

THESIS

ACTIVITY AND BIONOMIC ASPECTS OF PRIMARY AND
SECONDARY VECTORS FOR DENGUE TRANSMISSION IN
GAMPAHA DISTRICT, SRI LANKA

Submitted by

C.P.R.D.DALPADDU

(FGS/ 01/PhD/07/2016/50)

A thesis submitted to the Faculty of Graduate Studies, University of Kelaniya
in fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Entomology



February 2022



This thesis has been accepted by the University of Kelaniya for the award of the Degree of Doctor of Philosophy (2022...) It is not allowed to Publish this as a thesis accepted for the Degree of Doctor of Philosophy without the authority of the University

Deputy Registrar / Examinations

208804

THESIS

ACTIVITY AND BIONOMIC ASPECTS OF PRIMARY AND
SECONDARY VECTORS FOR DENGUE TRANSMISSION IN
GAMPAHA DISTRICT, SRI LANKA

Submitted by

C.P.R.D.DALPADDO

(FGS/ 01/PhD/07/2016/50)

A thesis submitted to the Faculty of Graduate Studies, University of Kelaniya

in fulfillment of the requirements for the degree of

Doctor of Philosophy in Medical Entomology




February 2022

To my family members and teachers

DECLARATION


I declare that the work embodied in the thesis is my own and has not been submitted for any degree in this university or any other institute, and to the best of my knowledge and belief, it does not contain any material previously published or written or orally communicated by another person except, where due reference is made in the text.


..... Signature of the candidate

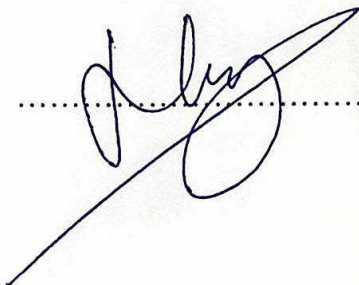
12/09/2022
..... Date

To the best of our knowledge, we endorse the declaration by the candidate.

Main supervisor


..... Prof. Mrs L.D. Amarasinghe, Professor, Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Sri Lanka.

Co-supervisors


..... Prof. P.A.D.H.N. Gunanthilaka, Professor, Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka.

I. CONTENTS

	Page no.
DECLARATION	i
I. CONTENTS	ii
II. LIST OF FIGURES	x
III. LIST OF TABLES	xix
IV. LIST OF APPENDICES	xxiii
V. LIST OF ABBREVIATIONS	xxiv
VI. ACKNOWLEDGEMENTS	xxx
VII. ABSTRACT	xxxii
1.0. INTRODUCTION	1
1.1. Dengue infection and epidemiology	1
1.2. Justification for the present study	3
1.2.1. Bionomic aspects of dengue vector mosquitoes in Sri Lanka	3
1.2.2. Assessment of the spatial variation of insecticide resistance status amongst <i>Aedes aegypti</i> and <i>Aedes albopictus</i>	5
1.2.3. Development of area-specific threshold values for Gampaha district dengue vector indices.	7

1.3	Objectives	11
	1.3.1. General objective	11
	1.3.2. Specific objectives	11
2.0.	LITERATURE REVIEW	12
2.1.	The burden of mosquito-borne infections	12
2.2.	Public health burden of Dengue and Dengue hemorrhagic fever	13
2.3.	Transmission of dengue virus	15
2.4.	Dengue vector mosquitoes	18
	2.4.1. Global distribution of Dengue vectors	19
	2.4.1.i. <i>Aedes aegypti</i> (Linnaeus, 1762)	19
	2.4.1. ii. <i>Aedes albopictus</i> (Skuse, 1894)	20
	2.4.2. Morphology and Life cycle of <i>Aedes</i> mosquitoes	21
	2.4.2.i. Egg stage	22
	2.4.2. ii. Larval stage	24
	2.4.2. iii. Pupal stage	27
	2.4.4.iv Adult mosquitoes	27
2.5.	Bionomics of dengue vector mosquitoes	29
	2.5.1. Oviposition behaviour and site selection	30
	2.5.2. Mating behaviour of <i>Aedes</i> mosquitoes	33
	2.5.3. Feeding behaviour of <i>Aedes</i> mosquitoes	35
	2.5.4. Host-seeking behaviour of mosquitoes	37
	2.5.5. Resting behaviour	40

2.6.	Water quality characteristics of dengue vector breeding habitats.	41
2.7.	Integrated Vector Management (IVM) in dengue control	44
	2.7.1. Environmental management	44
	2.7.2. Biological and chemical control	
	2.7.3. Chemical control of dengue vectors	46
	2.7.3.i. Use of insecticides for immature control	47
	2.7.3. ii. Insecticides for adult control	50
2.8.	Resistance to insecticides by <i>Aedes</i> mosquitoes	53
2.9.	Dengue vector surveillance and incidence modeling	56
	2.9.1. Entomological surveillance	56
	2.9.2. Forecasting dengue epidemics based on entomological indices	58
3.0.	METHODOLOGY	62
3.1.	Work plan	62
3.2.	Study area	63
	3.2.1. Selection of study sites	66
	3.2.2. Selection of sentinel sites for field surveys	67
3.3	Entomological investigations and bionomics aspects of dengue vectors; <i>Aedes aegypti</i> and <i>Aedes albopictus</i>	70
	3.3.1. Collection of mosquito larvae from breeding habitats	70
	3.3.1.i. Taxonomic classification of mosquito larval stages	70
	3.3.2. Adult mosquito collection from resting places and species identification	71

3.3.3	detection of host-seeking hours of adult mosquitoes	72
3.4	Water quality characteristics of dengue vector breeding habitats	73
3.5.	Determination of the insecticide resistance in dengue vectors	74
3.5.1.	Collection of mosquitoes	75
3.5.2.	Establishment of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adult mosquito colony	75
3.5.3.	Larval susceptibility test	77
3.5.4.	Adult susceptibility assay	79
3.5.4.i.	Resistance frequency bioassay for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adults	79
3.5.4. ii.	Resistance intensity bioassay for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adults	81
3.6.	Dengue prediction modelling and development of area-specific thresholds based on entomological indices for dengue epidemic management	82
3.6.1.	Collection of meteorological data	82
3.6.2.	Collection of epidemiological and entomological data	82
3.6.3.	Climatic effect on density and abundance of <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	83
3.6.4.	Prediction modeling for dengue incidence	84
3.6.4.i.	Development of model I; based on both climatic variables and vector indices	85
3.6.4.i. a.	The model I: urban	85
3.6.4.i. b.	The model I: suburban	86

3.6.4.i. c. The model I: rural	87
3.6.4. ii. Development of model II; based on vector indices	89
3.6.4.iii. Development of model III; based on vector indices and relative humidity	
3.6.4. iv. Development of model IV; based on vector indices and monthly average rainfall	95
3.6.4.v. Development of model v; based on climatic variables	98
3.6.5. Validation of models	101
3.7. Development of area-based threshold values based on the Breteau Index (BI)	101
3.8. Data analysis	102
3.8.1. Entomological investigations and vector bionomics aspects	102
3.8.1.i. Oviposition preference of <i>Aedes</i> mosquitoes	102
3.8.1. ii. Resting preferences of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adult mosquitoes	102
3.8.1. iii. Biting behaviors and peak biting hours of <i>Aedes</i> Mosquitoes	103
3.8.2. Water quality characteristics of dengue vector breeding habitats	103
3.8.2.i. Diversity of breeding habitats and vector density	103
3.8.2.ii. Water quality index	103
3.8.3. Determination of the insecticide resistance in dengue vectors	105
3.9. Ethical clearance	105

4.0. RESULTS	
4.1. Bionomics aspects of <i>Aedes aegypti</i> and <i>Aedes albopictus</i>	106
4.1.1. Oviposition preferences <i>Aedes</i> mosquitoes at different environmental setup	106
4.1.2. Habitat preference of <i>Aedes</i> mosquitoes	110
4.1.3. Productivity of habitats for vector breeding in terms of Container Index	112
4.1.4. Resting preferences of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adult mosquitoes	116
4.1.4.i. Resting habitat preference by adult <i>Aedes</i> mosquitoes	118
4.1.4. ii. Resting material and height of the resting surface	119
4.1.5. Biting behaviours and peak biting hours of <i>Aedes</i> mosquitoes	120
4.2. Water quality characteristics of dengue vector breeding habitats	124
4.2.1. Diversity of breeding habitats and vector density	124
4.2.2. Physico-chemical characteristics of the breeding habitats of <i>Aedes</i> mosquitoes	127
4.2.2.i. Temperature in water	129
4.2.2. ii. The pH in the water	131
4.2.2.iii. Turbidity and Total Dissolved Solids (TDS) in water	133
4.2.2. iv. Conductivity in water	137
4.2.2.v. Mean Dissolved Oxygen level in the water	140

4.2.3. Physical nature of the vector breeding habitats	143
4.2.4. Water quality index	145
4.3 Determination of the insecticide resistance in dengue vectors	148
4.3.1. Insecticide susceptibility of <i>Aedes</i> larvae	148
4.3.2. Determination of 24-hour LC50 and LC99 for exposed <i>Aedes</i> larvae	151
4.3.3. Resistance frequency bioassay for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adults	153
4.3.4. Resistance intensity bioassay in <i>Ae. aegypti</i> and <i>Ae. albopictus</i> adults	155
4.4. Dengue prediction modelling and development of area-specific thresholds based on entomological indices for dengue epidemic management	157
4.4.1. Temporal distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	157
4.4.1. Climatic effect on density and abundance of <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	161
4.4.3. Prediction modelling of Dengue	169
4.4.3.i. Development of prediction model for urban areas.	172
4.4.3. ii. Development of prediction model for suburban areas.	177
4.4.3.iii Development of prediction model for rural areas	183
4.4.4. Model validation	186

5.0.	DISCUSSION	191
5.1.	Entomological investigations on bionomics aspects of <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	191
5.2.	Water quality characteristics of dengue vector breeding habitats	199
5.3.	Determination of the insecticide resistance in dengue vectors	207
5.4.	Dengue prediction modelling and development of area-specific thresholds based on entomological indices for dengue epidemic management	212
5.5.	Concluding remarks	221
6.0	REFERENCES	225
7.0.	APPENDICES	290
8.0.	RESEARCH PUBLICATIONS AND COMMUNICATIONS	355

II. LIST OF FIGURES

		Page no.
Figure 2.1	Transmission of Dengue virus by <i>Aedes</i> mosquitoes.	18
Figure 2.2	The life cycle of <i>Aedes</i> Mosquitoes (Source; CDC, Centre for disease control and prevention).	22
Figure 2.3	Scanning electron micrographs show the detailed structure of eggs of (a): <i>Ae. aegypti</i> and (b): <i>Ae. albopictus</i> (Source; Win <i>et al.</i> , 2018).	24
Figure 2.4	Drawing illustrating the significant morphological differences between <i>Ae. albopictus</i> and <i>Ae. aegypti</i> larval stages. (Source; Modified illustration from Franco and Craig, 1995).	26
Figure 2.5	Lateral view of <i>Aedes</i> pupa (Source; Neslon, 1986).	27
Figure 2.6	Dorsal surfaces of the thorax, (a); <i>Ae. albopictus</i> , with a diagnostic median white stripe, (b); <i>Ae. aegypti</i> , with black scales and typical lyre-shaped silvery markings (Source; Service 2012).	29
Figure 2.7	The epidemic channel for infectious disease, defined by the World Health Organization (Source; WHO, 2016b).	61
Figure 3.1	The map of the Gampaha district, Sri Lanka, showing the selected MOH areas for entomological surveillance.	65
Figure 3.2	Selected sentinel sites at each selected MOH area in Gampaha, Sri Lanka.	69

Figure 3.3	Resting mosquito collection (a) Prokopack aspirator (model: 140), (b) Outdoor Collection of mosquitoes, (c) Indoor Collection of mosquitoes.	72
Figure 3.4	A sample of ovi-trap used for surveys (Black color; 250 mL ; Diameter 12 cm and Height 10 cm).	74
Figure 3.5	<i>Ae. aegypti</i> and <i>Ae. albopictus</i> larval trays at the insectary.	75
Figure 3.6	Adult <i>Ae. aegypti</i> and <i>Ae. albopictus</i> rearing cages at the insectary.	77
Figure 3.7	Susceptibility trial setup for temephos larvicide.	79
Figure 3.8	Testing insecticide susceptibility status in adult <i>Aedes</i> mosquitoes	81
Figure 3.9	The procedure used in the modelling approach I	88
Figure 3.10	The procedure used in developing the dengue prediction Model II.	90
Figure 3.11	The procedure used in developing the dengue prediction Model III.	93
Figure 3.12	The procedure used in developing the dengue prediction Model IV.	96
Figure 3.13	The procedure used in developing the dengue prediction model V	99
Figure 4.1	Percentage positive premises for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> at different environmental setups in Gampaha district, Sri Lanka.	107
Figure 4.2	(a) Bar chart showing the significant variability of Container Indexes (CI) for immature stages <i>Ae. aegypti</i> , (b) pairwise comparisons of mean ranks of CI values across	108

	different spatial setups in Gampaha district at 0.05 level of significance of independent sample Kruskal Wallis test.	
Figure 4.3	(a) Bar chart showing the significant variability of Container Indexes (CI) for immature stages <i>Ae. albopictus</i> , (b) pairwise comparisons of mean ranks of CI values across different spatial setups in Gampaha district at 0.05 level of significance of independent.	109
Figure 4.4	Prevalence of breeding sites for both <i>Ae. aegypti</i> and <i>Ae. albopictus</i> at different environmental setups in the Gampaha district.	111
Figure 4.5	Breeding site categories observed during the field surveys;	112
Figure 4.6	Resting place preference of <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	118
Figure 4.7	The bar chart showing the host-seeking and peak biting cycle of <i>Ae. albopictus</i> mosquitoes.	122
Figure 4.8	The bar chart showing the host-seeking and peak biting cycle of <i>Ae. aegypti</i> mosquitoes.	123
Figure 4.9	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with temperatures in the breeding habitat.	130
Figure 4.10	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test shows the temperature distribution across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	131
Figure 4.11	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with pH in the water.	132

Figure 4.12	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test showing the distribution of pH across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	133
Figure 4.13	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with turbidity.	134
Figure 4.14	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with Total dissolved Solids.	135
Figure 4.15	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test shows the TDS level distribution across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	136
Figure 4.16	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test showing the distribution of turbidity level across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	137
Figure 4.17	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with conductivity in water.	138
Figure 4.18	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test shows conductivity distribution across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	139
Figure 4.19	Frequency distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with Dissolved Oxygen level in the water.	140

Figure 4.20	Box-plot (median and quartiles) of the Kruskal-Wallis rank-sum test showing the distribution of Dissolved Oxygen levels across different breeding habitats for <i>Aedes</i> mosquitoes followed by Dunn's posthoc test, $P < 0.05$.	141
Figure 4.21	Percentage distribution of positive containers for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> with appearance and condition of breeding water	145
Figure 4.22	Water Quality Index of in different breeding categories that were positive for the breeding of <i>Aedes</i> mosquitoes.	147
Figure 4.23	Percentage mortality rates of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females that were exposed to deltamethrin (0.03%), and malathion (0.8%) in the studied MOH areas.	154
Figure 4.24	Percentage mortality rates of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females exposed to deltamethrin (0.15%) and malathion (4.0%) in the studied MOH areas.	156
Figure 4.25	Temporal distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> in rural settings of the District of Gampaha for the period of 2014-2019.	158
Figure 4.26	Temporal distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> in suburban settings of the District of Gampaha for the period of 2014-2019.	159
Figure 4.27	Temporal distribution of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> in rural settings of the District of Gampaha for the period of 2014-2019.	160

Figure 4.28	Cross-correlation analysis (CCF) between climatic variables [(a) monthly average rainfall / mm, (b) monthly total rainfall / mm, (c) monthly maximum rainfall / mm, (d) monthly average relative humidity, (e) number of rainy days per month (f) monthly average temperature] with <i>Ae. aegypti</i> Breteau Index (urban).	164
Figure 4.29	Cross-correlation analysis (CCF) between climatic variables [(a) monthly average rainfall / mm, (b) monthly total rainfall / mm, (c) monthly maximum rainfall / mm, (d) monthly average relative humidity, (e) number of rainy days per month, (f) monthly average temperature] with <i>Ae. albopictus</i> Breteau Index (urban).	165
Figure 4.30	Illustration of the dengue epidemic level selected MOH areas of Gampaha district	170
Figure 4.31	Regression plot of dengue case incidence with Breteau Index for <i>Ae. aegypti</i> at one month lag in an urban setting.	174
Figure 4.32	Regression plot of dengue case incidence with monthly average relative humidity at a two-month lag in an urban setting.	175
Figure 4.33	Regression plot of dengue case incidence with Breteau Index for <i>Ae. albopictus</i> at one month lag in a suburban setting	179
Figure 4.34	Regression plot of dengue case incidence with Breteau Index for <i>Ae. aegypti</i> at one month lag in a suburban setting.	180

Figure 4.35	Regression plot of dengue case incidence with Breteau Index for <i>Ae. albopictus</i> at one month lag in a rural setting.	184
Figure 4.36	Residual normality plots of the developed models; a, b; urban setting, c, d; suburban settings, e; rural settings.	187
Figure 4.37	The goodness of fitness of model developed for urban settings of Gampaha district. Fitted and forecast dengue case incidence from the developed model illustrated for a period of 2014-2018 of Jaela MOH area.	188
Figure 4.38	The goodness of fitness of model developed for suburban settings of Gampaha district. Fitted and forecast dengue case incidence from the developed model illustrated for a period of 2014-2018 of Biyagama MOH area. Epidemic threshold level is denoted with a dotted line on the graph at 26 cases per 100,000 population.	199
Figure 4.39	The goodness of fitness of model developed for rural settings of Gampaha district. Fitted and forecast dengue case incidence from the developed model illustrated for a period of 2014-2018 of Katana MOH area. Epidemic threshold level is denoted with a dotted line on the graph at 18 cases per 100,000 population.	190

III. LIST OF TABLES

		Page no.
Table 2.1	Insecticides used for cold fogging (ULV) and thermal fogs control mosquitoes (Source; WHO,2006b).	52
Table 3.1	Selected sentinel sites (GN divisions).	68
Table 3.2	Preparation of Temephos concentration series for larval bioassays.	78
Table 3.3	Regression equations used for dengue prediction model II	91
Table 3.4	Regression equations used for dengue prediction model III.	94
Table 3.5	Regression equations used for dengue prediction model IV.	97
Table 3.6	Regression equations used for dengue prediction model V.	100
Table 4.1	Container Index (CI) for <i>Aedes aegypti</i> mosquitoes encountered at different study setups in Gampaha District, Sri Lanka.	114
Table 4.2	Container Index (CI) of <i>Aedes albopictus</i> mosquitoes encountered at different study setups in Gampaha District Sri Lanka	115
Table 4.3	Frequency and relative prevalence of adult mosquitoes collected at different indoor and outdoor resting places.	117
Table 4.4	Frequency and relative percentage of adult mosquitoes collected at different resting heights.	120

Table 4.5	Mean density of immature stage across different habitat categories for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> .	125
Table 4.6	Frequency occurrence of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> containers across different habitat categories according to the level of larval density.	126
Table 4.7	Asymptotic significance of physicochemical characteristics across different breeding habitats of <i>Aedes</i> mosquitoes.	127
Table 4.8	Asymptotic significance of physico-chemical characteristics across <i>Ae. aegypti</i> and <i>Ae. albopictus</i> .	128
Table 4.9	Asymptotic significance of physico-chemical characteristics across indoor and outdoor breeding localities for <i>Aedes</i> mosquitoes.	129
Table 4.10	Physicochemical parameters of different breeding habitats of <i>Aedes spp.</i> in Gampaha District, Western Province, Sri Lanka.	142
Table 4.11	Mean density of immature stages of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> in habitat categories under different water conditions.	144
Table 4.12	Percentage mortality rates of <i>Ae. aegypti</i> larvae exposed to different concentrations of temephos.	148
Table 4.13	Percentage mortality rates of <i>Ae. albopictus</i> larvae exposed to different concentrations of temephos	150
Table 4.14	LC ₅₀ and LC ₉₉ values of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> larvae exposed to 24 hours for temephos	152

Table 4.15	Pearson's correlation coefficient (R) for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquitoes with climatic variables significant (p) value at three different environmental settings in the District of Gampaha.	162
Table 4.16	Cross-Correlation Function summary for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquitoes with climatic variables at urban environmental settings with one to five lag periods in the District of Gampaha.	166
Table 4.17	Cross-Correlation Function summary for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquitoes with climatic variables at suburban environmental settings with one to five lag periods in Gampaha.	168
Table 4.18	Cross-Correlation Function summary for <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquitoes with climatic variables at rural environmental settings with one to five lag periods in Gampaha.	168
Table 4.19	Cross-Correlation Function summary for case incidence with climatic variables and vector indices at one to five lag periods in the District of Gampaha.	171
Table 4.20	Pearson correlation coefficient values and regression equations for prediction models for urban setup	173
Table 4.21	Predicted case incidences for urban areas of Gampaha District based on the selected model.	176

Table 4.22	Pearson correlation coefficient values and regression equations for prediction models for suburban setup.	178
Table 4.23	Predicted case incidences for suburban areas of the Gampaha district based on the selected model.	182
Table 4.24	Pearson correlation coefficient values and regression equations for prediction models for rural setup.	185

IV. LIST OF APPENDICES

		Page no.
Annexure 1	Work plan	290
Annexure 2	Data forms	291
	Annexure 2.1. Larval entomological surveillance data entry form	291
	Annexure 2.2. Data form of water quality characteristics of breeding sites	292
	Annexure 2.3. Adult resting mosquito collection form	293
Annexure 3	Consent forms	294
	Annexure 3.1. Consent form for adult landing collection and double trap net collection – English	294
	Annexure 3.2. Consent form for adult landing collection and double trap net collection - Sinhala	295
Annexure 4	Ethical clearance	296
Annexure 5	Research publications	297
	Annexure 5.1. Full Paper publications	297
	Annexure 5.2. Conference papers	297

V. LIST OF ABBREVIATIONS

<i>Ae.</i>	<i>Aedes</i>
A/C	Air Conditioner
ARIMA	Auto Regressive Integrated Moving Average
BI	Breteau Index
BIA _(t-1)	Breteau Index for <i>Ae. aegypti</i> at one month lag
BIB _(t-1)	Breteau Index for <i>Ae. albopictus</i> at one month lag
BIAR _(t-1)	Breteau Index for <i>Ae. aegypti</i> in rural areas at one month lag
BIAS _(t-1)	Breteau Index for <i>Ae. aegypti</i> in suburban areas at one month lag
BIAU _(t-1)	Breteau Index for <i>Ae. aegypti</i> in urban areas at one month lag
BIBR _(t-1)	Breteau Index for <i>Ae. albopictus</i> in rural areas at one month lag
BIBS _(t-1)	Breteau Index for <i>Ae. albopictus</i> in suburban areas at one month lag
BIBU _(t-1)	Breteau Index for <i>Ae. albopictus</i> in urban areas at one month lag
BIMR _(t-1)	Cumulative Breteau Index in rural areas at one month lag
BIMS _(t-1)	Cumulative Breteau Index in suburban areas at one month lag
BIMU _(t-1)	Cumulative Breteau Index in urban areas at one month lag
Bti	<i>Bacillus thuringiensis</i>
°C	Celsius
CCF	Cross-Correlation Function
CI	Container Index
CDC	Centre for disease control and prevention
DDT	Dichlorodiphenyltrichloroethane
CFR	Case Fatality Rate

ChE	Cholinesterase
cm	centimeter
DENV	Dengue Virus
df	degree of freedom
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DI	Disease Incidence
DIR	Disease Incidence; Rural
DIS	Disease Incidence; Suburban
DIU	Disease Incidence; Urban
DO	Dissolved Oxygen
DS	Divisional Secretariats
DSS	Dengue Shock Syndrome
EC	Emulsifiable concentrate
ECDC	European Centre for Disease Prevention and Control
EIP	Extrinsic incubation period
F0	Zeroth generation/ wild generation
F1	First generation
FAO	Food and Agriculture Organization
GR	Granules
GN	Grama Niladhari
HI	House Index
IIP	Intrinsic incubation period
IVM	Integrated Vector Management
l	liter

LC	Lethal concentrations
L:D	Light: Dark
Lg DI	Log-transformed disease incidence
m	meter
mg	milligrams
ml	milliliter
mm	millimeter
MOH	Medical Officer of Health
MLR	Multiple Linear Regression
NDCU	National Dengue Control Unit
NTU	Nephelometric Turbidity unit
Ops	Organophosphates
PHI	Public Health Inspector
ppm	parts per million
RD _(t-2)	Rainy days at two-month lag
RFavg _(t-2)	Average monthly rainfall at lag two months
RFmax _(t-2)	Monthly maximum rainfall at lag two months
RFtot _(t-2)	Total monthly rainfall at lag two months
RHavg _(t-2)	Monthly average relative humidity a two-month lag
RH	Relative Humidity
RNA	Rhibo Nucleic Acid
SD	Standard Deviation
SG	Sand granules
SPSS	Statistical Package for the Social Sciences
Tavg _(t-3)	Monthly average atmospheric temperature at three-month lag

TDS	Total dissolved solids
TSS	Total suspended solids
ULV	Ultra-Low Volume
WDP	water-dispersible powders
WP	Wettable powders
WQI	Water Quality Index
WHO	World Health Organization
μs	microsiemens

VI. ACKNOWLEDGEMENTS

My deepest gratitude and sincere appreciation is extended to my main supervisor Prof. (Mrs) L. D. Amarasinghe, Professor, Department of Zoology and Environmental Management, University of Kelaniya, for her guidance, encouragement, and many useful suggestions during my Ph.D. research activities and writing of the dissertation. I appreciate all her contributions of time, ideas, and funding to make my Ph.D. productive and stimulating.

I am extremely grateful to Prof. P. A. D. H. Nayana Gunathilaka, Professor, Department of Parasitology, Faculty of Medicine, University of Kelaniya who is my co-supervisor, for his guidance, encouragement, and many useful suggestions during my Ph.D. research activities and writing of the dissertation. I also thank Prof. P. A. D. H. Nayana Gunathilaka for his technical co-operation for the laboratory studies.

My sincere thanks also go to Prof. (Mrs.) Annista Wijayanayake, Professor, Department of Industrial Management, University of Kelaniya for her guidance for the statistical analysis and modelling part of the current study.

I gratefully acknowledge Dr. A. L. Fareed, The Provincial Director of Health Service, Western province and Dr. Nalin Ariyaratna, The Regional Director of Health Service, Gampaha district, who has permitted me to utilize the district entomological teams, laboratory and research facilities for conducting the study and allowing me to plan all the district entomological activities base on the requirements of the current research study. I would also like to thank all the Health Entomological Officers and other staff members of

the Gampaha district entomology team for their kind cooperation and generous help is given throughout the study period. Further, I would like to acknowledge the National Dengue Control Unit of Sri Lanka for providing standard WHO susceptibility test kits and technical cooperation for laboratory studies.

I would also like to thank Dr Tharaka Ranathunge, Former Lecturer, Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya and Dr. Lahiru Udyanga, Lecturer Department of Biosystems Engineering, Faculty of Agriculture & Plantation Management, Wayamba University of Sri Lanka for their technical guidance and many useful suggestions during my Ph.D. research activities.

My gratitude goes to Dr. Subashini Aryaprema, Senior Entomologist, Colombo district and Mrs. Sakunthala Janaki, Entomologist, National Dengue Control Unit and all other regional entomologists for their technical guidance and generous help extended throughout the study.

I would like to thank to all my colleagues for their kind cooperation. I acknowledge the co-operation and generous help given by staff members of the Department of Zoology and Environmental Management, University of Kelaniya, and Department of Parasitology, Faculty of Medicine, University of Kelaniya for their generous help throughout the study.

I gratefully acknowledge Prof. G.A.S.M Ganehiarachchi, Head of the Department and Professor, Department of Zoology and Environmental Management, University of Kelaniya for his guidance, encouragement throughout the study. I would also like to acknowledge to Prof. Sudath R D Kalingamudali, Dean, Faculty of Science, University of

Kelaniya, Dean, Faculty of the Graduate Studies University of Kelaniya and Vice-Chancellor, University of Kelaniya, for accepting my candidature for the Degree of Doctor of Philosophy.

Finally, I would also like to show my gratitude to my parents and husband, for their encouragement and support extended throughout the study.

VII. ABSTRACT

Sri Lanka had experienced periodic dengue epidemics every two to three years for the last two decades. In this scenario, understanding bionomics aspects, and the prevalence of dengue vector species in varied settings will help to develop more accurate and focused vector control approaches. Thus, the current study is intended to elucidate the bionomics of *Aedes aegypti* and *Ae. albopictus*, in urban (Negombo, Wattala, Kelaniya), suburban (Attanagalla, Gampaha, Minuwangoda) and rural (Dompe, Meerigama, Divulapitiya) areas of the Gampaha District, Sri Lanka from April 2017 to December 2019. The effectiveness of the insecticide space spraying depends on susceptibility status and the behaviour of the targeted species, hence a part of this study included testing susceptible levels of *Ae. aegypti* and *Ae. albopictus* against malathion and deltamethrin insecticides. The study also sought to establish area-specific threshold values for determining the risk of dengue transmission based on larval indices, and a forecasting model for predicting impending dengue outbreaks within the district.

Total 19,835 possible breeding habitats were investigated at 13,563 premises where *Aedes* larvae were identified in 11.03% of premises and 1856 habitats were positive for larvae (*Ae. aegypti*; 9.8%; *Ae. albopictus*; 90.2 %). Results showed that *Ae. aegypti* prefers urban locations while *Ae. albopictus* is the predominant vector in all spatial settings. *Ae. albopictus* (54.5%; n= 999) dominated the adult collection, followed by *Ae. aegypti* (45.5%; n= 835), with a 1:4 male to female ratio. *Ae. aegypti* mosquitoes demonstrated endophilic resting behaviour, whereas *Ae. albopictus* were exophilic (Chi-square analysis between the two species; $P < 0.001$). Resting places of *Aedes aegypti* recorded as on cloth hangings (36.9%, n=308) and under furniture (40.4%) predominantly in bedrooms (40.4%) and living rooms (24.8%) while *Ae. albopictus* preferred to rest on outdoor vegetation

46%(n=460). The majority (43.7 %; n=801) of *Aedes* mosquitoes rest 1–2 m above ground, and 34.4 % (n=399) rested 1 m or less. The host-seeking cycle of *Ae. albopictus* was bimodal, with morning peak occurring between 05:00 and 11:00 and afternoon peak between 14:00 and 19:00. While that of *Aedes aegypti* was with a minor peak between 05:00 and 09:00 and a major peak between 13:00 and 19:00.

Physicochemical parameters of mosquito breeding water changed significantly across breeding categories (Kruskal–Willi's statistics, $p < 0.05$), but not among species. Concrete slabs, temporary removals, and water storage items were the key breeding habitats of *Ae. aegypti*, while those for *Ae. albopictus* were concrete slabs, gutters, and natural habitats. Deltamethrin and malathion exposure to adults and temephos exposure to larvae of *Ae. aegypti* and *Ae. albopictus* showed a considerable geographical variation of mortality ($P < 0.001$) showing the change of mosquito susceptibility status.

There was a strong positive association between rainfall, larval vector density, and the likelihood of high dengue incidence. When Breteau Index for *Ae. aegypti* (BIA) exceeds 3.00 and relative humidity exceeds 80%, an early epidemic alert is triggered while BIA > 6.0, case incidence reached an epidemic level in urban areas. In suburban areas, when BI for *Ae. albopictus* (BIB) > 14.0 an early epidemic alert is triggered. At the BIB > 20, case incidence reached epidemic levels even in absence of *Ae. aegypti*. Case incidence exceeds the epidemic level in rural areas when the BI for *Ae. albopictus* is 10 with a one-month latency. In conclusion, area-based entomological thresholds and rational use of insecticides can be proposed for future control of rising dengue epidemics in the Gampaha district based on vector biology/bionomic related evidence through regular monitoring.

Keywords ,

Dengue , *Ae. aegypti* , *Ae. albopictus* , Gampaha , bionomics

xxx