

A comparative study of *Aedes albopictus* becoming a global pest and its native relative *Aedes flavopictus*

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Dissertation

**A comparative study of *Aedes albopictus* becoming a global pest and its
native relative *Aedes flavopictus***

世界的な害虫となっているヒトシジシマカとその近縁種ヤマダシマカの比較研究

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This research is dedicated to my mother and departed soul of my father. I also dedicate this work to my beloved wife, and my two lovely sons, for their great sacrifice to complete this study.

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CONTENTS

| Chapter | Contents | Page |
|---------|---|------|
| | Acknowledgements | |
| | Table of contents | |
| | List of tables | |
| | List of figures | |
| | Abstract | |
| 1. | General introduction | |
| | 1.1. Organization of the study | 11 |
| | 1.2. Background of the study | 16 |
| | 1.2.1. <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> | |
| | 1.2.2. Distribution patterns of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> | |
| | 1.2.3. Global climate change impact on <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> | |
| | 1.2.4. Effects of larval environments on adult biology and vectorial capacity of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> | |
| | 1.3 Objectives of the study | 23 |
| | References | 25 |

| | | |
|--------|---|----|
| 2. | A comparative study of <i>Ae. albopictus</i> becoming a global pest and its native relative <i>Ae. flavopictus</i> | |
| 2.1. | Introduction | 32 |
| 2.2. | Materials and Methods | 34 |
| 2.2.1. | Mosquito sources | |
| 2.2.2. | Experimental design | |
| 2.2.3. | Measurement of wings | |
| 2.2.4. | Per Capita Performance | |
| 2.2.5. | Data analysis | |
| 2.3. | Results | 39 |
| 2.4. | Discussion | 43 |
| | References | 48 |
| 3. | Seasonal fluctuation in distribution and habitat of <i>Ae. albopictus</i> (Diptera: Culicidae) and <i>Ae. flavopictus</i> in Kanazawa, Japan. | |
| 3.1. | Introduction | 57 |
| 3.2. | Materials and methods | 59 |
| 3.2.1. | Study area | |
| 3.2.2. | Adult collection | |
| 3.2.3. | Larval collection | |
| 3.2.4. | Mosquitoes identification | |
| 3.2.5. | Oviposition patterns | |
| 3.3. | Results | 61 |

| | | |
|--------|---|----|
| 3.3.1. | Adult Spatial distribution | |
| 3.3.2. | Adult Seasonal Distribution | |
| 3.3.3. | Larval abundance and seasonal trends | |
| 3.3.4. | Larval abundance among microhabitat | |
| 3.3.5. | Oviposition patterns | |
| 3.4. | Discussion | 64 |
| | References | 68 |
| 4. | Influence of vegetation type on population growth of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> | |
| 4.1. | Introduction | 72 |
| 4.2. | Materials and methods | 74 |
| 4.2.1. | Leaf litter source | |
| 4.2.2. | Mosquitoes strain | |
| 4.2.3. | Experimental design | |
| 4.3. | Results | 77 |
| 4.4. | Discussion | 78 |
| | References | 81 |
| 5. | Summary and conclusions | |
| 5.1. | Author summary | 86 |
| 5.2. | Concluding remarks | 89 |
| 5.3. | Scope for further research | 90 |
| 5.4. | Limitations of this study | 91 |

LIST OF TABLES

| | Page |
|--|-------------|
| Table-2.1. Mortality ratio of <i>Ae. albopictus</i> (albo) and <i>Ae. flavopictus</i> (flavo) with result of logistic regression analysis and comparisons (Fisher's exact test) under same conditions on the mortality rate in developmental stage (first instar to emergence) | 92 |
| Table- 2.2. The developing time (2A) and wing size (2B) of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> under different rearing temperatures. | 93 |
| Table- 2.3. Fecundity of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> under different rearing temperatures with one or two blood meals. | 94 |
| Table- 3.1. Status of Adult catch mosquitoes in Kanazawa 2017- 2018 | 95 |
| Table- 4.1. Wing size of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> under the influence of different vegetation type. | 96 |

LIST OF FIGURES

| | Page |
|---|------|
| Fig. 2.1. Female ratio of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> at 22, 25, var. 25 and 28°C. | 97 |
| Fig. 2.2. Estimated per capita performance index <i>I</i> of <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> with observed sex ratio under different rearing temperatures (22, 25, var. 25 and 28 °C). | 97 |
| Fig. 2.3. Relationship of fecundity vs wing length of <i>Ae. albopictus</i> A, B, C & D are at 22, 25, 28°C and var. 25°C respectively. | 98 |
| Fig. 2.4. Relationship of fecundity vs wing length of <i>Ae. flavopictus</i> A, B, C & D are at 22, 25, 28°C and var. 25°C respectively. | 98 |
| Fig. 3.1. Locations maps of the study sites in Kanazawa | 99 |
| Fig. 3.2. 30 Years mean temperature and precipitation for the city of Kanazawa. | 99 |
| Fig. 3.3. Seasonal trends of <i>Stegomyia</i> adults at different sites on Kanazawa. | 100 |
| Fig. 3.4. Seasonal variation of <i>Stegomyia</i> larval abundance in Kanazawa. | 101 |
| Fig. 3.5. Microhabitat type preference as ovipositioning site by <i>Ae. albopictus</i> and <i>Ae. flavopictus</i> female. | 101 |
| Fig. 4.1. Larval mortality from 1 st instar to Pupa A) <i>Ae. albopictus</i> B) <i>Ae. flavopictus</i> . | 102 |

Fig. 4.2. Developmental duration from 1st ~ 4th instar A) *Ae. albopictus* 102
B) *Ae. flavopictus*

Title: A comparative study of *Aedes albopictus* becoming a global pest and its native relative *Aedes flavopictus*

Abstract

Aedes albopictus, a well-known vector of some globally important viral diseases like dengue, zika or chikungunya, has first been reported in Asia and expanded its distribution worldwide in the last three decades. In Japan, this species occurs from the Ryukyu islands in Tohoku district, whereas its sibling species *Ae. flavopictus* is distributed throughout Japan, including Hokkaido. On the other hand, the former species generally inhabits in rural and urban areas, whereas the latter species does natural environments such as bamboo groves and forests.

To understand how they differ in habitat use, firstly their performance was compared under various temperature regimes, i.e., constant temperature regimes of 22°C, 25°C and 28°C and a fluctuating temperature regime of 20-30°C (var. 25°C). Mortality from the first instar stage to adult emergence was significantly higher in *Ae. flavopictus* than in *Ae. albopictus* at constant temperatures of 25 and 28°C. Development time was significantly longer in *Ae. flavopictus* than in *Ae. albopictus* at 28°C. The proportion of females that did not oviposit was significantly higher in *Ae. flavopictus* at a constant temperature of 28°C and a fluctuating temperature regime. Fecundity was positively correlated with wing size in *Ae. albopictus* but in *Ae. flavopictus* positive correlation at a lower temperature (22°C, 25°C) and a negative correlation of higher temperature (28 &

var. 25°C). Per capita performance was higher in *Ae. flavopictus* at a constant temperature of 25°C, but in *Ae. albopictus* at the other temperature regimes.

Furthermore, to understand the seasonal fluctuation and distribution, we studied the temporal and spatial distribution of adult and larvae of *Ae. albopictus* (Skuse) and *Ae. flavopictus* (Yamada) in Kanazawa city, Ishikawa, Japan. Adults of *Ae. albopictus* attracted to human bait were distributed uniformly in the urban part of the city, an area disturbed by humans, whereas *Ae. flavopictus* was confined to the bamboo forest. Adults of both species occurred from May through September. Both *Ae. albopictus* and *Ae. flavopictus* were much more abundant in July and August. Larval *Ae. flavopictus* occurred primarily bamboo stumps, but also were found in larger artificial containers in forests. Although the abundance of adult *Ae. albopictus* in the urban site, larvae occurred in the forest less frequently than *Ae. flavopictus*; *Ae. albopictus* larvae mostly found in the forest during hot summer.

In order to determine how vegetation change influences the population growth of *Ae. albopictus* and *Ae. flavopictus* being fed with three types of leaf, deciduous broad-leaved trees, evergreens, bamboo leaves in laboratory condition. Overall mortality was higher in all three-leaf type, but mortality from the fourth instar stage to pupation is higher in *Ae. albopictus* compared with *Ae. flavopictus*. Bamboo leaf shows the rapid larval development than evergreen and deciduous leaf up to 4th instar larvae in both the species.

Thus, *Ae. albopictus* is at least more adapted to temperatures, food resource and some others ecological parameter than *Ae. flavopictus*. So, the adaptation of

Ae. albopictus to a wide range of temperature and its high ecological plasticity, might result in their different geographic distribution and habitat use which might play important role in established *Ae. albopictus* as a one of most important vectors globally.

CHAPTER 1

General introduction

1.1. Organization of the study

This dissertation is the outcome of 3 years of research into the effect of larval environment, such as, different temperature regimes on the larval development, adult biology of most important invasive vector mosquito *Ae. albopictus* Skuse and its relative mosquito species *Ae. flavopictus*. In addition to that, we also describe the seasonal fluctuation and different common vegetation as a larval food with population performance of both the species. The materials presented in this dissertation are organized into 5 chapters.

This chapter 1 begins with a brief introduction reflecting the background, and the objectives of the study.

Chapter 2 provides a detailed account on Effects of temperature on development and reproduction of *Ae. albopictus* and *Ae. flavopictus* (Diptera: Culicidae). How constant and variable temperature affects the larval mortality, developmental duration, body size of emerging adults, adult life history traits, such as, multiple blood feeding, fecundity and hatchability were examined, and a detail on the background, materials and methods, results with the discussion are presented there.

Chapter 3 discusses about Seasonal fluctuation in distribution and habitat of *Ae. albopictus* and *Ae. flavopictus* in Kanazawa, Japan. Temporal and spatial distribution of both the species abundance and seasonal fluctuation data has been presented here.

Chapter 4 examines Influence of vegetation type on population growth of *Ae. albopictus* and *Ae. flavopictus*. how the vegetation change influences the population size of them, the performances of the two species were fed with major three type vegetation, deciduous broad-leaved trees, evergreens, bamboo leaves were evaluated in laboratory and describe the background, materials and methods, results with the discussion.

Chapter 5 provides a summary of all experiments. From this discussion, I draw some concluding remarks and scope for further research. I put some limitations of my study in this chapter.

1.2. Background of the study

1.2.1. *Ae. albopictus* and *Ae. flavopictus*

Ae. albopictus Skuse, belonging to *Stegomyia* species commonly known as the Asian tiger mosquito/forest day mosquito, is an epidemiological important vector for several arboviruses such as dengue, yellow fever, Zika, and Chikungunya (Kow et al. 2001; Gubler 2002; Lounibos 2002; Maurice et al. 2010). A number of other viruses affecting human health have also been identified from field-collected *Ae. albopictus* in different countries. Moreover, its recent involvement in the localized transmission of Chikungunya virus in Africa, India, Bangladesh, Southeast Asia, Western Pacific and Americas and dengue virus in most Asian and Latin American countries focuses on the importance of monitoring this invasive species. During the last 3–4 decades, *Ae. albopictus* has spread from native Asian area to all continents except Antarctica (Paupy et al 2009), becoming the most invasive mosquitoes, which imposed an extensive public health threat to human beings throughout the world.

Opposite to, some *Stegomyia* spp. in the scutellaris group (Huang 1979) are confined to local distribution in Asia. *Ae. flavopictus* an indigenous species, belonging to the *Stegomyia* species in subtropical and warm temperate zones of southwestern Japan, have restricted local distributions, compared with *Ae. albopictus*.

There may exist significant biological differences between globally invasive *Ae. albopictus* and locally distributed *Ae. flavopictus* that are related to their dispersal and colonizing abilities. Relative studies of the behavior, ecology,

physiology and biological features of *Ae. albopictus* and its associated *Stegomyia* species may help to clarify divergent environmental adaptations among *Stegomyia* that may be significant in understanding of different *Stegomyia* species evolutionary behavior which may further help to envisage the distribution and abundance of *Ae. albopictus* in human-disturbed habitats as well as adaptation to wider range of area from tropical to temperate zone.

1.2.2. Distribution patterns of *Ae. albopictus* and *Ae. flavopictus*

Ae. albopictus, is an important vector for some viral disease like dengue, Chikungunya which are common in Asian countries. These species are important to man as the vectors of DENV and CHIKV because of their competence to breed in artificial containers in and around the house, close to human habitats (Cheong, 1967). *Ae. albopictus* is commonly found in urban, suburban and rural settings with vegetation. The predicted distribution of *Ae. albopictus* includes most of Asia and coverings tropical and subtropical regions worldwide (Howley, 1987), with a temperate distribution in North America, Europe and in the United States (Hobbs *et al*, 1991, Benedict *et al* 2007, Mogi *et al* 2017). *Ae. albopictus* is a generalist that freely adjusts to the diverse environmental conditions in both hot, humid area to cold temperate zone (Rai, 1991). The adaptation of *Ae. albopictus* to a wide range of temperature conditions and its high ecological plasticity, are important characteristics of populations persisting in seasonally changing environments (Tsuda & Takagi, 2001).

Ae. albopictus has experienced an intense global expansion accelerated by human activities, especially the trading of used tires and ‘lucky bamboo’. Together with passive transit via public and private transport, this has resulted in a widespread global distribution of *Ae. albopictus*. It is now recorded as one of the top 100 invasive species by the Invasive Species Specialist Group.

The accomplishment of the conquest of *Ae. albopictus* is due to a number of elements, including: its ecological flexibility, robust competitive fitness, globalization, i.e. increase of trade and travel, lack of surveillance, and lack of effective control. Climate change projections suggest that *Ae. albopictus* will continue to be a successful invasive species that will spread elsewhere its current geographical margins. This mosquito is now exhibiting the signs of adaptation to cooler waters which may result in disease transmission in new areas. Invasion of *Ae. albopictus* in new areas, is one of the several hypotheses for the recent intensified outbreaks, and expansion of DENV and CHIKV into new regions (Paupy *et al.*, 2010).

Last 3-4 decades *Ae. albopictus* have experienced a dramatic global expansion of its geographic distribution which continues till date (Benedict *et al.*, 2007). This mosquito originated from the Southeast Asia, but human movement towards the Malay Peninsula and the Indian Ocean islands, including Madagascar, may have led to an early invasion of *Ae. albopictus* out of its native Asian range (Paupy *et al.*, 2009). *Ae. albopictus* extended its distribution in the southern United States, it appeared to displace *Ae. aegypti* (Hobbs *et al.*, 1991). Between

1985 and 1998, *Ae. albopictus* was recovered from many countries in the Americas and the Caribbean (Benedict *et al.*, 2007).

On the other hand, *Ae. flavopictus* is extensively found throughout mainland Japan (Palearctic region) and Korea. The type locality of the species is Tokyo, Japan. It is a local mosquito's species exclusively found in the forest and its larvae mostly breed in bamboo stumps and tree holes (Tanaka *et al.* 1979, Toma and Miyagi 1986). Females of *Ae. flavopictus* shown strong biting tendency in day time in the forest. Experimentally *Ae. flavopictus* can replicate and transmit dengue virus, but there is no evidence as a vector in nature (Eshita 1982, Eshita *et al.* 1982, Tang *et al.* 1987).

1.2.3. Global climate change impact on *Ae. albopictus* and *Ae. flavopictus*

Global warming and Climate change may influence on the risk of the world-wide distribution of vector-borne diseases (Hales *et al.*, 2002; Liu-Helmersson *et al.*, 2014; McMichael, 2013; Murray *et al.*, 2013; World Health Organization (WHO), 2015). Changes in temperature variation have great impacts on mosquito populations, and possibly as important as changes in mean temperatures, if not more (Lambrechts *et al.*, 2011;). Since 1950, diurnal temperature limits have increased throughout the globe and magnitudes of annual temperature cycles increased by 0.4 °C in temperate regions. This could result in the spread of vector of different viral disease are introduced and re-establish temperate part of the globe.

Both *Ae. albopictus* and *Ae. flavopictus* are susceptible to temperature changes as larval stages in the aquatic environment and as adults. In higher temperature the larvae hastily grow to adults (Reuda *et al.*, 1990, Tun-lin *et al.*, 2000, Delatte *et al.*, 2009 and Farjana *et al.*, 2012) and subsequently there is a greater capacity to produce more offspring (Alto & Juliano, 2001a, 2001b). Temperature changes and variation in precipitation affect the population dynamics of *Ae. albopictus*, which indicates that climate change is expanding the limits of its northern distribution (Alto & Juliano, 2001b). Pumpuni *et al.* (1992) found that higher temperatures significantly reduce or stop diapause incidences in *Ae. albopictus* specimen that was exposed to critical photoperiods. In warmer climates, increase vector breeding rate and biting tendency, faster blood ingestion further boosting the spread of infection (Gillies, 1953).

According to recent forecasting, by the end of the next century, average world temperature will have risen by 0.3 to 4.8 °C and rainfall patterns also change in irregular orders, such as drought or inundation (Collins *et al.* 2013) which may create a condition which may be unfavorable for some insects, but some are adapted and behave like extreme insect pest and transmitting more intense than previous trends that may increase the probability of these vector borne diseases. The temporal and spatial changes in temperature, precipitation and humidity that are expected to occur under different climate change scenarios will affect the biology and ecology of vectors, and consequently the risk of disease transmission (Githeko *et al.*, 2000). In the range of 30–32°C, vectorial capacity of *Ae. spp* for dengue can increase substantially owing to a reduction in

the extrinsic incubation period (Watts *et al.*, 1987), despite a reduction in the vector's survival rate (Reuda *et al.*, 1990). Warming above 34°C generally has a negative impact on the disease transmission, because the mortality rate of *Ae. albopictus* is too high at this high temperature (Reuda *et al.*, 1990; Tun-lin *et al.*, 2000; Delatte *et al.*, 2009). By modeling the effect of temperature, Barbazan *et al.* (2010) predicted that global warming will increase the probability of dengue virus transmission by *Ae. albopictus*.

1.2.4. Effect of larval environment on adult biology and vectorial capacity of *Ae. albopictus* and *Ae. flavopictus*

The effect of larval environment on adult life history traits of *Ae. albopictus* such as developmental duration, age of maturity, biting tendency, lengths of gonotrophic cycle, Adult body size, oviposition, and life span are well documented (Briegel, 1990; Rueda *et al.*, 1990; Tun-lin *et al.*, 2000, Delatte *et al.*, 2009) but few reports about *Ae. flavopictus*. Body size of adult *Ae. flavopictus* is comparatively larger than *Ae. albopictus* when they grow in the same temperature regimes. With the increasing temperature body size are decreasing in both species. Larval food supply is also an important factor to emergence of larger *Ae. spp* (Briegel, 1990). Body size has been interrelated to numerous features influencing the capacity of a mosquito population to spread disease, such as fecundity and gonotrophic cycle (Briegel, 1990), longevity (Naschi, 1986, 1988), biting contact with the host and feeding success (Naschi, 1988, 1991; Briegel, 1990), and vector competence (Grimstad & Haramis, 1984).

The larval habitat environment may also stimulate adult *Aedes*-pathogen interactions, by altering a vector competence for a virus resulting in variations in the distribution and transmission power of an arbovirus. Larval habitat factors, like temperature, food availability, larval density and inter and intraspecific competition can affect vectorial capacity in certain *Aedes*-arboviral systems (Grimstad & Walker, 1991; Turell, 1993; Sumanochitrapon *et al.*, 1998; Alto *et al.*, 2005; Alto *et al.*, 2008). Changes in larval nutrition are related to the different vector competence of *Ae.* species (Grimstad & Walker 1991). Higher levels of intra- or interspecific competition among larvae increased the vulnerability of *Ae. albopictus* to dengue virus infection and the potential for transmission, as indicated by disseminating infections (Alto *et al.*, 2008). *Ae. albopictus* with the largest body size were emerging from the 18°C temperature, and six times more likely to be infected with CHIKV than females reared at 32°C (Westbrook *et al.*, 2010). There was also a significant effect of rearing temperature on CHIKV dissemination, resulting in an increase in the population dissemination at the coolest temperature (Westbrook *et al.*, 2010).

1.3. Objectives of the study

Ae. albopictus is now globally distributed and most invasive species compared with *Ae. flavopictus*, why such type of distribution difference occur between the two species is our main focusing point. Anticipation of a disease outbreak such as DENV and CHIKV requires one or more of the factors which are responsible for causes the occurrence of disease are required to control/stop, i.e. the pathogen, vector, reservoir and/or susceptible host. The most realistic way to prevent epidemic or dissemination of the disease is to target the vectors. Before any attempt like chemical or biological controls, the vector ecology and disease transmission pathways must be understood. Most studies have emphasized on the adult environment with fewer studies investigating about the influence of larval ecology of *Aedes* arbovirus interactions. Likewise, previous research has mainly focused on the effect of a single factor in the larval setting but not considering some other factor. So, therefore, the purpose of this study is to determine the probable effects of temperature on the development and adult stage performance of *Ae. albopictus* and *Ae. flavopictus*, that will help to clarify the invasive and widely distributional behavior of the former species and mysterious distribution which is the restricted distribution of the latter species. However, the specific objectives of this study are as follows:

1. To determine the effect of constant and fluctuating temperature effects on larval mortality, developmental duration and adult body size of *Ae. albopictus* and *Ae. flavopictus*.

2. To determine the effect of adult body size on multiple blood feeding and fecundity of *Ae. albopictus* and *Ae. flavopictus*.
3. To determine the effect of different temperature regimes throughout the larval stage on adult's performance, such as, multiple blood feeding, fecundity, length of gonotrophic cycle and sex ratio of *Ae. albopictus* and *Ae. flavopictus*.
4. To evaluate the population size of *Ae. albopictus* and *Ae. flavopictus* in various temperature regimes.

References

- Alto, B.W. & Juliano, S.A. (2001a) Temperature effects on the dynamics of *Ae. albopictus* (Diptera: Culicidae) populations in the laboratory. *Journal of Medical Entomology*, **38**, 548-556.
- Alto, B.W. & Juliano, S.A. (2001b) Precipitation and temperature effects on populations of *Ae. albopictus* (Diptera: Culicidae): Implications for range expansion. *Journal of Medical Entomology*, **38**, 646-656.
- Alto, B.W., Lounibos, L.P., Higgs, S. & Juliano, S.A. (2005) Larval competition differentially affects arbovirus infection in *Ae.* mosquitoes. *Ecology*, **86**, 3279–3288.
- Alto, B.W., Lounibos, L.P., Mores, C.N. & Reiskind, M.H. (2008) Larval competition alters susceptibility of adult *Ae.* mosquitoes to dengue infection. *Proceedings of Royal Society of London-B Biological Science*, **275**, 463–471.
- Barbazan, P., Guiserix, M., Boonyuan, W., Tuntaprasart, W., Pontier, D. & Gonzalez, J.P. (2010) Modelling the effect of temperature on transmission of dengue. *Medical and Veterinary Entomology*, **24**, 66-73.
- Benedict, M.Q., Levine, R.S., Hawley, W.A. & Lounibos, L.P. (2007) Spread of the tiger: global risk of invasion by the mosquito *Ae. albopictus*. *Vector-borne Zoonotic Disease*, **7**, 76–85.
- Briegel, H. (1990) Metabolic relationship between female body size, reserves, and fecundity of *Ae. aegypti*. *Journal of Insect Physiology*, **36**, 165-172.

- Chan, K.L., Chan, Y.C. & Ho, B.C. (1971a) *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse) in Singapore City. 4. Competition between species. *Bulletin of the World Health Organization*, **44**, 643-649.
- Cheong, W.H. (1967) Preferred *Ae. aegypti* larval habitats in urban areas. *Bulletin of Health Organization*, **36**, 586-589.
- Collins, M., R. Knutti, J. Arblaster, J. -L. Dufresne, T. Fichet, P. Friedlingstein, X. Gao, W. J. Gutowski, T. Johns, G. Krinner, et al. 2013. Long-term Climate Change: Projections, Commitments and Irreversibility. In: T. F. Stocker, D. Qin, G. -K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley (eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Delatte, H., Gimonneau, G., Triboire, A. & Fontenille, D. (2009) Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Ae. albopictus*, vector of chikungunya and dengue in the Indian Ocean. *Journal of Medical Entomology*, **46**, 33-41.
- Gillies, M.T. (1953) The duration of the gonotrophic cycle in *Anopheles gambiae* and *An. funestus* with a note on the efficiency of hand catching. *East African Medical Journal*, **30**: 129–135.

- Githeko, A.K., Lindsay, S.W., Confalonieri, U.E. & Patz, J.A. (2000) Climate change and vector-borne diseases: a regional analysis. *Bulletin of World Health Organization*, **78**, 1136-47.
- Gratz, N.G. (2004) Critical review of the vector status of *Ae. albopictus*. *Medical and Veterinary Entomology*, **18**, 215–227
- Grimstad, P. R. & Haramis, L. D. (1984) *Ae. triseriatus* (Diptera: Culicidae) and La Crosse virus. III. Enhanced oral transmission by nutrient deprived mosquitoes. *Journal of Medical Entomology*, **21**, 249-256.
- Grimstad, P.R. & Walker, E.D. (1991) *Ae. triseriatus* (Diptera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. *Journal of Medical Entomology*, **28**, 378–386.
- Gubler, D.J. (2002) Epidemic dengue/ dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Microbiology*, 10(2): 100 – 103.
- Hawley, W.A, Reiter, P, Copeland, R.S., Pumpuni, C.B, Craig, G.B.J.R. (1987) *Ae. albopictus* in North America: Probable introduction in used tires from Northern Asia. *Science*, **236**, 1114-1116.
- Hobbs, J.H., Hughes, E.A. & Eichold, B.H. II. (1991) Replacement of *Ae. aegypti* by *Ae. albopictus* in Mobile, Alabama. *Journal of the American Mosquito Control Association*, **7**, 488-489.
- Juliano, S.A. & Lounibos, L.P. (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology Letters*, **8**, 558-574.

- Kow CY, Koon LL, Pang FY. (2001) Detection of dengue viruses in field-caught male *Ae. aegypti* and *Ae. albopictus* (Diptera: Culicidae) in Singapore by type-specific PCR. *Journal of Medical Entomology*, 38: 475 – 479.
- MaDonald, W.W. (1956) *Ae. aegypti* in Malaya. II. Larval and adult biology. *Annals of Tropical Medicine and Parasitology*, 50, 399–414.
- Maurice D, Herve JP, Leroy E, Simard F. (2010) Comparative Role of *Ae. albopictus* and *Ae. aegypti* in the Emergence of Dengue and Chikungunya in Central Africa. *Vector borne and Zoonotic Diseases*, 10(3), 259 – 266.
- Mogi, M., Armbruster, P. A., Tuno, N., Aranda, C., & Yong H. S. (2017) The Climate Range Expansion of *Aedes albopictus* (Diptera: Culicidae) in Asia Inferred from the Distribution of Albopictus Subgroup Species of *Aedes* (Stegomyia). *Journal of Medical Entomology*, 54(6):1615-1625.
- Naschi, R.S. (1988) Biology of *Ae. triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. *Journal of Medical Entomology*, 25, 402-405.
- Naschi, R.S. (1991) Influence of larval and adult nutrition on biting persistence in *Ae. aegypti* (Diptera: Culicidae), *Journal of Medical Entomology*, 28, 522-526.
- Naschi, R. S. & Mitchell, C. J. (1994) Larval diet, adult size, and susceptibility of *Ae. aegypti* (Diptera, Culicidae) to infection with Ross River virus. *Journal of Medical Entomology*, 31, 123–126.
- Naschi, R. S. (1986) The size of emerging and host-seeking *Ae. aegypti* and the relationship of size to blood feeding success in the field. *Journal of American Mosquito Control Association*, 2, 61-62.

- Paupy, C., Delatte, H., Bagny, L., Corbel, V. & Fontenille, D. (2009) *Ae. albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection*, **11**, 1177-1185.
- Paupy, C., Ollomo, B., Kamgang, B., Moutailler, S., Rousset, D., Demanou, M., Herve, J.P., Leroy, E. & Simard, F.D.R. (2010) Comparative Role of *Ae. albopictus* and *Ae. aegypti* in the Emergence of Dengue and Chikungunya in Central Africa, *Vector Borne Zoonotic Diseases*, **10**, 259-266.
- Pumpuni, C.B., Knepler, J. & Craig, G.B.J.R. (1992) Influence of temperature and larval nutrition on the diapause inducing photoperiod of *Ae. albopictus*. *Journal of the American Mosquito Control Association*, **9**, 223-227.
- Rai, R.S (1991) *Ae. albopictus* in the Americas. *Annual Review of Entomology*, **36**, 459-484.
- Ratsitorahina, M., Harisoa, J., Ratovonjato, J., Biacabe, S. & Reynes, J.M. (2008) Outbreak of dengue and chikungunya fevers, Tasmania, Madagascar, 2006. *Emerging Infectious Disease*, **14**, 1135–1137.
- Reiskind, M.H., Pesko, K., Westbrook, C.J. & Mores, C.N. (2008) Susceptibility of Florida Mosquitoes to Infection with Chikungunya Virus. *American Journal of Tropical Medicine and Hygiene*, **78**, 422-425.
- Rueda, L.M., Patel, K.J., Axtell, R.C. & Stinner, R.E. (1990) Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Ae. aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **27**, 892-898.

- Stanton, A. T. (1920) The mosquitoes of far East ports with special reference to the prevalence of *Stegomyia fasciata*. *Bulletin of Entomological Research*, **10**, 333-344.
- Sumanochitrapon, W., Strickman, D., Sithiprasasna, R. & Kittayapong, P. (1998) Effect of size and geographic origin of *Ae. aegypti* on oral infection with dengue-2 virus. *American Journal of Tropical Medicine and Hygiene*, **58**, 283–286.
- Tsuda, Y. & Takagi, M. (2001) Survival and development of *Ae. aegypti* and *Ae. albopictus* (Diptera: Culicidae) larvae under a seasonally changing environment in Nagasaki, Japan. *Environmental Entomology*, **30**, 855-860.
- Tun-Lin, W., Burkot, T.R. & Kay, B.H. (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector *Ae. aegypti* in north Queensland, Australia. *Medical and Veterinary Entomology*, **14**, 31-37.
- Turell, M.J. (1989) Effects of environmental temperature on the vector competence of *Ae. fowleri* for Rift Valley fever virus. *Research in Virology*, **140**, 147–154.
- Turell, M.J. (1993) Effect of environmental temperature on the vector competence of *Ae. taeniorhynchus* for Rift Valley fever and Venezuelan equine encephalitis viruses. *American Journal of Tropical Medicine and Hygiene*, **49**, 672–676.
- Watts, D.M., Burke, D.S., Harrison, B.A., Whitmire, R.E. & Nisalak, A. (1987) Effect of temperature on the vector efficiency of *Ae. aegypti* for dengue 2

virus. *American Journal of Tropical Medicine and Hygiene*, **36**, 143–152.

Westbrook, C.J., Reiskind, M.H., Pesko, K.N., Greene, K.E. & Lounibos, L.P. (2010) Larval environmental temperature and the susceptibility of *Ae. albopictus* Skuse (Diptera: Culicidae) to Chikungunya virus. *Vector Borne Zoonotic Diseases*, **10**, 241-247.

CHAPTER 2

A comparative study of *Ae. albopictus* becoming a global pest and its native relative *Ae. flavopictus*

2.1. Introduction

Now a day's global climate change is an important dispute for worldwide distribution of vectors and vector-borne infectious diseases. Dengue fever, zika, and chikungunya are major problems in tropical, subtropical and even in the temperate regions, and are now the most common arthropods borne viral diseases worldwide (Gubler, 2002). The most prevalent mosquito species, commonly known as Asian tiger mosquitoes *Ae. albopictus* (Skuse) are competent vectors in Asian countries and Southeast Asia is the native habitat of *Ae. albopictus*. The immature stages of *Ae. albopictus* inhabited in natural water containers such as bromeliads, bamboo stumps, and tree holes in addition to artificial containers (Hawley 1988) and they can establish in urban and semi-urban areas of Asia (Hawley 1988). *Ae. albopictus* mostly observed in forest areas with more vegetation and sometimes coexisted with its related *Stegomyia* species like *Ae. flavopictus*, *Ae. riversi*, *Ae. aegypti* etc. (Sota et al. 1992, Chaves 2016). Moreover, *Ae. albopictus* is one of the most common mosquito species on the main (Honshu) and southern (Kyushu) islands of Japan, and *Ae. flavopictus* are locally distributed all over Japan and Korea.

For last few decades, *Ae. albopictus* (Lounibos 2002, Juliano and Philip Lounibos 2005) has expanded its distribution and its typically regulated by temperature, locally in Japan (Kobayashi et al. 2002, Mogi and Tuno 2014), but

also globally (Proestos et al. 2015). It has also been observed that microclimatic changes might lead to a survival rate in invasive species, as well as, changes in biological interactions between invasive species and native resident fauna (Lounibos et al. 2010). Furthermore, climate changes were also motivated altitudinal and latitudinal variations which might affect the distribution of many invertebrate species, where their tendency to both the expansion and contraction of habitat ranges (Parmesan 2006). Regional photoperiod and temperature regimes may limit the distributions of the two mosquitoes (Hawley 1988), and the degree of urbanization (Chan et al., 1971a) or vegetation/detritus type (Murrell & Juliano, 2008) may affect the outcome of interspecific competition where they are sympatric.

The vector *Ae. albopictus* is known to be able to acclimatize to various ecological conditions due to its poikilothermic characters and has shown seasonal increases in the vector population and seasonal variability in vector competence (Paupy et al., 2003). The rate and larval developmental duration mostly governed by a series of internal and external factors (Christophers, 1960; Clements, 1999) of which temperature is one of them. Higher temperatures reduce the development duration of *Ae.* species (Tun-lin et al., 2000; Alto & Juliano, 2001a, 2001b; Delatte et al., 2009), which results in the production of smaller adults (Rae, 1990; Rueda et al., 1990; Tun-lin et al., 2000). Variation in temperature can strongly impact mosquito population dynamics (Beck-Johnson et al. 2013), mosquito life history traits, the pupation age and adult size of various mosquito species which may reflect the environmental conditions during growth of the

larval stages (Reisen et al., 1984; Fish, 1985; Haramis, 1985; Lyimo et al., 1992). At high temperature and under low nutrient conditions reared larvae to develop into small adults and experience high mortality (Reisen et al., 1984; Siddiqui et al., 1976, T. Farjana et al. 2012). Conversely, larvae reared at low temperatures and fed optimally developed into large adults (Tun-Lin et al., 2000, T. Farjana et al. 2012). In addition to environmental temperature, variation in precipitation (Bomblies 2012, Johansson et al. 2009, Chowell et al. 2006) also enhance vector-borne disease transmission.

In Kanazawa, *Ae. albopictus* have expanded into the mountains during hot summer where populations of *Ae. flavopictus* occur. Knowledge of the effect of different temperatures on *Ae. albopictus* and *Ae. flavopictus* with a focus on their biology and reproduction, including dispersion helps to predict about the realities. So, in this study, we investigate the comparative effects of different temperature regimens on the different life history attributes of two related *Stegomyia* species to explain the micro- and macro habited and distribution patterns that may help to clarify the temperature as a one of the main abiotic factors for their abundance and distribution.

2.2. Materials and Methods

This study was conducted to determine the effect of different temperature regimes on the mosquito's life-cycle to evaluate the entomological parameters of two vector mosquito *Ae. albopictus* and *Ae. flavopictus* during the period from 2017 to 2018 in the Laboratory of Ecology, Kanazawa University, Japan.

2.2.1. Mosquito source

Ae. albopictus and *Ae. flavopictus* used in this experiment were obtained from a laboratory colony established for two months from field-collected mosquitoes. *Ae. albopictus* were collected at larval stage in ovitrap set up at residential area, Kanazawa City, Japan and *Ae. flavopictus* were collected at the larval stage from the bamboo forest near Kanazawa, and both were shifted to Kanazawa university ecology laboratory Insectarium. The Insectarium was maintained under $25\pm 1^{\circ}\text{C}$ and 70–90% relative humidity under 14L/10D photoperiod conditions. Adults were kept in cages at same Insectarium had free access to 3% sucrose solution, and offered a blood meal (human) five days after emergence. For maintenance of the population, one of the authors provided the blood meal. Mosquitoes of generations F2/F3 were used for the different experiments.

2.2.2. Experimental design

We examined the effect of temperature regimes on a number of life-history traits of *Ae. albopictus* and *Ae. flavopictus* comprises constant temperature regimes of 22°C , 25°C , 28°C and a fluctuating Temperature profiles were set to follow a sinusoidal series during the day, and exponential reduction at night, with an interval of temperature input. Temperature ramped between set temperatures each interval is 20°C at 8:00p. m. - 6:00a. m., 25°C at 6:00a. m. -10:00a. m., 30°C at 10:00a. m. -6:00p. m. and again 25°C at 6:00p. m. -8:00p. m. with the peak temperature of each day reached at 10:00a. m. -6:00p. m., and the minimum at 8:00p. m. -6:00a. m. with a mean of 25°C (var. 25°C). We programmed a

photoperiod of 12:12 (L: D) h cycle, with alternations occurring at 6:00 a. m. and 6:00 p. m. Relative humidity was maintained between 70 and 90% across all experiments. We measured larval development time and mortality and some female reproductive features, including the length of the gonotrophic cycle, and the number of eggs in the first gonotrophic cycle. Experimental microcosms were created using plastic cups (8cm x 8cm x 3.5cm) length x width x height, containing 50ml of chlorine-free water and placing 20 1st instar larvae in each cup x five replicates with 100 larvae for each temperature treatment and fed ad libitum daily with sufficiency of tetramin[®] fish food. The instar period was regularly monitored by larvae observation and confirmed by the appearance of exuviae at every stage.

Larvae were fed daily with the quantity of food increasing, according to the stage of development. The larval development was recorded daily for each temperature; Larval development, survival, molting, and pupation were checked daily. Dead larvae were removed, and rearing water was changed every second day to prevent scum formation and accumulation of metabolites. Pupae were isolated in vials until they emerged. Pupation rate was calculated for each treatment temperature, based on the total number of pupae obtained at the end of the development period. The male and female numbers were counted to determine the sex ratio for surviving adults for each treatment temperature. For fecundity mosquitoes allowed to lay eggs in each experiment were all reared under the temperatures at which they were tested, with the same numbers in experimental cages. Between 20 and 30 females raised per temperature were each

placed with ten males with-in 24 hours of emergence. We measured the time length where it took from the first blood meal to the first day eggs were observed (length of the first gonotrophic cycle) and the number of eggs that were laid (clutch size/fecundity). Human blood was offered for 15 minutes for every experiment, and until seven days after the first blood meal of each female, which were laid no eggs in first observation we offer a second blood meal. Females at each temperature were allowed a minimum of seven days to lay eggs.

2.2.3. Measurement of wings

Emerged adults were killed by freezing to allow measurement of one wing per insect. The wing was measured using a micrometer under a stereomicroscope. One wing from every mosquito (right or left wing) was selected randomly to measure the wing length, because the differences between the length of the right or left wing were rare. The wing length was measured from the distal end of the axial inclusion to the apical margin, not including the fringe (Van Den Heuvel, 1963).

2.2.4. Per Capita Performance

We determined the condition-specific population performance parameter “*I*” of Livdahl and Sugihara (1984), with some modifications:

$$I = \frac{\ln \left(\left(\frac{1}{N_o} \right) \sum f(m_x) \right)}{\bar{D} + \sum \bar{x}}$$

Where N_o represents the observed initial number of females, \bar{D} the delay between female emergence and first oviposition (average first gonotrophic cycle

of respective temperature), and $f(m_x)$ predicts the numbers of eggs produced by females of day x . The definition of N_0 in the original paper (Livdahl and Sugihara 1984) is the initial number of females; however, it is impossible to know the initial number of female unless all of the eggs emerged into adult stages. To deal with the problem, some studies define N_0 as half of eggs/larvae applied to assume even sex ratio in mosquitoes to determine, “I” or similar population growth index (Livdahl & Sugihara, 1984; Lounibos *et al.*, 2002; Alto *et al.*, 2005). To our knowledge, there were no data to judge if the assumption is rational; therefore, we determined “I” using the number of egg production of each female for a first blood meal and including average days \bar{x} to adults for each temperature, under the assumption of absolute sex ratio.

2.2.5. Data analysis

Effects of temperature on Mortality ratio in *Ae. albopictus* and *Ae. flavopictus* were analyzed using logistic regression analysis and comparisons by Fisher's exact test under the same conditions on the mortality rate in the developmental stage (first instar to emergence). Developing time and wing size of *Ae. albopictus* and *Ae. flavopictus* under different rearing temperatures were analyzed using an ANOVA and Tukey Kramer analysis to determine whether any significant differences occurred between and among different temperatures. The relationship between the fecundity and the number of blood meals taken, first and second in a gonotrophic cycle were analyzed by one-way ANOVA.

2.3. Results

Most mortality occurred in *Ae. albopictus* 64.7% of total mortality occurred in second instars and 17.64% in fourth instars stages. In *Ae. flavopictus*, Table 1. Mortality ratio of *Ae. albopictus* (albo) and *Ae. flavopictus* (flavo) with result of logistic regression analysis and comparisons (Fisher's exact test) under same conditions on the mortality rate in developmental stage (first instar to emergence) 34.78% of total mortality occurred in the first instars and 23.2% in second instars stages. The logistic regression analysis of mortality (first instar to emergence) effects on temperature, species, and interaction between species x temperature showed a significant result on both *Ae. albopictus* and *Ae. flavopictus* (Table 1). Overall larval mortality rates between *Ae. albopictus* and *Ae. flavopictus* were significantly different under the constant temperature at 25°C and 28°C (Fishers exact test $p=0.032$ and $p<0.0001$ respectively). Mortality rates in *Ae. flavopictus* were higher at 22°C, 25°C and var. 25°C (26%, 21%, 15%, and 7% respectively) than *Ae. albopictus* (12%, 5%, and 1%, respectively) but most significantly effects found at 28°C between the two species (Table 1).

The female ratio of *Ae. albopictus* was 0.44, with significant departure from 0.5 in any treatment, whereas in *Ae. flavopictus*, the ratio was 0.498, which was not significantly different from 0.5 (χ^2 test, $P = 0.76$. Fig. 1). Furthermore, the sex ratio differed considerably among treatments. Fewer females of *Ae. albopictus* emerged at 22°C, 25°C and var. 25°C were (female ratio: 0.44, 0.40 and 0.40 respectively) than at high temperature 28°C (female ratio: 0.50, χ^2 test, $P < 0.01$).

In *Ae. flavopictus* female ratio was lower at 25°C (female ratio 0.43) but higher female ratio observed at 22°C (0.57) but in at 25°C and var. 25°C female ratio was 0.5.

We recorded developmental duration by sex in all test temperature treatment. Males generally had shorter development times than females in both species (Table 2A), and Increasing temperature reduced the developmental duration. Overall Development time was significantly longer in *Ae. flavopictus* than in *Ae. albopictus* at 28°C. In *Ae. albopictus* male at 22°C was 13.12 ± 0.14 days (Mean \pm SE) significantly deferred its development than at 25°C, var. 25°C and 28°C were 9.89 ± 0.13 , 9.92 ± 0.13 and 8.16 ± 0.14 days respectively, same trends observed in *Ae. albopictus* female (one-way ANOVA df=3, 163, F=219.4, $p < 0.0001$) at 22°C was 14.0 ± 0.16 days, which also significantly different from 25°C, var. 25°C and 28°C were 10.2 ± 0.16 , 10.8 ± 0.16 and 8.7 ± 0.14 days respectively.

In *Ae. flavopictus* at 22°C male developmental duration 13.5 ± 0.24 days significantly varies with different rearing temperature at 25°C, var. 25°C and 28°C which were 9.6 ± 0.20 , 9.7 ± 0.20 and 9.2 ± 0.24 days respectively. The female also showed a similar difference in developmental duration (one-way ANOVA df=3, 161, F=81.456, $p < 0.0001$) under lower temperature, at 22°C was 13.9 ± 0.21 days significantly different from 25°C, var. 25°C and 28°C were 10.1 ± 0.23 , 10.7 ± 0.21 and 9.6 ± 0.23 days respectively.

All temperatures showed a significant decrease in wing length with increasing temperature (Table 2B). *Ae. albopictus* Males wing size (mean \pm SE) was 2.18 ± 0.02 mm (one-way ANOVA $df=3$, 213, $F=96.7$, $p<0.0001$) at 28°C which were significantly smaller than those developed at 22°C , 25°C and var. 25°C were 2.58 ± 0.02 mm, 2.48 ± 0.02 mm and 2.52 ± 0.02 mm respectively. In the case of *Ae. flavopictus* male (one-way ANOVA $df=3$, 162, $F= 58.04$, $p<0.0001$) smaller wing size was 2.40 ± 0.02 mm observed at 28°C which were significantly different from 2.77 ± 0.02 mm, 2.76 ± 0.02 mm and 2.54 ± 0.02 mm at 22°C , 25°C , var. 25°C respectively. Females also showed a significant decrease in average wing length with increasing temperatures in both species. Wing length at 28°C and 25°C produced females with significantly smaller wings 2.85 ± 0.02 mm and 2.91 ± 0.03 mm, respectively, than those developed at 22°C and var. 25°C were 3.12 ± 0.03 mm and 3.08 ± 0.03 mm respectively in *Ae. albopictus* (one-way ANOVA $df=3$, 161, $F=26.8$, $p<0.0001$). In *Ae. flavopictus* female (one-way ANOVA $df=3$, 161, $F=148.9$, $p<0.0001$) developed smaller wing at 28°C was 2.95 ± 0.03 mm, and larger wing length was 3.65 ± 0.02 mm at 22°C (Table 2B).

Number of eggs produced by female by the different sized mosquitoes was positively correlated in *Ae. albopictus* in all test temperature (22 , 25 , 28 & var. 25°C were $R^2= 0.009$, 0.21 , 0.03 & 0.15 respectively) (Figure 2) but in case of *Ae. flavopictus* body size (wing size) was positively correlated at lower temperature (22 and 25°C were $R^2= 0.022$ & 0.04 respectively) and negatively

correlated at higher temperature (28, and var. 25°C were $R^2= 0.03$ & 0.005 respectively) (Figure 3).

In all, 56.2% *Ae. albopictus* (n=105) and 58.18% of *Ae. flavopictus* (n=110) females oviposit with their first blood meal (Table 3). Of 46 *Ae. albopictus* females that did not oviposit with their first blood meal lay eggs (n=30) 28.57% with the second blood meal and 15.23% (n=16) have not laid any eggs and didn't take a further blood meal. In *Ae. flavopictus* only 12 of 46 oviposited with the second blood meal at 22°C, and 23 of the remaining 34 were not taken further blood meal and died 7 at 28°C after second blood meal. Of those that oviposit with the first blood meal highest number of *Ae. albopictus* females oviposit at 28°C {n=20, fecundity= (mean±SE) 44.6 ± 6.0 } which was 74.04%, followed by at var. 25°C {n=18, (49.8 ± 7.1)}, at 22°C {n=12, (40.2 ± 10.8)} and at 25°C {n=09, (37.8 ± 6.8)} were 64.28%, 46.15% and 37.5% respectively. In case of *Ae. flavopictus* with their first blood meal all females oviposit at 25°C {n=28, (39.2 ± 5.6)} was 100%, followed by at var. 25°C {n=17, (16.5 ± 2.9)}, at 22°C {n=12, (17.6 ± 5.4)} and at 28°C {n=7, (35.7 ± 10.8)} which were 60.71%, 42.85% and 26.92% respectively. Fecundity did not considerably differ in *Ae. albopictus* with the first blood meal (one-way ANOVA df= (3, 55) $F = 0.43$, $P=0.73$) but significantly differ with the second blood meal (one-way ANOVA df=2, 27, $F=8.12$, $P<0.01$). In the case of *Ae. flavopictus* fecundity significantly changed between the first (one-way ANOVA df= 3, 60, $F = 4.338$, $P<0.05$) and second blood meal (one-way ANOVA df= 3, 45, $F = 26.76$, $P<0.0001$).

We calculated the per capita performance index, I , to combine the effects of temperature on population growth. It would further allow us to define which species would be favoured by the temperature (Figure: 1). The I value of *Ae. flavopictus* decrease with the increasing temperature, but favoured by only at temperature 25°C where it was 57.14% higher than *Ae. albopictus*; however, the I value of *Ae. albopictus* almost 100% increase compares to *Ae. flavopictus* at 22°C, var. 25°C and 28°C. So, The I value for *Ae. flavopictus* favoured at 25°C temperature and significantly varies from *Ae. albopictus*; Nonetheless, *Ae. albopictus* significantly increases its population all temperature ranges (22°C-28°C including var. 25°C) whereas *Ae. flavopictus* population performance reduced drastically.

2.4. Discussion

Mosquitoes are well known poikilothermic organisms which are liable to environmental temperature differences that have stimulated on their body temperature (Hawley 1988). Our results importantly showed that temperature affects the population bionomics at larval and adults' stages of both the *Ae. albopictus* and *Ae. flavopictus*. We showed that the temperature had variable effects on the population growth of the two species. Although *Ae. albopictus* performed better at temperatures 25°C and the population growth in both species was quite similar. Therefore, there should be no difference in their population growth under the same breeding conditions. However, there is striking sympatric variation in water temperatures in the field, ranging from 10 to 30°C (Tun-Lin et al. 2000, Tuno et al. 2005). There is also extreme variation in the larval survival

rate at similar water temperatures, indicating the degree of temperature response is different between the two species. The variable temperatures observed in the field may enhance species coexistence, since other factors may also affect the population growth of the two species. Sota *et al.* (1992) reported that *Ae. albopictus* was uniformly both urban and rural areas, but *Ae. flavopictus* only distributed in the forest part (Sota *et al.* 1992, Chaves 2016 and Hawley *et al.* 1987 & 1988), although the habitats used by the two species are remarkably similar to the forest part during hot summer. The inconsistent distribution of the two species cannot be explained only by the one factor examined in our study: temperature. The stability of breeding sites may be an essential factor to consider (Alto and Juliano 2001b). *Ae. albopictus* has been reported to be prevalent in the urban and suburban area. Usually, urban and suburban regions interpreted as an exceedingly disturbed unpredictable environment.

Across the four temperatures (22°C, 25°C, var. 25°C and 28°C), mortality differed between the two species and higher mortalities shows at 28°C and 22°C treatment group in *Ae. flavopictus*. Most deaths in this study occurred at the first instar and second instar stages in *Ae. flavopictus* whereas second and fourth instars larval death occur in *Ae. albopictus*. Holometabolous insects, such as mosquitoes, must attain a critical mass during larval development (Clements, 1992). They also require sufficient mass for Ecdysis, and the potential attainable mass decreases with increasing temperature (Chambers & Klowden, 1990). During the present study, the immatures were fed optimally, and care was taken to prevent overcrowding so, therefore, the results reported here represent the

effects of temperatures ranging from 22 °C to 28°C including var. 25°C for development of the immature stages of the *Ae. albopictus* and *Ae. flavopictus* mosquitoes.

The average development time for *Ae. albopictus* reared under different temperature regimen was 8–14 days, which were consistent with the results by Kamimura et al., 2002 and Tun-Lin et al., 2000. Similarly, these trends of development also observed in *Ae. flavopictus* under the same temperature ranges. The results showed that there were distinct differences in developmental times and size of both *Ae. albopictus* and *Ae. flavopictus* mosquitoes reared at constant temperatures vs. diurnal temperature regimens. The decrease in wing lengths for both sexes and species of equally showed a strong negative correlation with temperature, suggesting a direct relationship between temperature and size of adults, that is, higher temperatures produce significantly ($P < 0.0001$) smaller adults.

Many reports have shown wing length (Nasci, 1986; Packer & Corbet, 1989; Briegel, 1990; Lounibos *et al.*, 1990; Livdahl & Wiley, 1991; Blackmore & Lord, 2000; Briegel & Timmermann, 2001) to be correlated with fecundity in mosquitoes. The result of this study was consistent for *Ae. albopictus* and *Ae. flavopictus* at a lower temperature with the previous studies but slightly different in *Ae. flavopictus* at a higher temperature. However, the chain of events from larval development to actual lifetime fecundity is complex, and there is not likely to be a simple relationship between resources accumulated as larvae and lifetime fecundity (Blackmore & Lord, 2000).

Ae. albopictus has higher population growth than *Ae. flavopictus* if conditions allow. Based on our observations, the former species always perform better specially fecundity without considering temperature variation while the latter species were better at 25°C but drastically affected by increasing temperature. These features, i.e., higher productivity that's lead to rapid population growth, may enable *Ae. albopictus* to establish colonies in highly disturbed environments in urban areas, in addition to their closer association to humans (Hawley 1988) and similarly this feature may also apply for the *Ae. flavopictus* distribution restriction to forest part. By the end of the 21st century, world temperature will have increased by 1.1 to 5.8 °C (IPCC 2007). Based on our data, we predict that, with an elevated world temperature, the population growth of *Ae. albopictus* will increase in regions where the average temperature is in the range of 20 to 30 °C. With global climate change, the distribution patterns of both species will likely to be changed, and there may be trouble in present habited equilibrium between the species when the temperature is considered alone. However, climate change also affects precipitation patterns (IPCC 2007) and interspecies competition (Ives and Gilchrist, 1993), which may affect the distributions of both species.

The performances of the two species are somewhat similar under low temperature, but the performance was completely reversed when we considered their performance under high temperature (28°C and var. 25°C). We predict that this alternative reaction with temperature may help to predict/explain the mystifying distribution of the two species. We need to consider other parameters,

such as environmental stability, breeding site preference, reaction to the sunny intensity and interspecific competition between the species to better understand the location-specific dominance out-comes in the two species.

Reference:

- Alto, B.W. & Juliano, S.A. (2001a) Temperature effects on the dynamics of *Ae. albopictus* (Diptera: Culicidae) populations in the laboratory. *Journal of Medical Entomology*, **38**, 548-556.
- Alto, B.W. & Juliano, S.A. (2001b) Precipitation and temperature effects on populations of *Ae. albopictus* (Diptera: Culicidae): Implications for range expansion. *Journal of Medical Entomology*, **38**, 646-656.
- Alto, B.W., Lounibos, L.P., Higgs, S. & Juliano, S.A. (2005) Larval competition differentially affects arbovirus infection in *Ae.* mosquitoes. *Ecology*, **86**, 3279–3288.
- Beck-Johnson, L., Nelson, W., Paaijmans, K., Read, A., Thomas, M. & Bjørnstad, O. (2013) The effect of temperature on anopheles mosquito population dynamics and the potential for malaria transmission. *PLoS One*, **8**(11): e79276.
- Blackmore, M.S. & Lord, C.C. (2000) The relationship between size and fecundity in *Aedes albopictus*. *Journal of Vector Ecology*, **25**, 212-217.
- Blanford, J.I., Blanford, S., Crane, R.G., Mann, M.E., Paaijmans, K.P. & Schreiber, K.V., (2013) Implications of temperature variation for malaria parasite development across Africa. *Scientific Reports*, **3**:1±11. 1300
- Bomblies, A. (2012) Modeling the role of rainfall patterns in seasonal malaria transmission. *Climate Change*, **112**(3±4):673±85.

- Briegel, H. & Timmermann, S.E. (2001) *Aedes albopictus* (Diptera: Culicidae): Physiological Aspects of Development and Reproduction, *Journal of Medical Entomology*, **38**, 566-571.
- Briegel, H. (1990) Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *Journal of Insect Physiology*, **36**, 165-172.
- Chadee, D.D., Shivnauth, B., Rawlins, S.C. & Chen, A.A. (2007) Climate variability, mosquito density and epidemiology of Dengue fever in Trinidad (2002–2004): a prospective study. *Annals of Tropical Medicine and Parasitology*, **101**, 68–77.
- Chambers, G.M. & Klowden, M.J. (1990) Correlation of nutrition reserves with a critical weigh for pupation in larval *Ae. aegypti* mosquitoes. *Journal of the American Mosquito Control Association*, **6**, 394–399.
- Chan, K.L., Chan, Y.C. & Ho, B.C. (1971a) *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse) in Singapore City. 4. Competition between species. *Bulletin of the World Health Organization*, **44**, 643-649.
- Chaves, F. L. (2016) Globally invasive, withdrawing at home: *Ae. albopictus* and *Ae. japonicus* facing the rise of *Ae. flavopictus*. *International Journal of Biometeorology*, **60**:1727–1738.
- Chowell, G. & Sanchez, F. (2006) Climate-based descriptive models of dengue fever: the 2002 epidemic in Colima, Mexico. *Journal of Environmental Health*, **68**(10):40±4, 55.
- Christophers, S.R. (1960) *Ae. aegypti* (L): *The yellow fever mosquito. Its life history, bionomics and structure*. Cambridge University Press, Cambridge.

- Clements, A. & Paterson, G. (1981) The analysis of mortality and survival rates in wild populations of mosquitoes. *Journal of Applied Ecology*, 18(2):373–399.
- Clements, A.N. (1992) *The biology of mosquitoes. Vol. 1. Development, nutrition and reproduction*. Chapman and Hall, New York.
- Clements, A.N., (1999) *The Biology of Mosquitoes*, vol. 2. CABI Publishing, Wallingford, Oxon.
- Delatte, H., Gimonneau, G., Triboire, A. & Fontenille, D. (2009) Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Ae. albopictus*, vector of chikungunya and dengue in the Indian Ocean. *Journal of Medical Entomology*, 46, 33–41.
- Farjana, T., Tuno. N. & Higa, Y. (2012) Effects of temperature and diet on development and interspecies competition in *Ae. aegypti* and *Ae. albopictus*. *Medical and Veterinary Entomology*, 26: 210–217.
- Fish, D. (1985) An analysis of adult size variations within natural mosquito populations. In: Lounibos, L.P., Rey, J.R., Frank, J.H. (Eds.), *Ecology of Mosquitoes: Proceedings of a Workshop. Florida Medical Entomology Laboratory*, pp. 419–429.
- Gilotra, S.K., Rozeboom, L.E. & Bhattacharya, N.C. (1967) Observation on possible competitive displacement between populations of *Ae. aegypti* and *Ae. albopictus* Skuse in Calcutta. *Bulletin of the World Health Organization*, 37, 437-446.

- Gubler, D.J. (2002) Epidemic dengue/dengue haemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Microbiology*, 10, 100–103.
- Gubler, D.J. & Kuno, G. (1997) *Dengue and Dengue Haemorrhagic Fever*. University Press, CAB International, Cambridge.
- Guha-Sapir, D. & Schimmer, B. (2005) Dengue fever: new paradigms for a changing epidemiology. *Emerging Themes in Epidemiology*, 2, 1–10.
- Haramis, L.D. (1985) Larval nutrition, adult body size and biology of *Ae. triserriatus*. In: Lounibos, L.P., Rey, J.R., Frank, J.H. (Eds.), *Ecology of Mosquitoes: Proceedings of Workshop. Florida Medical Entomology Laboratory*, pp.
- Hawley, W.A. (1988) The biology of *Ae. albopictus*. *Journal of the American Mosquito Control Association*, 4, 1-39.
- Hawley, W.A., Reiter, P., Copeland, R.S. & Pumpini, C.B. (1987) *Ae. albopictus* in North America: Probable introduction in used tires from northern Asia. *Science* 1987; 236:1114–1116.
- Hobbs, J.H., Hughes, E.A. & Eichold, B.H. II. (1991) Replacement of *Ae. aegypti* by *Ae. albopictus* in Mobile, Alabama. *Journal of the American Mosquito Control Association*, 7, 488-489.
- I.P.C.C. (2007) *Climate Change 2007: Synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change.*

Ives, A.R. & Gilchrist, G. (1993) Climate change and ecological interactions.

Biotic Interactions and Global Change (ed. by P. J. Kareiva, J. G.

Kingsolver & R. Huey), pp. 120–146. Sinauer, Sunderland.

Johansson, M.A., Dominici, F. & Glass, G.E. (2009) Local and global effects of climate on dengue transmission in Puerto Rico. *PLoS Neglected Tropical Diseases*, 3(2):17.

Juliano, S.A. & Lounibos, L.P. (2005) Ecology of invasive mosquitoes: Effects on resident species and on human health. *Ecology Letters*, 8:558–574.

Kamimura, K., Matsuse, I., Takahashi, H., Komukai, J., Fokuda, T., Suzuki, K., Aratani, M., Shirai, Y. & Mogi, M. (2002) Effect of temperature on the development of *Ae. aegypti* and *Ae. albopictus*. *Medical Entomology and Zoology*, 53:53–58.

Kobayashi, M., Nihei, N. & Kurihara, T. (2002) Analysis of northern distribution of *Ae. albopictus* (Diptera: Culicidae) in Japan by geographical information system. *Journal of Medical Entomology*, 39:4–11.

Lambrechts, L., Paaijmans, K.P., Fansiri, T., Carrington, L.B. & Kramer, L.D. (2011) Impact of daily temperature fluctuations on dengue virus transmission by *Ae. aegypti*. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 7460–7465.

Livdahl, T.P. & Sugihara, G. (1984) Non-linear interactions of populations and the importance of estimating per capita rates of change. *Journal of Animal Ecology*, 53, 573-580.

- Livdahl, T. P. & Willey, M. S. (1991) Prospects for an invasion: competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science*, **253**, 189-191.
- Lounibos, L. P., Larson, V. L. & Morris, C. D. (1990) Parity, fecundity and body size of *Mansonia dyari* in Florida. *Journal of American Mosquito Control Association*, **6**, 121- 126.
- Lounibos, L.P., O'Meara, G.F., Juliano, S.A., Nishimura, N., Escher, R.L. & Reiskind, M.H. (2010) Differential survivorship of invasive mosquito species in South Florida Cemeteries: do site-specific microclimates explain patterns of coexistence and exclusion? *Annals of the Entomological Society of America*, 103:757–770.
- Lounibos, L.P. (2002) Invasions by insect vectors of human disease. *Annual Review of Entomology*, **47**, 233-266.
- Lounibos, L.P., Suarez, S., Menendez, Z., Nishimura, N., Escher, R.L., Connell, S.M.O. & Rey, J.R. (2002) Does temperature affect the outcome of larval competition between *Ae. aegypti* and *Ae. albopictus*? *Journal of Vector Ecology*, **27**, 86-95.
- Lyimo, E.O., Takken, W. & Koella, J.C. (1992) Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. *Entomologia Experimentalis et Applicata*, 63: 265–271.
- Mekuria, Y. & Hyatt, M.G. (1995) *Ae. albopictus* in South Carolina. *Journal of the American Mosquito Control Association*, **9**, 352-355.

- Mogi, M. & Tuno, N. (2014) Impact of climate change on the distribution of *Ae. albopictus* (Diptera: Culicidae) in northern Japan: retrospective analyses. *Journal of Medical Entomology*, 51:572–579.
- Murrell, E.G. & Juliano S.A. (2008) Detritus type alters the outcome of interspecific competition between *Ae. aegypti* and *Ae. albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, **45**, 375-383.
- Nasci, R. S. (1990) Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae). *Journal of Medical Entomology*, **27**, 716-719.
- Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S. & Murdock, C.C. (2013) Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19(8):2373± 80.
- Packer, M.J. & Corbet, P.S. (1989) Size variation and reproductive success of female *Aedes punctor* (Diptera: Culicidae). *Ecological Entomology*, **14**, 297–309.
- Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37:637–669.
- Paupy, C., Chantha, N., Vazeille, M., Reynes, J.M., Rodhain, F. & Failoux, A.B. (2003) Variation over space and time of *Ae. aegypti* in Phnom penh (Cambodia) genetic structure and oral susceptibility to a dengue virus. *Inf. Genet. Res. Camb.* 82, 171–182.

- Proestos, Y., Christophides, G.K., Ergüler, K., Tanarhte, M., Waldock, J. & Lelieveld, J. (2015) Present and future projections of habitat suitability of the Asian tiger mosquito, a vector of viral pathogens, from global climate simulation. *Philosophical Transaction of the Royal Society of London B; Biological Science*, 370:20130554.
- Rae, D.J. (1990) Survival and development of the immature stages of *Culex annulirostris* (Diptera: Culicidae) at the Ross River Dam in tropical eastern Australia. *Journal of Medical Entomology*, 27, 756–762.
- Reisen, W.K., Milby, M.M. & Bock, M.E., (1984) The effects of immature stress on selected events in the life history of *Culex tarsalis*. *Mosquito News*, 44, 385–395.
- Rueda, L.M., Patel, K.J., Axtell, R.C. & Stinner, R.E. (1990) Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Ae. aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 27, 892–898.
- Siddiqui, T.F., Aslam, Y. & Reisen, W.K. (1976) The effect of larval density on selected immature and adult attributes of *Culex tritaeniorhynchus*. *Tropical Medicine (Nagasaki)* 18, 195–200.
- Sota, T., Mogi, M. & Hayamizu, E. (1992) Seasonal distribution and habitat selection by *Ae. albopictus* and *Ae. riversi* (Diptera, Culicidae) in Northern Kyushu, Japan. *Journal of Medical Entomology*, 29:296–304.
- Tun-Lin, W., Burkot, T.R. & Kay, B.H. (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector *Ae. aegypti* in

north Queensland, Australia. *Medical and Veterinary Entomology*, **14**, 31-37.

Tuno, N., Okeka, W., Minakawa, N., Takagi, M. & Yan, G. (2005) Survivorship of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. *Journal of Medical Entomology*, **42**, 270-277.

Van Den Heuvel, M.J. (1963) The effect of rearing temperature on the wing length, thorax length, leg length and ovariole number of the adult mosquito, *Ae. aegypti* (L.). *Transactions of the Royal Entomological Society of London*, **115**, 197-216

Chapter 3

Seasonal fluctuation in distribution and habitat of *Ae. albopictus* and *Ae. flavopictus* in Kanazawa, Japan.

3.1. Introduction

Asian tiger mosquitoes, *Ae. albopictus* (*Stegomyia*) is a mosquito's native to south east Asia and breeding in temporary water body in tree holes, bamboo stump, etc. and they are able to establish in urban and semi-urban areas of Asia (Hawley 1988). *Ae. albopictus* has the ability to expand its population to human habitats and overwintering diapause (Hawley *et al.* 1987, Hawley 1988, Rai 1991). *Ae. flavopictus* (*Stegomyia*) are restricted to local distribution and found in evergreen broad leaf and bamboo forest (Mogi 1976, 1990; Eshita & Kurihara 1979; Miyagi & Toma 1980; Toma & Miyagi 1981; Miyagi *et al.* 1983)

Recent temperature changes have been anticipated that invasive vectors of disease like Asian tiger mosquitoes *Ae. albopictus* (Lounibos 2002; Juliano and Lounibos 2005) have extended its dispersal and its often regulated by temperature, locally in Japan (Kobayashi *et al.* 2002; Mogi and Tuno 2014), but also worldwide (Proestos *et al.* 2015). It has also been observed that the survival rate in invasive species depends on microclimatic changes as well as, changes in biological interactions between invasive species and native resident fauna (Lounibos *et al.* 2010). Climate changes have directed to altitudinal and latitudinal changes which might affect the distribution of many invertebrate species, where their tendency to both the expansion and contraction of habitat ranges (Parmesan 2006).

There is a significant biological difference between widely distributed *Ae. albopictus* and locally confined *Ae. flavopictus* that are related to their dispersal and colonizing abilities. Moreover, *Ae. albopictus* most common in urban and suburban area, but also been observed forested area with more vegetation and sometimes coexist with its related *Stegomyia* species like *Ae. flavopictus*, *Ae. riversi*, *Ae. aegypti* etc. By considering meteorological data to evaluate the role that changing weather patterns could have on the abundance and coexistence of these two-mosquito species. So, the coexisting behavior of *Ae. flavopictus* might be deleterious interacting with *Ae. albopictus*. However, before we can predict these species responses to climate change, we need to better understand the ecology and evolutionary patterns of these insect vectors of pathogens. Relative ecological studies of mosquitoes of *Stegomyia* spp (*Ae. albopictus* and *Ae. flavopictus*) help to understand the evolutionary changes between the two species and also give information about the abundance and distribution patterns in human disturbed habitats. So, a study on the habitat differences between adult and larval *Ae. albopictus* and *Ae. flavopictus* on suburban and urban areas of Kanazawa, Japan will help to clarify the influence of macro and microhabitat on adult and larval populations, seasonal abundance and the association between the two species.

3.2. Materials and Methods

3.2.1. Study area

The main study area was Kanazawa city, located northwestern part of Ishikawa prefecture in the Hokuriku region where the western part is surrounded by the Sea of Japan and the eastern part is occupied by Japanese Alps (36° 33' N, 136° 39' E) with about 466000 populations. The climate of this area is warm and humid summers and cold winters with heavy snowfall with a 30-yr mean annual temperature of 14.6°C and annual precipitation of 2399 mm. The natural flora of this area is a broadleaf evergreen tree, deciduous tree and most of the forests are invaded by bamboo. The experiments were done in the Kakuma Bamboo forest, Kanazawa where *Ae. flavopictus* is a dominant species and *Ae. albopictus* co-occur during the hot summer and different park, shrine, cemetery and residential area of Kanazawa city where *Ae. albopictus* is a dominant species to some others Culicidae species.

3.2.2. Adult Collection

To explain seasonal and spatial distribution of adult, human bait collection was done during daytime 11am-6pm twice a month from June-October/2017 and June-September/2018 in Kanazawa at Six sites, including Kakumamachi bamboo forest at a rural site, human residential area and different Park and shrine in the city area.

The adult mosquito catch was performed under the tree canopy by human bait collection, allowing both bare legs to feed on them. Mosquitoes coming to feed were detected and collected with plastic tubes and placed in a screened pint-size container with labeled location. The number of mosquitoes collected was performed by 8 min x 2 in two different places (50-100 meter apart) in the same location.

3.2.3. Larval Collections:

Larval mosquitoes were collected from 36 natural and artificial containers on Bamboo forest and residential area in Kanazawa city from March-October/2017 and March-September/2018. Mosquito larvae were collected from each container by using a siphon and a pipette and reared to adults in the laboratory at 25°C and photoperiod of 14:10 (L: D) on a Tetramin[®] commercial fish food. Rearing was necessary because the two *Stegomyia* species were difficult to identify in the larval stage.

To describe the seasonal trends in the distribution of the two *Stegomyia* larvae in the bamboo stump, 20 stumps that had harbored mosquito larvae frequently found were selected for monthly sampling in the Kakuma Bamboo forest from March-October 2017. Mosquito larvae were collected by pipette and were reared to adults in the laboratory.

3.2.4. Mosquito identification:

Identification of adult mosquitoes and larvae were done morphologically under a stereomicroscope to species level using the identification keys for adult

and fourth-instar larvae of mosquito species from Japan, where Morphology was observed methods described by Tanaka *et al* 1979 and the size of the PCR product in the ITS region of rDNA (Higa *et al* 2010) were studied for confirmation of *Stegomyia* species (*Ae. albopictus* and *Ae. flavopictus*)

3.2.5. Oviposition Pattern:

To describe the oviposition patterns of the two *Stegomyia* species we select 20 bamboo stumps at natural habited in the bamboo forest and also set up each site with (Forest and Urban) four large ovitrap (16cm diameter and 20 cm high) painted black initially adding 500ml water with some dry bamboo leaves and four small ovitrap (10cm diameter and 11cm high plastic cup) containing 200 cc tap water for an oviposition site. Ovitrap were set above the ground in the forest and residential area. The larvae in each trap were collected twice in a month and reared to adults in the laboratory.

3.3. Results

3.3.1. Adult Spatial Distribution

A total 362 adult mosquitoes were caught from different location in the figure-1 by human bait in Kanazawa during July-September 2017 and June-September 2018. *Ae. albopictus* was collected almost all collection sites, but *Ae. flavopictus* was found only in Kakumamachi bamboo forest (rural site). In Table 1 compares the abundance of females collected per 8 min per person among three habitat types: Bamboo forest (Kakumamachi), evergreen forest (Shrine, cemetery, Park) and less vegetative part (Residential area). In Kanazawa city, *Ae. albopictus*

was distributed uniformly among the three habitat types, whereas *Ae. flavopictus* was confined to the forest area in rural sites (Figure 3) In the Kenmin park (coastal area) where only the evergreen forest and Teramachi forest part (bamboo forest) was selected for a sampling site and in both places only *Ae. albopictus* was found.

3.3.2. Adult Seasonal Distribution

Monthly human bait sampling at six sites revealed that the annual total capture in 2017 was greater for *Ae. albopictus* (57.73%) than *Ae. flavopictus* (17.40%). The incidence of *Ae. flavopictus* was higher in the bamboo forest and its occurrence only restricted to this part. Both species occurred from June through September (Figure 3). Although the seasonal distribution in the bamboo forest, where *Ae. flavopictus* occurred abundantly and *Ae. albopictus* dominantly found in the urban area from June to September and *Ae. flavopictus* equally distributed in the forest part June through September. Table 1 represents the relative abundance of females collected per 8 min per person among habitat types: Bamboo forest, evergreen forest, urban area having dispersed bamboo forest, and human-disturbed habitat (residential area, cemetery, park and shrine).

3.3.3. Larval abundances and seasonal trends

Analyses of larval survey and seasonal variation showed that, considering all species, mosquitoes were more abundant in natural sites (Bamboo forest $n > 9181$) than in Urban area ($n \approx 3893$, $P < 0.001$). There was an effect of the site: *Ae. flavopictus* were significantly found in the forest ($P < 0.001$) and *Ae. albopictus* mostly found in the urban area (Residential area, cemetery, shrine, etc.) showed that abundances typically affected by the environment ($P < 0.001$) and during July

and August they are dispersed at the forest site (Figure 4). As for seasonal activity, *Stegomyia* species were more abundant in 'June through September ($P < 0.001$). For specific species abundance and seasonality *Ae. albopictus* were found only in July-September at the forest site (Figure 4), so we considered the two-mosquito species *Ae. albopictus* and *Ae. flavopictus* for analysis. Results showed that *Ae. albopictus* from bamboo forest accounted for only 12.20% of *Stegomyia* recovered ($n = 3385$). The presence of *Ae. flavopictus* did not differ among months, whereas *Ae. albopictus* was collected significantly more frequently in August ($X^2 = 767.11$ $df = 3$, $P < 0.001$) and *Ae. flavopictus* were present significantly and more frequently than *Ae. albopictus* in forest site from April - October ($P < 0.001$) with seasonal pick up in July-August (Figure 4). Nevertheless, if we considered others dominating mosquito's species like *Ae. japonicus* in the natural habited which were profoundly present in March-June compare to *Ae. flavopictus* which usually increases its population in July-September (Figure 4).

3.3.4. Larval abundance among micro habited

Figure 5 shows the occurrences of the two *Stegomyia* species in different types of containers. *Stegomyia* mosquitoes were collected from bamboo stumps and ovitrap installed in the bamboo forest and evergreen tree sites where *Ae. flavopictus* (89 of 200) were collected significantly more frequently than *Ae. albopictus* (11 of 200) ($X^2 = 81.12$, $df = 1$, $P < 0.001$). Although *Ae. albopictus* occurred in forest sites in the bamboo stumps only in August (among the season, $X^2 = 1.534$, $df = 1$, $P > 0.005$) and the frequency of occurrence of *Ae. flavopictus*

did not vary among seasons ($P > 0.05$) but significantly affected by microhabitat type ($P < 0.001$).

3.3.4. Oviposition Patterns

In the bamboo forest in natural habitats larval sampling in March-October/2017 from 20-mark bamboo stumps were sampled at Kakumamachi, total 4189 larvae recovery (*Ae. albopictus* 131, *Ae. flavopictus* 2019, *Ae. japonicus* 1439, *Tripteroides bambusa* 488, *Culex pipiens* 100, *Ur. novobscura* 9 and *Ar. subalbatus* 3). In August when two species were found in forest area the number per trap was greater for *Ae. flavopictus* (53.8 ± 13.62) [mean \pm SE]/ per trap) than for *Ae. albopictus* (15.28 ± 7.14), (Mann-Whitney *U* test, $U = 92.5$, $P < 0.001$). Of 20 bamboo stumps, 3 yielded both species, 12 yielded *Ae. flavopictus*, 4 yielded *Ae. albopictus* and 1 yielded neither species. The frequency of occurrence of *Ae. flavopictus* (15 of 20) was significantly greater than that of *Ae. albopictus* (5 of 20) ($X^2 = 6.465$, $df = 1$, $P < 0.005$). Oviposition activity influenced by container type- *Ae. albopictus* significantly influenced by large size ovitrap containing more water (Fig: 5) where *Ae. flavopictus* like to oviposit in natural habited (bamboo stumps) ($X^2 = 640.17$, $df = 2$ and $P < 0.001$).

3.4. Discussion

The experimental habitation differences between *Ae. albopictus* and *Ae. flavopictus* showed the same findings with some previous findings in Kyushu and the Ryukyu Islands, southwestern Japan (Eshita & Kurihara 1979, Miyagi & Toma 1980, Toma & Miyagi 1981, Miyagi et al. 1983, Sota et al 1992). *Ae. flavopictus* usually were confined to forest and bamboo stumps in the adult and

larval stages, although it also oviposited in various containers other than bamboo stumps if these were within or adjacent to forest habitat. *Ae. albopictus* breeds in both forest and disturbed habitations, although the larvae occurred occasionally in bamboo stumps in summer.

Seasonal abundance of adult populations exhibited that both *Ae. albopictus* and *Ae. flavopictus* were much higher in number from July to September where former species found in the urban and forest part, but latter species only found in the forest area. Such type of variation in location indicates that response to temperature and light intensity between the two species may be considerate as important cues. The population growth and dispersal of *Ae. flavopictus* may be restricted because of its dependency upon natural habitats and preference on cooler weather in comparing with *Ae. albopictus*, which exploit a wider range of habitats and adapted to varying degree of temperature.

Why is the adults of *Ae. flavopictus* confined to forest areas? Why do *Ae. flavopictus* not using the any containers outside the forest as breeding sites? There may be some biological and behavioral distinctions between the two species that influence the use of open environments. *Ae. flavopictus* prefer to breed in natural habitat and artificial container with a larger volume of water which is comparatively cooler than exposed habitat. For example, the larvae of *Ae. flavopictus* are more vulnerable to temperature changes than *Ae. albopictus* larvae (unpublished data), and females may prefer not to lay eggs in any containers outside the forest (Sota *et al* 1992)

Why do *Ae. albopictus* occasionally use bamboo stumps as larval habitats? *Ae. albopictus* females may prefer different types of containers, including bamboo stumps as oviposition sites. Actually, *Ae. albopictus* prefer to avoid hot weather during humid summer, which damage eggs and reduce hatchability (unpublished data). Bamboo stumps may be somewhat favorable throughout hot seasons compared with containers outside the forest in terms of temperature, water quantity and quality. The higher mean temperature of aquatic habitats outside the forest causes higher larval mortality. Bamboo stump harbored complex mosquito communities with numerous leaf litter as a food source that may favoured a suitable site for mosquitoes breeding specially for *Ae. flavopictus* compare to *Ae. albopictus*. Consequently, the use of containers outside the forest may have no advantageous effects, as *Ae. flavopictus* have not adapted to the adverse weather condition. Do interspecific interactions (larval competition and mating interference) between the two species influence their population dynamics when they cohabit during summer? Experimental interspecific competition studies have not been done for *Ae. albopictus* and *Ae. flavopictus*. Independent or positive association of the distributions in container types used by both species indicated that competitive interactions may not eliminate either species. In the adult stage, interaction through interspecific mating has been hypothesized as a mechanism of exclusion of one species by a closely related species or their parapatric distributions (Ribeiro 1988, Kuno, 1992; Yoshimura & Clark, 1994; Feng et al., 1997; Kishi & Nakazawa, 2013). There is an evidence about cross-insemination take place under laboratory conditions between *Ae. albopictus* and

Ae. flavopictus from Kanazawa, Ishikawa (unpublished data). But exclusion or large scale female damage is not observed between the two species and within a shorter period of time they can recover and both species can regenerate their population. Thus, the effect of mating interference can be excluded for *Ae. albopictus* and *Ae. flavopictus*.

In conclusion, it seems required to clarify the behavioral changes in the selection of oviposition site by females and larval developmental success in different containers to realize the differential habitat selections of the two species. The interactions in the larval stage between the two species and between the *Stegomyia* and other cohabiting mosquito species are also important subjects for future study.

Reference

- Eshita, Y. & T. Kurihara. 1979. Studies on the habitats of *Ae. albopictus* and *Ae. riversi* in the southwestern part of Japan. *Jpn. J. Sanit. Zool.* 30:181-185.
- Feng, W., Lu, X. and Liu, W. (1997) Comparison and numerical simulations for diffusive models of resource and sexual competition. *Nonlinear Analysis: Theory, Methods & Applications*, 30, 2765–2774
- Hawley, W.A. (1988) The biology of *Ae. albopictus*. *Journal of the American Mosquito Control Association*, 4, 1-39.
- Hawley, W.A., Reiter, P., Copeland, R.S. & Pumpini, C.B. (1987) *Ae. albopictus* in North America: Probable introduction in used tires from northern Asia. *Science* 1987; 236:1114–1116.
- Higa, Y., Toma, T., Tsuda, Y., and MUYAGI, I. (2010) A Multiplex PCR-Based Molecular Identification of Five Morphologically Related, Medically Important Subgenus *Stegomyia* Mosquitoes from the Genus *Ae.* (Diptera: Culicidae) Found in the Ryukyu Archipelago, Japan, *Jpn. J. Infect. Dis.* 63 (5), 312-316.
- Juliano, S.A. & Lounibos, L.P. (2005) Ecology of invasive mosquitoes: Effects on resident species and on human health. *Ecology Letters*, 8:558–574.
- Kishi, S. and Nakazawa, T. (2013) Analysis of species coexistence co-mediated by resource competition and reproductive interference. *Population Ecology*, 55, 305–313.

- Kobayashi, M., Nihei, N. & Kurihara, T. (2002) Analysis of northern distribution of *Ae. albopictus* (Diptera: Culicidae) in Japan by geographical information system. *Journal of Medical Entomology*, 39:4–11.
- Kuno, E. (1992) Competitive exclusion through reproductive interference. *Researches on Population Ecology*, 34, 275–284.
- Lounibos, L.P. (2002) Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47, 233-266.
- Lounibos, L.P., O’Meara, G.F., Juliano, S.A., Nishimura, N., Escher, R.L. & Reiskind, M.H. (2010) Differential survivorship of invasive mosquito species in South Florida Cemeteries: do site-specific microclimates explain patterns of coexistence and exclusion? *Annals of the Entomological Society of America*, 103:757–770.
- Lounibos, L.P., Suarez, S., Menendez, Z., Nishimura, N., Escher, R.L., Connell, S.M.O. & Rey, J.R. (2002) Does temperature affect the outcome of larval competition between *Ae. aegypti* and *Ae. albopictus*? *Journal of Vector Ecology*, 27, 86-95.
- Miyagi, I. & T. Toma. 1980. Studies on the mosquitoes in Yaeyama Islands, Japan 5. Notes on the mosquitoes collected in forest areas of Iriomotejima. *Jpn. J. Sanit. Zool.* 31: 81-91. 1989. Experimental crossing of *Ae. albopictus* *Ae. flavopictus downsi* and *Ae. riversi* (Diptera: Culicidae) occurring in Okinawajima, Ryukyu Islands, Japan. *Jpn. J. Sanit. Zool.* 40: 87- 95.

- Miyagi, I., T. Toma & S. Iha. 1983. Studies on the mosquitoes in Yaeyama Islands, Japan 8. On the mosquitoes collected in Yonagunijima. *Jpn. J. Sanit. Zool.* 34: 16.
- Mogi, M. & Tuno, N. (2014) Impact of climate change on the distribution of *Ae. albopictus* (Diptera: Culicidae) in northern Japan: retrospective analyses. *Journal of Medical Entomology*, 51:572–579.
- Mogi, M. 1976. Notes on the northern records of *Ae. (Stegomyia) riversi* Bohart and Ingram. *Mosq. Syst.* 8: 347-352.
1982. Variation in oviposition, hatching rate and setal morphology in laboratory strains of *Ae. albopictus*. *Mosq. News* 42: 196-201.
1990. Further notes on the northern distribution of *Ae. (Stegomyia) riversi* (Diptera: Culicidae). *Mosq. Syst.* 22: 47-52.
- Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37:637–669.
- Proestos, Y., Christophides, G.K., Ergüler, K., Tanarhte, M., Waldoock, J. & Lelieveld, J. (2015) Present and future projections of habitat suitability of the Asian tiger mosquito, a vector of viral pathogens, from global climate simulation. *Philosophical Transaction of the Royal Society of London B; Biological Science*, 370:20130554.
- Rai, K. S. (1991) *Ae. albopictus* in the Americas. *Annu. Rev. Entomol.* 36: 459-484.

- Rai, K. S. 1991. *Ae. albopictus* in the Americas. *Annu. Rev. Entomol.* 36: 459-484.
- Ribeiro, J.M.C. 1988. Can satyrs control pests and vectors? *Journal of Medical Entomology.* 25: 431-440.
- Sota, T., Mogi, M. & Hayamizu, E. (1992) Seasonal Distribution and Habitat Selection by *Aedes albopictus* and *Ae. riversi* (Diptera: Culicidae) in Northern Kyushu, Japan. *Journal of Medical Entomology*, 29(2): 296-304.
- Tanaka, K., K. Mizusawa, and E. S. Saugstad. (1979) A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib. Am. Entomol. Inst.* 16: 1-987.
- Toma, T. & I. Miyagi. 1981. Notes on the mosquitoes collected at forest areas in the northern part of Okinawajima, Ryukyu Islands, Japan. *Jpn. J. Sanit. Zool.* 32: 271-279.
- Yoshimura, J. and Clark, C.W. (1994) Population dynamics of sexual and resource competition. *Theoretical Population Biology*, 45, 121-131.

Chapter 4

Influence of vegetation type on population growth of *Ae. albopictus* and *Ae. flavopictus*

4.1. Introduction

Resource diversity of nutritional stuffs can have important effects for the population performance of many macroinvertebrates which are inhabiting freshwater biomes and utilize allochthonous leaf litter as food. Several invertebrates ingest leaf debris directly by mincing coarse particulate detritus, or by assembling finer litter, but for these guilds, the productivity of alteration of leaf material into invertebrate tissue is usually low (Berrie 1976; Slansky and Scriber 1985). Other invertebrates, including mosquito larvae, exploit leaf debris by filtering, scraping, or looking microbes in the biofilm on the leaf litter surface (Cummins and Klug 1979; Fish and Carpenter 1982; Walker and Merritt 1991; Merritt et al. 1992). The growth rate of invertebrate on vegetation varies with feeding mode, quantity of leaf decomposition, chemical properties of leaf detritus, rate of leaf decomposition, and microbial contents of the leaf material (Kaushik and Hynes 1971; Berrie 1976; Anderson and Sedell 1979; Cummins and Klug 1979; Merritt et al. 1984; Dieng et al 2002).

Water-filled containers, both natural ("phytotelmata") and artificial (e.g., discarded tires), are usual habitations of mosquitoes and other insects in temperate and tropical areas (Frank and Lounibos 1983). *Aedes* mosquitoes of the subgenus *Stegomyia* use these containers types (Sota et al. 1992) that stipulate the same typical food, principally comprised of detritus (Clements 1999).

Ae. albopictus is an invasive mosquito in North America (Hawley et al., 1987), and may face a various array of larval resources over its expanding range and a well-known vector of dengue in Southern Asia (Chan et al. 1971, Jumali et al. 1979), is expanding its distribution throughout the globe (Rai 1991, Reiter 1998). The capability of this species to succeed on a variety of resources may help its rapid expansion and competitive advantage at the larval stage, shown in common garden experiments with *Ae. triseriatus* and *Ae. aegypti* (Livdahl & Willey, 1991; Braks et al., 2004). In spite of the competitive superiority of *Ae. albopictus* in laboratory experiments, it appears to coexist with its related species *Ae. flavopictus*, *Ae. triseriatus* and *Ae. aegypti* under natural conditions (Juliano et al., 2004; Costanzo et al., 2005; Griswold & Lounibos, 2005). Probable causes of the coexistence of *Ae. albopictus* and *Ae. flavopictus*, *Ae. triseriatus* or *Ae. aegypti* may be resource diversity in terms of plant versus animal debris (Yee et al., 2007; Murrell & Juliano, 2008). Leaf litter variation may also be important, but little is known about how larval mosquitoes respond to specific leaf species as a larval food.

The adults of *Ae. albopictus* occur in both forested and urban areas while *Ae. flavopictus* remain only in the forest part. The larvae of former species breed in tree holes, bamboo stumps and various artificial containers (Hawley 1988) but the latter species only breed in the natural micro habited (bamboo stumps, tree hole, bromeliads etc.). So Larval development of both *Ae. albopictus* and *Ae. flavopictus* is highly sensitive to the ration of leaf litter available per larva (Fish and Carpenter 1982; Carpenter 1983; Leonard and Juliano 1995).

In Japan, forestry workers are on a downward trend and bamboo forests invading and expand in abandoned forests in various places. Now we are not able to predict how such changes in vegetation affect the distribution of two species. So, in this paper, we determine the leaf litter from three vegetation type that have diverse abilities to provide larval development. These affect larval dynamics and the differences interact with population density. However, the relative importance of variation in litter quantity, litter quality, and microbial colonization to larval growth remains poorly understood. We tested the hypothesis that detritus quality and microbial populations will interact with food ration to affect the survival, development time of *Ae. albopictus*.

4.2. Materials and Methods

This study was done in order to determine how the vegetation change influences the population growth of *Ae. albopictus* and *Ae. flavopictus* being fed with three types of vegetation, deciduous broad-leaved trees, evergreens, bamboo leaves in laboratory condition.

4.2.1. Leaf Litter source

Recently dropped leaves of bamboo *Phyllostachys heterocykla*, evergreen tree *Castanopsis* sp. and deciduous tree *Cerasus* × *yedoensis* were collected at different location points at the study site. Only whole leaves that have no soil contacts were collected and used for the experimental purposes. Leaves collected from multiple locations were pooled together and dried. Prior to the experiment, leaves were dried at incubator for 7-14 days, weighed into leaf packs of 0.20g.

Dried leaf placed in the microcosms containing 200ml dechlorinated water one week before the start of the experiment.

4.2.2. Mosquito strain

Ae. albopictus and *Ae. flavopictus* used in this experiment were obtained from a laboratory colony established for two months from field-collected mosquitoes. *Ae. albopictus* were collected at larval stage in ovitrap set up at residential area, Kanazawa City, Japan and *Ae. flavopictus* were collected at the larval stage from the bamboo forest near Kanazawa, and both were shifted to Kanazawa university ecology laboratory Insectarium. The Insectarium was maintained under $25\pm 1^{\circ}\text{C}$ and 70–90% relative humidity under 14L/10D photoperiod conditions. Adults were kept in cages at same Insectarium had free access to 3% sucrose solution, and offered a blood meal (human) five days after emergence. For maintenance of the population, one of the authors provided the blood meal. Mosquitoes of generations F2/F3 were used for the different experiments.

4.2.3. Experimental Design

We examined different leaf litter as a larval food on growth performance of *Ae. albopictus* and *Ae. flavopictus*. Newly hatched first instars larvae of both species, taken from a laboratory colony. For each species 50 (1st instar larvae) X 2 replicates for each experimental treatment group with 0.2g of each leaf as a larval food in 200 ml of dechlorinated water in 500 ml food grade, plastic cups (size diameter 12cm by height 5cm) were used. Leaf detritus was provided as a resource of food for larvae with 0.2gm (dry weight) of fallen leaves (Bamboo,

deciduous and evergreen leaves) soaked in 200ml of water 7 days before the start of the experiment. Entire experiment was conducted under $25\pm 1^{\circ}\text{C}$ and 70–90% RH and 14L:10D condition.

We measured larval development time from 1st instar to 4th instar and larval mortality, adult body size and some female reproductive features, like fecundity. The instar period was regularly monitored by larvae observation and confirmed by the appearance of exuviae at every stage. We observed Female fecundity for each temperature group, so female and male from each experimental group after emerging were kept in a box and after 4-5 days provide blood meal. Then separate, individually in a plastic vial and facilitate for egg laying and maintain with 3% sugar solution. For wing length measurement the wing length of both males and females were dissected out after killing in freezers to measure wing length from the distal end of the axial inclusion to the apical margin. Every week during the experimental period, we measured COD, Polyphenols, Chlorophyll a and Turbidity of the water sample from each experimental group and considering one sample from each group.

4.3. Results

The mortality rate of *Ae. albopictus* during development was consistently lower in the all vegetation types at young stage (1st and 2nd instar) but higher mortality observed at the old stage of larval instar (3rd and 4th instar). Significantly Most mortality occurs at 4th instar larvae by all vegetation types in *Ae. albopictus* (Figure 1).

Both *Ae. albopictus* and *Ae. flavopictus*, mortality in the 4th instar larvae were significantly different in all the stages of larval development. Clearly, therefore, the mortality is affected by larval instar level, but such kind of effect is mostly seen due to nutrient required for Ecdysis. So, there was no significant variation of mortality between the three-leaf species (Figure 1). Leaf litter considerably influence the larval developmental period of both *Ae. albopictus* and *Ae. flavopictus* (Fig 2). Bamboo leaf litter had shorter developmental periods than those fed on the evergreen and deciduous leaf in both species (Figure 2). Larval development period is significantly shorter by vegetation type but have no significant effects on species ((Fig 2; Two-way ANOVA, vegetation: $F_{2,332} = 59.39$, $P < 0.0001$; species: $F_{1,366} = 0.9543$, $P = 0.3293$; species \times vegetation: $F_{2,662} = 3.373$, $P = 0.0353$))

Leaf litter have no significant effects on wing size of both male and female of *Ae. albopictus* but *Ae. albopictus* Males wing size (mean \pm SE) was 2.11 ± 0.04 mm and 2.10 ± 0.04 mm (one-way ANOVA $df=2$, 47 , $F=6.49$, $p=0.0032$) with bamboo leaf and evergreen leaf respectively significantly differ which

developed with deciduous leaf were 1.85 ± 0.05 mm. Overall evergreen leaf produces larger wing size in both male and female in *Ae. albopictus* and *Ae. flavopictus* but female wing size in *Ae. flavopictus* female was larger in deciduous leaf treatment (Table 1)

4.4. Discussion

The most principal observation of our results is that bamboo leaf litter is a more suitable food source for *Ae. albopictus* larval development than others leaf litters type. The developmental period was clearly shorter in the bamboo microcosms, especially in the larval development period up to 4th instar larvae followed by deciduous leaf and evergreen leaf litter. In *Ae. flavopictus*, bamboo leaf had similar effects like *Ae. albopictus* but there was no difference in deciduous and evergreen leaf litter on development from 1st instar to 4th instar. Larval mortality rates were higher after 4th instar of larval development in both species, but higher mortality observed in *Ae. albopictus* in all three-leaf litter type where *Ae. flavopictus* mortality found higher in evergreen leaf. Mortality at 4th larval instar indicates that Holometabolous insects, such as mosquitoes, must attain a critical mass during larval development (Clements, 1999). They also require sufficient mass for Ecdysis, and the potential attainable mass especially Poly unsaturated fatty acid is required for ecdysis (Riddiford 2012) but laboratory colony with only leaf litter having less amount of fatty acid compared to natural habitat where poly unsaturated fatty acid source is dead insects.

The increased longer larval instar period on leaf litter food resource will therefore lead to smaller population sizes of mosquitoes. The adult size of mosquitoes become smaller which is also possibly to have an effect on the population density of the mosquito. Size is often correlated with fitness in the field, because it can affect both the reproductive potential and feeding behavior of adult mosquitoes. Fewer number of eggs produced by smaller female which leads to slower post-emergence pre-blood meal ovarian development (Jalil 1974, Livdahl 1984). In *Ae. albopictus* small males' size can cause deferred spermatogenesis (Smith and Hartberg 1974).

Females should take longer to develop if that can lead to increased size, while male mosquitoes should favor faster development (Kleckner et al., 1995; Bedhomme et al., 2003). This is because of the correlation between size and female fitness, and between early reproduction/emergence and male fitness. In view of such observations, the smaller size of female mosquitoes in leaf litter may be due to lack of some essential nutrient. There may be some reasons why mosquitoes performed better under field condition than the laboratory condition because in natural micro habitat like bamboo stumps or tree hole itself act as a source for larval food and aggregate many insects which may favor quick larval development. Multiple leaf species may act as a more diverse resource base for micro-organisms in aquatic (Lecerf *et al.*, 2005; Cardinale *et al.*, 2006) and terrestrial systems (Cardinale et al., 2006), and therefore may provide either increased abundance or diversity of microbes, resulting in a superior diet for mosquito larvae. Mosquitoes are active filter feeders and can make adaptations

about where to feed and on what materials, even in comparatively small containers.

In the study, we revealed a major effect of both nutritional resources and development time where different leaf litter input influences the survival, development time, and fecundity of tree-hole mosquitoes by manipulating the release of biomass to the microbial community (decomposition rate), the efficacy with which microorganisms alter the decaying portions of the litter to new microbial tissue, and the dietary feature of the microbial community for grazing mosquito larvae.

Reference

- Anderson, N.H. & Sedell, J.R. (1979). Detritus processing by macroinvertebrates in stream ecosystems. *Annu. Rev. Entomol.* 24: 351 -377.
- Bedhomme, S., Agnew, P., Sidobre, C. & Michalakis, Y. (2003) Sex-specific reaction norms to intraspecific larval competition in the mosquito *Aedes aegypti*. *Journal of Evolutionary Biology.* 16:721–730.
- Berrie, A.D. (1976). Detritus, microorganisms, and animals in fresh water. In The role of terrestrial and aquatic organisms in decomposition processes. Edited by J. Andersen and A. Mac-Fadyen. *Blackwell Scientific Publications*, Oxford. pp. 323 - 328.
- Braks, M., Honorio, N., Lounibos, L.P., Lourenco-de-Oliveira, R. & Juliano, S.A. (2004). Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. *Annals of the Entomological Society of America.* 97:130–139.
- Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M. & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature.* 443:989–992.
- Carpenter, S. (1983) Resource limitation of larval tree hole mosquitoes subsisting on beech detritus. *Ecology.* 64:219–223.
- Chan, Y. C., B. C. Ho. & K. L. Chan. (1971). *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore city. Observations in relation to dengue haemorrhagic fever. *Bull. Wld. Hlth. Org.* 44: 651-658.

- Clements, AN. (1999) *The Biology of Mosquitoes, Sensory Reception and Behaviour*. CABI Publishing; Wallingford, UK.
- Costanzo, K.S., Kesavaraju, B. & Juliano, S.A. (2005). Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology*. 86:3289–3295.
- Cummins, K.W. & Klug, M.J. (1979). Feeding ecology of stream invertebrates. *Annu. Rev. Ecol. Syst.* 10: 147-172.
- Dieng, H., Mwandawiro, C., Boots, M., Morales, R., Satho, T., Tuno, N., Tsuda, Y., & Takagi, M. (2002) Leaf litter decay process and the growth performance of *Aedes albopictus* larvae (Diptera: Culicidae). *Journal of Vector Ecology*; 27:31–38.
- Fish, D. & Carpenter, S. (1982) Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology*. 63:283–288.
- Frank, J.H. & Lounibos, L.P. (1983). *Phytotelmata: terrestrial plants as hosts for aquatic insect communities*. Plexus, Medford, N.J.
- Griswold, M.W. & Lounibos, L.P. (2005). Competitive outcomes of aquatic container diptera depend on predation and resource levels. *Annals of the Entomological Society of America*. 98:673–681
- Hawley, W.A., Reiter, P., Copeland, R.S. & Pumpini, C.B. (1987) *Ae. albopictus* in North America: Probable introduction in used tires from northern Asia. *Science* 1987; 236:1114–1116.
- Hawley, W.A. (1988) The biology of *Ae. albopictus*. *Journal of the American Mosquito Control Association*, 4, 1-39.

- Jalil, M. (1974) Observations of the fecundity of *Aedes triseriatus* (Diptera: Culicidae). *Entomol. Exp. Appl.* 17: 223-233.
- Juliano, S.A., Lounibos, L.P. & O'Meara, G.F. (2004). A field test for competitive effects of *Aedes albopictus* on *A. aegypti* in South Florida: differences between sites of coexistence and exclusion? *Oecologia*. 139:583–593.
- Jumali, Suarto, D. J., Gubler, S., Nalim Bram, S. & Saroso, J. S. (1979). Epidemic dengue haemorrhagic fever in rural Indonesia III. Entomological studies. *Am. J. Trop. Med. Hyg.* 28: 717-724.
- Kaushik, N.K. & Hynes, H.B.N. (1971). The fate of dead leaves that fall into streams. *Arch. Hydrobiol.* 68: 465-515.
- Kleckner, C.A., Hawley, W.A., Bradshaw, W.E., Holzapfel, C.M. & Fisher, I.J. (1995) Protandry in *Aedes sierrensis* – the significance of temporal variation in female fecundity. *Ecology*; 76:1242–1250.
- Lecerf, A., Dobson, M., Dang, C.K. & Chauvet, E (2005). Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia*. 146:432–442.
- Leonard, P. M. & Juliano, S. A. (1995). Effect of leaf litter and density on fitness and population performance of the tree hole mosquito *Aedes triseriatus*. *Ecol. Entomol.* 20: 125-136.
- Livdahl, T., Koenekoop, R. & Futterweit, S. G. (1984) The complex hatching response of *Aedes* eggs to larval density. *Ecol. Entomology*. 9: 437-442.

- Livdahl, T.P. & Willey, M.S. (1991). Prospects for an invasion – competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science*. 253:189–191.
- Merritt, R., Dadd, R. & Walker, E. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*. 37:349–376.
- Merritt, R.W., Cummins, K.W. & Burton, T.M. (1984). The role of aquatic insects in the processing and cycling of nutrients. In *The ecology of aquatic insects*. Edited by V. Resh and D. Rosenberg. Praeger Scientific, New York. pp. 134-163.
- Murrell, E.G. & Juliano, S.A. (2008). Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*. 45:375–383.
- Rai, K. S. (1991) *Ae. albopictus* in the Americas. *Annu. Rev. Entomol.* 36: 459-484.
- Reiter, P. (1998). *Aedes albopictus* and the world trade in used tires, 1985-1995: the shape of things to come? *J. Am. Mosq. Control. Assoc.* 14: 83-94.
- Riddiford, L. M. (2012). How does juvenile hormone control insect metamorphosis and reproduction? *General and Comparative Endocrinology*, 179(3); 477–484.
- Slansky, F. Jr. & Scriber, J.M. (1985). Food consumption and utilization. In *Comprehensive insect physiology, biochemistry, and pharmacology*.

Edited by G. Kerkut and L. Gilbert. Pergamon Press, New York. pp. 88-163.

Smith, R. P. & Hartberg, W. K. (1974) Spermatogenesis in *Aedes albopictus* (Skuse). *Mosq. News* 34: 42-47.

Sota, T. (1993) Performance of *Aedes albopictus* and *A. riversi* larvae (Diptera: Culicidae) in waters that contain tannic acid and decaying leaves: is the tree hole species better adapted to tree hole water? *Annals of the Entomological Society of America*; 86:450–457.

Sota, T., Mogi, M. & Hayamizu, E. (1992) Seasonal distribution and habitat selection by *Aedes albopictus* and *Aedes riversi* (Diptera: Culicidae) in Northern Kyushu, Japan. *J. Med. Entomol.* 29: 296-304.

Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T. & Klug, M.J. (1991). Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, 72: 1529-1546.

Yee, D.A., Kaufman, M.G. & Juliano, S.A. (2007) The significance of ratios of detritus types and micro-organism productivity to competitive interactions between aquatic insect detritivores. *Journal of Animal Ecology*. 76:1105–1115.

CHAPTER 5

Summary and conclusion

5.1. Author summary

Aedes albopictus Skuse, are the major vectors of dengue or Chikungunya in Asia, and many other countries of the world, and *Ae. flavopictus* a sibling species of former species are locally found in Japan. The purpose of this study was to determine the effect of larval environment, such as, different temperature regimes on the development, and adult biology of *Ae. albopictus* and *Ae. flavopictus* under laboratory conditions. The objective of this study was to estimate the population dynamics of *Ae. albopictus*, as well as *Ae. flavopictus* and their vector potential for dengue and chikungunya due to global climate change. This final chapter is describing the combination of the results of the previous chapters of this dissertation. Many topics have been considered in the individual chapters, so only the summary and conclusions of previous chapters will be considered here.

The vector ecology, developmental biology, behavior, survival and the transmission dynamics of the diseases they spread are strongly dependent on climatic factors. Temperature, rainfall, and humidity are especially important, but others, such as, diet, conditions of breeding sites are also significant. In mosquito vectors, larva rearing under different constant temperature and temperature with variation can impact on larval mortality, development time, and adult body size. In this study, the effect of different temperature was studied in the development on two mosquitoes *Ae. albopictus* and *Ae. flavopictus*. Among four temperatures

(22, 25, var. 25 and 28°C), larval mortality was found higher in 22°C for *Ae. albopictus* and 28°C for *Ae. flavopictus*. Most mortality occurred at on the old stage (4th) of larvae in *Ae. albopictus* but young stage (1st and 2nd instar) in *Ae. flavopictus*. These results indicate that early triggering of metamorphosis occurred which further would not allow sufficient time for ecdysteroid accumulation for complete metamorphosis, resulting death. Development duration was decreased with increase of temperature. So, there was an inverse relationship between adult body size and temperature. Adult body size was decreased with increase of temperature.

The size of a female mosquito, determined by its larval environment, has a relationship with their biological and behavioral traits. The multiple blood feeding frequency, fecundity of *Ae. albopictus* and *Ae. flavopictus* were studied under laboratory condition. Most of the females (56.2% and 58.18% of *Ae. albopictus*, and *Ae. flavopictus* respectively) oviposited with only single blood meals. This indicates that a single complete blood meal is not sufficient for both *Aedes* species to develop eggs in the first gonotrophic cycle, but when we compared with temperature under 25°C *Ae. flavopictus* laid eggs 100% of female after taking their first blood meal and In *Ae. albopictus* female it was 74.05% at 28°C after first blood meal. So, multiple blood meal was required for mosquitoes when the temperature is changing and it's also varies between the species. These results suggest that multiple blood meals in *Ae. albopictus* and *Ae. flavopictus* might be due to interruption of feeding by defensive host in nature, whereas, *Ae. albopictus* is likely to feed more than once in a single gonotrophic cycle.

Mosquito population growth was mostly dependent on the temperature during their developing stage. By population dynamics models, per capita rate of growth can be measured by developmental stage parameters, such as, larval mortality, developmental duration, body size of emerging adults with or without the information of adult fecundity, and mean maturation time. This study estimated the population growth in for per capita performance index, I , to integrate the effects of temperature. The population growth in both species varied with temperature changes, though *Ae. albopictus* showed better performance at 22°C, var. 25°C and 28°C but *Ae. flavopictus* only showed better performance under 25°C with a decline in population growth under higher temperature. This study suggests that in higher temperature *Ae. flavopictus* retarded its population growth, but *Ae. albopictus* increases its population with various temperature ranges from higher to lower.

We studied the temporal and spatial distribution of adult and larval *Ae. albopictus* (Skuse) and *Ae. flavopictus* (Yamada) in Kanazawa city, Ishikawa, Japan. In this study Adults of both species occurred from May through September. Both *Ae. albopictus* and *Ae. flavopictus* were frequently found in July and August. Larval *Ae. flavopictus* choose primarily bamboo stumps, but also were found in larger artificial containers with higher volume of water in the forests. Although the abundance of adult *Ae. albopictus* in the urban site, larvae occurred in the forest less frequently than *Ae. flavopictus*; *Ae. albopictus* larvae mostly found in the forest during hot summer.

Leaf litter variation may also be studied for larval growth and development, but little is known about how larval mosquitoes respond to specific leaf type as a larval food. So, we observed how the different vegetation type influences the population growth of *Ae. albopictus* and *Ae. flavopictus*, and we use three types of leaf, deciduous broad-leaved trees, evergreens, bamboo leaves in laboratory condition. Overall mortality was higher in all three-leaf type, but mortality from the fourth instar stage to pupation is higher in *Ae. albopictus* compared with *Ae. flavopictus*. Bamboo leaf shows the rapid larval development than evergreen and deciduous leaf up to 4th instar larvae in both the species.

5.2. Concluding remarks

1. Mosquito developmental period, and adult body size and mortality were mainly manipulated by larva rearing temperature.
2. Mosquito oviposition, hatchability was also governed by larva rearing temperature.
3. More than 50% of female of *Ae. albopictus* and *Ae. flavopictus* can oviposit with a single complete blood meal but for more population growth they need second blood meal.
4. Population dynamics of mosquito was also manipulated by the temperature during larval stage.

5. Mosquito vector efficiency was affected by larva rearing temperature by the way of changing vector population size, and/or by altering the adult body size (which influence the survival of adults).
6. This study will help to predict the causes of distribution variation between *Ae. albopictus* and *Ae. flavopictus* with their population dynamics and transmission dynamics of dengue and chikungunya in response to the global climate change.

5.2. Scope for further research

1. Based on the information on this study, interspecific competition and reproductive interference between the two species is required to investigate which may give more clarification about their habitat difference and distribution.
2. Response to extreme higher temperature with their consequence in life history trait of both the species in laboratory condition are required to explain for better understanding of hot summer effects on the mosquito population.
3. I did not manipulate the quantity and quality of the food in my study. In nature, quality and quantity of food varied with habitat and location. Therefore, the quantity and quality of nutrient in natural food for *Ae. albopictus* and *Ae. flavopictus* is needed to measure.

4. For multiple blood feeding phenomena in *Ae. albopictus* and *Ae. flavopictus* a detailed study is necessary in field condition. it is necessary to investigate if they take a multiple meal within a shorter time, even after a first full blood meal.

5.4. Limitation of this study

During my study into the effect of temperature on the development of *Ae. albopictus* and *Ae. flavopictus* (Chapter 2), I mixed up recorded individual female of a different individual in a cage, for blood feeding in a group. So, in that case use the average age of female in individual temperature regimes for analyzing per capita performance index I in both species.

Another limitation of my study was a few numbers of natural habitat samples, analyze for water quality which is not sufficient to compare in my seasonal distribution experiment and the influence of vegetation on the larval development study. So. Lack of data for water quality assessment in my experiment.

Table 2.1. Mortality ratio of *Ae. albopictus* (albo) and *Ae. flavopictus* (flavo) with result of logistic regression analysis and comparisons (Fisher's exact test) under same conditions on the mortality rate in developmental stage (first instar to emergence)

| Summary of logistic regression analysis on mortality | | | |
|--|----|------------|--------|
| N=800, df=7, Chi square=73.2697, p<0.0001 | | | |
| Factor | df | Chi square | p |
| Temperature | 3 | 24.988 | <.0001 |
| Species | 1 | 39.802 | <.0001 |
| Species*Temperature | 3 | 17.633 | 0.0005 |

| Mortality (N=100 in respective treatment) | | | |
|---|------|-------|-------------------------|
| Rearing temperature (°C) | albo | flavo | p (Fisher's exact test) |
| 22 | 12 | 21 | 0.127 |
| 25 | 5 | 15 | 0.032 |
| 25 var. | 1 | 7 | 0.065 |
| 28 | 0 | 26 | <0.0001 |

Table 2.2. The developing time (2A) and wing size (2B) of *Ae. albopictus* and *Ae. flavopictus* under different rearing temperatures.

| A. Developing time (day) | <i>Ae. albopictus</i> | | | | | <i>Ae. flavopictus</i> | | | |
|--------------------------|------------------------------|----|-------|-------|-----------------------|-------------------------------|------|-----------------------|---|
| | Rearing temperature (°C) | n | mean | SE | Steel-Dwass test | mean | SE | Steel-Dwass test | |
| Summary of ANOVA | df=3, 163, F=219.4, p<0.0001 | | | | | df=3, 161, F=81.456, p<0.0001 | | | |
| Female | 22 | 39 | 14.0 | 0.16 | A | 45 | 13.9 | 0.21 | A |
| | 25 | 38 | 10.2 | 0.16 | B | 37 | 10.1 | 0.23 | B |
| | 25 var. | 40 | 10.8 | 0.16 | B | 46 | 10.7 | 0.21 | B |
| | 28 | 50 | 8.7 | 0.14 | C | 37 | 9.6 | 0.23 | C |
| Summary of ANOVA | df=3, 211, F=219.5, p<0.0001 | | | | | df=3, 162, F=74.2, p<0.0001 | | | |
| Male | 22 | 49 | 13.12 | 0.14 | A | 34 | 13.5 | 0.24 | A |
| | 25 | 57 | 9.89 | 0.13 | B | 48 | 9.6 | 0.20 | B |
| | 25 var. | 59 | 9.92 | 0.13 | B | 47 | 9.7 | 0.20 | B |
| | 28 | 50 | 8.16 | 0.14 | C | 37 | 9.2 | 0.23 | B |
| B. Wing size (mm) | <i>Ae. albopictus</i> | | | | | <i>Ae. flavopictus</i> | | | |
| | Rearing temperature (°C) | n | mean | SE | Tukey-Kramer HSD test | mean | SE | Tukey-Kramer HSD test | |
| Summary of ANOVA | df=3, 161, F=26.8, p<0.0001 | | | | | df=3, 161, F=148.9, p<0.0001 | | | |
| Female | 22 | 39 | 3.12 | 0.03 | A | 45 | 3.65 | 0.02 | A |
| | 25 | 38 | 2.91 | 0.03 | B | 37 | 3.38 | 0.03 | B |
| | 25 var. | 40 | 3.08 | 0.03 | A | 46 | 3.22 | 0.02 | C |
| | 28 | 50 | 2.85 | 0.02 | B | 37 | 2.95 | 0.03 | D |
| Summary of ANOVA | df=3, 213, F=96.7, p<0.0001 | | | | | df=3, 162, F= 58.04, p<0.0001 | | | |
| Male | 22 | 49 | 2.58 | 0.019 | A | 34 | 2.76 | 0.02 | A |
| | 25 | 57 | 2.48 | 0.017 | B | 48 | 2.77 | 0.02 | A |
| | 25 var. | 59 | 2.52 | 0.017 | AB | 47 | 2.54 | 0.02 | B |
| | 28 | 50 | 2.18 | 0.018 | C | 35 | 2.40 | 0.02 | C |

Table 2.3. Fecundity of *Ae. albopictus* and *Ae. flavopictus* under different rearing temperatures with one or two blood meals.

| Species | Rearing temperature (°C) | First Blood meal | | | | | Second Blood meal* | | | | |
|------------------------|--------------------------|---------------------------------|---------------------|---------------------|------|------------------|-------------------------------|---------------------|---------------------|-------|------------------|
| | | N of female | N female oviposited | Number of eggs laid | | | N of female | N female oviposited | Number of eggs laid | | |
| | | | | Mean | SE | Steel-Dwass test | | | Mean | SE | Steel-Dwass test |
| | | df= 3, 55, F = 0.4319, P=0.7310 | | | | | df = 2, 27, F = 8.125, P<0.01 | | | | |
| <i>Ae. albopictus</i> | 22 | 26 | 12 | 40.2 | 10.8 | | 14 | 7 | 28.0 | 12.0 | A |
| | 25 | 24 | 9 | 37.8 | 6.8 | | 15 | 15 | 35.3 | 7.0 | B |
| | 25 var. | 28 | 18 | 49.8 | 7.1 | | 10 | 8 | 79.4 | 9.7 | C |
| | 28 | 27 | 20 | 44.6 | 6.0 | | 7 | 0 | 0 | 0 | |
| | | df= 3, 60, F = 4.338, P<0.05 | | | | | df=3,45, F=26.76, P<0.0001 | | | | |
| <i>Ae. flavopictus</i> | 22 | 28 | 12 | 17.6 | 5.4 | A | 16 | 12 | 70.5 | 13.83 | |
| | 25 | 28 | 28 | 39.2 | 5.6 | B | | | | | |
| | 25 var. | 28 | 17 | 16.5 | 2.9 | A | 11 | 0 | 0 | 0 | |
| | 28 | 26 | 7 | 35.7 | 10.8 | B | 19 | 0 | 0 | 0 | |

* Females that did not lay any eggs with the first blood meal were supplied with the second blood meal with 07 days interval from the first one,

Table 3.1. Status of Adult catch mosquitoes in Kanazawa 2017- 2018.

| Year | Location and habitat | Total number | Frequency | | No. females/person/8 min, ($x \pm SE$) | |
|------|------------------------------------|--------------|-----------------------|------------------------|--|------------------------|
| | | | <i>Ae. albopictus</i> | <i>Ae. flavopictus</i> | <i>Ae. albopictus</i> | <i>Ae. flavopictus</i> |
| 2017 | Kakumamachi Bamboo forest | 11 | 0 | 11 | | 5.5±2.5 |
| | Residential Area (less vegetation) | 39 | 39 | 0 | 6.5±2.65 | |
| | Shrine (evergreen tree) | 16 | 16 | 0 | 2.5±1.02 | |
| | Park (evergreen tree) | 10 | 10 | 0 | 2.25±1.125 | |
| | Cemetery (evergreen tree) | 7 | 7 | 0 | 1.75±0.875 | |
| 2018 | Bamboo forest (Kakumamachi) | 52 | 0 | 52 | | 3.31± 0.705 |
| | Residential Area | 17 | 17 | 0 | 2 ± 0.655 | |
| | Shrine | 39 | 39 | 0 | 5 ± 1.322 | |
| | Park | 23 | 23 | 0 | 3 ± 1.149 | |
| | Cemetery | 49 | 49 | 0 | 2.625±1.742 | |
| | Bamboo Forest (Teramachi) | 21 | 21 | 0 | 4.5± 1.851 | |

Table 4.1: Wing size of *Ae. albopictus* and *Ae. flavopictus* under the influence of different vegetation type.

| 1. Wing size (mm) | <i>Ae. albopictus</i> | | | | | <i>Ae. flavopictus</i> | | | |
|-------------------|-----------------------------|----|-------|------|-----------------------|-------------------------------|-------|-----------------------|----|
| | Vegetation type | n | mean | SE | Tukey-Kramer HSD test | mean | SE | Tukey-Kramer HSD test | |
| Summary of ANOVA | df=2, 46, F=2.722, p=0.0763 | | | | | df=2, 40, F=3.29, p=0.0432 | | | |
| Female | Bamboo | 17 | 2.52 | 0.05 | A | 19 | 2.79 | 0.04 | A |
| | Deciduous | 5 | 2.48 | 0.05 | A | 15 | 2.97 | 0.04 | B |
| | Evergreen | 27 | 2.637 | 0.03 | B | 9 | 2.83 | 0.1 | A |
| Summary of ANOVA | df=2, 47, F=6.49, p=0.0032 | | | | | df= 2,40, F=0.8542, p= 0.4332 | | | |
| Male | Bamboo | 21 | 2.11 | 0.04 | A | 16 | 2.29 | 0.07 | A |
| | Deciduous | 10 | 1.85 | 0.05 | B | 15 | 2.327 | 0.05 | A |
| | Evergreen | 19 | 2.10 | 0.04 | A | 12 | 2.408 | 0.05 | AB |

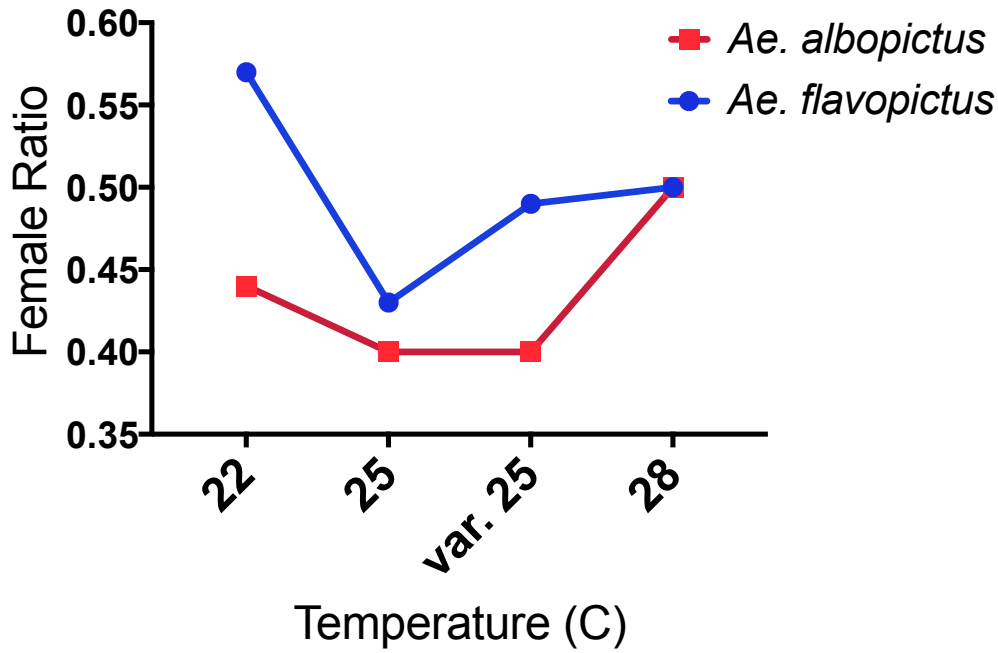


Fig 2.1. Female ratio of *Ae. albopictus* and *Ae. flavopictus* at 22, 25, var. 25 and 28°C.

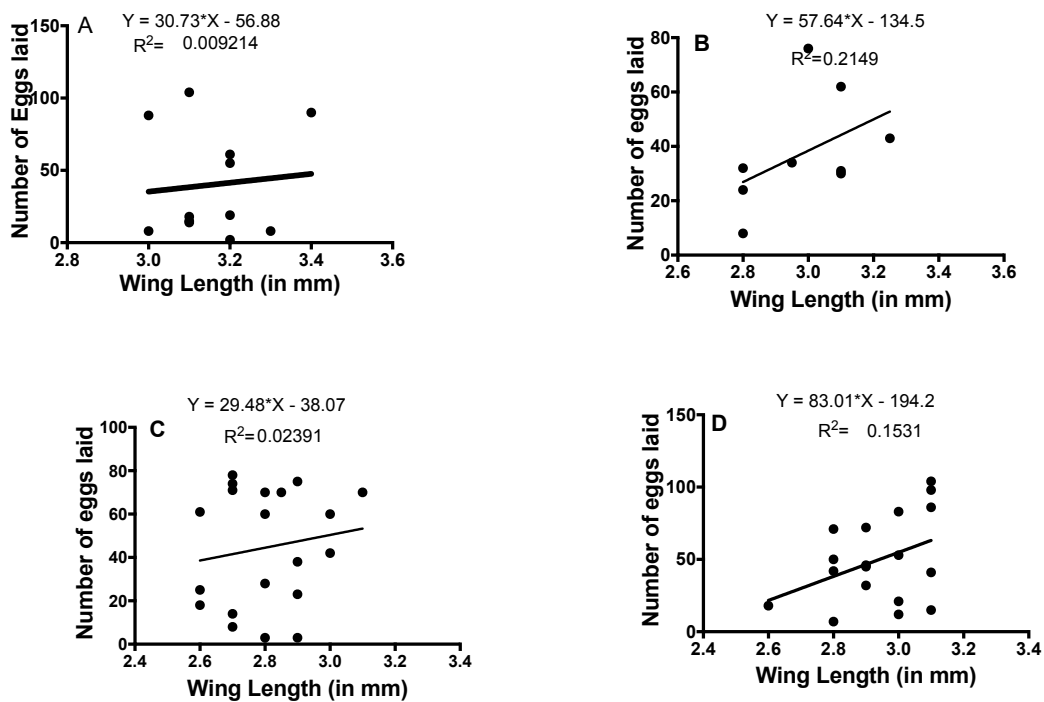


Fig 2.2. Relationship of fecundity vs wing length of *Ae. albopictus* A, B, C & D are at 22, 25, 28°C and var. 25°C respectively.

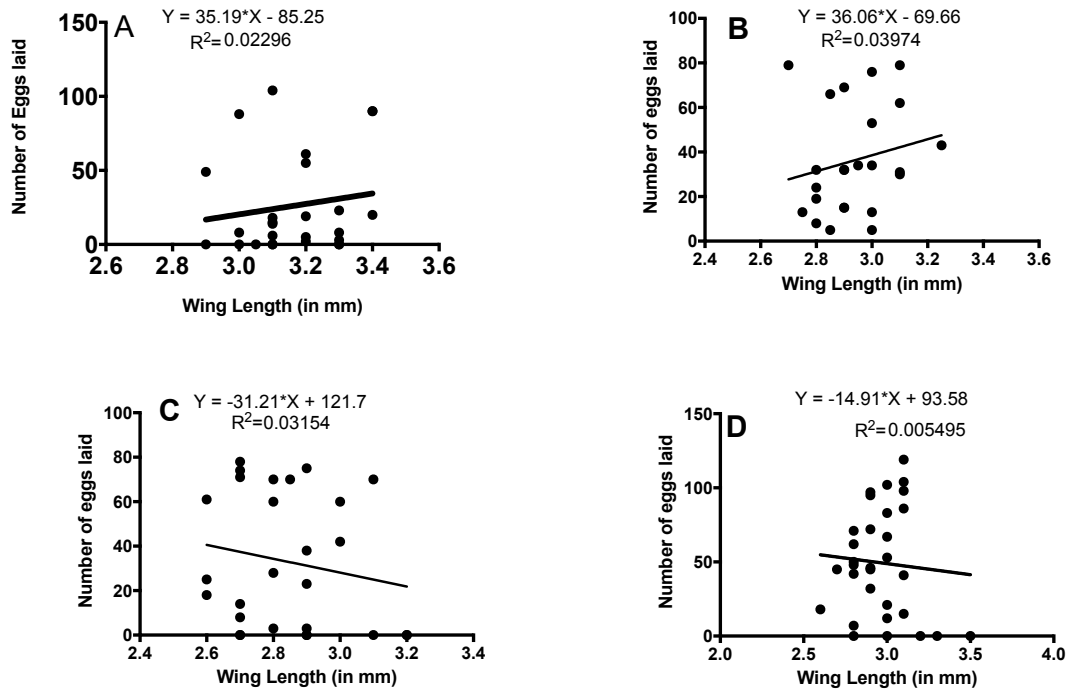


Fig 2.3. Relationship of fecundity vs wing length of *Ae. flavopictus* A, B, C & D are at 22, 25, 28°C and var. 25°C respectively.

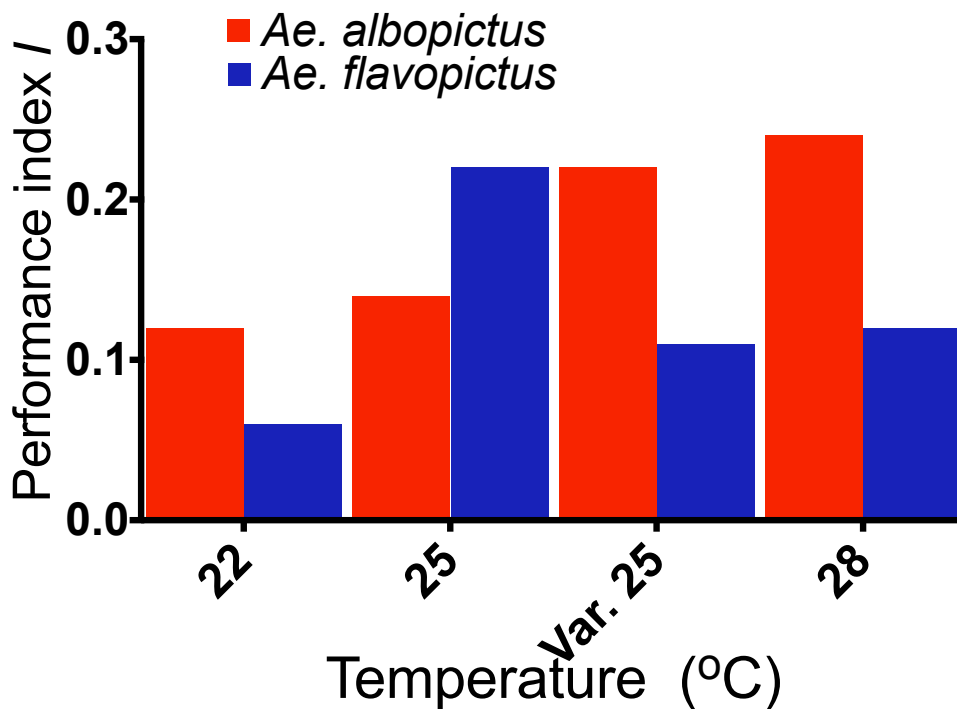


Fig 2.4. Estimated per capita performance index I of *Ae. albopictus* and *Ae. flavopictus* with observed sex ratio under different rearing temperatures (22, 25, var. 25 and 28 °C).



Fig 3.1. Locations maps of the study sites in Kanazawa.

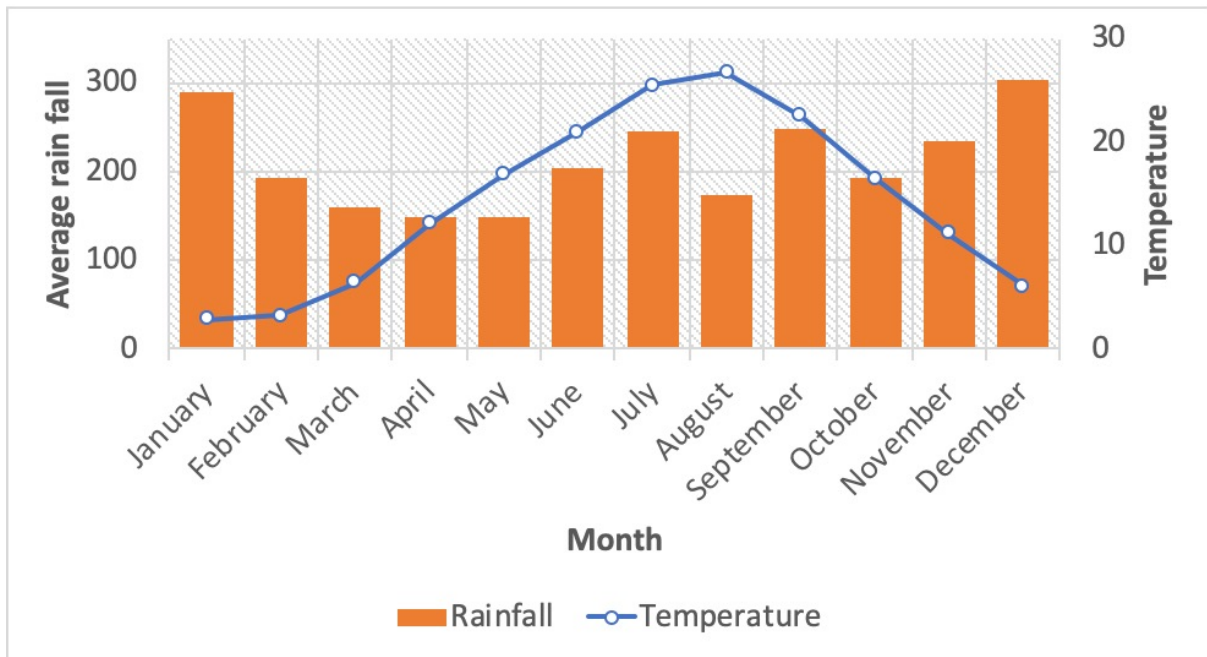


Fig 3.2. 30 Years mean temperature and precipitation for the city of Kanazawa.

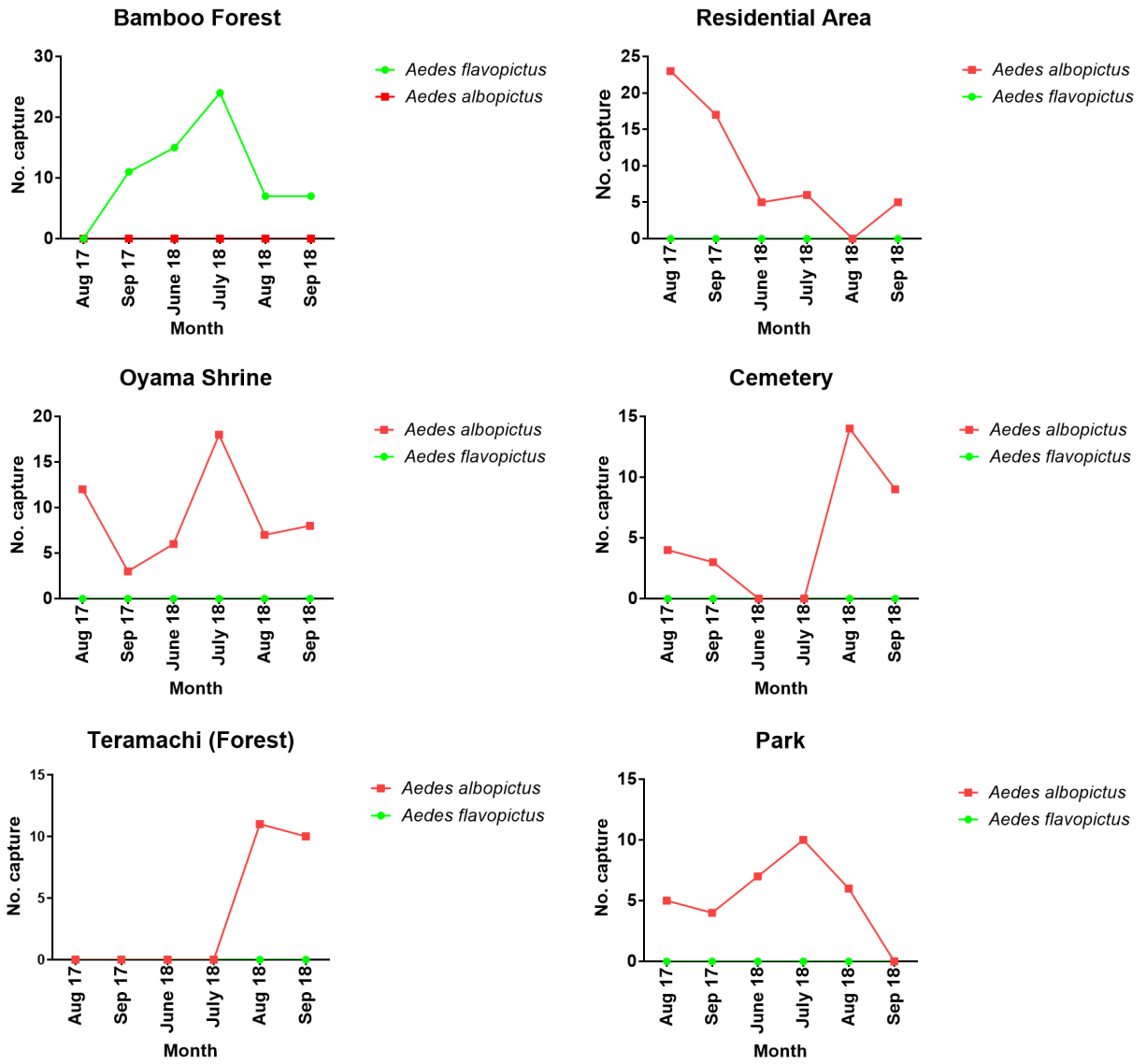


Fig 3.3. Seasonal trends of *Stegomyia* adults at different sites on Kanazawa.

Average number of larvae/ovitrap

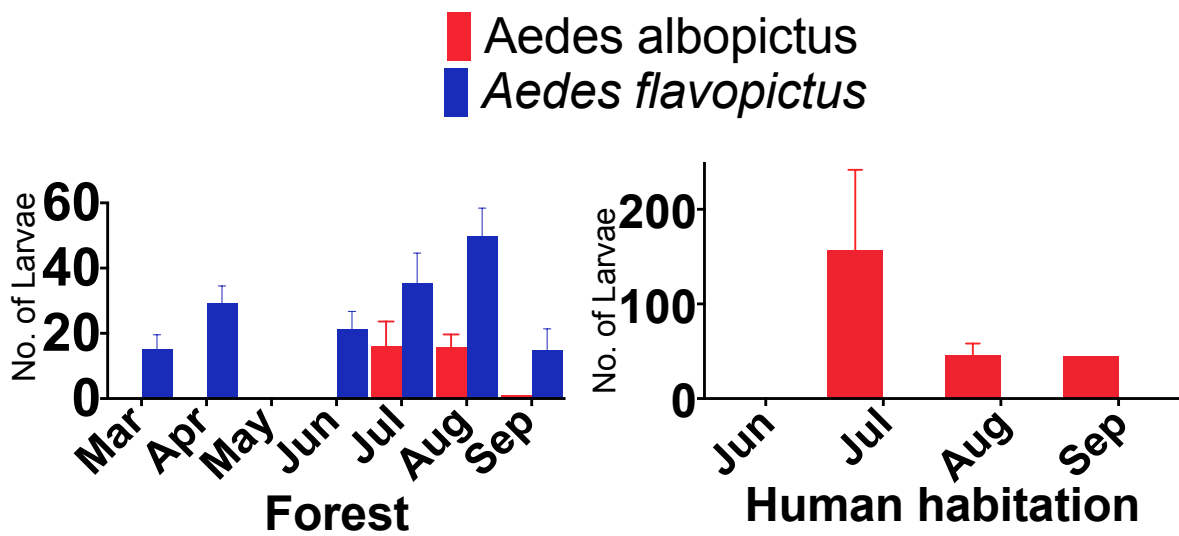


Fig 3.4. Seasonal variation of *Stegomyia* larval abundance in Kanazawa.

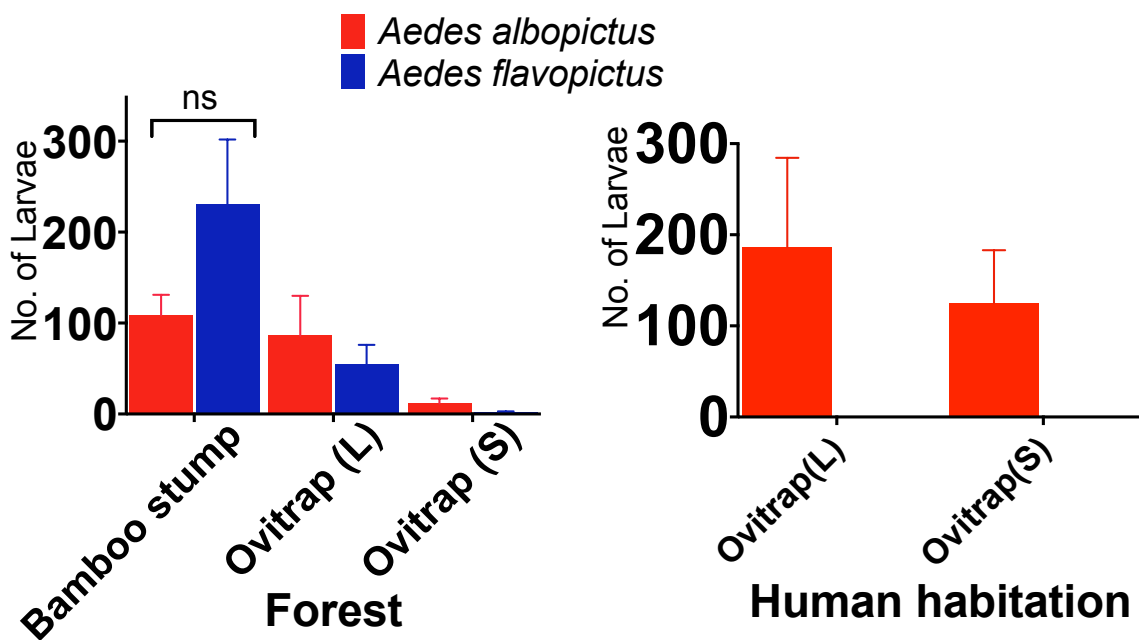


Fig 3.5. Microhabitat type preference as ovipositioning site by *Ae. albopictus* and *Ae. flavopictus* female.

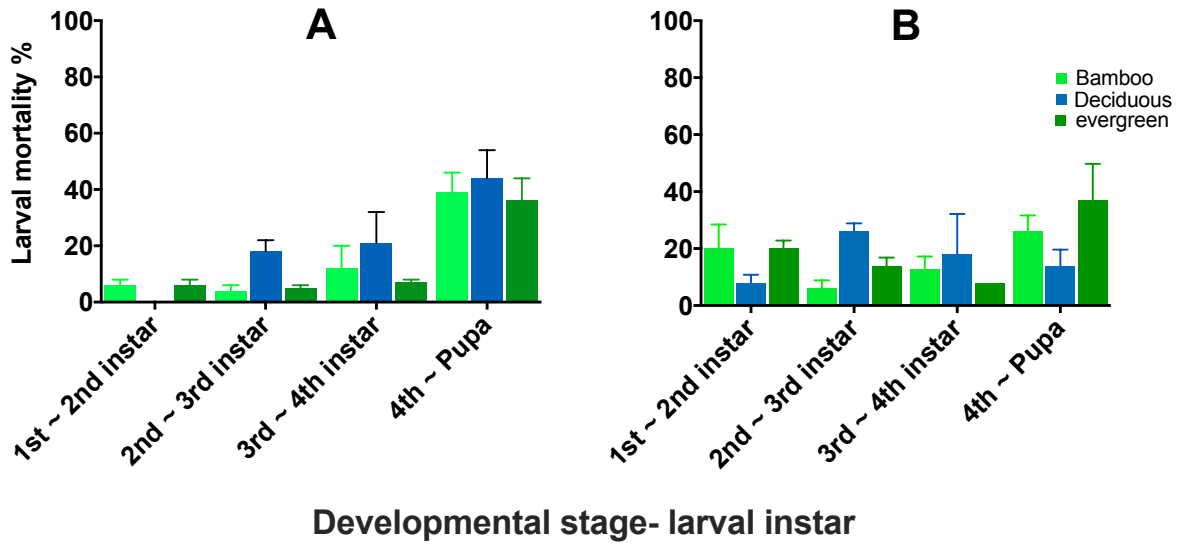


Fig 4.1. Larval mortality from 1st instar to Pupa A) *Ae. albopictus* B) *Ae. flavopictus*

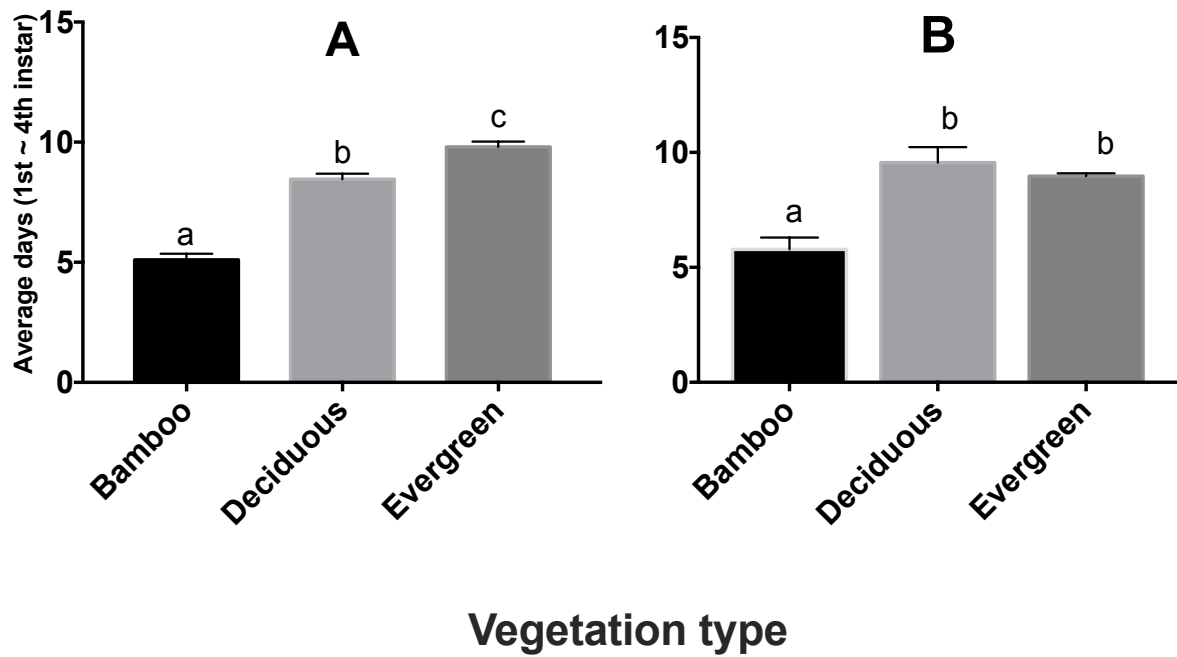


Fig 4.2. Developmental duration from 1st ~ 4th instar A) *Ae. albopictus* B) *Ae. flavopictus*