calliphoridae). *Journal of Medical Entomology*, 30, 338-352.
7. Beuter, L., & Mendes, J. (2013). Development of *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) in different pig tissues. *Neotropical entomology*, 42, 426-430.
8. Brundage, A., Benbow, M. E., &Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion blow fly (Diptera: Calliphoridae) species. *Ecological entomology*, 39, 539-547.
9. Byrd, J. H., Castner, J. L. (2009). Forensic entomology: the utility of arthropods in legal investigations.- CRC press.
10. Byrd, J. H., & Butler, J. F. (1997). Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development. *Journal of Medical Entomology*, 34, 353-358.
11. Clark, K., Evans, L., & Wall, R. (2006). Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. *Forensic Science International*, 156, 145-149.
12. Day, D. M., &Wallman, J. F. (2006). Width as an alternative measurement to length for post-mortem interval estimations using Calliphora augur (Diptera: Calliphoridae) larvae. *Forensic science international*, *159*(2-3), 158-167.
13. Dekeirsschieter, J., Frederick, C., Verheggen, F. J., Drugmand, D., &Haubruge, E. (2013). Diversity of forensic rove beetles (Coleoptera, Staphylinidae) associated with decaying pig carcass in a forest biotope. *Journal of forensic sciences*, 58, 1032-1040.

14. Faria, L. D. B., Trinca, L. A., & Godoy, W. A. C. (2004). Cannibalistic behavior and functional response in *Chrysomya albiceps* (Diptera: Calliphoridae). *Journal of Insect behavior*, *17*(2), 251-261.
15. Flores, M., Longnecker, M., &Tomberlin, J. K. (2014). Effects of temperature and tissue type on *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart) development. *Forensic science international*, 245, 24-29.
16. Goodbrod, J. R., & Goff, M. L. (1990). Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *Journal of Medical Entomology*, 27, 338-343.
17. Holdaway, F. G. (1933). The synonymy and distribution of *Chrysomyia rufifacies* (Macq.), an Australian sheep blowfly. *Bulletin of Entomological Research*, 24, 549-560.
18. Hu, G., Wang, Y., Sun, Y., Zhang, Y., Wang, M., & Wang, J. (2019). Development of *Chrysomya rufifacies* (Diptera: Calliphoridae) at Constant Temperatures Within its Colony Range in Yangtze River Delta Region of China. *Journal of Medical Entomology*, 56, 1215- 1224.
19. James, M. T. (1947). The flies that cause myiasis in man (No. 631). US Department of Agriculture.
20. Liyanage, S., Fernando, R., Kodagoda, N., and Samarawickrema, W.(1995). Estimation of time elapsed since death using entomological methods 1.*Sri Lanka Association for the Advancement of Science*. Proceeding part 1, 58.
21. Marchenko, M. I. (2001). Medicolegal relevance of cadaver entomofauna for the determination of the time of death. *Forensic science international*, 120, 89-109.
22. Minitab 14 Statistical Software. 2005. [Computer software]. Minitab, Inc., State College, PA. Available from www.minitab.com
23. O'Flynn, M. A. (1983). The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology*, 22, 137-148.
24. Shiao, S. F., & Yeh, T. C. (2008). Larval competition of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae): behavior and ecological studies of two blow fly species of forensic significance. *Journal of medical entomology*, 45, 785-799.
25. Siddiki, S., &Zambare, S. P. (2017). Studies on Time Duration of Life Stages of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) during Different Seasons. *Journal of Forensic. Research*, 8, 379.
26. Silva, J. O., Carvalho-Filho, F. D. S., Esposito, M. C., & Reis, G. A. (2012). First record of *Chrysomya rufifacies* (Macquart) (Diptera, Calliphoridae) from Brazil. *RevistaBrasileira de Entomologia*, 56, 115-118.
27. Smith, K. G. V. (1986). A Manual of Forensic Entomology. British Museum (Natural History), London, and Cornell University Press, Ithaca, NY.
28. Subramanian, H., & Mohan, K. R. (1980). Biology of the blowflies *Chrysomyia megacephala*, *Chrysomyia rufifacies* and *Lucilia cuprina*. *Kerala Journal of Veterinary Science*, 11, 252-261.
29. Sukontason, K. L., Narongchai, P., Sripakdee, D., Boonchu, N., Chaiwong, T., Ngern-Klun, R &Sukontason, K. (2005). First report of human myiasis caused by *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) in Thailand, and its implication in forensic entomology. *Journal of Medical Entomology*, 42, 702-704.

30. Sukontason, K. L., Narongchai, P., Sripakdee, D., Boonchu, N., Chaiwong, T., Ngern-Klun, R., &Sukontason, K. (2005). First report of human myiasis caused by *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) in Thailand, and its implication in forensic entomology. *Journal of Medical Entomology*, *42*(4), 702-704.
31. Sukontason, K., Piangjai, S., Siriwattanarungsee, S., &Sukontason, K. L. (2008). Morphology and developmental rate of blowflies *Chrysomya megacephala* and *Chrysomya rufifacies* in Thailand: application in forensic entomology. *Parasitology Research*, 102, 1207-1216.
32. Swiger, S. L., Hogsette, J. A., & Butler, J. F. (2014). Laboratory colonization of the blow flies, *Chrysomya megacephala* (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Diptera: Calliphoridae). *Journal of economic entomology*, 107, 1780-1784.
33. Thyssen, P. J., de Souza, C. M., Shimamoto, P. M., de BrittoSalewski, T., & Moretti, T. C. (2014). Rates of development of immatures of three species of *Chrysomya* (Diptera: Calliphoridae) reared in different types of animal tissues: implications for estimating the postmortem interval. *Parasitology research*, 113, 3373-3380.
34. Verma, K. (2013). Effects of Codeine, Sodium Pentothal and Different Temperature Factors on the Growth Rate Development of *Chrysomya rufifacies* for the Forensic Entomotoxicological Purposes. Journal of Bioanal Biomed, 5, 006-012.
35. Warren, J. (2006). The development of *Protophormia terraenovae* (Robineau) at constant and fluctuating temperatures. (MA thesis) Simon fraser University, Burnaby, British Colombia.
36. Yanmanee, S., Husemann, M., Benbow, M. E., &Suwannapong, G. (2016). Larval development rates of *Chrysomya rufifacies*Macquart, 1842 (Diptera: Calliphoridae) within its native range in South-East Asia. *Forensic science international*, 266, 63-67.