CHAPTER – 6

SUMMARY AND CONCLUSION

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The flies belonging to the Diptera, Calliphoridae are the first visitors to inhabit and colonize the dead body within a short period of time of cadavers found. The importance of generating location specific data of forensically important blow fly species for accurate assessment of PMI was evident from the previous works. The present investigation has recorded 17 blow fly species belonging to 4 subfamily and 8 genera from central Kerala. Of these, four forensically significant blow fly species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were prominently found to get attracted to carrion in summer, monsoon and winter seasons consistently.

Morphological identification of forensically significant blow flies; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were done based on the taxonomic characteristics of antennae, arista and palpi, bristles on the head, colour of parafrontalia, parafacialia, genae, colour and type of hairs and setulae on the parafacialia, colour of anterior spiracles, characteristics of sub costal sclerite, presence or absence of setulae on the stem vein, colour of suqama and the presence or absence of hairs, colour of the thorax and abdomen. Molecular diagnosis were done by amplifying the partial coding sequence of mitochondrial COI gene. The molecular analysis of the sequences showed strong boot strap support towards the corresponding nucletotide sequences representing *C. megacephala, C. rufifacies, C. chani,* and *H. ligurriens*.

Seasonal variation in the abundance of blow flies with special inference to summer, monsoon and winter seasons were done as such data is totally lacking in Southern India. The abundance of all the four species of blow flies were found to be significantly higher in monsoon. Developmental stages of *C. megacephala, C. rufifacies, C. chani, and H. ligurriens* were identified by studying the morphological features of eggs, the cephalopharyngeal skeleton, spinous bands on the lateral and ventral surfaces of larval body and posterior spiracles. SEM analysis was done for the morphology based identification of different larval instar stages of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens*. The characterisitc ultra structural details studied were; dorsal organ, terminal organ, ventral organ, labium, mouth hooks, oral cirri, number of spine clusters present dorso-medial to the functional mouth opening, post spiracular discs, tubercles on larval body and types of spines on the anal and thoracic segment.

The average mating time (hrs), pre oviposition period (days), periodicity of egg laying (days), number of eggs laid in a day and during the total life span in *C. megacephala, C. rufifacies*, *C. chani* and *H. ligurriens* were studied. Life cycle related parameters of larval instars like length, weight and duration of life of different larval instars and pupation were studied for all the four species of blow flies. The survival rate (%) in egg, first, second, third instars and pupae were studied and it was found that higher survival rate was seen in the egg and instar I. Effect of temperature and humidity on the pre-oviposition period, eggs laid in a day, periodicity of egg laying (days) and the number of eggs laid by four species of blow flies in its life span have been investigated in this study. The pre-oviposition period was significantly higher in winter in all the four species. The number of eggs laid in a day and total number of eggs laid during the life span by all species were significantly higher in monsoon. The periodicity of egg laying was

significantly higher in winter in all species. 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. The length of I^{st}, II^{nd} and III^{rd} instar was significantly higher in monsoon in *C. megacephala* and *C. rufifacies,* higher in summer for *C. chani* and in winter for *H. ligurriens.* The weight was significantly higher in monsoon for II^{nd} instar, III^{rd} instar and post feeding stage in *C. megacephala,* II^{nd} instar and post feeding stage in *C. rufifacies.* The weight was significantly higher in summer for II^{nd} instar, III^{rd} instar and post feeding stage of *C. chani*, II instar and post feeding stage in *H. ligurriens.*

The total duration taken by the fly for its development from the egg stage till the emergence of adult fly was shorter in summer, followed by monsoon and winter. When the results of the laboratory rearing of the four species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens,* were compared with the outdoor rearing results, it was observed that the developmental duration of different stages in the outdoor rearing were higher than the laboratory results. The variations observed among the life cycle duration in the current study and also other reported works may be well explained by the changing weather patterns and environmental conditions like temperature and humidity in the outdoor rearing sites and the difference in the nature of the decomposing tissue used for rearing. The survival rate of all species were found to be significantly higher in monsoon in comparison to other seasons. The major outcome of this study is that the results of outdoor rearing cannot be simulated in the laboratory. During unexpected variations in climatic variables, there is always a possibility for the formation of microclimatic conditions which directly influence the biology of insects. The present investigation has analyzed the above perspectives by rearing *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* in

different seasons for three consecutive years. The data obtained in this investigation could be used for forensic research purposes in future as a reference data for Kerala, South India.

A high value of coefficient of determination (R^2) was obtained for all the four blow fly species for the predicted regression equation which indicated that higher percent of variability in length can be explained by duration. The major 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. This 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 this study emerged as the best suitable method for the estimation of PMI using life history of the blow flies. The changes observed in the rate of developmental data of the blow flies when compared to the previous works might be due to the changes in humidity, rainfall and temperature prevailing in these geographically different areas and genetic variations of blow flies. This cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area and signifies the importance of generating location specific data of forensically important species of blow flies. This is the first report on the developmental rate of these species during different seasons from South India and can be used for the PMI assessment of dead bodies under forensic investigations.

Present investigation 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 forensic application in medicolegal investigations in the study region. Further research can be conducted to augment the present study results and for exploring new dimensions in future.