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Eclosion Rate, Development and Survivorship of *Aedes albopictus* (Skuse) (Diptera: Culicidae) under Different Water Temperatures

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Eclosão, Desenvolvimento e Sobrevivência de *Aedes albopictus* (Skuse) (Diptera: Culicidae) sob Diferentes Condições de Temperatura da Água

RESUMO - Em áreas tropicais, onde as populações de insetos vetores são particularmente abundantes, as temperaturas usualmente variam entre 25°C e 35°C. Considerando a importância dessa variação na determinação da dinâmica populacional de mosquitos, neste trabalho, desenvolvimento e as taxas de eclosão e sobrevivência dos estágios imaturos de *Aedes albopictus* (Skuse) foram comparados sob temperaturas constantes de 25, 30 e 35°C (em câmaras climatizadas) e ambiente (25°C a 29°C). A taxa de eclosão foi considerada como o total de larvas obtidas após 24h. O período de desenvolvimento, assim como a taxa de sobrevivência larval e pupal foram avaliados diariamente. A taxa de eclosão foi significativamente mais elevada sob temperatura ambiente comparada às constantes, sugerindo que a variação da temperatura pode ser um fator estimulante da eclosão. O tempo médio de eclosão aumentou com a temperatura, variando de 2,8h (25°C) a 5,2h (35°C). A duração do período larval apresentou grande variabilidade dentro de cada grupo, embora não tenha diferido significativamente entre os mesmos (11,0 ± 4,19 dias), tendo sido mais longo para indivíduos mantidos na água a 35°C (12,0 ± 4,95 dias) e temperatura ambiente (13,6 ± 5,98 dias). Ao contrário, a sobrevivência das larvas foi fortemente afetada na temperatura mais elevada, onde apenas um indivíduo alcançou o estágio adulto. Esses resultados sugerem que a população de *Ae. albopictus* de Recife pode estar em processo de adaptação ao aumento de temperatura e que o limite para o desenvolvimento de estágios larvais se encontra próximo a 35°C.

PALAVRAS-CHAVE: Biologia, vetor, ciclo de vida, limite térmico

ABSTRACT - In tropical areas, where vector insects populations are particularly numerous, temperature usually range between 25°C and 35°C. Considering the importance of such temperature variation in determining mosquitoes population dynamics, in this work the developmental, eclosion and survival rates of the immature stages of *Aedes albopictus* (Skuse) were compared under constant 25, 30 and 35°C (using acclimatized chambers) and environmental (25°C to 29°C) temperatures. The hatching rate was considered as total number of larvae recovered after 24h. The development period as well as larval and pupal survival rate were evaluated daily. Eclosion rate was significantly higher under environmental temperature than under the studied constant temperatures, suggesting that temperature variation may be an eclosion-stimulating factor. The mean eclosion time increased with the temperature, ranging from 2,8h (25°C) to 5,2h (35°C). The larval period was greatly variable inside each group, although it did not differ significantly amongst groups (11,0 ± 4,19 days), with individuals showing longer larval stages in water at 35°C (12,0 ± 4,95 days) and environmental temperature (13,6 ± 5,98 days). Oppositely, survival was strongly affected by the higher temperature, where only one individual lived through to adult phase. The results suggest that population of *Ae. albopictus* from Recife may be adapting to increasing of environmental temperatures and that the limiting temperature to larval development is around 35°C.

KEY WORDS: Biology, vector, life cicle, temperature threshold

Geographical expansion of vector-borne diseases in several continents has been partially associated to changes in global warming (Githeko *et al.* 2000). Most of such diseases are transmitted by mosquitoes, whose developmental cycle tends to be reduced by temperature increase (Gomes *et al.* 1995, Navarro *et al.* 2002, Löwenberg Neto & Navarro
and 3.5°C around 2100 (IPCC 1997) is a critical aspect in the occurrence pattern of medically significant diseases such as malaria and dengue (Epstein 2000, WHO 2004).

*Aedes albopictus* (Skuse) is an important vector for arboviruses such as dengue and Japanese encephalitis in Asia (Gratz 2004), and was associated with dirofilariosis in dogs in Italy (Cancrini et al. 2003). Even though this mosquito species is not considered a competent vector of such diseases in the American continent, under experimental conditions it is shown to be competent to transmit, at least, 22 arboviruses, including the four serotypes of the dengue virus, yellow fever and the West Nile virus (Gratz 2004). Furthermore, this insect has been found naturally infected with several arboviruses (Gratz 2004, CDC 2005), including DENV-1 serotype isolated from a larva in Brazil (Serufo et al. 1993).

Although *Ae. albopictus* is a South Asian mosquito, it is spread through Africa, Europe, South and North America. Currently, its presence is registered in regions with temperatures ranging from -4.8°C (Rai 1991, OPS 1995) up to 33°C. In Brazil, infestations with *Ae. albopictus* have been recorded from Rio Grande do Sul (Cardoso et al. 2005), where the mean temperature ranges from 15°C and 18°C (Atlas Socioeconômico do Rio Grande do Sul) up to Amazônia (Fé et al. 2003), with highest temperature around 33°C during the hottest months. So far, only six Brazilian states have not registered *Ae. albopictus* Amapá, Roraima, Acre, Tocantins, Piauí, and Sergipe (Martins et al. 2006). The ability to survive in such different environmental conditions is likely to be influenced by differences in the strain populations (Ayres et al. 2002). This hypothesis is reinforced by the results from experimental trials performed in the field and laboratory conditions where development of *Ae. albopictus* has been evaluated under different temperature regimes. While, Gomes et al. (1995) found no effect of cyclic temperature (18°C to 22°C) on *Ae. albopictus* development from the periurban area of Tremembé, São Paulo State, Brazil, Löwenberg Neto & Navarro (2004) suggest that the lower temperature of a cyclic regime (18/25°C) is a limiting factor in mosquito development in a strain from Registro, Sao Paulo State. These findings suggest that the characteristics of the local strain in response to climate variation need to be investigated.

Although mosquito populations are predominantly numerous in tropical areas with average temperature ranging from 25°C to 35°C, there are no data on how strains of *Ae. albopictus* face temperature variation on the Northeast Brazil. Therefore, in this study the effect of temperature on egg hatching, developmental rate, survivorship and sex rate were compared under constant (25, 30 and 35°C) and environmental temperatures (25°C to 29°C). Understanding how the mosquito responds to climate changes would be of great contribution to indicate the best time to apply control measures and to predict disease transmission.

**Material and Methods**

**Mosquito source.** A laboratory colony of *Ae. albopictus* established in October 2004 from field collected mosquitoes, was used as mosquito source. Eggs were collected using ovitraps placed at the campus of the Universidade Federal de Pernambuco – Recife (8° 04' 03" S and 34° 55' 00" W). The mosquito colony was maintained under 27 ± 2°C, 60 ± 10% R.H. and natural photoperiod. Females were fed on mouse blood and 10% sucrose solution, *ad libitum*. Experimental design was based on Calado and Navarro-Silva (2002).

**Hatching under different temperatures.** The hatching rate was studied using groups of 100 and 200 eggs aged between three and 15 days from egg-laying. The eggs were divided into four experimental groups, three of which were kept under constant temperature (acclimatized chambers at 25, 30 and 35 ± 1°C) the other staying at environmental temperature (25°C to 29°C). In all experiments photoperiod was maintained at 12/12h light/dark. These conditions were maintained throughout the experiments. The total hatching rate was considered as the larvae number in the recipient after 24h, with the hatching pattern recorded every 60 min in the first 8h to confirm the hatching rate in absence of external stimuli and low oxygen content in the water.

**Larvae development.** Following hatch at environmental temperature (2h maximum), first-instar larvae (L1) were separated into three lots of 15 individuals for each experimental temperature. The larvae groups developed in plastic containers (12 cm height and 7.5 cm diameter) with distilled water in a 10 ml/individual proportion. Water volume was completed whenever needed in response to evaporation. Observations took place at each 12h, and the development was monitored until pupation. After each moult, identified by larval size and exuviae present in the water, specimens were transferred to a new container. Feeding was made on triturated Whiskas® cat food observing the proportion of 1.0 mg/larva at first and second instar (L2) and 0.25 mg/larva at third (L3) and fourth instar (L4). Food was weighted and distributed in the containers on daily basis. Food amount and distribution frequency were reduced depending on the amount of food still available in the containers. Larval and pupal carcases were counted and discharged.

Due to high mortality rate of late post-embryonary instar under 35°C, additional experiments were held and observations were performed at each 24h. For each experimental condition, 90 recently hatched L1 were randomly distributed into six containers filled with 150 ml of distilled water so that each container showed the same larvae number. Experimental groups were treated accordingly to the previously described pattern. Three replicates were made on a 7-day interval.

**Pupal development period and adult sex ratio.** The effect of temperature on the life-span and pupae survival was studied from individuals that developed under each experimental condition. Initially, groups of 300 L1 were kept at 25, 30, 35°C and environmental temperature until pupation. Growing conditions were similar to the previously described. Pupae obtained from each of these groups were divided as following: 25°C (three replicates with three replicates of 15 pupae each); 30°C (six replicates with three replicates of 15-16 pupae each); 35°C (one container with...
16 pupae); environmental temperature (five replicates with three replicates of 15-16 pupae each). After emergence, sex ratio was assessed. The mortality rate was estimated as the difference between the initial and final number of individuals at each developmental stage.

Statistical analysis. The average percentages for eclosion and surviving rates were transformed into arc sine and compared with chi-square (χ²) and Tukey tests. The average hatching and development times and the survivorship were analyzed using ANOVA parametric statistics and Tukey test (Zar 1984).

Results

Eclosion in response to temperature variation. The average percentages of larvae obtained from eggs maintained in the different temperatures are shown in Table 1. In the 24-hours period, the hatching rate was reduced with temperature elevation and varied from 28.3% (35°C) to 50.7% (25°C) at constant temperature experiments. The highest hatching rate was recorded at environmental temperature (72%). Although the highest average rate had been two and a half times greater than the lower one, there was no significant difference among hatching rates due to the great inner variation in each treatment.

In the studied temperatures, more than 80% of the total larvae recorded in the first 24 hours hatched within the first 8 hours (Table 1). At this time, the larvae proportion found in the lower temperature (25°C; 47.3%) was twice that found in the higher temperature (35°C; 23.7%). At the same moment, 39.6% of the larvae have hatched at 30°C. The average hatching time increased with temperature raising, varying between 2.8 (25°C) and 5.2 hours (35°C) (Fig. 1). At environmental temperature the hatching time was similar to that at 35°C (5.0 ± 3.0h), corresponding to twice the hatching time at 25°C. Independent of temperature and total hatching, most larvae hatched within the first hour from the beginning of the experiment, although a great variability was recorded among the different groups (Table 1). The hatching percentage at 25°C was 35.6%, followed by 30°C and 35°C, respectively 12.7% (± 8.51) and 6.9%. From the eggs kept at environmental temperature developed 36% of total larvae in the first hour of immersion, similarly to the lower temperature group.

Development time at different water temperature. The total larval development time did not significantly differ among the studied groups, averaging 13.6, 9.3, 8.8 and 12 days for environmental, 25, 30 and 35°C temperatures respectively. However, there was great variation on development stage within each stage and on the number of individuals that reached pupal stage, especially at 35°C (Table 2). The lasting of L1 instar was significantly greater for individuals kept under varying temperature in comparison to those under constant temperature. Significantly differences in L1 instar were also recorded in larvae reared at 25 °C and 30°C in comparison to those reared at 35°C (P < 0.001). The shorter L1 instar (1.7 ± 0.50 days) was recorded at 30°C (Table 2), where 60% of the larvae had already made the transition to the second stage 1.5 day after the hatching.

The second instar showed the shortest span in all tested treatments, while the fourth instar was the longest. L2 development time showed significant differences among all treatments according to Tukey test (F_{1,141} =16.596, P < 0.0001). The shortest time was recorded for 30°C, where an average 20.9 ± 9.51 h were necessary to reach L3, while the longest time was recorded for 35°C (64.3 ± 42 h) (Table 2). In the first 12h; the greater proportion of larvae (42%) reached L3 instar at 30°C (42%), being five to six times higher than the number of those larvae kept at 25°C (9%) and 35°C (7%).

A significant delay on the average span of the third instar was recorded with the raising of temperature (F_{3,120} =23.107, P < 0.0001), with the longest span occurring at 35°C (4.5 ± 2.20 days) and the shorter one at 25°C (2.0 ± 0.2 days). Larvae kept at environmental conditions with variable water temperature developed to L3 in 1.7 ± 1.0 days (Table 2). At constant 25 °C and 30°C temperatures, the larvae

Table 1. Mean percent (± S.E.) eclosion of *Ae. albopictus* larvae under constant (25, 30, 35°C) and environmental (25-29°C) temperature during 24h. (replicas = 6)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
<th>7h</th>
<th>8h</th>
<th>24h season 8h</th>
<th>Sum 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>14.56</td>
<td>2.24</td>
<td>0.30</td>
<td>0.63</td>
<td>0.82</td>
<td>0.82</td>
<td>0.00</td>
<td>0.26</td>
<td>1.80</td>
<td>14.64</td>
</tr>
<tr>
<td>S.E.</td>
<td>1.59</td>
<td>1.59</td>
<td>0.91</td>
<td>4.21</td>
<td>2.87</td>
<td>5.54</td>
<td>1.33</td>
<td>0.24</td>
<td>1.58</td>
<td>11.67</td>
</tr>
<tr>
<td>30°C</td>
<td>12.7</td>
<td>3.2</td>
<td>1.9</td>
<td>6.1</td>
<td>4.2</td>
<td>8.8</td>
<td>2.4</td>
<td>0.3</td>
<td>2.2</td>
<td>39.6</td>
</tr>
<tr>
<td>S.E.</td>
<td>8.51</td>
<td>1.59</td>
<td>0.91</td>
<td>4.21</td>
<td>2.87</td>
<td>5.54</td>
<td>1.33</td>
<td>0.24</td>
<td>1.58</td>
<td>11.67</td>
</tr>
<tr>
<td>35°C</td>
<td>6.9</td>
<td>5.4</td>
<td>1.2</td>
<td>4.6</td>
<td>2.6</td>
<td>1.1</td>
<td>0.4</td>
<td>1.4</td>
<td>4.6</td>
<td>23.7</td>
</tr>
<tr>
<td>S.E.</td>
<td>2.58</td>
<td>2.47</td>
<td>0.54</td>
<td>1.63</td>
<td>1.95</td>
<td>0.67</td>
<td>0.31</td>
<td>1.38</td>
<td>1.64</td>
<td>7.59</td>
</tr>
<tr>
<td>Environment</td>
<td>36.0</td>
<td>8.2</td>
<td>2.0</td>
<td>9.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.4</td>
<td>1.5</td>
<td>12.9</td>
<td>59.1</td>
</tr>
<tr>
<td>S.E.</td>
<td>11.41</td>
<td>2.01</td>
<td>0.65</td>
<td>6.90</td>
<td>0.51</td>
<td>0.58</td>
<td>0.24</td>
<td>0.76</td>
<td>3.31</td>
<td>10.76</td>
</tr>
</tbody>
</table>
development pattern was similar in the fourth stage, with 4.0 ± 1.0 days average span, and showed a significantly shorter L4 duration than that of the ones kept at environmental temperature, in which it lasted around seven days (F(2, 108) = 3.230, P < 0.0001). Pupae kept at 30ºC reached the adult stage faster (1.7 ± 0.20 day) than those reared at both 25ºC (2.5 ± 0.30 days) and environmental temperature (2.3 ± 0.30 days). Only one individual reached the adult stage at 35°C (Table 3).

Larval survival in response to temperature variation. The effect of temperature on individual survival was significant only at 35°C, where was recorded high mortality rates at all development stages, especially at the last larval instar. Intense microbial contamination was observed in larval development tests at 35°C. Thus, additional experiments were developed with reduced handling and 24h-interval observations. This adjustment reduced microbial presence resulting in a mortality rate between 18% and 70% (five replicas). At 25°C, 30°C and environmental temperature, the survival period varied between 86.7% and 91.2%. Each larval stage survival was different among treatments (Table 3). At 25°C and 30°C the higher mortality rate occurred in L1, and at 35°C it was in L4.

Temperature effect on adult sex rate. A total of 651 individuals were used to calculate sex ratio at the studied temperatures. Sexual proportion was similar in all temperatures. From 129 individuals at 25°C, 50.4% were males while 49.6% were females. At 30°C, 52.6% of pupae emerged males while 47.4% emerged females. At environmental temperature 47.4% males and 52.6% females were recorded.

Discussion

Several studies have shown the role of temperature on mosquito population dynamics (Alto & Juliano 2001, Glasser & Gomes 2002, Miciele & Campos 2003). In this study, under constant temperature, the local Ae. albopictus strain showed to be sensitive to warmness in the lower range (5°C) by reducing larval hatching and changing developmental pattern. Although adult production was similar at 25°C and 30°C, none was obtained from larvae reared at 35°C.

The higher eclosion rate recorded at environmental temperature (25-29°C) indicates that temperature variation may stimulate eclosion, like others reported in the literature, such as reducing water oxygen content (Judson 1963) and photoperiod (Nayar et al. 1973). Moreover, the asynchronous eclosion shown through the high standard deviation, suggests a great variety of individual responses. Response asynchrony can also be noticed during development period, when larvae of same age showed different development periods length. With the only exception of the group kept at 35°C, 40-50% individuals hatched within the first 24h thus showing that, under these conditions, half of the larvae hatch within the first day of immersion. This is of great importance when investigating Ae. albopictus success in exploring temporary habitats in which water availability is reduced by evaporation, e.g. synthetic containers abandoned in peridomestic areas. Disposable equipment deposits have been more frequently used as settling areas for this species than for Aedes aegypti (L.) (Chiavaralloti-Neto et al. 2002), which may favor its urbanization process.

The present results demonstrated no significant variation in the larval development length both in the controlled and environmental temperatures, with a mean period of eleven days from L1 to pupal stage. Analyzing the effect of the temperature on Ae. albopictus urban strain from São Paulo, a milder climate region (Southeastern Brazil), at controlled temperatures (15, 20, 25 and 30°C), Calado & Navarro-Silva (2002) recorded significant reduction on development duration of immatures related to increasing temperature. The

Table 2. Mean (± S.D) development time (days) of immature stages of Ae. albopictus under constant (25, 30, 35°C) and environmental (25-29°C) temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>2.0 ± 0.35</td>
<td>1.4 ± 1.00</td>
<td>2.1 ± 0.71</td>
<td>3.9 ± 0.72</td>
<td>9.3 ± 2.78</td>
</tr>
<tr>
<td>30°C</td>
<td>1.7 ± 0.46</td>
<td>0.9 ± 0.39</td>
<td>2.2 ± 0.79</td>
<td>4.1 ± 1.41</td>
<td>8.9 ± 3.05</td>
</tr>
<tr>
<td>35°C</td>
<td>2.3 ± 0.95</td>
<td>2.7 ± 1.75</td>
<td>4.5 ± 2.25</td>
<td>2.5 ± 0.00</td>
<td>12.0 ± 4.95</td>
</tr>
<tr>
<td>Environment</td>
<td>3.3 ± 1.25</td>
<td>1.6 ± 0.97</td>
<td>1.7 ± 1.02</td>
<td>6.9 ± 2.74</td>
<td>13.6 ± 5.98</td>
</tr>
</tbody>
</table>
shorter spans occurred at 25°C and 30°C and lasted for 7.7 and 5.9 days, respectively. So, it is possible that the Recife strain is more adapted to higher temperatures since the larval development did not present significant variation.

Based in the evidence that the best developing conditions occurred at 25°C and 30°C and that no individuals reached adult stage when raised at 35°C, we can infer that the limit temperature for *Ae. albopictus* larval stages development is close to 35°C. Opposed to that, *Ae. aegypti* survival at this temperature was 67% (Tun-Lin et al. 2000) suggesting that this species is more vulnerable to higher temperatures than the former. In the American continent, *Ae. albopictus* is an exotic species that spread long after *Ae. aegypti* (Consoli & Lourenço-de-Oliveira 1994) and can still be in the midst of its adapting process. Similar results were found by Bayoh & Lindsay (2004) for *Anopheles gambiae* (Giles), when they recorded high mortality rate at temperatures above 32°C and unviable larvae between 38°C and 40°C.

The high mortality rate found at 35°C, especially for pupae, may be related to cell damage by heat (Denlinger & Yocum 1998). Heat stress may cause abnormalities at the cellular level due to alterations in pH and ion concentration, and exerts a profound negative effect on the structure and function of macromolecules, resulting in the death of the individual. In addition, the accumulation of energetic supply and body mass are known to be necessary to molting. Thus, the death of individuals is enhanced by the reduced food supply due to the shortened development period at higher temperatures (Clements 1992). Although the development length at 35°C was similar to that at 25°C and 30°C, it is possible that increasing water temperature created unfavorable conditions for immature specimens by reducing the ability to find food. Moreover, high temperatures may raise metabolic and breathing rates to critical levels, reducing the ability to molt. Thus, the death of individuals is enhanced by the reduced food supply due to the shortened development period at higher temperatures (Clements 1992). Although the development length at 35°C was similar to that at 25°C and 30°C, it is possible that increasing water temperature created unfavorable conditions for immature specimens by reducing the ability to find food. Moreover, high temperatures may raise metabolic and breathing rates to critical levels, reducing the ability to molt. Thus, the death of individuals is enhanced by the reduced food supply due to the shortened development period at higher temperatures (Clements 1992).

### Table 3. Mortality rate (%) of *Ae. albopictus* during the immature development under different temperature conditions.

<table>
<thead>
<tr>
<th>Stages</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>6.5</td>
<td>5.9</td>
<td>12.8</td>
<td>8.8</td>
</tr>
<tr>
<td>L2</td>
<td>4.2</td>
<td>5.3</td>
<td>6.3</td>
<td>4.6</td>
</tr>
<tr>
<td>L3</td>
<td>2.9</td>
<td>3.2</td>
<td>7.7</td>
<td>2.2</td>
</tr>
<tr>
<td>L4</td>
<td>4.7</td>
<td>3.1</td>
<td>18.9</td>
<td>12.0</td>
</tr>
<tr>
<td>Pupa</td>
<td>0</td>
<td>0</td>
<td>99.7</td>
<td>0</td>
</tr>
</tbody>
</table>

The temperature is the most influential parameter on *Ae. albopictus* population dynamics (Alto & Juliano 2001), acting with other environmental factors as humidity and precipitation (Focks et al. 1994). Perspective is that by the end of the 21st Century world temperature will have increased 1°C to 3.5°C (IPCC 1997). In Northeastern Brazil the past few years have evidenced a tendency of elevated mean temperatures, frequently peaking 35°C in summertime. Our date suggest that *Ae. albopictus* from Recife is not adapted to such temperature yet, therefore high population increase would not be expected under temperatures about 35°C. Nevertheless, it is suitable to consider the hypothesis of gradual adaptation of the species to higher temperatures through a selective pressure on individuals.

### References


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