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Effects of temperature and diet on development and interspecies competition in Aedes aegypti and Aedes albopictus

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Title:

Effect of temperature and diet on the development and interspecific competition of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae)

Short title: Population growth of *Ae. aegypti* and *Ae. albopictus*

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Abstract

We asked whether climate change might affect the geographic distributions of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae). We tested the effects of temperature, diet, and the presence of congeneric species on the immature stage performance of these two aedine species in the laboratory. Mosquitoes in three different species-density combinations were reared at four constant temperatures (20, 25, 30, and 35°C) with low or high diets. Among the four temperatures tested, mortality increased only at 35°C in both species. Mortality was higher on the high diet than on the low one at 35°C, but not at other temperatures. Presence of the congeneric species had a significant positive effect on the mortality of *Ae. albopictus*, but not in case of *Ae. aegypti.* Both species developed more quickly at higher temperatures within the range 20–30°C; development was not enhanced at 35°C. Population growth of *Ae. albopictus* was more stable, regardless of diet and temperature; that of *Ae. aegypti* varied more with these two factors.

These species-specific attributes may help explain the latitudinal distribution of the mosquitoes and degree of species dominance where they are sympatric.

Key words: *Aedes aegypti*, *Aedes albopictus*, interspecific competition, temperature, diet

Introduction

The effect of global climate change on the geographic distributions of vectors and vector-borne infectious diseases is an important issue. Dengue fever and dengue hemorrhagic fever are expanding problems in tropical and subtropical regions, and are now the most frequent arboviral diseases worldwide (Gubler, 2002). Two common mosquito species, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), are competent vectors in Asian countries. Southeast Asia is the native habitat of *Ae. albopictus*, but *Ae. aegypti* has spread throughout the region and is increasing in abundance (Rudnick & Hammon, 1960; Gilotra *et al*., 1967; Jueco & Cabrera, 1969; Russell *et al*., 1969; Ho *et al*., 1973; Hawley, 1988). *Aedes albopictus* is a known vector of the dengue virus in parts of Asia (Smith, 1956; Russell *et al*., 1969; Chan *et al*., 1971a) where *Ae. aegypti* is rare or absent (Sunarto *et al*., 1979; Metselaar *et al*., 1980). In Asia, *Ae. aegypti* is more closely associated with human environments in which indoor and outdoor artificial containers, such as drums, tires, buckets, flowerpots, and vases, retain water and provide habitats for aquatic larval development (Focks *et al*., 1981; Service, 1992; Focks & Chadee, 1997; Gubler, 1998). In contrast, the immature stages of *Ae. albopictus* inhabit natural water containers such as bromeliads, bamboo stumps, and tree holes in addition to artificial containers (Hawley, 1988). *Aedes aegypti* is most prevalent in urbanized areas, whereas *Ae. albopictus* occurs in rural, suburban, and vegetated urban areas in Calcutta, India (Gilotra *et al.,*1967). In both urban and

rural areas in Singapore, it is uncommon for the two species to share breeding habitats (Chan *et al.*, 1971b). It has been hypothesized that the displacement of *Ae. albopictus* by *Ae. aegypti* in certain Asian cities is the result of urbanization (Chan *et al*., 1971; Hawley, 1988). In contrast to Asia, the abundances of the two species are similar in most suburban areas in southeastern Brazil and in Florida, United States (Braks *et al*., 2003).

Aedes albopictus was introduced into Texas in the 1980s (Hawley, 1988) and has since spread across southern North America (Hobbs *et al*., 1991; Mekuria & Hyatt, 1995), replacing *Ae. aegypti*, which was present in the southeastern United States for more than 100 years before the arrival of *Ae. albopictus* (Christophers, 1960; Lounibos, 2002). Regional photoperiod and temperature regimes may determine 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.

Aedes albopictus is one of the most common mosquito species on the main (Honshu) and southern (Kyushu) islands of Japan. *Aedes aegypti* is absent on these islands; although one report stated that it was temporarily present in a small southern Japanese town during the period 1944–1947 (Hotta, 1998). Unfortunately, we are unaware of the conditions that allowed the invasion of *Ae. aegypti* to occur or the reason why the species did not become successfully established, although interactions with *Ae. albopictus* may have played a crucial role.

The main environmental factors affecting mosquito population growth are temperature (Clements, 1992; Atkinson, 1994) and nutrition (Merritt *et al*., 1992). Higher temperatures shorten the developmental duration of *Aedes* species (Tun-lin *et al*., 2000; Alto & Juliano, 2001a; 2001b; Delatte *et al*., 2009), leading to the production of smaller adults (Reuda *et al*., 1990; Rae, 1990; Tun-lin *et al*., 2000). Nutrition and density dependent factors also influence developmental

time and survivorship in *Ae. aegypti* (Christophers, 1960) and *Ae. albopictus* (Teng & Apperson, 2000). Immature development in *Ae. aegypti* is governed by a combination of temperature and nutrition (Tun-lin *et al*, 2000).

Considerable literature has accumulated regarding competition between *Ae. aegypti* and *Ae. albopictus*. Several studies have suggested that *Ae. albopictus* has a competitive advantage over *Ae. aegypti* (Barrera *et al*., 1996; O'Meara *et al*., 1995; Juliano, 1998; Daugherty *et al.*, 2000; Lounibos, 2002; Braks *et al*., 2004; Juliano *et al*., 2004; Murrell & Juliano, 2008). Other studies report the competitive superiority of *Ae. aegypti* (Moore & Fisher, 1969; Sucharit *et al*., 1978; Serpa *et al*., 2008). Although these studies support the hypothesis that negative interactions exist between the two species, their relative superiority is variable depending on nutrition conditioning and the stability of breeding sites. We need to monitor invasions of alien species and their establishment under on-going global warming. However, the distributions of the two species are apparently not governed by a single factor, such as temperature. In this study, we manipulated nutrition and temperature to represent the geographic and local variation found among aedine breeding sites. This experiment allowed us to evaluate the outcome of competition between the two species by studying population growth rates of immature stages in the laboratory.

Materials and Methods

Mosquitoes

Laboratory stocks of *Ae. aegypti* (collected in Moshi, Tanzania, 3°20'38"S and 37°20'76"E) and *Ae. albopictus* (collected in Nagasaki, Japan, 32°46'20.35"N and 129°52'9.86"E) were used. Both colonies had been maintained at the Institute of Tropical Medicine, Nagasaki University, for over 3 years before they were transferred to our laboratory at Kanazawa University. Adults were

maintained at 25±1°C and 70–90% relative humidity under 14L/10D photoperiod conditions. They were fed with a 3% sucrose solution daily and blood fed on rats once weekly.

Experimental Design

Twenty-four (4 temperatures × 2 diets × 3 larval densities) treatment combinations were established. One experimental unit included 20 first instars (*Ae. aegypti* and *Ae. albopictus* in the following proportions: 0:20, 10:10, 20:0). Twenty larvae were transferred to a 500-ml vessel containing 200 ml dechlorinated tap water. Five replicates of single-species treatments and 10 replicates of mixed-species treatments made up 100 individual per species per treatment. Experimental vessels were kept at constant temperatures of 20, 25, 30, or 35°C under a 14L/10D cycle photoperiod. Larvae were fed a mixture of rat food (CE-2, CLEA Japan, Inc. Tokyo) and yeast extract powder (Ebios, Mitsubishi Tanabe Pharma Corporation, Osaka) (1:1 in weight). Two levels of the diet factor were tested: high and low. First and second instars were fed 0.2 mg and 0.05 mg/larva/day of larval food as the high and low diets, respectively; the respective feeding rates for third and fourth instars were 0.5 mg and 0.1 mg/larva/day as the high and low diets. 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. Emerged adults were killed by freezing to allow measurement of one wing per insect using a micrometer under a stereomicroscope. The wing was measured from the distal end of the axial inclusion to the apical margin, not including the fringe (Van Den Heuvel, 1963).

Per Capita Performance

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

$$I = \frac{Ln(1/N_0)(\sum w_x^3)}{\sum x\, w_x^3 / \sum w_x^3}$$

where N_0 is the initial number of larvae in an experimental treatment and w_x is the wing length of females that emerged on day x. The cubic value of the female wing length, w_x^3, a dimensionless expression of body volume, represents fecundity; this is a meaningful value for scaling metabolic parameters (Schmidt-Nielsen, 1984). A well documented positive correlation exists between wing length and fecundity, but the formula varies across the data range and by the method of data collection, possibly due to the trade-off between egg size and egg number (Berrigan, 1991; Hard & Bradshaw, 1993). Cubic values of wing length represent potential blood meal size and may be considered appropriate proxies of fecundity without considering the trade-offs between egg number and size. 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 eggs emerged into adult stages. To deal with the problem some studies define N_0 as a half of eggs/larvae applied assuming 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 was no data to judge if the assumption is rational, therefore, we determined "*I*" in two ways, one is to apply absolute data and the other is to calculate using average of wing length of females emerged day x, under the assumption of even sex ratio.

Statistical analysis:

Effects of temperature, diet and species size (single or mixed) on the mortality rate of *Ae. aegypti* and *Ae. albopictus* were analyzed with ANOVA. Mortality ratios were arcsin square-root transformed to meet the assumptions of normality and homogeneity of variances. We applied MANOVA to analyze the effect of temperature and diet on the development time and wing size of the aedine species respectively in mixed species treatment. Since we did not record the development time by sex in single species treatment, we did not analyze the data further except for mortality. By the same reason we calculate index "*I*" in mixed species but not in single species treatment.

To compare the mortality, development time and wing size among four temperature groups, we used Tukey's HSD test. We compared these parameters between high-low diet conditions by *t*-test adjusted by Bonferroni correction. We compared sex-specific wing size between single species and mixed species treatments using *t*-test adjusted by Bonferroni correction again. Statistical analyses were performed using JMP version 5.0.1.2 (SAS Institute, Cary, NC, USA).

Results

Most mortality occurred in the late larval stages: 44.0% of the total mortality at the fourth instars and 40.7% at the pupal stages in *Ae. aegypti*, and 31.0% at the fourth instars and 52.5% at the pupal stages in *Ae. albopictus*. The ANOVA results showed that species interactions had a significant negative effect of *Ae. aegypti* on *Ae. albopictus*, but not of *Ae. albopictus* on *Ae. aegypti* (Table 1). The interaction of temperature × diet was significant in both species, but that of temperature × species was only significant in *Ae. albopictus* (Table 1). Mortality rates were significantly higher at 35°C than at lower temperatures for both species ($P < 0.05$; Fig. 1). The

effect of diet level was significant only at 35°C for both species, where more mortality occur under high diet conditions (Fig. 1A, C). *Aedes albopictus* showed significantly higher mortality under mixed species treatment at 35°C (Fig. 1D). Comparisons of the effects of single/mixed-species treatment on mortality revealed complex effects. No effect of *Ae. albopictus* on *Ae. aegypti* was observed except under the treatment combination of high diet × 35°C ($P < 0.01$). The mortality of *Ae. albopictus* was higher in the presence of *Ae. aegypti*, but it was condition specific, i.e., mortality was reduced under the mixed species × low diet × 25°C ($P < 0.01$) treatment combination, and mortality was elevated under the mixed species × low diet × 30°C and the mixed species × high diet × 30°C treatments ($P < 0.01$).

We recorded developmental duration by sex in the mixed-species treatment only (Fig. 2A, B). Males generally had shorter development times than females. Increasing temperature reduced developmental duration in the temperature range of 20–30°C, but an increase in developmental duration was observed at 35°C in *Ae. albopictus* males, which took longer to develop at 35°C than at 30°C (Fig. 2B). Development times were reduced in both sexes of both species with the high diet at all temperatures (Fig. 2A, B).

Wing size was significantly reduced by increasing temperature (Fig. 3). Larger *Ae. aegypti* emerged under the high diet regardless of temperature or single/mixed species condition (Fig. 3A, B). However, the high diet did not contribute to larger *Ae. albopictus* at 35°C, except for males in the single-species treatment (Fig. 3C, D). Wing size was greatest under the low temperature × high diet combination, and it was smallest under the high temperature × low diet combination (Fig. 3). Each species was influenced by the presence of the other at 25°C and 30°C under the high diet; the effect was stronger under the low diet for *Ae. aegypti* than for *Ae. albopictus*. Both male and female *Ae. aegypti* were larger under mixed-species combinations

than under single species treatments at 20, 25, and 30°C (Fig. 3A, B). *Aedes albopictus* females were larger under mixed-species conditions at 20°C, whereas males were larger at 20°C and 35°C in mixed populations (Fig. 3C, D).

MANOVA indicated that the interaction of temperature × diet had a significant effect on the development time and wing size of females of both species and on males of *Ae. albopictus* (Table 2).

The overall female ratio of *Ae. aegypti* was 0.502, with no significant departure from 0.5 in any treatment (χ^2 test, $P > 0.1$), whereas in *Ae. albopictus,* the ratio was 0.469, which was significantly different from 0.5 (χ^2 test, $P = 0.03$). Furthermore, the sex ratio differed considerably among treatments. More females of *Ae. albopictus* emerged with low diets than with high diets (female ratio: 0.502 *vs.* 0.435, χ^2 test, $P < 0.01$) and under the mixed-species treatment than under the single-species treatment (female ratio: 0.525 *vs*. 0.419, χ^2 test, $P < 0.001$).

We calculated the per capita performance index, *I*, to integrate the effects of temperature and diet on population growth under a mixed-species treatment. This would further allow us to determine which species would be favored by the presence of the other species (Figure 4). The *I* values of the two species increased with temperature up to 30 °C; however, the *I* values declined at higher temperatures and were the lowest at 35 °C (Fig. 4). Diet also affected the *I* values of the two species. In *Ae. aegypti*, the *I* values were 61.2-93.4% higher with a high diet than with a low diet at the same temperature, while they were 48.3–56.7% higher with a high diet in *Ae. albopictus*. The *I* values of the two species were similar on a low diet (Fig. 4A). For *Ae. aegypti*, the *I* values were 35.1% higher at 30 °C (absolute sex ratio) than at 25 °C, whereas for *Ae*. *albopictus* the *I* values were only 4.4% higher (Fig. 4B). If we assume asymmetric diet

conditions, the species on a high diet would overwhelm that on a low diet at temperatures between 20 and 30 °C (Fig. 4C, D).

Discussion

We showed that temperature and diet combinations had variable effects on the population growth of the two species, and that interspecific competitive superiority can be condition dependent. Although *Ae*. *aegypti* performed better at temperatures higher than 25 °C on a high diet, the population growth in both species was quite similar. Therefore, there should be no difference in their population growth under the same breeding conditions. Nevertheless, 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 drastic variation in the larval survival rate at similar water temperatures, indicating large variation in terms of larval diet (Tun-Lin *et al*., 2000; Tuno *et al*., 2005). The variable temperatures and diet observed in the field may enhance species coexistence, since both factors markedly affect the population growth of the two species.

Brakes *et al*. (2003) reported that *Ae*. *aegypti* was most prevalent in highly urbanised areas, although the habitats used by the two species are remarkably similar in most suburban areas in southeastern Brazil and Florida, despite their hypothesis of habitat segregation. The paradoxical distribution of the two species cannot be explained by the three factors examined in our study: temperature, nutrition, and the presence of other species. The stability of breeding sites may be an important factor to consider (Alto & Juliano, 2001b). *Aedes aegypti* has been reported to be prevalent in highly urbanised areas. Highly urbanised areas may be interpreted as a highly disturbed unpredictable environment. *Aedes aegypti* has higher population growth than *Ae*. *albopictus* if conditions allow. Based on our observations, the former species always hatches

231 together, while the latter species hatches irregularly when the eggs were soaked in water. These
232 features, *i.e.,* immediate hatching and rapid population growth, may enable *Ae*. *aegypti* to
233 establish colonies in highly disturbed environments in urban areas, in addition to their closer
234 association to humans (Hawley, 1988). By the end of the 21st century, world temperature will
235 have increased by 1.1 to 6.4 °C (IPCC 2007). Based on our data we predict that, with an elevated
236 world temperature, the population growth of *Ae. aegypti* will increase in regions where the
237 average temperature is in the range of 20 to 30 °C. With global climate change, the distribution
238 of both species will likely expand when temperature is considered alone. However, climate
239 change also affects precipitation patterns (IPCC 2007) and interspecies competition (Ives and
240 Gilchrist, 1993), which may affect the distributions of both species. In addition, the combined
241 effect of temperature and diet makes it difficult to predict the expansion of these two species.
242 Counter to assumptions in previous studies, the sex ratio of the *Ae*. *albopictus* population was
243 not even. This result emphasises the need to evaluate the absolute sex ratio and to explore
244 whether environmental conditions affect aedine sex ratios for precise estimation of population
245 growth rates.
246 High nutrition reduced fitness at 35 °C, despite the fact that a high diet contributed to greater
247 fitness when the temperature was 20–30 °C. Across the four temperatures (20, 25, 30, and 35 °C),
248 mortality differed (was higher) only in the 35 °C treatment group in both species. Most deaths in
249 this study occurred at the fourth instar and pupal stages at 35 °C. Holometabolous insects, such
250 as mosquitoes, must attain a critical mass during larval development before pupation (Clements,
251 1992). They also require sufficient mass for emergence, and the potential attainable mass
252 decreases with increasing temperature (Chambers & Klowden, 1990). The effect of the high diet
253 on mortality was reversed at 35 °C in both species, and the developmental duration in *Ae.*

albopictus males was also reversed at this high temperature. Larval feeding duration affects ecdysteroid levels and nutritional reserves, which are crucial factors in the initiation of metamorphic moult in mosquito larvae (Telang *et al*., 2007). The high mortality rates among fourth instars and pupae on high diets at 35 °C may be explained by the early triggering of metamorphosis by elevated nutritional reserves. This early onset would not allow sufficient time for ecdysteroid accumulation to reach a level that would permit the completion of metamorphosis. Nevertheless, it is difficult to explain why the developmental duration was prolonged at 35 °C in *Ae. albopictus* males, although the cues triggering metamorphosis may be species specific (e.g., specific nutritional reserve or ecdysteroid levels).

We demonstrated that the competitive status of the two mosquito species is affected by temperature and nutrition. The performances of the two species are quite similar under the same conditions. We predict that considerable variation in their breeding sites enables coexistence of the two species. We also speculate that temperature and nutrition are not sufficient to explain the puzzling distribution of the two species. We need to consider other parameters, such as environmental stability, to better understand the location-specific dominance outcomes in the two species.

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Table 1. Summary of ANOVA on the mortality rate from hatching to emergence in single and mixed species treatment.

	Ae. aegypti			*Ae. albopictus*		
Source	df	F Ratio	P	df	F Ratio	P
Temperature	3	84.6	<0.0001	3	115.2	<0.0001
Diet	1	3.4	0.068	1	2.9	0.090
Mixed species	1	3.3	0.070	1	4.1	0.045
Temperature*Diet	3	12.1	0.001	3	8.3	0.005
Temperature*Mixes species	3	1.6	0.214	3	8.0	0.006
Diet*Mixed species	1	0.6	0.453	1	0.1	0.801
Temperature*Diet*Mixed species	3	0.9	0.345	3	0.2	0.676
C. total	119			119		

Table 2: Summary of MANOVA on the sex wise developing time and wing size in mixed species treatment.

		Ae. aegypti				*Ae. albopictus*			
Analysis	Source	F value	NumDF	DenDF	P	F value	NumDF	DenDF	P
Female									
	Temperature	540.9	2	71	<0.0001	353.9	2	67	<0.0001
	Diet	199.8	2	71	<0.0001	162.2	2	67	<0.0001
	Temperature*Diet	5.1	2	71	0.0087	9.0	2	67	0.0003
Male									
	Temperature	455.2	2	68	<0.0001	164.0	2	69	<0.0001
	Diet	131.2	2	68	<0.0001	242.5	2	69	<0.0001
	Temperature*Diet	0.1	2	68	0.8831	9.0	2	69	0.0003

Figure legends.

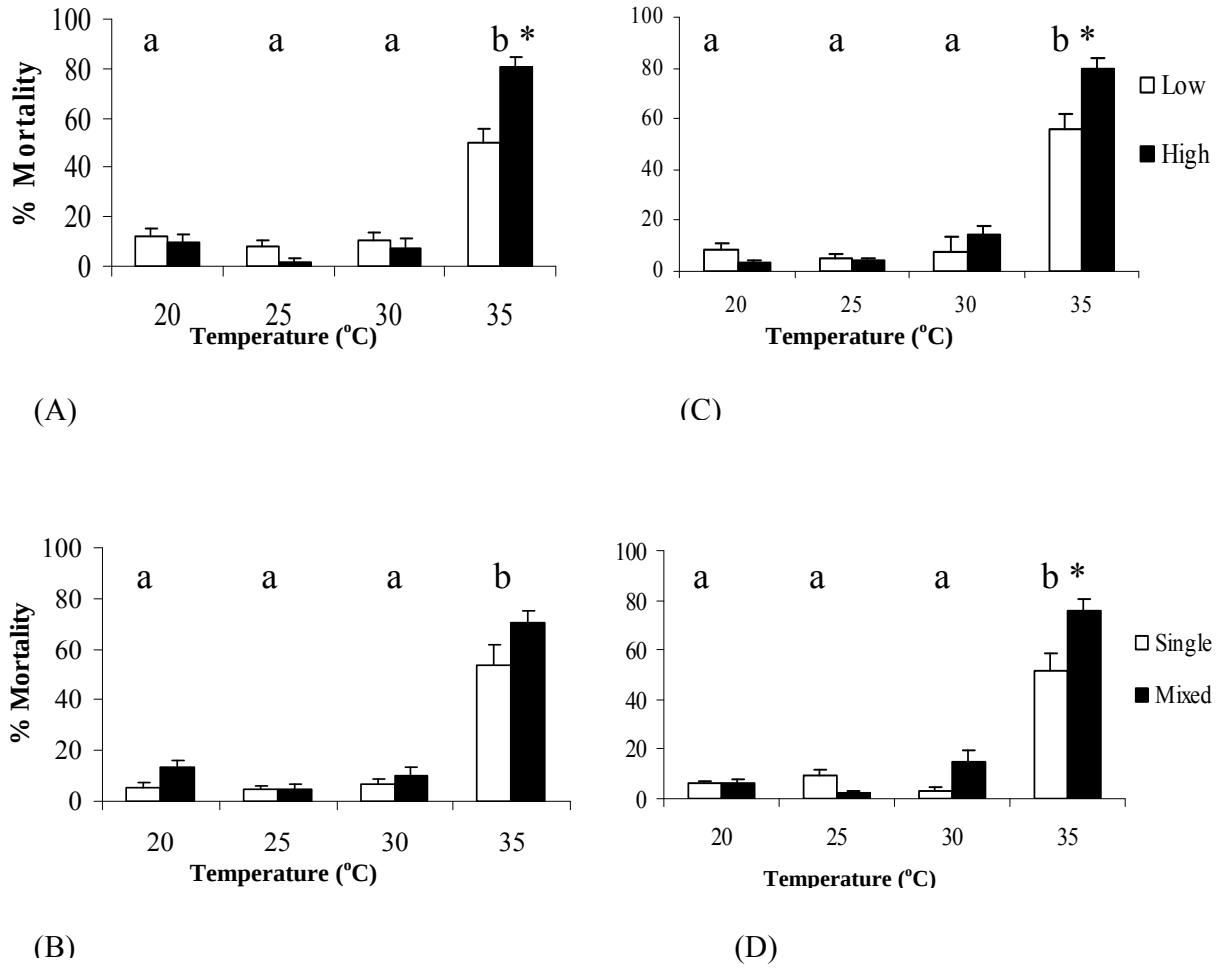
Fig. 1. Mortality rate (%) from second instars to emergence maintained at four temperatures: 20, 25, 30, and 35. (A) *Aedes aegypti* – Low *vs* high diet (B) *Ae. aegypti*-Single *vs* 2 species (C) *Ae. albopictus*- Low *vs* high diet (D) *Ae. albopictus* - Single *vs* 2 species. Different letters indicates significant differences between temperature treatments ($P < 0.05$; Tukey's HSD test). Significant differences between poor and rich nutritious conditions are indicated by asterisk ($P<0.05$, Paired *t*-test, Bonferroni corrected).

Fig. 2. Developing time from hatching to emergence (days) of male and female (A) *Ae. aegypti* and（B）*Ae. albopictus* in mixed species treatment.

Fig. 3. Wing length (mm) of *Ae. aegypti,* (A) female, (B) male, and *Ae. albopictus,* (C) female, (D) male at 20, 25, 30 and 35 °C.

Fig. 4. Estimated per capita performance index *I* of *Ae. albopictus* and *Ae. aegypti* with observed sex ratio and with assumed even sex ratio under combinations diet (low or high) and rearing temperatures(20, 25, 30 and 35 ℃) in mixed species treatment. (A) *Ae. aegypti*-Low diet & *Ae. albopictus*-Low diet (B) *Ae. aegypti* -High diet & *Ae. albopictus* –High diet (C) *Ae. aegypti*-High diet & *Ae. albopictus* –Low diet (D) *Ae. aegypti* -Low diet & *Ae. albopictus* -High diet. Abbreviations in the figure: *Ae. aegypti* (aeg), *Ae. albopictus* (albo), low diet (L), High diet (H), 1:1 sex ratio assumed (1:1).

468 **Fig. 1:**



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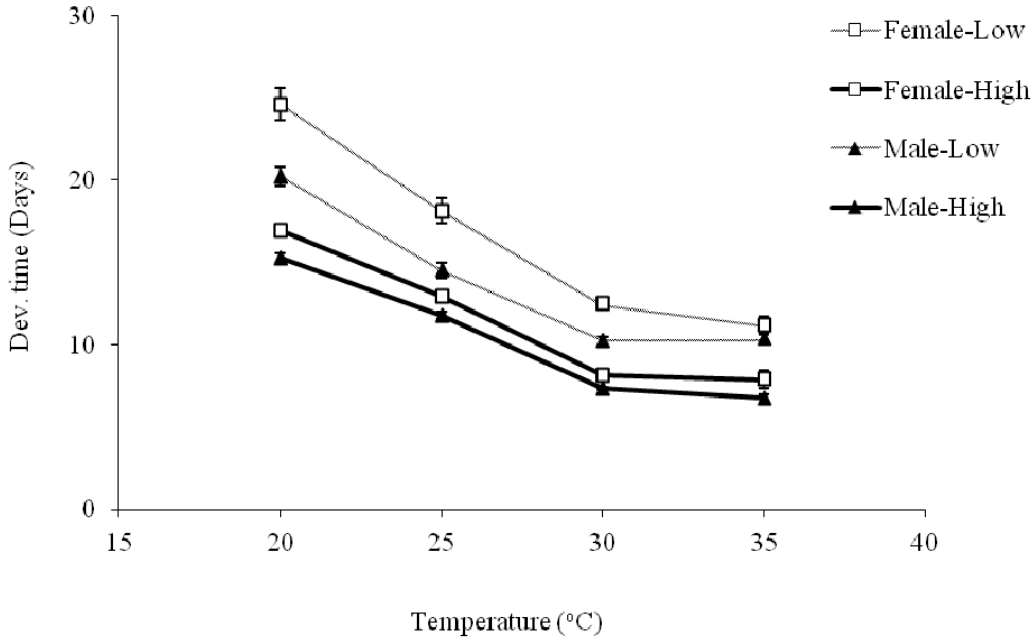
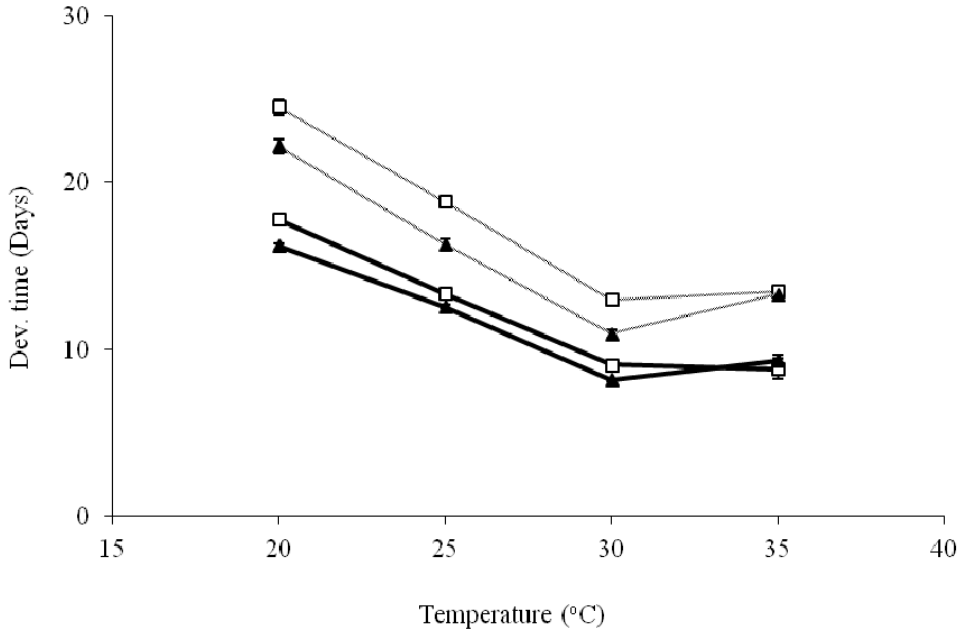
476 **Fig. 2.**



(A)



(B)

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Fig. 3.

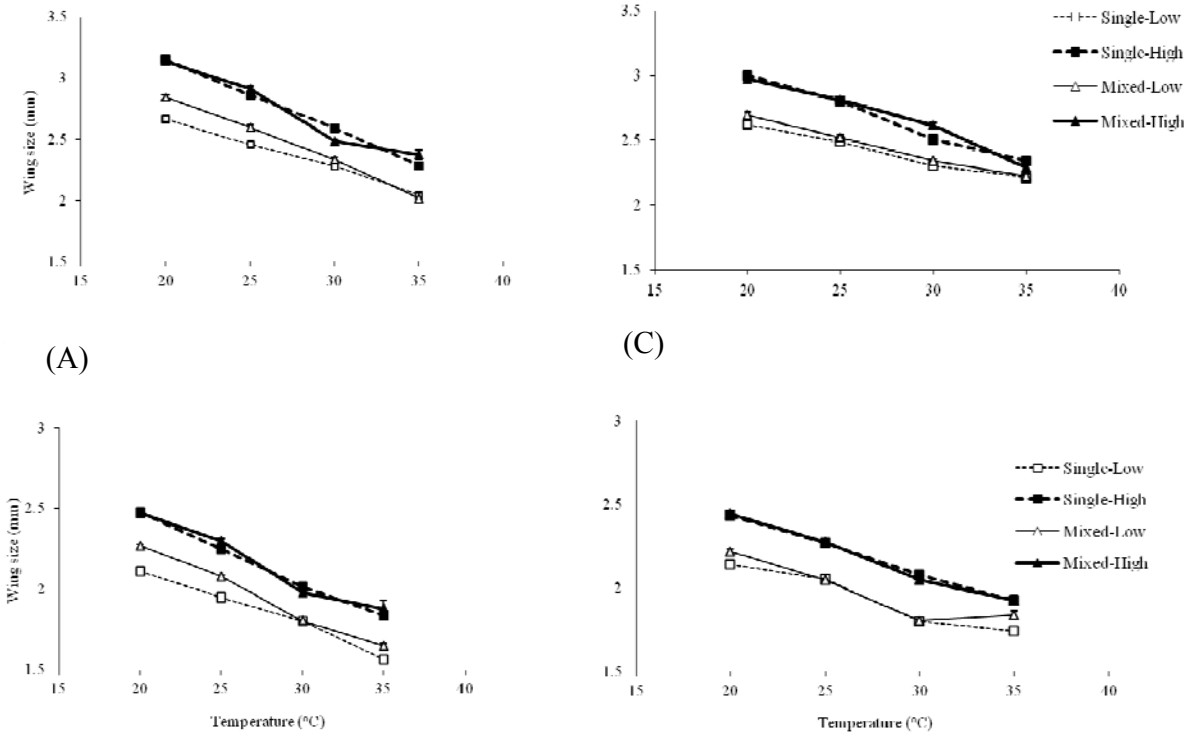
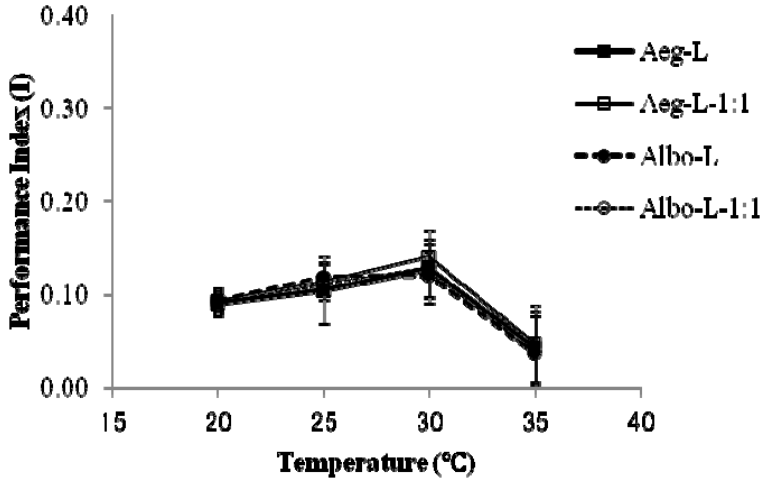
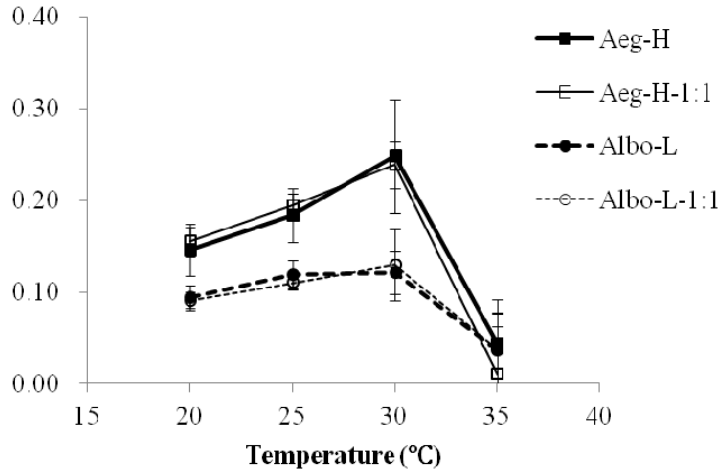

Wing size (mm)
3.5
3
2.5
2
1.5
15
20
25
30
35
40
Single-Low
Single-High
Mixed-Low
Mixed-High
(A)
(C)
Temperature (°C)
(B)
(D)

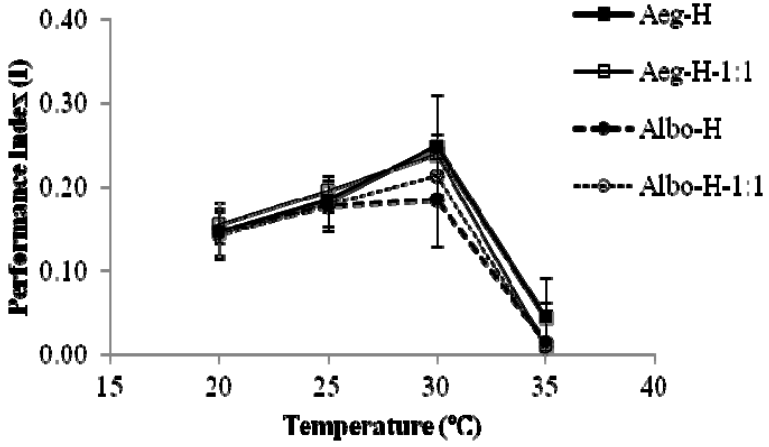
Fig. 4.


Performance Index (I)
0.40
0.30
0.20
0.10
0.00
15
20
25
30
35
40
Temperature (°C)
Aeg-L
Aeg-L-1:1
Albo-L
Albo-L-1:1

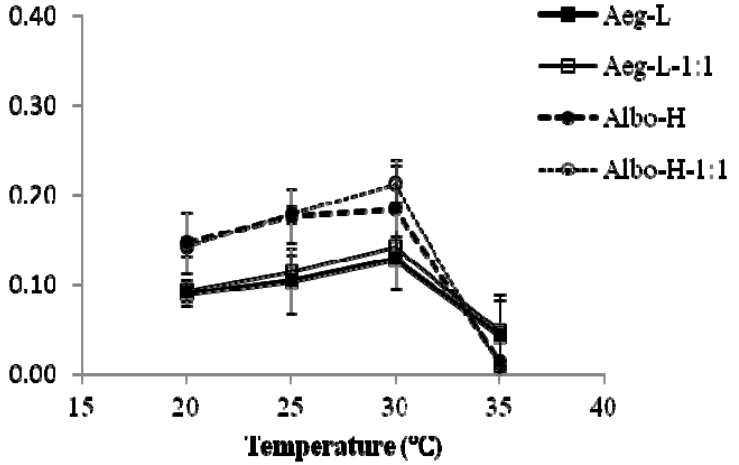
(A)


0.40
0.30
0.20
0.10
0.00
15
20
25
30
35
40
Temperature (°C)
Aeg-H
Aeg-H-1:1
Albo-L
Albo-L-1:1

(C)


Performance Index (I)
0.40
0.30
0.20
0.10
0.00
15
20
25
30
35
40
Temperature (°C)
Aeg-H
Aeg-H-1:1
Albo-H
Albo-H-1:1

(B)


0.40
0.30
0.20
0.10
0.00
15
20
25
30
35
40
Temperature (°C)
Aeg-L
Aeg-L-1:1
Albo-H
Albo-H-1:1

(D)

486