CHAPTER – 5

DISCUSSION

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Calliphorids are regarded as a prominent entomological evidence for estimating the PMI and ante mortem trauma. Hence, the identification of blow fly specimens are considered significant because of the pertinent evidences they offer for forensic research (Catts and Goff, 1992; Sukontason et al., 2007). The findings of the present study provide significant contribution towards the forensic science research by identifying four blow fly species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* based on the morphological and molecular characteristics with special inference on their life cycle.

5.1. Blow fly fauna of Central Kerala

Seventeen species belonging to 4 subfamily and 8 genera were recorded during this study. The genera present in Kerala are *Bengalia* (*B. jejuna* Fabricius, 1794, *B. surcoufi* Senior-White,1924), *Hemipyrellia* (*H. ligurriens* Wiedemann, 1830), *Lucilia* (*L. amplullacea* Villeneuve, 1922, *L. papuensis* Macquart, 1843, *L. sericata* Meigen, 1826), *Chrysomya* (*C. megacephala* Fabricius, 1794, *C. chani* Kurahashi, 1979, *C. nigripes* Aubertin, 1932, *C. rufifacies* Macquart, 1842, *C. albiceps* Wiedemann, 1819), *Idiella* (*I. euidielloides* Senior-White, 1922, *I. mandarina* Wiedemann, 1830), *Stomorhina* (*S. discolor* Fabricius, 1794), *Cosmina (C. simplex* Walker 1858, *C. bicolor* Walker, 1856) and *Strongyloneura* (*S. prolata* Walker, 1860).

Bharti, (2011) enlisted the various blow files of India. Bharti and Singh, (2017) reported the presence of *C. megacephala* Fabricius (1794), *C. rufifacies* Macquart (1842), *C. nigripes* Aubertin, (1932), and *C. chani* Kurahashi, (1979) from Calicut, Kerala, India. Radhakrishnan et al., (2012) have reported *C. albiceps* from Kerala. In addition to this, the

presence of various blow flies belonging to the central Kerala region were reported by previous workers (Subramanian & Mohan, 1980, Nandi, 2004, and Arce et al., 2020, Reject Paul and Binoy, 2021 & 2022).

5.1.1. Identification of blow flies

The examination of various morphological structures including the features of head, thorax, wings, egg, and the various developmental stages revealed the prominent taxonomic characters of blow flies.

C. megacephala has orange coloured antennae, arista and palpi. Outer vertical bristles were absent in males. Parafacialia and genae were also completely orange in colour. Hairs and setulae on the parafacialia were yellowish and anterior spiracles were dark brown in colour. Sub costal sclerite covered with brown felted pubescence and also with small erect hairs. A row of setulae were seen on the upper posterior margin on the stem vein. Upper calypter was with ventral hairs on the opaque white basal part. In addition to this, the colour of the thorax and abdomen was found to be bluish green .These observation were validated by previous works (Bharti et al., 2007, Bharti & Kurahashi, 2009, Sukontason et al., 2008, Sukontason et al., 2011, Ramaraj et al., 2014, Bharti, 2014, Bala and Singh 2015, Claver and Yaqub, 2015, Abd Al Galil & Zambare, 2015 , Siddiki & Zambare, 2017).

In the present investigation, adult flies of *C. rufifacies* were identified with the following characteristics. Parafrontalia was narrow with a black colour in the upper half. The lower half was covered with silver tomentum with upstanding white hairs. Frons was greyish in colour. Parafacialia and genae were light yellowish in colour and covered with

white hairs. Anterior spiracle was white in colour. The lower squama was slightly fuscous with white hairs. These obervations has been validated with the earlier reports. (Sukontason et al., 2008, Bharti, 2014, Bala and Singh, 2015, Abd Al Galil & Zambare, 2015, Siddiki & Zambare, 2017).

C. chani was having fuscous to black coloured genae and parafacialia .Seulae and hairs on parafacialia and parafrontalia were blackish in colour. 1st and 2nd antennal segments were brown to fuscous in colour. Thorax was bluish green coloured with anterior spiracle fuscous black in colour. Black setae were present on the upper margin of 3rd longitudinal vein. Base of alar squamae is white in colour. These observations were similar to the observation made earlier by Bharti et al., (2014) and Kurahashi (1997).

In the case of *H. ligurriens,* genae and parafrontalia was silver white in colour. Antennae were tawny yellow to brownish in colour. The upper squama was creamish white in colour with short cilia and lower squama has light brown cilia. Short setulae were present on the dorsal and ventral aspects of 3rd longitudinal vein. Previous works by Tumrasvin et al., (1979), Kurahashi and Chowanadisai (2001), Sinha and Nandi (2007) and Bunchu et al., (2012), have validated these observations.

Molecular characterization of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were done. The molecular analysis of the sequences such as SR1859-COI-F_E03, SR2284-COF_A07, SR2040-A-COF_D05, and SR1719-A-COF_B03 showed strong boot strap support towards the corresponding nucletotide sequences representing *C. megacephala, C. rufifacies, C.chani,* and *H. ligurriens* respectively. Many of the previous studies have substantiated the applicability of DNA-based strategies, especially the use of mitochondrial COI gene to identify blow flies (Sukontason et al., 2008, Mendonça et al.,

2010, Bharti & Singh, 2017, Qiu et al., 2017, Yusseff and Agnarsson., 2017, Abd Al Galil & Zambare, 2017).

5.2. Seasonal differences in blow fly population in Central Kerala

The present investigation has analyzed the seasonal variation in the abundance of blow flies with special inference to summer, monsoon and winter seasons as such data is totally lacking in Southern India.

Effect of season on the abundance was found to be significant for *C. megacephala* (F = 52.773; P = < 0.001) (Table 4.1), *C. rufifacies* (F = 3.935; 0.024; P = 0.022) (Table 4.4), *C chani* (F = 33.586; P =< 0.001) (Table 4.7) and *H. ligurriens* (F = 47.470; P = < 0.001) (Table 4.10). Wall et al., (2001) analyzed the seasonal abundance of C. *megacephala* in Kerala and their results showed peak population of the species during monsoon which was consistent with the present study.

Seasonal influence on the abundance of blow flies were studied in earlier works and their results showed pronounced seasonal fluctuation in the population of blow flies in response to climatic conditions (Evaldo et al., 2008, Brundage et al., 2011, Zabala et al., 2014, Sontigun et al., 2018, Jeong et al., 2022).

5.3. Life history of blow flies in carrion

Developmental stages of *C. megacephala* was studied in the current investigation in which the eggs were oblong, creamish white in colour and the caudal end was slightly wider than the anterior end. The cephalopharyngeal skeleton was present with prominent comma shaped dorsal sclerite. Dorsal cornua has uniform width and longer than the ventral cornua. Posterior spiracles were clearly seen with three spiracular slits. A dark pigmented

incomplete peritreme was seen surrounding the three slits with a bent in the middle slit. The spinous bands were found to be restricted to the lateral and ventral surfaces. These observations were consistent with the earlier reported works (Ishijima, 1967, Omar., 2002, Sukontason et al., 2007, Sukontason et al., 2008, Ramaraj et al., 2014).

Present studies on *C. rufifacies* revealed that the larval body was hairy with long tubercles with broad base and tapered with pointed spines at the tip. Spines were present on the anterior and posterior margins on the ventral and lateral surfaces of all the three thoracic segments. Cephalopharyngeal skeleton was with a shorter dorsal cornua. Parastomal and accessory sclerites were absent. Posterior spiracles were clearly seen with three spiracular slits with densly dark pigmented incomplete peritreme surrounding the three slits with a medial bent in the middle slit. The results obtained were in consonance with the previously reported works (Sukontason et al., 2006, Yanmanee et al., 2016, Abd Al Galil & Zambare, 2017).

Studies on the developmental stages of *C. chani* revealed eggs as creamish white in colour. Many tubercles were present on all the abdominal segments except the caudal segment of larval body. Cephalopharngeal skeleton has heavily sclerotized accessory sclerite, upwardly curved thin parastomal bar and intermediate sclerite. Posterior spiracles were having dark pigmented thick sclerotized complete peritremes. The button was indistinct. The features described were consistent with the earlier reports (Sukontason et al., (2018).

Developmental stages of *H. ligurriens* were studied in detail which showed moderately sized middle dorsal tubercles on the larval body in comparison to the inner and outer tubercles. Pigmentation of cephalopharyngeal skeleton was darker. Well-developed

comma shaped dorsal sclerite was present. Ventral cornua was seen equal in length to dorsal cornua. Posterior spiracles were dark brown with complete peritreme and button. The characteristics observed in the present study were consistent with the previous reports (Sinha and Nandi, 2007, Bunchu et al., 2012, Eliza and Zuha, 2018).

The present investigation has used SEM for the morphology-based identification of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens*. SEM examination of larval instars in the present study revealed characterisitc ultra structural details ; three lobed labium, semicircle shaped ventral organ and post spiracular discs with spiracular slits surrounded by fine spiracular hairs in *C. megacephala*, elongated tubercles with many sharp tipped fine spines on larval body and broad posterior spiracular hairs in *C. rufifacies*, branched mouth hooks with two to three rows of curved sharp tipped spines, bilaterally arranged oral cirri, filiform spines on the anal segment and fine tipped thoracic spines with flat triangular base in *C. chani* and conspicuous oral cirri in the shape of curved spines, trilobed labium with well-developed fleshy lateral lobes, three rows of spine clusters present dorso-medial to the functional mouth opening, slender tipped acuminate spines with bulbous base on the anterior and posterior margins of the ventral and lateral surfaces of thoracic segments and filiform spines of last anal segment in *H. ligurriens.*

The results were consistent with the earlier reported works (Sukontason et al., 2008, Klongklaew et al., 2012; Szpila et al., 2013, Sanit et al., 2012). Characteristic bilateral arrangement of medially curved spinulous oral cirri was not reported in *H. ligurriens* in earlier studies (Sukontason et al., 2008). Spines on the thoracic and last anal segment of *H. ligurriens* were different from the observations made by Sukontason et al., (2008), where

thoracic spines were acuminate with flat broad bases and anal spines were verrucate and echinate.

The average mating time (hrs), pre oviposition period (days), periodicity of egg laying (days), number of eggs laid in a day and during the life span in *C. megacephala, C. rufifacies*, *C.chani* and *H. ligurriens* were studied. It was 10 ± 04, 9.59 ± 0.89, 3.67 ± 0.37, 215.74 ± 22.29 and 1451.26 ± 83.71 in *C. megacephala,* 9 ± 3*,* 8.15 ± 0.99, 4.44 ± 0.38, 247.74 ± 28.43 and 1842.26 ± 97.99 in *C. rufifacies,* 10 ± 4, 8.74 ± 1.26 ,4.59 ± 0.31, 240.15 ± 19.6 and 1667.52 ± 49.78 in *C. chani* and 10 ± 04 , 9.59 ± 0.89, 3.67 ± 0.37, 215.74 ± 22.29 and 1451.26 ± 83.71 in *H. ligurriens* respectively. The results obtained were consistent with the earlier reported works (Rosatti et al., 2015, Lertthamnongtham et al., 2003, Yang & Shiao, 2012, Subramanian & Mohan, 1980).

Life cycle related parameters of larval instars like length, weight, life duration and pupation were studied for all the four species of blow flies. It was observed that the average length (mm) and weight (mg) of first, second, third and post feeding stage of instars for *C. megacephala* (Tables. 4.17 - 4.20; 4.22 - 4.25), *C. rufifacies* (Tables. 4.37 – 4.40; Tables. 4.42 – 4.45), *C. chani* (Tables. 4.57 – 4.60; 4.62 – 4.65) and *H. ligurriens* (Tables. 4.77 – 4.80; 4.82 - 4.85) were in consonance with the earlier reports (Gabre et al., 2005, Bharti et al., 2007, Sinha and Nandi., 2007, Sukontason et al., 2008, Bala and Singh, 2015, Bansode et al., 2016, Chakraborty et al., 2016, Siddiki & Zambare, 2017, Badenhorst and Villet, 2018, Zhang et al., 2019).

The life cycle duration (hrs) of all developmental stages such as egg, first, second and third instars, post feeding stage and pupa and the total life cycle from egg till the emergence of adult fly were studied. These were 18, 17, 22, 40, 30, 99 and 227 ± 59 for *C.*

megacephala, 16, 19, 23, 37, 27, 91 and 212.78 ± 8.98 for *C. rufifacies,* 21, 18, 23, 36, 36, 119 and 252.89 ± 17.16 for *C. chani* and 27, 17, 25, 59, 115, 153 and 395.88 ± 35.82 for *H. ligurriens.* The results were showing similarities with earlier works (Subramanian & Mohan, 1980, Bharti et al., 2007, Bala and Singh, 2015, Siddiki & Zambare, 2017). However, higher life cycle duration in all the stages were also reported in some previous works (Sukontason et al., 2008, Bunchu et al., 2012, Zhang et al., 2019).

The survival rate (%) of all the developmental stages for all the four species were studied. It was found that the survival rate in egg, first, second, third instars and pupae were 86.32 ± 6.50, 84.22 ± 7.27, 75.98 ± 8.03, 69.26 ± 4.82 and 69.4 ± 5.38 for *C. megacephala,* 82.47 ± 5.45, 81.90 ± 6.16, 76.03 ± 4.66, 72.27 ± 5.92 and 72.33 ± 6.14 for *C. rufifacies,* 75.84 ± 5.53, 76.04 ± 5.25, 76.45 ± 4.50, 68.49 ± 5.19 and 66.69 ± 3.81 for *C. chani* and 72.45 ±5.94, 72.57 ± 5.68, 70.78 ± 5.81, 69.78 ± 6.69, 69.77 ± 6.95 for *H. ligurriens.* The results of the study were in consonance with the earlier reports by Pitts et al., (2005), Mądra et al., (2017) and Zhang et al., (2019).

5.4. Effect of temperature and humidity on the life cycle of Calliphorid flies

Pre-oviposition period, eggs laid in a day, periodicity of egg laying (days) and the total number of eggs laid by four species of blow flies in its life span have been investigated.

Effect of season on the pre-oviposition period was found to be significant for *C .megacephala* (F = 19.73; P = < 0.001) (Table. 4.13), *C. rufifacies* (F = 23.444; P = < 0.001) (Table. 4.33), *C. chani* (F = 40.111, P = < 0.001) (Table. 4.53) and *H. ligurriens* (F = 13.727; P = < 0.001) (Table. 4.73). The pre-oviposition period was significantly higher in

winter in all the four species. Effect of year was found to be significant for *C. megacephala* (F = 11.545; P = < 0.001) (Table. 4.13). The interaction between years and seasons in *C. chani* were found to be significant (F = 4.778; P = 0.008) (Table. 4.53).

Effect of season on the number of eggs laid per day was found to be significant for *C. megacephala* (F = 74.306; P = < 0.001) (Table. 4.14), *C. rufifacies* (F = 223.63; P = < 0.001) (Table. 4.34), *C.chani* (F = 133.56; P = < 0.001) (Table. 4.54) and *H. ligurriens* (F = 417.585; P = < 0.001) (Table. 4.74). The number of eggs laid per day by all species were significantly higher in monsoon. Effect of year on egg laying was found to be significant for *C. rufifacies* (F = 3.67; P = 0.046) (Table. 4.34). The interaction between years and seasons were found to be significant for *H. ligurriens* (F = 3.562; P = 0.026) (Table. 4.74).

Effect of season on the periodicity of egg laying was found to be significant in *C. megacephala* (F = 6.300; P = < 0.008) (Table. 4.15), *C. rufifacies* (F = 5.727; P = 0.012) (Table. 4.35), *C. chani* (F = 901.52; P = < 0.001) (Table. 4.55) and *H. ligurriens* (F = 576.86; P = < 0.001) (Table. 4.75).

The periodicity of egg laying was significantly higher during winter in all species. Effect of year on the number of eggs laid was found to be significant in *C. megacephala* (F = 29.859; P = < 0.001) (Table. 4.15), *C. chani* (F=12.54; P =< 0.001) (Table. 4.55) and *H. ligurriens* (F=5.199; P =0.017) (Table. 4.75). The interaction between years and seasons were found to be non-significant in *C. rufifacies* (F = 3.28; P = 0.035) (Table. 4.35) and *C. chani* (F =3.65; P =0.024) (Table. 4.55).

Effect of season on the total number of eggs laid during its life span was found to be significant in *C. megacephala* (F = 323.32; P = < 0.001) (Table. 4.16), *C. rufifacies* (F =

835.8; P = < 0.001) (Table. 4.36), *C. chani* (F = 901.52; P = < 0.001) (Table. 4.56), and *H. ligurriens* (F = 576.86; P = < 0.001) (Table. 4.76). The number of eggs laid in all species were significantly higher in monsoon. Significant yearly fluctuations in egg laying were also noted in *C. chani* (F = 12.54; P = < 0.001) (Table. 4.56) and *H. ligurriens* ((F = 5.199; P = 0.017) (Table. 4.76). The interaction between years and seasons were found to be non-significant in *C. rufifacies* (F = 3.28; P = 0.035) (Table. 4.36) and *C .chani* (F = 3.65; P = 0.024) (Table. 4.56).

The time dependent data corresponding to the length and weight of developmental stages of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* during different seasons were investigated in this study. The seasonal data on the length and weight of larval instars were showing statistically significant differences due to significant interactions between years, seasons and larval stages in all the four species.

Interaction studies on year, season and stage was found to be significant for length in *C. megacephala* (F = 115.12; P = <0.001) (Table. 4.21), *C. rufifacies* (F = 0.95; P = < 0.001) (Table. 4.41) and *C. chani* (F = 0.335; P = <0.001) (Table. 4.61). However only the interaction between season and stage was significant for *H. ligurriens* (F = 9.15, P < 0.001) (Table. 4.81).

This indicated that seasonal variations in length of each stage were different in different years. The length of I^{st}, II^{nd} and III^{rd} instar were significantly higher in monsoon in *C. megacephala and C. rufifacies,* higher in summer for *C. chani* and in winter for *H. ligurriens.* The earlier studies (Singh et al., 1999, Bala and Singh, 2015) justified the strong relation between the temperature and humidity on the length and weight of larval instars.

Interaction studies on year, season and stage was found to be significant for the weight of *C. megacephala* (F = 14.365; P <0.001) (Table. 4.26), *C. rufifacies* (F = 10.095; P = < 0.001) (Table. 4.46), *C. chani* (F = 7.656; P = < 0.001) (Table. 4.66) and *H. ligurriens* (F = 61.121; P < 0.001) (Table. 4.86) indicating that seasonal variations in weight of each stage are different in different years.

The weight was significantly higher in monsoon for IInd and IIIrd instar and post feeding stage in *C. megacephala,* IInd and post feeding stage in *C. rufifacies.* The weight was significantly higher in summer for IInd and IIIrd instar and post feeding stage of *C. chani*, IInd instar and post feeding stage in *H. ligurriens.*

Recent studies also confirmed the effect of various environmental parameters including temperature and humidity on the life cycle of Calliphorid flies (Salimi et al., 2018, Reject Paul and Binoy, 2021& 2022). In a study conducted in Thailand, the length of the *C. megacephala* and *C. rufifacies* increased during the summer season which may be due to the changes in the biogeoclimatic conditions (Sukontason et al., 2008).

Length and weight of the larval instars were found to be increasing in all the four species in the current study. However, the length and weight got reduced during the post feeding stage in all species. These results were consistent with the previous reports from India and from other countries (Acosta et al., 2021, Yang et al., 2016, Bala and Singh, 2015, Siddiki & Zambare, 2017).

Rearing data on duration (hrs) of life stages related to *C. megacephala* revealed that there was significant interaction between seasons and incubation (F = 408.40; P < 0.001), second instar stage (F = 8.555; P < 0.036), post feeding stage (F = 8.704; P < 0.035) and

pupation stage (F = 480.82; P < 0.001) (Table. 4.27). These results were significantly different from the observations made by Bala and Singh (2015) as it took 10, 40, 60 and 5 at 32°C, 10, 40, 60 and 5 at 29°C and 25, 55, 85, and 5 at 25°C.

Present study revealed that total duration taken by the fly from egg stage till the emergence of adult fly was 168.00 ± 5.29 in summer, 227.00 ± 22.52 in monsoon and 286.00 ± 23.26 in winter which were significantly different from 211 in summer, 239 in monsoon and 263 in winter in Maharashtra (Siddiki & Zambare, 2017). The duration of 241.33±1.15 in monsoon (Nordin et.al., 2020) and 224 at 25.6°C (Subramanian and Mohan, 1980) were similar to the current observation.

Rearing data on duration (hrs) of life stages related to *C. rufifacies* revealed that there was significant interaction between seasons and the second instar stage (F = 12.250; P < 0.020), third instar stage (F = 491.46; P < 0.001), post feeding stage (F = 45.953; P < 0.002) and pupation stage (F = 115.71; P < 0.001) (Table. 4.47). These results were significantly different from the observations made by Bala and Singh (2015) as it took 5, 25, 45 and 5 at 32°C, 10, 55, 35 and 5 at 29°C and 15, 40, 75 and 5 at 25°C.

Present study revealed that total duration taken by the fly from the egg stage till the emergence of adult fly was 148.33 ± 6.43 in summer, 223.00 ± 13.45 in monsoon and 286.00 ± 23.26 in winter which were significantly different from the duration of 216 in summer, 239 in monsoon in Maharashtra (Siddiki & Zambare, 2017). But the duration of 286 in winter reported by the same authors were similar to the present results. Life cycle duration during monsoon was significantly lesser than that reported by Nordin et al., (2020) in Malaysia which was 266. Duration of 193 reported by Subramanian and Mohan (1980) at 25.6°C was significantly shorter than the present result.

The seasonal life cycle data on *C. chani* were found to be extremely scanty and the data obtained in this investigation could be recognized as the pioneering study. The data on duration (hrs) of life stages on rearing of *C. chani* revealed that there was significant interaction between seasons and incubation period ($F = 42.08$; $P < 0.002$) and post feeding stage ($F = 15.69$; $P= 0.013$) (Table. 4.67). Total duration taken by the fly from the egg stage till the emergence of adult fly was 192.33 ± 8.15 in summer, 246 ± 20.08 in monsoon and 320.33 ± 24.03 in winter.

The data on duration (hrs) of life stages on rearing of *H. ligurriens* revealed that there were significant interactions between seasons and incubation period ($F = 84.88$; $P<0.001$), second instar stage ($F = 8.269$; $P < 0.038$) and pupation stage ($F = 128.58$; $P < 0.001$) (Table. 4.87). The durations observed during winter season was found to be significantly different from the earlier observations made by Sinha and Nandi, (2007) in which the egg, first instar, second instar, third instar and pupal stages took 15, 6, 14, 104, and 186 respectively for completing the developmental process.

The total duration taken by the fly from the egg stage till the emergence of adult fly was 300.00 ± 23.07 in summer, 430.33 ± 33.5 in monsoon and 457.33 ± 54.31 in winter. The total life cycle duration observed in the summer was found to be significantly higher when compared to the duration of 288.4 (Yang et.al, 2015) and the duration in monsoon was significantly lesser, when compared with 532 reported by Nordin et al, (2020). The total duration observed in the winter season was significantly higher than 325 (Sinha and Nandi, 2007) and 321.9 (Yang et al., 2015).

Laboratory rearing under controlled conditions has been done to validate the outdoor results. It was observed that there existed a significant difference in the total life

cycle duration (hrs) observed in outdoor rearing and laboratory rearing in *C. megacephala* (Table. 4.95), *C. rufifacies* (Table. 4.98), *C. chani* (Table 4.100) and *H. ligurriens* (Table. 4.103). Similar observations were also reported by earlier workers (Acosta et al., 2022, Chen et al., 2019).

The variations observed in the life cycle duration in the current study and previous works may be explained by the changing weather patterns and environmental conditions and also due to the nature of the decomposing tissue used for rearing. Other reasons could be the intrinsic and extrinsic factors which may be explored in future research.

The survival rate of different developmental stages during the summer, monsoon and winter seasons was analysed and found that there exists a significant interaction between various seasons and years in *C. megacephala* (Table. 4.31), *C. rufifacies* (Table. 4.51), *C. chani* (Table. 4.71) and *H. ligurriens* (Table. 4.91). The survival rate of all species was significantly higher in monsoon in comparison to other seasons.

The results of the study were in consonance with the earlier reports by Pitts et al., (2005) who investigated the survival rate of various developmental stages of blow flies and found that seasons imposed significant contribution towards their survival. The present results were also consistent with the previous works by Mądra et al., (2017) and Zhang et al., (2019).

The major outcome of this study is that the results of outdoor rearing cannot be simulated in the laboratory (Acosta et al., 2021, 2022). Therefore a methodology was conceptualized to conduct outdoor rearing in different seasons in the study area. The results of the present study differed from the earlier works where varying seasonal climatic

variables were simulated in the laboratory using different temperature and humidity (Faris et al., 2020, Niederegger et al., 2010). Badenhorst et al., (2018) suggested to compare the laboratory rearing data with the outdoor results as it can provide sufficient information for the practical implementation of observed data for forensic science research.

Speight et al., (2008) indicated that microclimatic conditions may influence the biology of insects. The present investigation has analyzed the above perspectives for C. *megacephala, C. rufifacies, C. chani* and *H. ligurriens* and found that the data obtained in this investigation could be used for forensic research purposes in future as a reference data for Kerala, South India.

5.5. Estimation of PMI

The insect specimens, specifically the egg, larvae and pupae found in cadavers at death scenes have been previously used as strong evidence to determine the PMI (Catts and Goff, 1992). Accurate estimation of PMI demands the assessment to the level of minimum one hour duration for any stage of insect development.

In the current investigation, regression equation method using curve estimation was used for all larval instars. The results showed that in the case of *C. chani*, coefficient of determination (R^2) for the predicted equation is almost equal to 0.94 which indicates that about 94 percent of variability in length can be explained by duration and R^2 for the predicted equation in the case of *C. megacephala, H. ligurriens* and *C. rufifacies* were above 0.894, 0.855 and 0.771 respectively.

Another advantage of this method is that instars of any length could be applied in the equation without the requirement of the length of the largest instar. The regression

equation was found to give accurate estimate of developmental duration to the level of specific hour corresponding to the length of any particular instar.

The regression equation method developed in the current study is found to be really important as it emerged as the best suitable method for the estimation of PMI using the life history of the blow flies. This is in agreement with the earlier observations of Yang et al., (2014) on the PMI estimation using native species of blow flies in that particular geographical region.

The limitation of the use of isomegalen and isomorphen diagram method and thermal summation method for the estimation of PMI from the length of post feeding larval instars were proved by Yang et al., (2015). The study also suggested the necessity of applying the length of the largest instar in these models for determining the PMI. The simple additive methods proposed by Siddiki and Zambare (2017) could estimate the postmortem interval only up to stage specific development time of blow flies.

Similarly, the estimation of accumulated degree hours (ADH) of *C. megacephala* and *C. rufifacies* at different constant temperatures could reveal only the stage specific duration (Bala and Singh, 2015) which is inadequate for the accurate estimation of PMI in forensic investigation. ADH and ADD (Accumulated degree days) methods for PMI estimation have been criticized for not having any consideration for the post feeding stage (Arnott and Turner, 2008).

In recent studies, forensic entomologists use the data of developmental stages of insects to get precise information concerning the minimum PMI for forensic needs. For this purpose, it is essential to find a reference database of the respective species. Acosta et al.,

(2021, 2022) constructed growth models followed by isomegalen diagrams for the specific variables of the body weight and length of the larvae belonging to *Lucilia* genus.

Tachibana et al, (2006) revealed that the blowflies belonging to the *Chrysomya* genus usually have a preference for certain specific regions as well as seasons. For instance in Japan, blowflies belonging to the *Chrysomya* genus were found in mountainous zones during summer and lowlands during autumn.

This indicates that the variations in temperature have strong influence on the life cycle of blow flies and region specific studies are needed to reveal such inferences for different geographical areas and such findings have significant value for estimating the PMI for forensic research purpose (Muskan et al., 2022). In this study, such data was collected for C. *megacephala, C. rufifacies, C. chani* and *H. ligurriens* in Kerala region, India.

The differences in the developmental data of the blow fly species when compared to the previous works might be due to the changes in humidity, rainfall and temperature prevailing in these geographically different areas. The differences in developmental rate under constant temperatures are probably due to genetic variations (Tourle et al., 2009).

The changes in the developmental rate of species during different seasons cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area (Gallagher et al., 2010). This signifies the importance of generating location specific seasonal data of forensically important species for accurate assessment of postmortem interval.

In this regard, the data generated on the life cycle of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* and the regression equation model constructed for the estimation of PMI has been found to be useful for the forensic application in medicolegal investigations and for future forensic research in the study region. As this is the first report on the developmental rate of the above mentioned blow fly species, these findings can be used as forensic reference data for Kerala in future.