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Chapter

Post-Embryonic Development of *Aedes (Stegomyia) aegypti* Linnaeus, 1762 at Different Temperatures and CO₂ Concentrations, and Their Influences on Hatching and Development of Stabilized Population

Ediane Oliveira do Amaral, André Felipe da Silva, William Ribeiro da Silva, Karina Rossi da Silva, Rosemary Aparecida Roque, Wanderli Pedro Tadei and João Antonio Cyrino Zequi

Abstract

This research aimed to verify biological parameters of *Aedes aegypti* Linnaeus, from Londrina, Paraná, in an incubator chamber (BOD) with different temperatures, and to analyze biological aspects of this mosquito from Manaus, Amazonas, in environments simulating the climatic conditions provided by the IPCC. In Londrina, the eggs were incubated for 10 days in BOD at different temperatures. The viability of eggs, number of adults, and mortality rate were analyzed later. In Manaus, the biological cycle time, number of adults, and mortality rate were analyzed in environmental rooms with different temperatures and CO₂ concentrations. The viability of eggs and the number of adults from Londrina was greater at 5 and 25°C, while the mortality rate of immatures was greater at 0°C; eggs incubated at 45°C did not hatch. Mosquitoes from Manaus completed the fastest biological cycle in room 4. The mortality percentage in the different instars for rooms 1, 2 and 4 was: 14.4; 28 and 53.6%, respectively. Thus, temperatures from 5 to 29.74°C were more appropriate since values outside these limits can cause deleterious effects on the species during its development, but the *A. aegypti* mosquitoes from Londrina and Manaus can benefit from the increase in temperature stipulated by the IPCC.

Keywords: arbovirus, mosquitoes, control, climate, quiescence

1. Introduction

Dengue is one of the main arboviruses in the world, exposing more than half of the population to the risk of contracting the disease [1–3]. In addition, the severe dengue is one of the main causes of child deaths in countries in Latin America and Asia [3]. The circulation of the dengue virus is ruled as a major problem due to the genetic plasticity of the mosquito under environmental conditions, and due to the selection of resistant insects because of chemical control, coupled with the fact that the mosquito is anthropophilic and domiciled. The occurrence of vertical transmission in epidemic times also helps in the dynamics of maintaining the virus in the urban environment [4]. Murillo et al. [5] reported that increased vertical transmission in times of epidemic makes outbreaks more difficult and expensive to control.

Aedes (Stegomyia) aegypti (Diptera: Culicidae) is the transmitter of dengue, urban yellow fever, chikungunya and Zika viruses in Brazil [6, 7]. The spread of chikungunya and Zika viruses has caused public health problems because the former causes severe recurrent joint pain and the latter may involve neurological complications such as microcephaly in children, being very severe, in addition to Guillain-Barré Syndrome [8–11].

Females of this mosquito species practice hematophagy and prefer to breed in artificial breeding sites, where competition with other species is practically absent [12–15]. The proliferation of this mosquito has been intensifying with increasing urban perimeter, lack of basic sanitation, deforestation and lack of proper treatment of solid waste, as these are factors that increase breeding sites [16, 17]. *A. aegypti* eggs can be dehydrated for more than a year and are viable in late-hatching post-embryonic development, causing an imminent danger in the proliferation of this mosquito [18, 19].

Mosquito development, especially those with high adaptive genetic plasticity, may be affected by various abiotic factors, with temperature being the most relevant [20]. According to the Intergovernmental Panel on Climate Change [21], the Earth will experience an average temperature increase of approximately 2°C, and by 2099 this could rise to 6.5°C, a situation that may favor the cycle of these Culicidae, as well as increased viral circulation. These data are confirmed by analyzing the statistics from 1955 to 2007, a time when temperature increases have been added to one of the world's largest viral propagations, with a 30-fold increase in the last 50 years, and over 2 million of reported cases annually on the American continent each year [3].

In the current perspective, there is a need to intensify the monitoring and control of mosquitoes, as well as the monitoring of early viral circulation, since almost half of the world's population is exposed to the risk of dengue contamination with an estimated 50–100 million cases annually in more than 100 endemic countries [3]. This situation can worsen with global warming, which has been increasing each year, due to anthropic factors [17]. In this sense, understanding the life cycle of *A. aegypti* in stabilized insectaries kept in environmental rooms simulating the IPCC climate predictions, is a strategy that can help to optimize the alternatives for monitoring and controlling of this insect, given the possibility of the emergence of this scenario.

In this context, the present work aimed to: (i) verify the influence of different temperatures on egg viability, number of adults and mortality rate of *A. aegypti* from Londrina, Paraná, under laboratory conditions and, (ii) check the time of the biological cycle, number of adults and mortality rate of *A. aegypti* from Manaus, Amazonas, kept in environmental rooms that simulate the temperatures and concentrations of carbon dioxide predicted by the IPCC.

2. Methods

2.1 Study area

The study was carried out in incubator chambers (BOD) with different temperatures, kept at the Medical Entomology Laboratory of Londrina State University (UEL), Londrina, Paraná, and in insects kept at the Laboratory of Ecophysiology and Molecular Evolution (LEEM), located at *Campus I* of the National Institute of Amazonian Research (INPA), Manaus, Amazonas.

2.2 Collection of *A. aegypti* eggs from Londrina, Paraná, under field conditions

The sample of *A. aegypti* population from Londrina, Paraná, used in this study, was obtained from eggs collected in the field, with the aid of traps called ovitraps [22]. The main attraction in the traps was a solution containing grass infusion (10%) [23], with a total volume of 300 ml. Twenty traps were set up and distributed at the UEL campus, at ground level, protected from sun and rain, and in places with little movement of people and animals. Samples were taken for 2 weeks and every 7 days, the reeds were replaced and sent to the insectarium with controlled temperature, humidity and photoperiod conditions ($27 \pm 2^\circ\text{C}$, 80–90% and 12L/12E).

2.3 Maintenance of immature and adult of *A. aegypti* from Londrina, Paraná, under laboratory conditions

The reeds containing eggs were immersed in plastic trays ($45 \times 30 \times 7.5$ cm) containing distilled water to stimulate the larvae to hatch. The immatures obtained were kept until adults by means of food containing a mixture of cat food (Whiskas®) and rodents (Teklab global®) in a 1:1 ratio, ground into fine particles (1 mm). All trays were covered with a nylon fabric to prevent the escape of mosquitoes. After emergence, the males and females were collected with a Castro catcher and the species identification was performed using external morphological characters, mainly from the chest, with the aid of a stereoscopic microscope ZEISS Stemi 2000 50× and identification keys proposed by Forattini [14], Harbach [24], and WRBU [25].

After the identification stage, the adults were placed to copulate in cardboard cages (17×20 cm), containing two plastic cups lined with strips of filter paper and filled with 70 mL of distilled water, which were used as a substrate for oviposition. As a source of carbohydrates, an Erlenmeyer was introduced containing a roll of gauze with pieces of cotton in the center, soaked with 12% sugar water. The blood meal was carried out using an anesthetized hamster for 30min, according to the procedure approved by the Ethics Committee on the Use of Animals at UEL (“Breeding of mosquitoes in laboratory conditions”).

2.4 Biological aspects of *A. aegypti* from Londrina, Paraná, in incubator chambers (BOD) with different temperatures

At UEL, the experiment was carried out in four incubator chambers (BOD) that had different temperatures (0, 5, 25 and 45°C) with $\pm 2^\circ\text{C}$, as well as at ambient temperature, where the eggs remained in dry incubation in 500 ml capacity pots containing only moistened cotton over a period of 10 days, with a 12/12 h photoperiod.

Subsequently, 150 ml of distilled water, 25 eggs of *A. aegypti*, F1 field generation and 0.055 g of larval food were introduced into each of the five pots, which were placed in BOD with temperature of $25 \pm 2^\circ\text{C}$. The monitoring of the hatching rate of eggs, the rate of immature deaths and the number of adults were carried out daily.

2.5 Stabilization of a sample of the population of *A. aegypti* from Manaus, Amazonas, in environmental rooms with temperature and CO₂ conditions according to the provisions of the IPCC

The same process of creating and maintaining the immature and adults of *A. aegypti* from Londrina, Paraná, mentioned in items 2.2 and 2.3, was carried out with a sample of the population of *A. aegypti* from Manaus, Amazonas, collected in the same way on *Campus I* of INPA. However, the blood meal carried out followed the procedure approved by the Ethics Committee on the Use of Animals at INPA (CEUA/INPA: 04/2013-“Breeding of mosquito vectors, under laboratory conditions”).

After the stabilization of the three insectaries in the LEEM, the F28 generation was obtained in the different environmental rooms that started the experiment for post-embryonic development, using three different environments. Rooms 1, 2 and 4 suffer from interference from different temperatures and levels of carbon dioxide, and rooms 2 and 4 refer to 50 and 100 years in front of room 1, which presents ambient temperature.

These rooms are equipped with technological devices that guarantee, respectively, the maintenance of ambient temperature, 2.5°C (400 ppm CO₂) and 4.5°C (850 ppm CO₂) above ambient temperature. The natural conditions of the respective environmental rooms are collected in real time by sensors isolated in the forest and the humidity of all remains constant in relation to the environmental conditions.

2.6 Biological aspects of *A. aegypti* from Manaus, Amazonas, in insectaries kept in environmental rooms that present simulated conditions of climate forecast by the IPCC

After stabilization of insectaries in environmental rooms 1 (ambient temperature), 2 (mild temperature) and 4 (extreme temperature), 125 eggs from each room were counted with the aid of a stereoscopic microscope. These eggs were separated into 5 groups (replicates) of 25 eggs, kept in pots with capacity of 750 mL, containing 300 mL of distilled water and 0.055 g of larval food added every 3 days.

The hatching rate was monitored daily for 19 days under these conditions. The average time of the biological cycle, number of adults (males and females) and larval mortality rate were verified. Exuvia and dead immatures were removed from the pots and the volume of distilled water was replaced.

2.7 Statistical analysis

Data on the hatching rate of eggs, number of adults (males and females) and mortality rate of immature *A. aegypti* from Londrina, Paraná, were expressed as a percentage. The number of emerged adults was subjected to a Kruskal-Wallis test, at the level of 5% significance, in order to verify differences between the data in the temperatures analyzed, using the BioEstat[®] 5.3 program for Windows [26].

The results on the number of adults (males and females) and the larval mortality rate of *A. aegypti* from Manaus, Amazonas, were also analyzed by means of percentage. On the other hand, the data regarding the average time of the biological cycle of mosquitoes kept in the different environmental rooms, were analyzed using averages, standard deviation and standard error. At first, these data underwent a Lilliefors normality test (K samples) to find out whether they have a normal distribution or not, and subsequently subjected to Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test ($p \leq 0.05$), with the aid of the statistical program SPSS[®] 14.0 package for Windows[®] (SPSS Inc. 2005 Headquarters, Chicago, Illinois, USA).

3. Results

3.1 Viability of eggs and total adults of *A. aegypti* from Londrina, Paraná, in incubator chambers (BOD) with different temperatures

The highest hatching rates of eggs were obtained at temperatures of 5 and 25 ± 2°C, respectively, where 48% for both was verified, followed by ambient temperature with 36% and at 0 ± 2°C presenting 7%. On the other hand, there was no hatching of eggs at 45 ± 2°C (Figure 1).

The adults obtained in the proportion of males and females are shown in Table 1.

At 0 ± 2°C, seven of nine immatures reached adulthood, which indicates a mortality of 22.2% of the larvae at this temperature. Considering the 60 immatures obtained at a temperature of 5 ± 2°C, 56 reached the adult stage indicating a larval mortality of 6.6%, while at a temperature of 25 ± 2°C only 50 immatures among 60 reached the adult stage, indicating a larval mortality of 16.6%. At ambient temperature, it was observed that of 45 immatures obtained, 44 reached adulthood, representing a larval mortality of only 2.2%. On the other hand, there was no adult emergency at 45 ± 2°C.

There is no statistical difference between hatching rates at 0, 5, 25 and ambient temperatures (Table 1), while at 0°C there is difference in relation to the hatching rate at other temperatures.

Of the total 625 eggs incubated at different temperatures, 157 eggs reached adulthood, and of these, 98 were males and 59 females, making up a male:female ratio of 1.66:1. The proportion of emerged adults was 5.6% at 0°C, 44.8% at 5°C, 40% at 25°C and 35.2% at room temperature (Table 1).

3.2 Average biological cycle time and total adults of *A. aegypti* from Manaus, Amazonas, in environmental rooms that simulate the climatic conditions provided by the IPCC

The averages of climatic variations during the experiment in °C, increase in ppm of CO₂ and percentage (%) of humidity for the different environmental rooms are represented in Table 2.

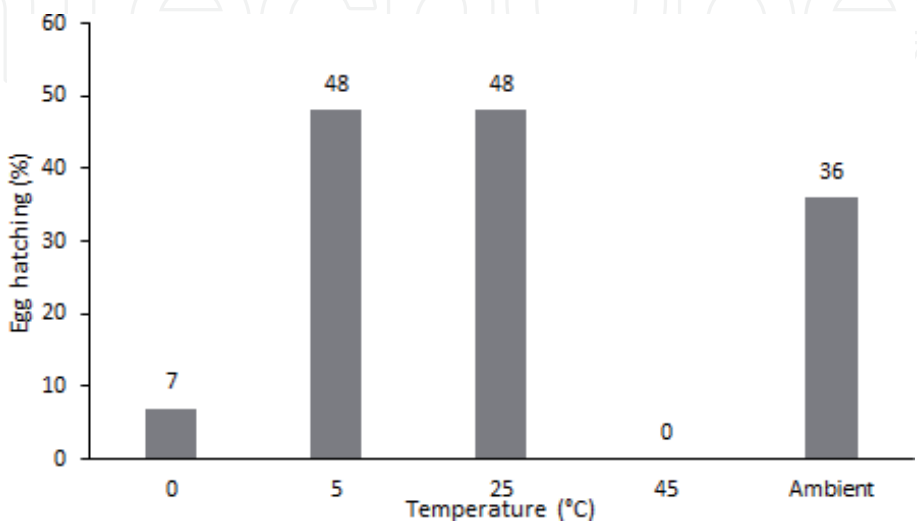


Figure 1. Egg hatch percentage of *A. aegypti* from Londrina, Paraná, in incubator chambers (BOD) with different temperatures with ±2°C limits and ambient temperature (16.7–24.1°C), observed for 10 days.

Adult emergence rate	Temperature ($\pm 2^{\circ}\text{C}$)									
	0°C		5°C		25°C		45°C		Ambient (16.7–24.1°C)	
Replicas	M	F	M	F	M	F	M	F	M	F
1	4	1	8	7	10	4	0	0	6	5
2	0	1	5	4	8	6	0	0	0	0
3	0	0	8	4	5	2	0	0	0	0
4	0	1	9	7	12	3	0	0	10	6
5	0	0	4	0	0	0	0	0	9	8
Total	4	3	34	22	35	15	0	0	25	19
Total M-F*	7a*		56a		50a		0b		44a	
Total %	5.60%		44.80%		40%		0%		35.20%	

*M-F: M = male and F = female.
*Equal letters on the same line do not differ from each other by the Kruskal-Wallis $p = 0.0261$, $H = 11.0428$.

Table 1.
Emergence of Aedes aegypti adults with eggs incubated for 10 days at different temperatures and placed for post-embryonic development at a temperature of $25 \pm 2^{\circ}\text{C}$.

Rooms	Climate variations		
	Temperature ($^{\circ}\text{C}$)	CO ₂ (ppm)	Humidity (%)
1	27.23	434.4	77.01
2	29.74	864.6	78.66
4	32.18	1279.87	76.96

Table 2.
Averages of temperature $^{\circ}\text{C}$, increase in ppm of CO₂ and percentage (%) of humidity for the different environmental rooms.

There was a statistical difference ($p < 0.05$) between the average time of the biological cycle of *A. aegypti* in the different environments, with room 4 being the environment that had the greatest influence on the life cycle of mosquitoes, since they developed more faster than the insects kept in the other environmental rooms (Table 3).

In the room 1, it was found that adults emerged on the sixth and seventh day, with 45.79% females and 54.20% males. In the room 2, adults emerged on the sixth day, with 58.88% of females and 41.11% of males. In the room 4, adults began to emerge on the fifth and sixth day of the experiment, with 60.34% females and 39.66% males. The mortality percentage in the different instars for rooms 1, 2 and 4 was: 14.4; 28 and 53.6%, respectively.

4. Discussion

Temperature is one of the main environmental variables responsible for changes in the biology of the mosquito *A. aegypti*, directly influencing the reproductive cycle of this species [27–31]. The exposure of *A. aegypti* eggs to temperatures out of their normal range can cause physiological stress, interrupting the development [32]. Therefore, the temperature must be in a suitable range, in order to the larvae hatch and the generations develop and multiply.

Parâmetros	Room 1 (TA)	Room 2 (TM)	Room 4 (TE)
Averages	7.3430 ^{a,*}	6.6780 ^{b,*}	6.0510 ^{c,*}
Standard deviation	0.43756	0.65009	0.60656
Standard error	0.13837	0.20558	0.19181

TA = ambient temperature; TS = mild temperature; TE = extreme temperature. For the Tukey test, the averages were transformed into a root of $x + 0.5^*$. Different letters on the same line, indicate a statistically significant difference according to the Tukey test ($p < 0.05$), CV = 4%; F = 12.64 and DMS = 0.120.

Table 3.
Average life cycle time of adults kept in different environmental rooms that simulate the climatic conditions provided by the IPCC.

In this study, the high hatching rate of larvae (48%) maintained at a temperature of 25°C, in Londrina, Paraná, corroborates what was observed in the laboratory by Farnesi et al. [33] and Mohammed and Chadee [34], who verified larvae hatching rates above 90% at this temperature. In addition, it is in accordance with the optimal temperature range for the development of the *A. aegypti* mosquito, mentioned in the literature.

Beserra et al. [35] observed that this range is between 21 and 29°C, when studying the thermal requirements of the species in four bioclimatic regions of Paraíba, Brazil. Later, Beserra et al. [36] observed that the optimal temperature for the development of *A. aegypti* is between 22 and 32°C, when studying the mosquito's biological cycle in air-conditioned chambers with six different temperatures. For Marinho [37], the ideal temperature range for the development of the vector is between 22°C and 36°C, according to results obtained after analyzing the influence of climatic factors on the pattern of oviposition, distribution and population development of *A. aegypti* in the field and the laboratory. More recently, Galavíz-Parada et al. [29] conclude that hatching and survival of *A. aegypti* in Mexico can occur in a temperature range between 15°C and 32°C under laboratory conditions.

Thus, the third highest rate of hatching of larvae (35%), as well as the lowest mortality of larvae (2.2%) occurring at ambient temperature (16.7–24.1°C) in Londrina, is also in line with the range optimal temperature for the development of *A. aegypti*, mentioned in the literature, and corroborates with a study by Yang et al. [38], where they observed that the mortality rate of adult females of *A. aegypti* was lower between 15°C and 30°C and increases rapidly at temperatures below or above this range, thus corroborating with the mortality rate observed at 0°C (22.2%) in Londrina. On the other hand, Costa et al. [39] reported that the longevity in *A. aegypti* decreased from 25 to 35°C, corroborating with the second highest mortality rate, observed at 25° C (16.6%) in Londrina.

Assessing the influence of ambient temperature on the longevity and fertility of *A. aegypti* in the city of Guarapuava, Paraná, Ajuz et al. [40] found that the vector's survival zone is wide and that only the minimum temperatures below 5°C limit the proliferation and super infestation of the species in the city. Thus, it is evident that the life cycle of the mosquitoes in Londrina is also affected only in extreme temperatures, in view of the greater number of emergencies of adults of *A. aegypti* observed at 5°C (44.80%), as well as a relatively low mortality rate on this temperature (6.6%). The ability of *A. aegypti* larvae and adult to tolerate low temperatures also was demonstrated in the study by Jass et al. [41].

The low hatching of larvae (7%) and emergence of adults (5.6%) at 0°C, as well as the absence at 45°C observed in Londrina, is justified by the fact of extreme temperatures (very low or very high) are harmful to the development of *A. aegypti*,

according to Buriol et al. [42], who stated that temperatures below 5°C and above 40°C are lethal to mosquito development. Similar data were observed by De Majo et al. [43], Marinho et al. [44], Mohammed and Chadee [34] in studies about the effect of different temperatures on the development of *A. aegypti*. More recently, Sukiato et al. [32] also noted in their study that there was no hatching of *A. aegypti* eggs at 40°C, and the larval and pupal mortality was higher at 37°C, compared to other lower temperatures (34, 31 and 28°C).

In Manaus, Amazonas, the greatest influence of the room 4 (with the highest temperature - 32.18°C) on the life cycle of the mosquitoes, where they developed faster than mosquitoes kept in other lower temperature environmental rooms, is in line with Carrington et al. [45], who found that temperatures around 30°C are ideal for the development of *A. aegypti* and that the development was faster under a temperature of 35°C and impaired above this range. Similar results were also obtained by Brady et al. [46], when evaluating the survival of the species at different temperatures, located between 0°C and 40°C. Sukiato et al. [32] also observed that the *A. aegypti* development time was shorter at higher temperatures (37 and 34°C).

In relation to the mortality rate, the lower mortality (14.4%) that occurred at the room 1, where the temperature was lower, as well as the higher mortality (53.6%) of different instars that occurred at the room 4, also corroborate with the results reported by Yang et al. [38] and Sukiato et al. [32] already compared with the results obtained in Londrina. A study by Farjana et al. [47] also demonstrated that the mortality of *A. aegypti* increased at a higher temperature (35°C).

The lowest and the highest mortality rate at the rooms 1 and 4, respectively, may have happened due to the influence of CO₂ concentration—which was lower at the room 1 (similar to the current atmospheric concentration) and much higher at the room 4—because CO₂ atmospheric is also related to the biological cycle of living beings, influencing their ecological interactions. High CO₂ rates have an impact on ecological communities, causing a reduction in nitrogen concentration. Furthermore, it can reduce the quality and quantity of food in breeding sites, compromising larval growth and survival [48].

However, in a study by Azevedo et al. [49], higher concentrations of CO₂ had no significant influence on the results obtained in relation to the biological cycle of *A. aegypti*.

5. Conclusion

In the South region of the country, the hatching of eggs and emergence of adults of *A. aegypti* only did not occur at 45°C, while the lowest rate of hatching and emergence occurred at 0°C, indicating that the development of the mosquitoes in Londrina is affected only in very extreme temperatures, since the temperature of 5°C still proved beneficial to the development of *A. aegypti*. In the North region of the country, the development of the immature in Manaus is faster as the temperature in the environmental rooms increases; however, at the same time, death rates also increase.

Therefore, it is concluded that temperatures from 5 to 29.74°C are more appropriate, since values outside these limits can cause deleterious effects in biological aspects related to the reproductive success of the species. Thus, temperature has a great influence on these aspects, with medium temperatures being more beneficial to this species.

The results obtained show that both *A. aegypti* mosquitoes from the South and North regions of Brazil have adaptive potential in face of the increase in the average temperature stipulated by the IPCC, in view of the unviability of eggs only at

extreme temperatures and considering the shorter average duration of the life cycle observed at high temperatures. Thus, the predicted climate changes may favor the development and proliferation of *A. aegypti*, and consequently the viral circulation, in addition to make possible the occurrence of a geographical expansion of *A. aegypti*. However, it is important to mention that other environmental variables can also influence the biology of mosquitoes, as well as viruses, requiring, therefore, more studies related to the various environmental variables and viruses in order to be able to affirm whether there will be a greater occurrence of arboviruses due to global warming.

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Conflict of interest

The authors declare no conflict of interest.

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