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Effects of constant and fluctuating low temperatures on the development of *Aedes aegypti* (Diptera: Culicidae) from a temperate region

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Abstract

Most studies of the effects of low temperature on the development of immature stages of *Aedes aegypti* have been performed at constant temperatures in the laboratory, which may not accurately reflect the variable environmental conditions in the field. Thus, the aim of this study was to assess the effect of constant temperatures (CT) and fluctuating low temperatures (FT) on the fitness of *Ae. aegypti* of Buenos Aires, Argentina. Three CT treatments (12, 14, 16°C) and three FT treatments (12, 14, 16°C ± 4ºC) were performed and then survival, development time and size of adults analyzed for each treatment. The immature stages completed development in all the treatments, with an average survival of 88% at 16°C, 85% at 14°C and 22% at 12°C, and showed no differences between the CT and FT treatments. Development times were similar between the CT and FT treatments at 16°C (average±SD: 22.7±2.0 days) and at 14°C (average±SD: 30.5±2.5 days), whereas at 12°C they lasted longer under CT (average±SD: 46.6±5.1 days) than under FT (average±SD: 37±6.5 days). The sizes of the adults at 12 and 14°C were similar but larger than those at 16°C, and showed no differences between the CT and FT treatments. Compared to populations of other geographical regions assessed in previous studies, the shorter development times and the high survival at 14 and 16°C, and the ability to complete development at 12°C, a fact not previously reported, suggest that the *Ae. aegypti* population of Buenos Aires city has a higher tolerance to these conditions.

Key words: low temperature, thermal condition, survival, development time, adult size

Introduction

In temperate regions, low temperature is one of the most important environmental factors affecting many aspects of the life cycles of insects. Certain ranges of temperatures during the winter season can cause important mortality to ectotherms. This mortality is considered a selective agent (Willmer et al. 2000, Clarke 2003, Hoffmann et al. 2003) that affects the ecology and evolution of species (Clarke 2003, 2006, Hoffmann et al. 2003, Sinclair et al. 2003, Angilletta 2009).

Temperature affects the metabolic activity (Kingsolver and Woods 1997, Renault et al. 2002), growth rates (Sinclair et al. 2003, Angilletta 2009), and development of many insects, affecting, for example, adult body size (Atkinson and Sibly 1997) and development time of immature stages (Knies and Kingsolver 2010). This implies that individuals reared at higher temperatures have smaller body size and faster development times than those reared at lower temperatures. Lower temperatures generally reduce the metabolic rates and thereby the growth rate. Therefore, development requires more time and a large body size is obtained (Atkinson 1994). The variation in the development time and body size related to a variation in temperature can modulate fitness characteristics such as survival, time of reproduction, fecundity, and resistance to extreme temperatures (Hoffmann et al. 2003, Sorensen et al. 2003, Zeilstra and Fischer 2005, Rajamohan and Sinclair 2009).

Aedes aegypti (Diptera: Culicidae) is a mosquito species of tropical origin that has a worldwide distribution, ranging from tropical to temperate regions (Eisen et al. 2014). Buenos Aires city, Argentina, is located near the southern limit of its world distribution (approximately 35° S latitude, at the winter isotherm of 10°C) (Christophers 1960, Sabattini et al. 1998). In the last decades, two mayor dengue outbreaks occurred in Argentina, one in 2009 and the other in 2016. Within this time frame, no association to El Niño events was detected, whereas a positive association with a high number of dengue cases in the neighboring countries was reported (Carbajo et al. 2018). The high number of locally transmitted cases of dengue recorded in Buenos Aires during these outbreaks (Ministry of Health of Argentina 2010, 2016) demonstrates that the abundance of *Ae. aegypti* and the local weather conditions are appropriate for the completion of the extrinsic cycle of the virus.

Buenos Aires city is characterized by a temperate climate with a pronounced thermal seasonality, and the temporal dynamics of *Ae. aegypti* also exhibits a pronounced seasonality associated with temperature (Bejarán et al. 2000, de Garín et al. 2000, De Majo et al. 2013, Fischer et al. 2017). The development of the immature stages, and the survival and reproduction of adults of *Ae. aegypti* are mainly limited by the low temperatures that take place during the cold season. However, recent studies in laboratory

conditions have shown that eggs of *Ae. aegypti* from Buenos Aires can hatch at temperatures as low as 12ºC (Byttebier et al. 2014), previously considered unfavorable for the development of the immature stages of this species (Headlee 1941, Bar Zeev 1958, Richardson et al. 2011, Carrington et al. 2013). In addition, during an experimental study under natural temperature conditions, we have previously observed egg hatching and successful development of some individuals during the winter, at a mean temperature of 13.2ºC (De Majo et al. 2017).

The effect of temperature on the development of *Ae. aegypti* has been studied for populations of different regions. Laboratory studies at constant temperatures have shown increased survival of *Ae. aegypti* associated with an increase in temperature (Bar Zeev 1958, Rueda et al., 1990, Tun Lin et al. 2000) from 15-16°C to 25-27ºC, temperature at which they reach optimal survival. In the range from 14ºC to 20ºC, survival can vary significantly, from below 25% at 15ºC to 90% at 20ºC (Rueda et al. 1990, Tun Lin et al. 2000). The lowest temperature at which *Ae. aegypti* has been found to complete development is 14°C, with a survival rate of 24% (Bar Zeev 1958).

The development time of immature stages of *Ae. aegypti* from different populations is about 60 days at 14°C, between 40 and 58 days at 15ºC, between 34 and 29 days at 16ºC, and between 12 and 17 days at 20°C (Headlee 1942, Bar Zeev 1958, Rueda et al. 1990, Tun Lin et al. 2000, Kamimura et al. 2002, Richardson et al. 2011).

Most studies of the effects of temperature on the development of *Ae. aegypti* have been performed under constant temperature conditions, because they are easier to control in the laboratory (Brakefield and Kesbeke 1997, Petavy et al. 2001). However, these conditions are unrealistic, since, in natural conditions, individuals are exposed to temperature fluctuations.

Several studies in different insect taxa have shown that individuals exposed to fluctuating low temperature treatments within the viable range for the development of the species (Hagstrum and Hagstrum 1970) have higher survival (Chen and Denlinger 1992, Nedved et al. 1998) and a reduced development time (Brakefield and Kesbeke 1997, Petavy et al. 2001, Fantinou et al. 2003) than under constant temperatures with the same mean.

In addition, individuals exposed to larger fluctuations around the mean temperature exhibit larger differences in development time compared to those exposed to smaller fluctuations (Fantinou et al. 2003).

However, despite the importance of the developmental response at low fluctuating temperatures to understand the population dynamics in temperate regions (Eisen et al. 2014), very few studies have evaluated the development of *Ae. aegypti* at low temperatures (e.g. Bar Zeev 1958, Rueda et al. 1990, Tun Lin et al. 2000, Kamimura et al. 2002, Richardson et al. 2011), and even less compared the development at constant and fluctuating temperatures (e.g. Headlee 1940, 1941, Carrington et al. 2013). Furthermore, most of the studies at low temperatures worked with long lasting laboratory colonies, usually of tropical origin, despite of the fact that an adaptation within few generations to low temperatures has been documented for *Ae. aegypti* (Chang et al. 2007).

This highlights the need to study the survival and development of field collected individuals of *Ae. aegypti* populations from temperate regions at low temperatures under different thermal conditions.

Thus, the aim of the current study was to assess the effect of constant and fluctuating low temperatures representative of the cold season in Buenos Aires city, Argentina, on the survival and the development of the immature stages of the local *Ae. aegypti* population.

Materials and Methods

Temperature conditions and treatments

The temperature conditions used for the study were achieved by means of thermal baths: cuvettes with water inside and a copper serpentine through which cold water circulated. Thermal baths were regulated through programmable thermostats, both for the constant and fluctuating regimes. The air temperature remained constant throughout the experiment (23 ± 1°C), without interfering with the temperatures of the thermal baths.

The experiment consisted of the combinations of two factors: mean temperature (12, 14 and 16°C) and the thermal regime (constant temperatures and fluctuating temperatures). Larvae and pupae of *Ae. aegypti* were exposed to one of the different treatments.

The fluctuating temperature treatments consisted in fluctuations around the same mean temperatures as those of the constant temperature treatments (i.e. 12, 14 and 16°C) ± 4ºC. In these treatments, daily cycles consisted of a decrease in temperature during the night for 2 hours, followed by a period of 6 hours with constant low temperature. After that, temperature increased during the day for approximately 7 hours, until reaching the maximum temperature, which was maintained for approximately 9 hours (the profile of the daily cycles was an average of the entire study period) (**Figure 1**).

The temperatures used correspond roughly to the average thermal range characteristic of winter and the beginning of spring in Buenos Aires city. This was calculated as the hourly average temperatures of 20 years for the coldest winter month (July) and the early spring (September), with data obtained from the National Meteorological Service for the Villa Ortúzar weather station (**Figure 1**).

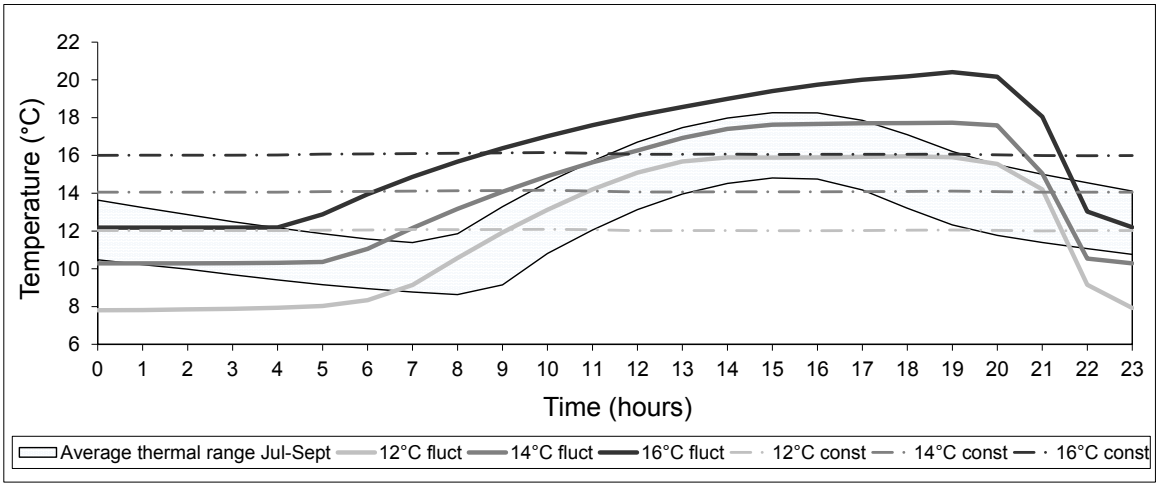

Figure 1. Hourly temperatures profile for different temperature conditions and the 20 year hourly average temperatures of the winter (July) and the beginning of spring (September) in Buenos Aires City (National Meteorological Service: SMN).

Egg collection and conditioning

Eggs of *Ae. aegypti* were collected from the field by means of ovitraps placed in different neighborhoods of Buenos Aires city, Argentina, from February to May 2015. All the eggs were assumed to correspond to *Ae. aegypti* because this is the only container-breeding Aedine mosquito species in this region (Rubio et al. 2012). After collection, eggs were stored in the laboratory at 16°C ± 2°C for 2 to 5 months. Then, all eggs were immersed together at the same time for 7 hours at room temperature (23°C). The newly hatched larvae were randomly assigned to one of the treatments.

At the beginning of the experiment, each larva was placed individually in a plastic tube, which was acclimated at decreasing temperatures: 18, 16 and 14°C in thermal baths until reaching the temperature of the assigned treatment. This was done to gradually decrease the temperature without affecting the normal development of the larvae. The total period of acclimation (passage through the temperatures mentioned above) was approximately 10 minutes and this procedure was carried out for both thermal regimes.

Experimental design

For each treatment, 30 recently hatched larvae in their respective individual plastic tube were used. Plastic tubes contained 10 ml of a nutrient solution (0.47 mg of dry baker´s yeast/ 10 ml of dechlorinated water), which allowed *ad libitum* feeding (Romeo Aznar et al. 2018). The nutrient solution was changed every two days to maintain relatively constant food availability and avoid decomposition. The new solution was prepared 24 hours before use to stabilize temperature conditions. The solution was first changed on the third day after the beginning of the experiment, to reduce excessive manipulation of the first-instar larvae.

The plastic tubes with pupae were conditioned with a support to facilitate the resting of the newly emerged adults. Regardless whether or not the nutrient solution was changed, each individual was inspected daily, and living and dead larvae were counted and their instar recorded. The emerged adults were killed by placing them in a freezer for 2 h. The sex of adults and time of adult emergence were recorded.

Both wings of each individual were removed, placed on a slide and coverslip, and photographed, using a stereoscopic microscope (Leica® S8 APO) equipped with a digital camera (Leica® DFC 295) at a resolution of 0.001 mm. The wings were measured from the wing incision to the apex, excluding the marginal scales (Schneider et al. 2011), by using the application of Leica Suite V 4.0.0. The length of the wing was used as an estimate of the total body size of the adult (Van Handel and Day 1989, Clements 1992).

The temperatures in each thermal bath were recorded with two HOBO® Pendant Temperature Dataloggers every five minutes during the whole duration of the experiment. The experiment was performed under controlled photoperiod (12:12 light-dark).

Data analyses

Survival during development

For each treatment, total survival was estimated as the number of individuals that reached the adult stage divided by the initial number of larvae. A categorical variable of survival was constructed, with individuals that reached the adult stage coded as 1 and individuals that did not complete the development coded as 0. Survival between treatments was compared with Generalized Linear Models (GLM) using the R software, Version 3.2.3 (R CoreTeam 2014), accessed through a user-friendly interface in the Infostat software (Di Rienzo et al. 2015). The factors considered were: temperature (three levels: 12, 14 and 16°C), thermal regime (constant and fluctuating temperatures), and the interaction between both variables; the binary family (Bernoulli) with the logit link function was selected. *A posteriori* comparisons were made with Fisher's LSD test on ranks (Conover 1999), adjusting the significance of the test with the Holm–Bonferroni correction for multiple comparisons (Holm 1979).

For treatments with mortality higher than 20%, the mortality was analyzed by stage. The mortality for each larval instar and pupal stage was calculated as: $1 - (N_{xf} / N_{xi})$, where N_{xi} = number of individuals at the beginning of stage x, N_{xf} = number of individuals at the end of stage x.

Development time

For each individual, the total development time was estimated from first-instar larvae (seven hours after the immersion of eggs) to adult emergence, and the duration of each stage (for individuals which completed development) was estimated as the difference between the day on which a certain stage was reached and the day on which it passed to the next. Total development time between treatments was compared with GLM. The factors considered were: temperature (three levels), thermal regime (two levels), sex (two levels), and double and triple interactions between variables. The gamma family and the logarithm link function were selected. To evaluate significant differences, *a posteriori* comparisons were made with the Fisher LSD test of ranges, adjusting the Bonferroni correction for multiple comparisons.

Wing length

The average length of the left and right wing was calculated for each individual. For the statistical analysis, the length of the wings was compared between temperatures, thermal regimes and sexes by GLM, using the normal distribution. The factors considered were: temperature (three levels), thermal regime (two levels), sex (two levels), and their interactions. The temperature*regime*sex interaction was deleted from the model because it was not significant. To evaluate the differences between treatments, *a posteriori* comparisons were made with the Fisher LSD test of ranges, adjusting Bonferroni correction for multiple comparisons.

Comparison with survival and development time data from previous studies

The results of survival and development times obtained in the present study were compared with those obtained in previous studies from other regions, under similar rearing conditions at constant laboratory temperatures for the thermal range of 12 to 16°C. This comparison includes the following studies: Headlee 1942, Bar Zeev 1958, Rueda et al. 1990, Tun Lin et al. 2000, Kamimura et al. 2002 (only for development time) and Richardson et al. 2011.

Results

Survival during development

Survival during the development was significantly affected by the temperatures (GLM: $X^2 = 28.8$, df = 2, $P < 0.001$) but not by the thermal regimes ($X^2 = 1.3$, df = 1, $P = 0.3$) or the interaction between both variables ($X^2 = 0.3$, df = 2, $P = 0.71$). For both the constant and fluctuating temperature treatments, the survival of the emerged adults was on average 88% at 16°C and 85% at 14°C. Survival at these temperatures was significantly higher than at 12°C, which was 22% on average (**Table 1**).

Table 1. Instar-specific development time (L: larval instar) and pupa stage of *Ae. aegypti* in laboratory conditions. Mean time is expressed in days ± standard deviation. Number of surviving males and females and percent survival under constant and fluctuating treatments.

Temperature (°C)	Larval instar				Pupa stage	N° of individuals		% Survival
Constant	L1	L2	L3	L4		♂	♀	
12	6.6 ± 1.5	5.1 ± 1.1	5.6 ± 1.3	20.6 ± 3.5	8.7 ± 0.8	4	3	23
14	4.2 ± 0.9	3.6 ± 0.5	4.0 ± 0.6	12.1 ± 1.1	6.7 ± 0.6	14	12	87
16	3.4 ± 0.9	2.7 ± 0.5	3.1 ± 0.7	8.8 ± 1.3	5.2 ± 0.6	15	13	93
Fluctuating								
12	4.8 ± 1.0	5.5 ± 2.3	5.7 ± 2.2	13.3 ± 2.1	7.3 ± 1.0	4	2	20
14	4.3 ± 1.1	3.7 ± 0.7	4.0 ± 0.6	12.1 ± 2.2	6.3 ±0.6	9	16	83
16	3.1 ± 0.5	2.5 ± 0.5	2.9 ± 0.6	8.6 ± 1.4	5.2 ± 0.6	13	12	83

Regarding instar-specific mortality at 12ºC, the highest mortality at constant temperature was recorded for pupae (72%), followed by the fourth (11%) and first larval instars (8%), and no mortality was recorded during the second and third larval instars.

On the other hand, at fluctuating temperature the highest mortality was recorded during the fourth (50%) larval instar, followed by the second larval instar (38%), the pupal stage (33%), the third (11%) and first larval instars (7%). The highest mortality occurred a few

days after the occurrence of extreme minimum temperatures with short periods near to 6°C (**Figure 2**).

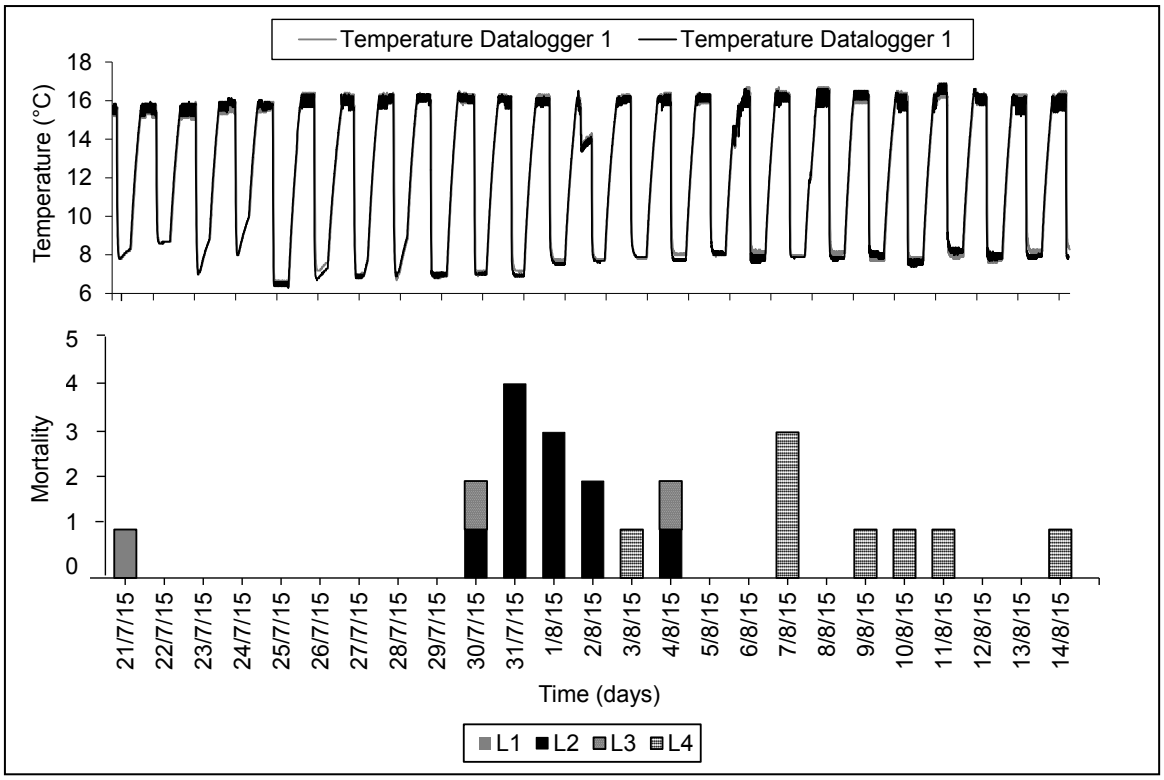

Figure 2. a) Temperature profile at 12°C under fluctuating temperature treatment during the larval development period b) Mortality of larval instars at 12°C under fluctuating temperature treatments.

Development time

Total development time was significantly affected by the temperature*regime*sex interaction (X^2 =7.02, df = 2, P <0.001). For females, the development times were significantly different between temperatures but not between thermal regimes. The development times ranged from a mean of 22 days at 16°C, to a mean of 31 days at 14°C and a mean of 43.5 days at 12°C, for both thermal regimes (**Figure 3a**). For males, the development times were also significantly different between temperatures from a mean of 21.5 days at 16°C, to a mean of 29.8 days at 14°C and a mean of 41.4 days at 12°C, for both thermal regimes. Although differences in development time between thermal

regimes were observed, with a trend toward a decrease under the fluctuating temperature treatment, these differences were significant only at 12°C, with a mean development time of 15 days less under the fluctuating temperature than under the constant temperature treatment (**Figure 3b).** Development times were significantly shorter for males than for females only at 12ºC ($P < 0.05$) and 16ºC ($P < 0.05$) fluctuating temperatures. Although it was not significant, in most of the remaining treatments (except at 12ºC constant temperature) a trend towards shorter development times in males was observed (**Figure 3**).

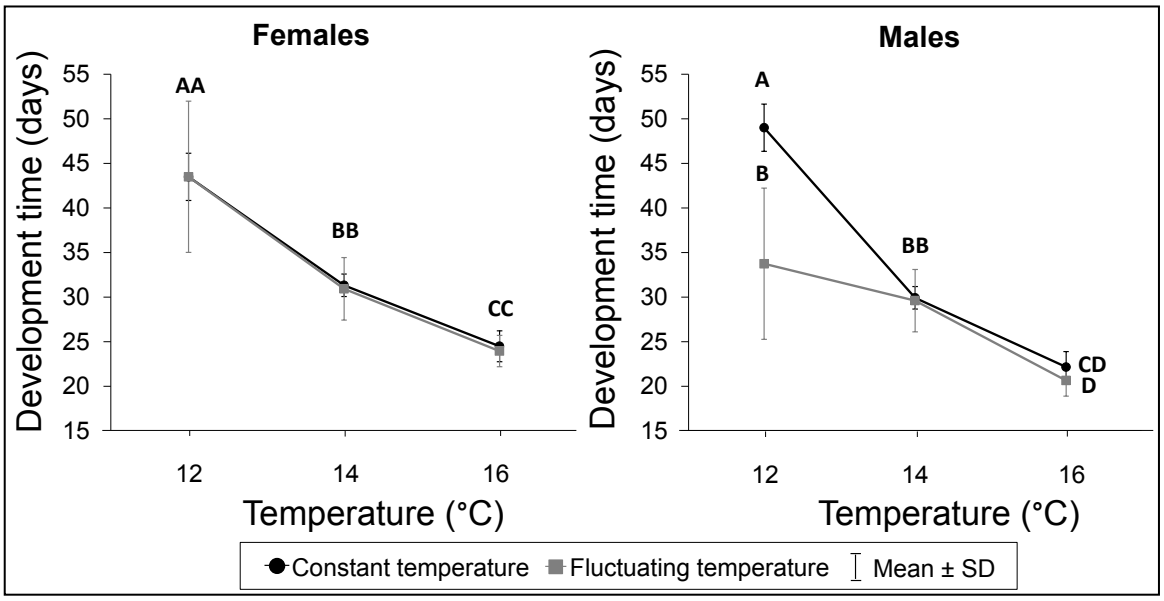

Figure 3. Development times of *Ae. aegypti* under constant and fluctuating temperature treatments for: **a)** females and **b)** males. The different letters indicate significant differences within each graph and between graphs ($P < 0.001$).

The duration of each larval instar showed similar responses between the different thermal regimes, except for the first and fourth larval instar and pupal stage at 12°C (**Table 1**).

The duration under the constant temperature treatment was longer than under the fluctuating temperature treatment for the first larval instar (1.8 days), the fourth larval instar (7.3 days) and the pupal stage (1.4 days).

Wing length

The mean wing length was significantly affected by the temperature (GLM: $X^2 = 17$, df = 2, $P < 0.001$) and sex ($X^2 = 494$, df = 1, $P < 0.001$), but not by the thermal regime ($X^2 = 3.7$, df = 1, P = 0.06) or the temperature*regime ($X^2 = 2.8$, df = 2, P = 0.07), regime*sex ($X^2 = 0.04$, df = 1, P = 0.8), or temperature*sex interactions ($X^2 = 0.4$, df = 2, P = 0.7).

No statistical differences in wing lengths were detected between individuals reared at 12 and 14°C, but these were significantly longer than those of individuals reared at 16°C. Females showed longer wings than males ($P < 0.05$) (**Figure 4**).

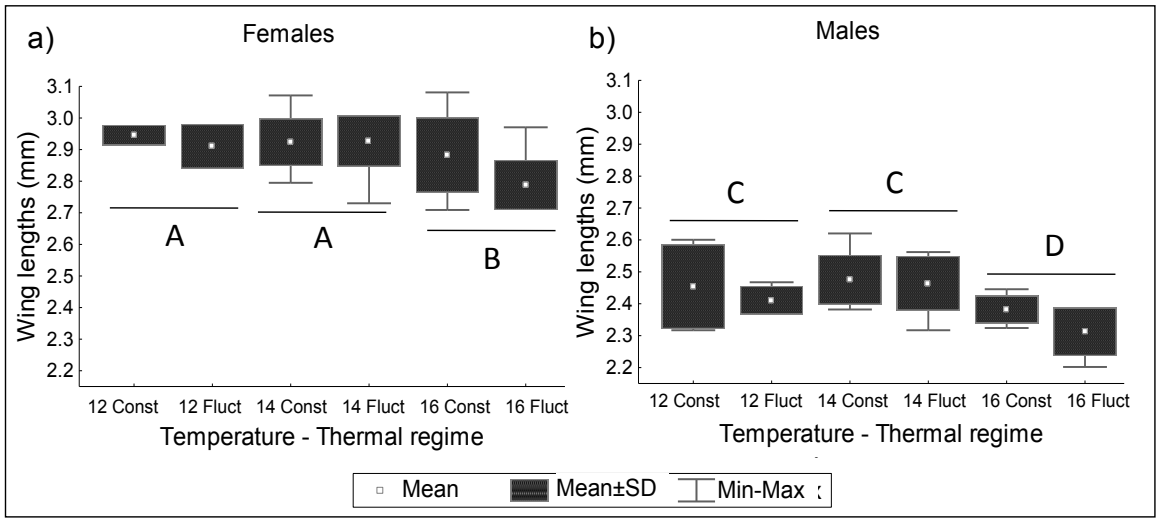

Figure 4. Wing length of *Ae. aegypti* under constant and fluctuating temperature treatments for **(a)** females **(b)** males. Different letters indicate significant differences.

Comparison with survival and development time data from previous studies

The results of the present study at constant temperatures showed higher survival than that observed in other studies. At 12°C, some individuals completed the development with a survival of 23%, whereas, in previous studies, the lowest constant temperature at which *Ae. aegypti* individuals completed development was 14°C (**Figure 5.a**). In line with this result, at 14 and 16°C, the development times in our study were shorter than those observed in previous studies at constant temperatures (**Figure 5.b**).

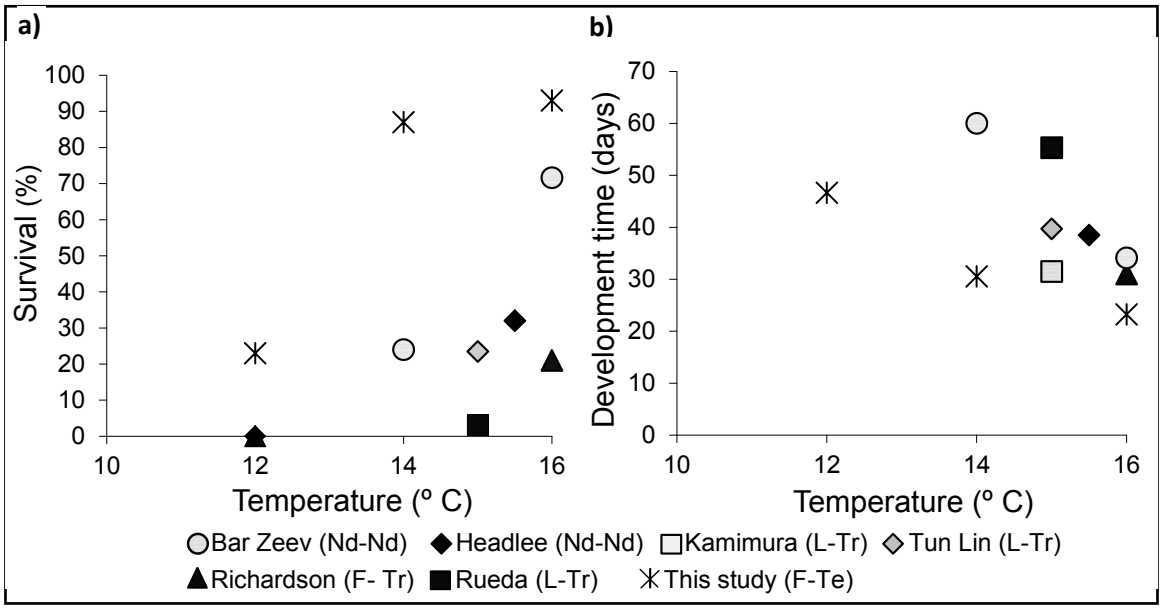

Figure 5. Data from the current study and the results of previous studies in the range of 12 to16°C **a)** survival of immature stages and **b)** development time. Codes for origin climate: Te (temperate), Tr (tropical). Codes for source of individuals: L (laboratory colony), F (field collected). Nd (no data available).

Discussion

This is the first study assessing the effects of constant and fluctuating low temperature treatments on the development of the immature stages of field collected *Ae. aegypti* from a temperate region. The similarity in survival and development time between the two thermal regimes obtained in the present study (except for males at 12°C) is consistent with the results of Carrington et al. (2013) at a mean temperature of 16°C (range: 12.2-19.8°C). In contrast, for larger fluctuations a reduction in development time and an increase in survival has been observed at a mean of 18ºC (range: 10-26.7ºC) (Headlee 1941) and of 16ºC (range 6.7-25.3ºC) (Carrington et al. 2013). This suggests that within certain temperature range, the effects of the temperature fluctuations on survival and development depend on the amplitude of these fluctuations.

At 12ºC, a longer development time was observed under constant temperature than under fluctuating temperature, caused by a longer duration of the first and fourth larval instars and the pupal stage. These differences could be attributed to physiological responses such as a lower temperature threshold at fluctuating as compared to constant temperatures,

observed previously in Lepidopterans (Fantinou et al. 2003). Furthermore, the reduced development time under fluctuating temperatures, compared to constant temperatures near to the threshold where feeding activity is prevented, could be related to an increase in food intake during the day at higher temperatures, and a slow decrease in energy reserves by metabolic losses during the night at lower temperatures (Fischer et al. 2011).

The longer development time and the late emergence of males (difference of 15 days) at 12ºC under constant temperature as compared to fluctuating temperatures, suggest that these conditions are especially unfavorable for males, thus reducing their fitness. This could be at least partly explained by the observation of a higher development threshold temperature for males (9.45ºC) than for females (8.52) (Tsuda and Takagi 2001), although the low number of surviving males and females in both treatments at 12ºC prevents from drawing definitive conclusions.

The trend to an earlier emergence of males compared to females in the entire range studied (except for 12ºC at constant temperatures) is caused by protandry, which maximizes male mating opportunities with virgin females (Wormington and Juliano 2014). In contrast, the later emergence of females might be related to a higher storage and utilization of nutrients for increasing body mass (Briegel et al. 2001) and for reproduction (Honek 1993).

The high mortality of the pupal stage under constant temperature at 12ºC is consistent with results from previous studies, where a higher mortality of late development stages (fourth instar larvae and pupae) was observed at 15ºC (Rueda et al. 1990) and at 16ºC (Bar Zeev 1958).

Furthermore, the higher mortality during the larval instars at 12ºC under fluctuating temperature treatment might be related to the low minimum temperature events that occurred in that treatment during the experiment. The delayed mortality after exposure to low temperatures has been previously observed in several insect species (Koštál et al. 2019). Different mechanisms have been proposed for this pattern in different species (Lee 2010), and in the case of *Ae. aegypti* the mechanism involved deserves further investigation.

The low survival observed at 12°C, the higher mortality of larval instars under the fluctuating temperature treatment (with minimum temperatures of 8ºC during 6 hours and temporary minimums of 6ºC), the later emergence of males under constant temperatures suggest that these thermal conditions are unfavorable for development and that individuals are subjected to thermal stress.

On the other hand, the inverse relationship between wing length and temperature (at 12 and 14°C wing length was longer than at 16°C) for both sexes could be due to an extended time for food intake and a higher efficiency in converting ingested food into body matter at low temperatures (Karl and Fischer 2008). At low temperatures the larger body size of adults due to the extension of development increases food intake during larval instars, this may be related to increased tolerance to unfavorable conditions, for example short photoperiods, low temperatures or low food availability (Costanzo et al. 2015).

The similar wing length at 14°C and at 12°C is consistent with the results of Tun Lin et al. (2000) at constant temperatures of 15 and 20°C. This result was unexpected because, in general, insects reared at increasing temperatures have smaller body size (Atkinson 1994). This relationship is assumed to be linear between approximately 14 and 30°C (Couret et al. 2014). However, the fact that wing length did not increase at 12°C, despite the extension of the development time, could indicate extreme conditions or cold stress, which would be consistent with the higher mortality recorded at that temperature (Kingsolver and Huey 2008).

A striking result of this study was the higher survival and the shorter development time at low temperatures compared to that observed in previous studies (Bar Zeev 1958, Rueda et al. 1990, Tun Lin et al. 2000, Richardson et al. 2011). The differences could be attributed to the use in previous studies of long lasting laboratory colonies (except Richardson et al. 2011), and their subtropical or tropical origin. The possibility that other factors related with the experimental design are involved cannot be rejected.

An unexpected result was that some individuals completed development at 12°C under both the constant and the fluctuating temperature treatments, a fact not observed

previously (Headlee 1941, Bar Zeev 1958, Richardson et al. 2011, Carrington et al. 2013). Furthermore, the high survival observed in this study in the treatments at 14 and 16°C indicates that these temperatures are not limiting for the *Ae. aegypti* population of Buenos Aires city.

In summary, the shorter development times, the high survival at 14 and 16°C and the ability to complete development at 12°C suggest that the *Ae. aegypti* population of Buenos Aires city has a higher tolerance to these conditions than populations of other regions previously studied. This could be an adaptation of the population studied to the local winter conditions during part of the year. This ability to adapt to low temperatures has been demonstrated in laboratory experiments for other populations of this species (Tun Lin et al. 2000, Chang et al. 2007). Future studies comparing the development time and the survival of the population of Buenos Aires with those of tropical regions at low temperature would provide support for this hypothesis.

In conclusion, the results of the present study indicate that the *Ae. aegypti* population of Buenos Aires could have a higher tolerance to the cold temperatures of late autumn, winter and early spring of Buenos Aires city. These results are consistent with those obtained under natural temperature conditions during the winter season in Buenos Aires for the same population (De Majo et al. 2017). This would allow the population to shorten the period of dormancy and start their reproductive activity earlier.

These results imply the possibility of an expansion in the geographic distribution of *Ae. aegypti* towards colder areas, continuing the pattern observed in Buenos Aires province during the last years (Zanotti et al. 2015).

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