CONTENTS

		Page No.
	List of figures	i
	List of tables	v
	Abbreviations	viii
	Abstract	X
1	Introduction	1
	1.1. Insects as evidence for forensic investigation	1
	1.2. Relevance of the Study	13
	1.3. Objectives of the present study	13
2	Literature Review	14
3	Methodology	42
	3.1. Blow fly fauna of Central Kerala	42
	3.1.1. Collection and Identification of blow flies	43
	Morphological identification	44
	Molecular characterization	44
	3.2. Seasonal abundance in blow fly population in Central Kerala	52
	3.3. Life history of blow flies in carrion	55
	3.3.1. Outdoor rearing of blow flies	55
	3.3.2. Preservation	57
	3.3.3. Morphological identification of eggs and different larval instars	57
	3.3.4. Ultrastructure study of larvae using Scanning Electron Microscopy	58
	3.3.5. Life cycle	59
	3.4. Effect of temperature and humidity on the life cycle of Calliphorid flies	61
	3.4.1. Assessment of development rate	61
	3.4.2. Laboratory rearing of blow flies under controlled conditions and validation	64
	3.5. Relation between life cycle and time since death assessment for forensic application	65
	3.5.1. Estimation of Post Mortem Interval (PMI)	65
	3.6. Statistical analysis of data	66
4	Results	68
-	4.1. Blow fly fauna	68
	4.1.1. Identification	75
	Morphological identification	, 5

	Molecular characterization		
	4.2. Seasonal abundance in blow fly population in Central Kerala	92	
	4.3. Life history of Blow flies	99	
	4.3.1. C. megacephala	99	
	4.3.2. C. rufifacies	104	
	4.3.3. <i>C. chani</i>	110	
	4.3.4. H. ligurriens	116	
	4.4. Effect of temperature and humidity on the life cycle	123-204	
	Effect on fecundity		
	Effect on length of larvae		
	Effect on weight of larvae		
	Effect on life cycle duration		
	Effect on survival rate		
	Laboratory rearing		
	4.5. Relation between life cycle of flies and time since death assessment	205	
	4.5.1. Estimation of Post Mortem Interval (PMI)	205	
5	Discussion	209	
6	Summary & Conclusion		
7	Recommendations	231	
	References	233	
	Appendix	247	
	I. Publications	247	
	II. NCBI GenBank submission details	250	
III. Seasonal life cycle data of C. megacephala, C. rufifacies, C. chani and H.			
	ligurriens	251	

LIST OF FIGURES

	LIST OF FIGURES		
Fig.	Title		
No.			
3.1.	Blow fly collection sites in Thrissur, Palakkad and Ernakulam districts		
3.2.	Collection methods of blow flies		
3.3.	Blow fly collection sites in Ernakulam		
3.4.	Blow fly collection sites in Thrissur		
3.5.	Blow fly collection sites in Palakkad		
3.6.	Outdoor rearing facility		
3.7.	Rearing methods I		
3.8.	Rearing methods II		
3.9.	Laboratory rearing showing brood chamber with culture replicates of blow flies		
4.1.	Characteristics of C. megacephala		
4.2.	Partial coding sequence of mitochondrial COI gene of C. megacephala		
4.3.	Translational product of mitochondrial COI gene of <i>C. megacephala</i>		
4.4.	Agarose Gel Electrophoresis of the PCR product		
4.5 a.	Electropherogram of mitochondrial COI gene of <i>C.megacephala</i>		
	using forward primer		
4.5 b.	Electropherogram of mitochondrial COI gene of <i>C.megacephala</i> using reverse primer		
4.6.	Phylogenetic tree based on COI sequence, SR1859-COI-F E03 of <i>C</i> .		
т.0.	megacephala by Maximum Likelihood method		
4.7.	Characteristics of <i>C. rufifacies</i>		
4.8.	Partial coding sequence of mitochondrial COI gene of <i>C. rufifacies</i>		
4.9.	Translational product of mitochondrial COI gene of <i>C. rufifacies</i>		
4.10.	Agarose Gel Electrophoresis of the PCR product		
4.10. 4.11 a.	Electropherogram of the mitochondrial COI gene of <i>C. rufifacies</i>		
4.11 a.	using forward primer		
4.11 b.	Electropherogram of the mitochondrial COI gene of <i>C. rufifacies</i>		
4.11 0.	1 0		
4.12	using reverse primer Phylogopatic tree based on COI seguence SP2284 COE A07 of C. muffraise		
4.12.	Phylogenetic tree based on COI sequence SR2284-COF_A07 of <i>C. rufifacies</i> by Maximum Likelihood method		
4.13.	Characteristics of <i>C. chani</i>		
4.14.	Partial coding sequence of mitochondrial COI gene of <i>C. chani</i>		
4.15.	Translational product of mitochondrial COI gene of <i>C. chani</i>		
4.16.	Agarose Gel Electrophoresis of the PCR product		
4.17 a.	Electropherogram of the mitochondrial COI gene of <i>C. chani</i> using forward primer.		
4.17 b.	Electropherogram of the mitochondrial COI gene of <i>C. chani</i> using reverse primer		
4.18.	Phylogenetic tree based on COI sequence, SR2040-A-COF_D05 of <i>C. chani</i> by Maximum Likelihood method		
4.19.	Characteristics of <i>H. ligurriens</i>		
4.20.	Partial coding sequence of mitochondrial COI gene of <i>H. ligurriens</i>		
4.21.	Translational product of mitochondrial COI gene of <i>H. ligurriens</i>		
4.21.	Agarose Gel Electrophoresis of the PCR product		
4.22. 4.23 a.	Electropherogram of the mitochondrial COI gene of <i>H. ligurriens</i>		
7.23 a.	using forward primer		
4.23 b.	Electropherogram of the mitochondrial COI gene of <i>H. ligurriens</i>		
	using reverse primer		

- 4.24. Phylogenetic tree based on COI sequence, SR1719-A-COF_B03 of *H. ligurriens* by Maximum Likelihood method
- 4.25. Eggs, different larval instars and pupal case of *C. megacephala*
- 4.26. Cephalopharyngeal skeleton and posterior spiracle of *C. megacephala* larvae
- 4.27. SEM micrographs of first instar larvae of *C. megacephala*
- 4.28. SEM micrographs of second instar larvae of *C. megacephala*
- 4.29. SEM micrographs of third instar larvae of *C. megacephala*
- 4.30. SEM micrographs of post feeding stage of *C. megacephala*
- 4.31. Eggs, different larval instars and pupa of *C. rufifacies*
- 4.32. Cephalopharyngeal skeleton and posterior spiracle of *C. rufifacies* larvae
- 4.33. SEM micrographs of first instar of *C. rufifacies*
- 4.34. SEM micrographs of second instar of *C. rufifacies*
- 4.35. SEM micrographs of third instar of *C. rufifacies*
- 4.36. SEM micrographs of post feeding instar of *C. rufifacies*
- 4.37. Eggs, different larval instars and pupa of *C. chani*
- 4.38. Cephalopharyngeal skeleton and posterior spiracle of *C.chani* larvae
- 4.39. SEM micrographs of first instar larvae of *C. chani*
- 4.40. SEM micrographs of second instar larvae of *C. chani*
- 4.41. SEM micrographs of third instar larvae of *C.chani*
- 4.42. SEM micrographs of post feeding instar larvae of *C.chani*
- 4.43. Eggs, different larval instars and pupal case of *H. ligurriens*
- 4.44. Cephalopharyngeal skeleton and posterior spiracle of *H. ligurriens* larvae
- 4.45. SEM micrographs of first instar of *H. ligurriens*
- 4.46. SEM micrographs of second instar of *H. ligurriens*
- 4.47. SEM micrographs of third instar of *H. ligurriens*
- 4.48. SEM micrographs of post feeding instar stage of *H. ligurriens*
- 4.49. Seasonal developmental rate (Length (mm) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation
- 4.50. Developmental rate (Length (mm) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation during the study period
- 4.51. Seasonal developmental rate (Weight (mg) Vs. Age (hrs) of *C.megacephala* from hatching upto pupation
- 4.52. Developmental rate (Weight (mg) Vs Age (hrs) of *C.megacephala* from hatching upto pupation during the study period
- 4.53. Seasonal developmental rate (Length (mm) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation
- 4.54. Developmental rate (Length (mm) Vs. Age (hrs) of *C. rufifacies* from hatching up to pupation during the study period
- 4.55. Seasonal developmental rate (Weight (mg) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation
- 4.56. Developmental rate (Weight (mg) Vs. Age (hrs) of *C.rufifacies* from hatching upto pupation during the study period
- 4.57. Seasonal developmental rate (Length (mm) Vs. Age (hrs) of *C.chani* from hatching upto pupation
- 4.58. Developmental rate (Length (mm) Vs. Age (hrs) of *C.chani* from hatching upto pupation during the study period
- 4.59. Seasonal developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani* from hatching upto pupation
- 4.60. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani* from hatching upto pupation during the study period
- 4.61. Seasonal developmental rate (Length (mm) Vs. Age (hrs) of *H.ligurriens* from hatching upto pupation

- 4.62. Developmental rate (Length (mm) Vs. Age (hrs) of *H.ligurriens* from hatching upto pupation during the study period
- 4.63. Seasonal developmental rate (Weight (mg) Vs. Age (hrs) of *H.ligurriens* from hatching upto pupation
- 4.64. Developmental rate (Weight (mg) Vs. Age (hrs) of *H.ligurriens* from hatching upto pupation during the study period
- 4.65. Developmental rate (Length (mm) Vs. Age (hrs) *C. chani*, *C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation during the study
- 4.66. Developmental rate (Length (mm) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation under laboratory conditions
- 4.67. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation under laboratory controlled conditions
- 4.68. Developmental rate (Length (mm) Vs. Age (hrs) of *C.rufifacies* from hatching upto pupation under laboratory conditions
- 4.69. Developmental rate (Weight (mg) Vs. Age (hrs) of *C.rufifacies* from hatching upto pupation under laboratory conditions
- 4.70. Developmental rate (Length (mm) Vs. Age (hrs) of *C.chani* from hatching upto pupation under laboratory conditions
- 4.71. Developmental rate (Weight (mg) Vs. Age (hrs) of *C.chani* from hatching upto pupation under laboratory conditions
- 4.72. Developmental rate (Length (mm) Vs. Age (hrs) of *H. ligurriens* from hatching upto pupation under laboratory conditions
- 4.73. Developmental rate (Weight (mg) Vs. Age (hrs) of *H.ligurriens* from hatching upto pupation under laboratory conditions
- 4.74. Developmental rate (Length (mm) Vs. Age (hrs) of *C. chani, C. megacephala, C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions
- 4.75. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani*, *C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions
- 4.76. Growth curves of *C. chani* for the estimation of the PMI
- 4.77. Growth curves of *C. megacephala* for the estimation of the PMI
- 4.78. Growth curves of *C. rufifacies* for the estimation of the PMI
- 4.79. Growth curves of *H. ligurriens* for the estimation of the PMI

LIST OF TABLES

Table	The state of the s
No.	Title
4.1.	Abundance (%) of <i>C. megacephala</i> between seasons and between years
4.2.	Abundance (%) of <i>C. megacephala</i> male flies between seasons and between years
4.3.	Abundance (%) of <i>C. megacephala</i> female flies between seasons and between years
4.4.	Abundance (%) of <i>C. rufifacies</i> between seasons and between years
4.5.	Abundance (%) of <i>C. rufifacies</i> male flies between seasons and between years
4.6.	Abundance (%) of <i>C. rufifacies</i> female flies between seasons and between years
4.7.	Abundance (%) of <i>C. chani</i> between seasons and between years
4.8.	Abundance (%) of <i>C. chani</i> male flies between seasons and between years
4.9.	Abundance (%) of <i>C. chani</i> female flies (%) between seasons and between years
4.10.	Abundance (%) of <i>H. ligurriens</i> between seasons and between years
4.11.	Abundance (%) of <i>H. ligurriens</i> male flies between season and between years
4.12.	Abundance (%) of <i>H. ligurriens</i> female flies between season and between years
4.13.	Pre-oviposition period (days) of <i>C. megacephala</i>
4.14.	Eggs laid by the C. megacephala in a day
4.15.	Periodicity of egg laying (days) by C. megacephala
4.16.	Eggs laid by the C. megacephala during its life span
4.17.	Seasonal changes in length (mm) of 1st instar larvae of C. megacephala
4.18.	Seasonal changes in length (mm) of IInd instar larvae of C. megacephala
4.19.	Seasonal changes in length (mm) of IIIrd instar larvae of <i>C. megacephala</i>
4.20.	Seasonal changes in length (mm) of post feeding stage of <i>C. megacephala</i>
4.21.	ANOVA for comparing length of larval instars of <i>C. megacephala</i>
4.22.	Seasonal changes in weight (mg) of 1st instar larvae of C. megacephala
4.23.	Seasonal changes in weight (mg) of IInd instar larvae of <i>C. megacephala</i>
4.24.	Seasonal changes in weight (mg) of IIIrd instar larvae of C. megacephala
4.25.	Seasonal changes in weight (mg) of post feeding stage of <i>C. megacephala</i>
4.26.	ANOVA for comparing weight of larval instars of <i>C. megacephala</i>
4.27.	Seasonal changes in duration (hrs) of life cycle of <i>C.megacephala</i>
4.28.	Changes in duration (hrs) of life cycle of <i>C.megacephala</i>
4.29.	Seasonal changes in survival rate (%) of C. megacephala
4.30.	Survival rate (%) of life cycle of <i>C.megacephala</i>
4.31.	Seasonal changes in survival rate (%) of life cycle stages of <i>C. megacephala</i>
4.32.	ANOVA for comparing the survival rate of <i>C. megacephala</i>
4.33.	Pre-oviposition period (days) of <i>C.rufifacies</i>
4.34.	Eggs laid by the C. rufifacies in a day
4.35.	Periodicity of egg laying (days) by <i>C. rufifacies</i>
4.36.	Eggs laid by the <i>C. rufifacies</i> during its life span
4.37.	Seasonal changes in length (mm) of 1st instar larvae of <i>C. rufifacies</i>
4.38.	Seasonal changes in length (mm) of IInd instar larvae of <i>C. rufifacies</i>
4.39.	Seasonal changes in length (mm) of IIIrd instar larvae of <i>C. rufifacies</i>
4.40.	Seasonal changes in length (mm) of post feeding stage of <i>C. rufifacies</i>
4.41.	ANOVA for comparing length of larval instars of <i>C. rufifacies</i>
4.42.	Seasonal changes in weight (mg) of 1st instar larvae of <i>C. rufifacies</i>
4.43.	Seasonal changes in weight (mg) of IInd instar larvae of <i>C. rufifacies</i>
4.44.	Seasonal changes in weight (mg) of IIIrd instar larvae of <i>C. rufifacies</i>
4.45.	Seasonal changes in weight (mg) post feeding stage of <i>C. rufifacies</i>
4.46.	ANOVA for comparing weight of larval instars of <i>C. rufifacies</i>
4.47.	Seasonal changes in duration (hrs) of life cycle of <i>C.rufifacies</i>
4.48.	Changes in duration (hrs.) of life cycle of <i>C.rufifacies</i>

- 4.49. Seasonal changes in survival rate (%) of *C. rufifacies*
- 4.50. Survival rate (%) of life cycle of *C. rufifacies*
- 4.51. Seasonal changes in survival rate (%) of life cycle stages of *C. rufifacies*
- 4.52. ANOVA for comparing the survival rate of *C. rufifacies*
- 4.53. Pre-oviposition period (days) of *C. chani*
- 4.54. Eggs laid by the *C.chani* in a day
- 4.55. Periodicity of egg laying (Days) by *C. chani*
- 4.56. Eggs laid by the *C. chani* during its life span
- 4.57. Seasonal changes in length (mm) of Ist instar larvae of *C. chani*
- 4.58. Seasonal changes in length (mm) of IInd instar larvae of *C. chani*
- 4.59. Seasonal changes in length (mm) of IIIrd instar larvae of *C. chani*
- 4.60. Seasonal changes in length (mm) of post feeding stage of *C.chani*
- 4.61. ANOVA for comparing length of larval instars of *C. Chani*
- 4.62. Seasonal changes in weight (mg) of 1st instar larvae of *C.chani*
- 4.63. Seasonal changes in weight (mg) of IInd instar larvae of *C.chani*
- 4.64. Seasonal changes in weight (mg) of IIIrd instar larvae of *C.chani*
- 4.65. Seasonal changes in weight (mg) of post feeding stage of *C.chani*
- 4.66. ANOVA for comparing weight of larval instars of *C.chani*
- 4.67. Seasonal changes in duration (hrs) of life cycle of *C. chani*
- 4.68. Changes in duration (hrs) of life cycle of *C. chani*
- 4.69. Seasonal changes in the survival rate (%) of *C. chani*
- 4.70. Survival rate (%) of life cycle of *C.chani*
- 4.71. Seasonal changes in survival rate (%) of life cycle stages of *C. chani*
- 4.72. ANOVA for comparing the survival rate of *C. chani*
- 4.73. Pre-oviposition period (days) of *H. ligurriens*
- 4.74. Eggs laid by the *H. ligurriens* in a day
- 4.75. Periodicity of egg laying (days) by *H. ligurriens*
- 4.76. Eggs laid by the *H. ligurriens* during its life span
- 4.77. Seasonal changes in length (mm) of 1st instar larvae of *H. ligurriens*
- 4.78. Seasonal changes in length (mm) of IInd instar larvae of *H. ligurriens*
- 4.79. Seasonal changes in length (mm) of IIIrd instar larvae of *H. ligurriens*
- 4.80. Seasonal changes in length (mm) of post feeding stage of *H. ligurriens*
- 4.81. ANOVA for comparing length of larval instars of *H. ligurriens*
- 4.82. Seasonal changes in weight (mg) of 1st instar larvae of *H. ligurriens*
- 4.83. Seasonal changes in weight (mg) of IInd instar larvae of *H. ligurriens*
- 4.84. Seasonal changes in weight (mg) of IIIrd instar larvae of *H. ligurriens*
- 4.85. Seasonal changes in weight (mg) of post feeding stage of *H. ligurriens*
- 4.86. ANOVA for comparing weight of larval instars of *H. ligurriens*
- 4.87. Seasonal changes in duration (hrs) of life cycle of *H. ligurriens*
- 4.88. Changes in duration (hrs) of life cycle of *H. ligurriens*
- 4.89. Seasonal changes in the survival rate (%) of *H. ligurriens*
- 4.90. Survival rate (%) of life cycle of *H. ligurriens*
- 4.91. Seasonal changes in the survival rate (%) of life cycle stages of *H. ligurriens*
- 4.92. ANOVA for comparing the survival rate of *H. ligurriens*
- 4.93. Length (mm) of larval instars of *C. megacephala* under laboratory conditions
- 4.94. Weight (mg) of larval instars of *C. megacephala* under laboratory conditions
- 4.95. Duration (hrs) of life cycle of *C. megacephala* under laboratory conditions
- 4.96. Length (mm) of larval instars of *C. rufifacies* under laboratory conditions
- 4.97. Weight (mg) of larval instars of *C. rufifacies* under laboratory conditions
- 4.98. Duration (hrs) of life cycle of *C. rufifacies* under laboratory conditions
- 4.99. Length (mm) of larval instars of *C. chani* under laboratory conditions
- 4.99. Weight (mg) of larval instars of *C. chani* under laboratory conditions

4.100. Duration (hrs) of life cycle of *C. chani* under laboratory conditions
4.101. Length (mm) of larval instars of *H. ligurriens* under laboratory conditions
4.102. Weight (mg) of larval instars of *H. ligurriens* under laboratory conditions
4.103. Duration (hrs) of life cycle of *H. ligurriens* under laboratory conditions
4.104. Regression equation model for the estimation of PMI

ABBREVIATIONS

% percentage

 β_0, β_1 parameters of the regression model to be estimated

°C degree Celsius

μl microliter

ADD accumulated degree hours

ADH accumulated degree hours

ANOVA analysis of Variance

BLAST basic local alignment aearch tool

base pair

C.chani Chrysomya chani

C.megacephala Chrysomya megacephala

C.rufifacies Chrysomya rufifacies

CABI centre for agriculture and bioscience international

CO I cytochrome oxidase subunit I

CO II cytochrome oxidase subunit II

D₀ developmental threshold temperature

DNA deoxyribonucleic acid

E expectation

ft feet

H.ligurriens Hemipyrellia ligurriens

h/hr/hrs hour/s

K thermal summation constant

km kilometer

LSD least significance difference

m meter
M molar

mA milliampere

mg milligram

ml milliliter

mm millimeter

NCBI national center for biotechnology information

ns non-significant

ORF open reading frame

PCR polymerase chain reaction

PMI post mortem interval

R² coefficient of determination

RH/H relative humidity/humidity

RNase Ribonuclease

rRNA ribosomal RNA

SEM scanning electron microscopy

T temperature

t time

TBE tris borate EDTA

UV ultraviolet

v/v volume/volume

w/v weight/volume

 Y_t length (mm) at duration 't' (hrs