



Effect of temperature on life history traits during immature development of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) from Córdoba city, Argentina



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ABSTRACT

We investigated how ambient temperature under fluctuating conditions affects the larval–pupal immature traits of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes from Córdoba city, Argentina, and established each species development threshold and physiological time. Based on life tables, three cohorts of each mosquito species were reared in the laboratory under small fluctuating temperatures conditions of $15.2 \pm 1.7^\circ\text{C}$, $17.9 \pm 1.6^\circ\text{C}$, $21.6 \pm 0.7^\circ\text{C}$ and $25.3 \pm 0.4^\circ\text{C}$ for *Ae. aegypti*, and $16.6 \pm 1.7^\circ\text{C}$, $18.7 \pm 1.7^\circ\text{C}$ and $25.2 \pm 0.3^\circ\text{C}$ for *Cx. quinquefasciatus*. Immature development time and survival values, and also thermal development threshold and physiological time were estimated. Development times of all larval and pupal stages of *Ae. aegypti* and *Cx. quinquefasciatus* were significantly affected by the rearing temperatures, decreasing when temperature increased. Mean *Ae. aegypti* total (larva + pupa) development time ranged from 21.9 to 8.6 days, at 15.2 and 25.3 °C, whereas, for *Cx. quinquefasciatus* varied between 23.5 to 9.2 days at 16.6 and 25.2 °C, respectively. Larval and pupal survival of both species was affected by different rearing temperatures, increasing in general as temperature increased. For *Ae. aegypti* the total immature survival ranged from 26% at 15.2 °C to 92% at 21.6 °C; however, temperature did not have significant effect on this variable. The total immature survival of *Cx. quinquefasciatus* was significantly and positively affected by temperatures, ranging from 32 to 88%, at 16.6 and 25.2 °C. The temperature development threshold and the physiological time estimated for *Ae. aegypti* and *Cx. quinquefasciatus* were 11.11 °C and 93.74 degree-days, and 10.96 °C and 136.87 degree-days, respectively. The results of the present study showed that temperature significantly affects the larval–pupal immature traits of these mosquito species of sanitary importance, from the central region of Argentina. All the parameters recorded are useful for the development of mosquito management models.

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1. Introduction

Aedes aegypti (whose subgenus *Stegomyia* has been lately raised to the level of genus (Reinert et al., 2004)), and *Culex quinquefasciatus* (Diptera: Culicidae) are the major mosquito vectors of dengue (DENV) and St. Louis encephalitis virus (SLEV), respectively. In Argentina, a St. Louis encephalitis outbreak occurred in 2005 in

the Córdoba Province, where human cases and infected *Cx. quinquefasciatus* mosquitoes were recorded (Spinsanti et al., 2008). In 2009, the largest epidemic of dengue occurred in this country with more than 26,000 confirmed cases affecting the northern and central provinces of Argentina, including the Córdoba Province (M.S.N., 2009).

Temperature is one of the major extrinsic factors that affect many population parameters of insects. In mosquitoes of sanitary importance, information on the relationship between temperature and growth rates as well as development and survival of different stages is useful in designing vector control strategy models. Within the intermediate temperature range, immature development

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rate and survival increased with temperature, up to a point, and often lead to an increase of the number of adult mosquitoes in a population in a shortest time. Moreover, increased adult survival allows the vector to increase the chances of becoming infected, and deliver more infective bites, with consequences in the rate of diseases transmission (Clements, 1992). It has been reported worldwide that increases in temperature were associated with increases in mortality rates, as well as decreases in adult body size and oviposition activity of *Ae. aegypti* and *Cx. quinquefasciatus* (Bar-Zeev, 1958; Costa et al., 2010; Gunay et al., 2010; Mohammed and Chadee, 2011; Oda et al., 1999; Rueda et al., 1990). Similar relationships between thermal rearing conditions and life history traits of these species were observed in populations from Argentina at constant laboratory temperatures (Loetti et al., 2011), or at fluctuating temperatures under field conditions (Almirón and Brewer, 1996; Domínguez et al., 2000).

The temperature development threshold and physiological time are both ecological meaningful parameters in the study of ectotherm organisms that relate development time and temperature. Developmental rate is defined as the reciprocal of the development time, and is positively correlated with temperature. Extrapolation from the linear region to the temperature axis indicates the development thermal threshold, and is the temperature value below which immature stages do not develop (Clements, 1992). Physiological time is considered to be a species-specific thermal constant and is the product of mean ambient temperature above the developmental threshold and the time from start of development to completion. It expresses the total amount of degree-days required for an organism to develop from one point to another in its life cycle (Clements, 1992).

Recent studies have shown that the real effect of temperature on immature and adult life history traits could be better explained under more natural fluctuating temperature range than under constant laboratory temperature conditions (Carrington et al., 2013a,b). For *Cx. pipiens quinquefasciatus* and *Ae. aegypti* populations from central areas of Argentina, there is literature available on the life history traits under different fluctuating temperature range, and also on the development threshold (Almirón and Brewer, 1996; Domínguez et al., 2000). However, these works do not present detailed data on development time and survival for each instar larvae, or the physiological time of both species. Moreover, information on *Cx. pipiens quinquefasciatus* immature life history traits is given only for lower winter temperatures. The aims of this work were to study the effect of ambient temperature under fluctuating conditions on larval–pupal life history traits of *Ae. aegypti* and *Cx. quinquefasciatus* populations from Córdoba city (Central Argentina), and to determine the development threshold and physiological time of both species.

2. Materials and methods

2.1. Study area

Aedes aegypti and *Cx. quinquefasciatus* populations were from Córdoba city (31°22' S, 64°12' W). This is the main city and capital of Córdoba Province in Argentina with a resident human population of 1,330,023 (INDEC, 2010). The climate is temperate with distinctive hotter wet (October–April mean maximum and minimum temperatures 27.15° and 14.87 °C, respectively; mean monthly rainfall 111.43 mm) and cooler dry (May–September mean maximum and minimum temperatures 19.66° and 6.1 °C, respectively; mean monthly rainfall 17.98 mm) seasons (S.M.N., 2012).

In previous ecological studies performed in Córdoba city, larvae of *Cx. quinquefasciatus* were found in natural and artificial water bodies, including shallow wells, drains, retention ponds, canals and

ditches (Grech et al., 2013; Pires and Gleiser, 2010). Larvae of *Ae. aegypti* develop in a greater variety of artificial containers like buckets, tins, flower vases and discarded tires (Almirón and Asis, 2003), as well as share the same breeding habitats with *Cx. quinquefasciatus* (Grech, 2013). Studies of seasonal dynamics of both mosquito species in this city recorded *Ae. aegypti* ovipositional activity only during the hotter wet months (Domínguez et al., 2000; Grech, 2013), while *Cx. quinquefasciatus* larvae were collected during the whole year, being most abundant in the summer months (Grech et al., 2013).

2.2. *Ae. aegypti* and *Cx. quinquefasciatus* mass rearing

Material used in this study was obtained from laboratory colonies established from field immature stages collected in different houses and public places of Córdoba city. *Aedes aegypti* and *Cx. quinquefasciatus* larvae and pupae, and also *Cx. quinquefasciatus* egg rafts were obtained from artificial and natural breeding sites like discarded tires, buckets, tins, drains, canal and ditches. In Córdoba city, others species belonging to the genus *Culex* (*Cx. bidens-mollis*, *Cx. apicinus*, *Cx. eduardoi-dolosus*, and *Cx. saltanensis*), can share the same breeding habitat with *Cx. quinquefasciatus*; however these species represent less than 1% (Grech et al., 2013). To ensure that egg rafts field collected were *Cx. quinquefasciatus*, each raft was isolated individually and kept in plastic trays with water from natural habitat. Larvae hatched from each isolated raft were reared, and species identification was confirmed by examining the morphology of 4th instar larvae (Darsie, 1985). All collected material used in the colony was identified as *Cx. quinquefasciatus*. These collection methods were repeated several times to supplement the study with additional immature samples and minimize inbreeding or founder effects on the colony. Colonies of both species were developed under semi-controlled rearing conditions, and exposed to natural fluctuations of temperature and photoperiod, in the laboratory of the Centro de Investigaciones Entomológicas de Córdoba (CIEC). Groups of 50 larvae were kept in plastic trays (17 cm length, 14 cm width, 5 cm height) containing 500 ml of water from natural habitat, and fed with 0.25 mg daily/larva of liver powder (Gerberg et al., 1994). Water surface of larval trays was skimmed daily using a strip of filter paper to avoid subsequent fungal and bacterial development, and also to remove larval exuviae. When larvae reached pupae stage, they were individually picked from trays using a pipette, and transferred to plastic flasks with dechlorinated tap water put inside of adult cardboard cages (30 cm diameter, 50 cm high). Adults were provided with 10% sugar solution soaked in cotton wick on plastic flasks, as a nutritional source. A blood source was provided by placing a restrained mouse twice a week for 1 h during daylight hours for *Ae. aegypti*, and a restrained quail for 2 h at night for *Cx. quinquefasciatus*. For *Ae. aegypti* oviposition, plastic flasks (capacity 250 ml) with filter paper on the inside wall were filled approximately halfway with dechlorinated tap water and placed into the cages. Every day, flasks with eggs were removed from the cages and replaced. Eggs remained on moist filter paper for at least four days to ensure embryogenesis, and then were air-dried for storage until the experiment started. Females of *Cx. quinquefasciatus* oviposited in plastic flasks (capacity 250 ml) with dechlorinated tap water (125 ml), which were placed inside in the floor of the cages. Flasks with egg rafts were removed daily from the cages and replaced. Egg rafts were isolated individually and kept in plastic trays with dechlorinated tap water (500 ml), where larvae were allowed to hatch.

2.3. Experimental procedure

Life statistics of *Ae. aegypti* and *Cx. quinquefasciatus* populations, as well as development thermal threshold and physiological time

of both species, were studied based on horizontal life tables. Plastic flasks with water were placed inside the *Cx. quinquefasciatus* adult cages for oviposition, and papers with deposited *Ae. aegypti* eggs were submerged into trays filled with dechlorinated tap water. Within 12 h of eclosion, larvae of 1st instar were randomly selected, and 3 cohorts of 50 larvae for each species were established.

Cohorts were reared at different natural thermal profiles registered in Córdoba city, in the laboratory of CIEC. During each study period, temperature was allowed to vary freely, and daily maximum, minimum, and average temperatures were registered. Cohorts of *Ae. aegypti* and *Cx. quinquefasciatus* were not reared simultaneously. Three cohorts of *Ae. aegypti* were reared at $15.2 \pm 1.7^\circ\text{C}$ (June 2010; mean minimum: $13.3 \pm 1.9^\circ\text{C}$, mean maximum: $16.9 \pm 2.2^\circ\text{C}$, amplitude of fluctuation: 3.6°C ; photoperiod 10.2 h of light), $17.9 \pm 1.6^\circ\text{C}$ (September 2009; mean minimum: $15.7 \pm 1.7^\circ\text{C}$, mean maximum: $20.2 \pm 1.2^\circ\text{C}$ amplitude of fluctuation: 2.3°C ; photoperiod 11.9 h of light), $21.6 \pm 0.7^\circ\text{C}$ (November–December 2006; mean minimum: $20.04 \pm 0.9^\circ\text{C}$, mean maximum: $23.5 \pm 0.8^\circ\text{C}$ amplitude of fluctuation: 3.5°C ; photoperiod 13.9 h of light), and $25.3 \pm 0.4^\circ\text{C}$ (January–February 2009; mean minimum: $23.9 \pm 0.4^\circ\text{C}$, mean maximum: $26.5 \pm 0.6^\circ\text{C}$ amplitude of fluctuation: 2.6°C ; photoperiod 13.6 h of light). Whereas, three cohorts of *Cx. quinquefasciatus* were reared at $16.6 \pm 1.7^\circ\text{C}$ (July–August 2008; mean minimum: $15.1 \pm 1.9^\circ\text{C}$, mean maximum: $18.2 \pm 1.8^\circ\text{C}$ amplitude of fluctuation: 3.1°C ; photoperiod 10.4 h of light), $18.7 \pm 1.7^\circ\text{C}$ (September 2008; mean minimum: $16.7 \pm 2.1^\circ\text{C}$, mean maximum: $20.9 \pm 1.5^\circ\text{C}$ amplitude of fluctuation: 4.2°C ; photoperiod 11.9 h of light), and $25.2 \pm 0.3^\circ\text{C}$ (January–February 2009; mean minimum: $23.9 \pm 0.4^\circ\text{C}$, mean maximum: $26.6 \pm 0.5^\circ\text{C}$ amplitude of fluctuation: 2.7°C ; photoperiod 13.6 h of light).

Each cohort was kept in a 500 ml dechlorinated water-filled tray, the larvae fed with liver powder and monitored daily, as previously described. Every day, the number of larvae was counted in each cohort and categorized according to instars as determined visually. First and second instar larvae were considered together as one age class (larva 1 + 2) because it is difficult to discriminate between both instars. When larvae reached pupae stage, they were taken out of the tray and put into 500 ml plastic flasks containing 250 ml of dechlorinated tap water, where they were allowed to emerge as adults. Cohorts were reared until adult emergence, and emerged adults were frozen. The rearing trays and flasks were inspected daily, and water was added as needed to maintain constant volumes. Larval exuviae and dead larvae/pupae were removed and counted daily. To estimate the duration of each instar larvae and pupal stage development, the number of days spent in each preimaginal stage was recorded in each cohort. The presence of exuviae indicated changes of instar/stage. Total development time from egg hatch to adult emergence was recorded as the sum of days spent in each stage. Survival was expressed as the percentage of individuals that reached the next instar larvae or pupal stage.

2.4. Data analysis

The rearing temperature effect on development time and survival of each preimaginal stage of *Ae. aegypti* and *Cx. quinquefasciatus* was analyzed with general linear models. Statistical analyses were performed with R software, version 3.0.2 (R Core Team, 2013).

The development thermal threshold for each species was estimated by linear regression of the development rate, expressed as the reciprocal of development time, and as a function of temperature. The value of the x -intercept, below which the regression predicts that no development will occur, was determined as the development thermal threshold.

We studied whether there were significant differences in the slopes of the regression lines between pairs of consecutive

temperature values. For *Ae. aegypti*, we found that only the slope of the line between the highest values of temperature differed significantly from the previous ($t = 4.59$; $p < 0.01$). The slope in that interval was not significantly different from zero ($t = 1.68$; $p > 0.1$). For that reason the data (a rearing temperature of 25.3°C) was excluded from the analysis, because it was out of the range for linear development.

Physiological time was estimated as the product of mean ambient temperature above the developmental thermal threshold and the number of days required for completing the development, using the formula:

$$K = t(T - c)$$

where K is the physiological time measured in degree-days; t is the time from start of development to completion; T is the mean ambient temperature; and c is the estimated development thermal threshold (Clements, 1992). Statistical analyses were performed using InfoStat software, Version 2011 (Di Rienzo et al., 2011).

3. Results

3.1. Development time and survival

The *Ae. aegypti* development times of all considered immature instar larvae and the pupal stage were significantly affected by the rearing temperatures, decreasing as temperature increased. The mean *Ae. aegypti* larval development time ranged from 17.4 to 6.9 days, at 15.2 and 21.6°C , respectively, whereas both, the mean pupal and total (larva + pupa) development time ranged from 4.6 to 1.5, and 22 to 8.7 days, at 15.2 and 25.3°C , respectively (Table 1). The Table 1 also shows that an increase of 3 degrees from 15°C which produced a decrease of 7 days in total development time, between 18 and 21°C the decrease was somewhat smaller (5 days), and between 21 and 25°C was obtained only one day earlier in development time. The larval and pupal survival of *Ae. aegypti* were significantly affected by the rearing temperatures, increasing in general as temperature increased. Only the fourth instar-larvae and pupal stage were significant and negatively affected by temperature, increasing their mortalities as temperature increased. The larval–pupal survival ranged from 26% at 15.2°C to 92% at 21.6°C . However, temperature had no significant effect on this variable ($T = 1.85$; $p = 0.09$) (Table 1). At 15°C the survival of larvae 1 + 2 was less than 50%, but it exceeded 95% at temperatures above 18°C . The survival of larvae 3 was greater than 95% at all rearing temperatures. However, larvae 4 and pupae showed lesser survival at the highest rearing temperature (25°C).

The relationships observed between *Cx. quinquefasciatus* immature development time and rearing temperature were significantly shortened when temperature increased. The mean *Cx. quinquefasciatus* larval, pupal and total development time ranged from 20.4 to 7.4 days at 16.6 and 25.2°C , 3.2 to 1.6 days at 16.6 and 18.7°C , and 23.6 to 9.2 days at 16.6 and 25.2°C , respectively (Table 2). The larval–pupal survival of *Cx. quinquefasciatus* was also significantly and positively affected by temperatures, ranging from 32 to 88%, at 16.6 and 25.2°C . Relationships between both larval and pupal survival, and temperature were not observed ($T_L = 2.14$, $p = 0.07$; $T_P = 1.3$, $p = 0.24$) (Table 2).

3.2. Temperature development threshold and physiological time

Linear regression of the development rate as function of temperature was significant for *Ae. aegypti* ($R^2 = 0.9$; $p \leq 0.05$). Both the development thermal threshold and the physiological time calculated for this species were 11.1°C (Fig. 1A), and 93.7 degree-days, respectively. For *Cx. quinquefasciatus* linear regression of the

Table 1
Mean larval and pupal development time (days) and survival (%) ± standard error for *Aedes aegypti* registered at different temperatures (°C), and slope, y-intercept, R^2 adjusted, and p values obtained from general linear models.

Vital statistics	Rearing temperature				Slope	y-Intercept	R^2 adjusted	p-Value
	15.2 ± 1.7	17.9 ± 1.6	21.6 ± 0.7	25.3 ± 0.4				
<i>Development time</i>								
Larva 1 + 2	6.82 ± 0.01	4.23 ± 0.04	2.41 ± 0.09	3.19 ± 0.01	-0.39	12.08	0.66	0.0005
Larva 3	5.49 ± 0.09	4.26 ± 0.13	1.75 ± 0.28	1.26 ± 0.04	-0.47	12.63	0.89	7.9×10^{-07}
Larva 4	5.1 ± 0.27	3.22 ± 0.3	2.75 ± 0.24	2.7 ± 0.16	-0.25	8.53	0.75	7.4×10^{-05}
Total larva	17.41 ± 0.34	11.71 ± 0.29	6.91 ± 0.18	7.15 ± 0.19	-1.1	32.97	0.81	1.7×10^{-05}
Pupa	4.56 ± 0.53	2.75 ± 0.4	2.41 ± 0.22	1.52 ± 0.49	-0.26	8.21	0.66	0.0004
Larva + pupa	21.97 ± 0.19	14.46 ± 0.25	9.83 ± 0.59	8.67 ± 0.3	-1.4	41.72	0.85	4.1×10^{-06}
<i>Survival</i>								
Larva 1 + 2	40 ± 5.2	96 ± 1.15	100 ± 0.0	100 ± 0.0	0.06	-0.31	0.61	0.001
Larva 3	95 ± 0.0	95 ± 0.67	98 ± 2	100 ± 0.0	0.005	0.086	0.48	0.005
Larva 4	96 ± 2.2	91 ± 0.67	95 ± 1	81 ± 6.36	-0.01	1.17	0.29	0.03
Total larva	31 ± 3.33	82 ± 0.0	94 ± 3	81 ± 6.36	0.05	-2.28	0.47	0.006
Pupa	95 ± 2.89	91 ± 1.76	96 ± 3	77 ± 1.33	-0.02	1.22	0.36	0.02
Larva + pupa	26 ± 1.67	73 ± 1.76	92 ± 2	57 ± 7.51	0.04	-0.07	0.17	0.09

Table 2
Mean larval and pupal development time (days) and survival (%) ± standard error for *Culex quinquefasciatus* registered at different temperatures (°C), and slope, y-intercept, R^2 adjusted, and p values obtained from general linear models.

Vital statistics	Rearing temperature			Slope	y-Intercept	R^2 adjusted	p-Value
	16.6 ± 1.7	18.7 ± 1.7	25.2 ± 0.3				
<i>Development time</i>							
Larva 1 + 2	7.22 ± 0.15	5.82 ± 0.21	3.15 ± 0.13	-0.45	14.4	0.97	4.2×10^{-07}
Larva 3	5.52 ± 0.82	4.6 ± 0.71	1.63 ± 0.29	-0.46	13.13	0.73	0.002
Larva 4	7.61 ± 0.9	4.74 ± 0.12	2.66 ± 0.27	-0.52	15.5	0.72	0.002
Total larva	20.35 ± 1.77	15.16 ± 0.39	7.44 ± 0.23	-1.43	43.34	0.88	1×10^{-4}
Pupa	3.21 ± 0.2	1.62 ± 0.48	1.8 ± 0.07	-0.13	4.97	0.43	0.03
Larva + pupa	23.56 ± 0.2	16.77 ± 1.96	9.24 ± 0.2	-1.55	47.94	0.85	0.0004
<i>Survival</i>							
Larva 1 + 2	89 ± 0.33	99 ± 0.67	100 ± 0.0	0.01	0.75	0.45	0.03
Larva 3	74 ± 8.29	99 ± 0.67	99 ± 0.83	0.02	0.44	0.29	0.08
Larva 4	76 ± 10.04	93 ± 4.37	94 ± 2.2	0.02	0.53	0.15	0.16
Total larva	38 ± 16.29	91 ± 4.37	93 ± 2.2	0.05	-0.27	0.31	0.07
Pupa	94 ± 0.67	89 ± 1.33	95 ± 1.44	0.004	0.85	0.08	0.24
Larva + pupa	32 ± 15.76	80 ± 3.06	88 ± 3.63	0.05	-0.43	0.41	0.04

development rate as function of temperature was also significant ($R^2 = 0.98$; $p \leq 0.05$), and estimated development thermal threshold was 10.9 °C (Fig. 1B). The physiological time calculated for this species was 136.9 degree-days above the threshold.

4. Discussion

In this study we examined different fluctuating thermal profiles registered in Córdoba city, and evaluated its effect on immature life history traits of *Ae. aegypti* and *Cx. quinquefasciatus*. Our results show a general trend for both species towards decreasing development time with increasing temperature. Domínguez et al. (2000)

observed a similar trend for *Ae. aegypti* population in Córdoba city, reared under field conditions for one year (1996–1997). However, the mean development time (larva + pupa) registered by these authors were higher (19.9 days at 18.9 °C; 14.8 days at 20.7 °C; 11.8 days at 22.1 °C) than those observed for *Ae. aegypti* in the present study. Carrington et al. (2013b) showed that temperature effects on *Ae. aegypti* immature and adult life history traits depend on the combination of mean temperature and magnitude of fluctuations. While mean temperature rearing conditions were similar between studies (as well as the larval density, and type and amount of food provided), the amplitude of fluctuation for each temperature profile was not reported by Domínguez et al. (2000). Instead they

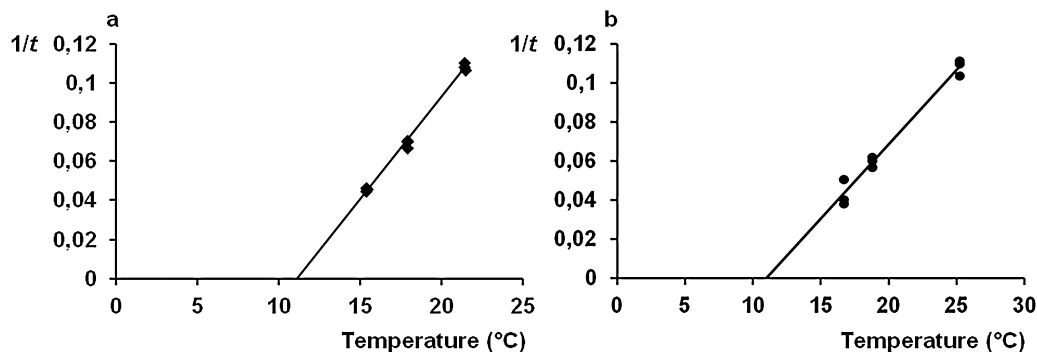


Fig. 1. Development rate ($1/t$) of *Aedes aegypti* (A), and *Culex quinquefasciatus* (B) reared under different temperature conditions.

only provided the temperature range for the whole study period of 12.8 °C (mean minimum: 13.2 °C, mean maximum: 26.1 °C). The smaller water volume (300 ml) used by these authors under field rearing conditions may suggest that these cohorts could have experienced greater water temperature variations than in our study. Tun-Lin et al. (2000) also observed that development under field conditions was generally slower, and the development rate had greater variability than under laboratory conditions, especially in smaller containers where temperature fluctuation is stronger. However, Carrington et al. (2013b) recently found that large fluctuations in temperature (amplitude of fluctuation: 18.6 °C) usually produce a decrease in *Ae. aegypti* larval development times at low mean temperatures (16 °C). The total development time for *Ae. aegypti* cohorts performed during the warmest months in the present study was similar to those observed for *Ae. aegypti* populations under semi-controlled conditions in the provinces of Misiones (8 days; 25.6 °C) (Tejerina et al., 2009), and Córdoba (9.3 days; 22.9 °C) (Grech et al., 2010).

In this study, the development of larvae and pupae of *Cx. quinquefasciatus* was also affected by different rearing temperatures. However, the *Cx. quinquefasciatus* development times were longer than those observed for *Ae. aegypti* under similar, but not simultaneous, temperature conditions. Contrary to the results obtained here, under constant laboratory temperatures, Rueda et al. (1990) reported that development time of *Ae. aegypti* were longer than those of *Cx. quinquefasciatus* for all instar larvae and pupae, being the greatest difference at the lowest rearing temperature (15 °C). Comparing the results obtained by Rueda et al. (1990) and those of the present study for the lowest rearing temperature condition their mean development times for *Ae. aegypti* (55.3 days) and *Cx. quinquefasciatus* (31.4 days) were 33.3 and 7.8 days, respectively, longer than those obtained here at small fluctuating temperature regimes. Carrington et al. (2013b), however, have found that there was no difference between *Ae. aegypti* development time obtained at small daily temperature fluctuations (amplitude of fluctuation: 7.6 °C) around low mean temperature (16 °C), and those at constant temperature regimes. Thus, the differences between constant (Rueda et al., 1990) and small fluctuating temperature regimes (present study) may be due to the effect of other factors such as larval density, size of rearing container, water volume and food, that could have influenced the results.

Low temperatures (15 °C) had a greater impact on *Ae. aegypti* overall survival because larvae 1 + 2 were killed by them. On the other hand, temperatures of 25 °C produced the highest larvae 4 and pupae mortality. The highest survival of larvae and pupae of this species was observed at 21.6 °C, however, this rearing temperature was lower than the temperature (25.3 °C) at which the total development time was fastest. For *Cx. quinquefasciatus* it has been recorded the highest survival and total development time values at the highest rearing temperature (25.2 °C). No significant differences were found in the effect of temperature on larval–pupal survival of *Ae. aegypti*, and larval and pupal survival of *Cx. quinquefasciatus*, even when the differences between survival values for the lowest and highest temperature rearing conditions were higher. The lack of significance may reflect large variability between the three cohorts, detected at 25.3 and 16.6 °C, for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Comparing our results with those of Rueda et al. (1990), larval–pupal survival values of both species were similar at 15–25 °C temperature range. Also, and according with our results, the highest survival reported by these authors were at 20 °C for *Ae. aegypti* (91.8%), and 25 °C for *Cx. quinquefasciatus* (90.3%). Only for *Ae. aegypti*, at the lowest temperature condition, survival values obtained by Rueda et al. (1990) (3%) were lower and differed from those of the present study (26%).

Although larval–pupal survival values obtained in this study at the lowest rearing temperatures (winter months in Córdoba city)

decreased until 26 and 32% for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively, both species were able to complete their development from first instar to adult emergence. However, these results do not imply that under field condition in Córdoba city these species can survive the winter season as adult and larvae. Whereas the presence of *Cx. quinquefasciatus* have been reported during the whole year (Almirón and Brewer, 1995; Grech et al., 2013); adults of *Ae. aegypti* cannot survive the adverse conditions and persist as eggs (Domínguez et al., 2000). The decline in mosquito activity by mid autumn was associated with a decrease in temperature. It seems that temperatures below the ovipositional thermal threshold of 17 °C reported for *Ae. aegypti* in Córdoba city (Domínguez et al., 2000), as well as in other temperate areas of Argentina (Campos and Maciá, 1996; Vezzani et al., 2004), can negatively affect its oviposition activity.

The estimated temperature development threshold in this study for *Ae. aegypti* (11.1 °C) was slightly lower than that obtained previously by Domínguez et al. (2000), for this mosquito species in Córdoba city. Other authors have been previously reported for this species temperature threshold value of 8.3 °C in Australia (Tun-Lin et al., 2000), and 13.3 °C in Israel (Bar-Zeev, 1958). Whereas, the development thermal threshold obtained here for *Cx. quinquefasciatus* was 10.9 °C, and differed from those obtained for *Cx. pipiens quinquefasciatus* of 9.5 °C in Córdoba city (Almirón and Brewer, 1996), and for *Cx. pipiens* of 5 °C in Buenos Aires city (this last species is closely related to *Cx. quinquefasciatus* belonging to the *Culex pipiens* L. Complex) (Loetti et al., 2011). In *Cx. quinquefasciatus* populations from New Zealand and Fiji Islands the estimated temperature development threshold was near 10 °C (Mogi, 1992).

The estimation of physiological time allows understanding the population dynamics of ectothermic organisms. The insect emergency is likely to occur when there were accumulated enough degree-days required for each species from the start of the development (Clements, 1992; Service, 1993). In the present study, the estimated *Ae. aegypti* physiological time was 93.7 degree-days, and differed from 181.2 degree-days observed in *Ae. aegypti* population from Australia (Tun-Lin et al., 2000). In temperate regions of Argentina, the *Ae. aegypti* seasonal activity begins after low winter temperatures, with larvae that probably hatched from eggs laid in the previous reproductive season. New adults emerging from these immature stages then begin the ovipositional activity (Campos and Maciá, 1996; Domínguez et al., 2000; Vezzani et al., 2004). Thus, in Córdoba city according with our results, once the ambient temperature has become greater than thermal threshold (11.1 °C), it will be necessary 93.7 degree-days, to complete successfully the larval–pupal development, and observe the emergence of adult of *Ae. aegypti* at the beginning of the season. For *Cx. quinquefasciatus* according to the estimated physiological time in this study (136.9 degree-days), this species will require more degree-days over its thermal threshold than *Ae. aegypti*, to observe the emergency of adult in the city. Loetti et al. (2011) estimated for *Cx. pipiens* population from Buenos Aires city a physiological time of 186.5 and 199.5 degree-days, for males and females, respectively. Whereas, Mogi (1992) estimated a lower value for *Cx. quinquefasciatus* population from New Zealand and Fiji Islands, of 139 degree-days for males and 150 degree-days for females.

The differences in the estimated thermal threshold values obtained here for *Ae. aegypti* and *Cx. quinquefasciatus* and those obtained by other authors, could be due to differences in the models applied to describe the relationship of developmental rate and temperature. There are available linear or many nonlinear models (e.g. Brière et al., 1999; Logan et al., 1976; Sharpe and DeMichele, 1977), that have different number of ecological parameters, and provide an upper and lower estimated developmental threshold. In the present study we applied a linear model like most of other authors (Almirón and Brewer, 1996; Bar-Zeev, 1958; Domínguez et al.,

2000; Mogi, 1992; Tun-Lin et al., 2000), and differed from that non-linear model proposed by Brière et al. (1999), and applied by Loetti et al. (2011). Moreover, despite the model used by each author, the development rate depend on development time, which in turn depend on experimental rearing conditions like constant or fluctuating temperature regimens, laboratory or field conditions, larval density, type and amount of food provided, and presence of predators. All these factors and its interactions could have influenced the estimated thermal thresholds in previous works. Regarding to the differences in the estimated physiological time, this parameter also depend on the development threshold considered by each author, and could be influenced by the factors mentioned above.

The results of this study show that small fluctuating temperature regimen affects the larval–pupal immature traits of *Ae. aegypti* and *Cx. quinquefasciatus* populations from Córdoba city. A general trend for both species was observed towards decreasing development time and increasing survival values with increasing temperature. Compared with other studies, our results show differences in life statistics, and also in temperature development threshold and physiological time, all of them useful parameters for the development of mosquito management models. This study was performed under semi-controlled temperature condition; however it would be interesting to repeat this study under wild conditions. Studies under semi-controlled conditions or at constant temperatures regimes in the laboratory are more difficult to interpret and discuss the real impact of temperature on life-history traits, and also on DENV and SLEV transmission, with regard to field conditions. Given that changes in temperature could affect mosquito adult parameters like survival, longevity and fecundity, and therefore the viruses they transmit, it would be also necessary from an epidemiological point of view, to evaluate the effect of temperature fluctuations on the adult stage in Córdoba city.

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