

Temperature and photoperiod effects on dormancy status and life cycle parameters in *Aedes albopictus* and *Aedes aegypti* from subtropical Argentina

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Abstract. *Aedes albopictus* (Diptera: Culicidae) distribution is bounded to a subtropical area in Argentina, while *Aedes aegypti* (Diptera: Culicidae) covers both temperate and subtropical regions. We assessed thermal and photoperiod conditions on dormancy status, development time and mortality for these species from subtropical Argentina. Short days (8 light : 16 dark) significantly increased larval development time for both species, an effect previously linked to diapause incidence. *Aedes albopictus* showed higher mortality than *Ae. aegypti* at 16 °C under long day treatments (16 light : 8 dark), which could indicate a lower tolerance to a sudden temperature decrease during the summer season. *Aedes albopictus* showed a slightly higher percentage of dormant eggs from females exposed to a short day, relative to previous research in Brazilian populations. Since we employed more hours of darkness, this could suggest a relationship between day-length and dormancy intensity. Interestingly, local *Ae. aegypti* presented dormancy similar to *Ae. albopictus*, in accordance with temperate populations. The minimum dormancy in *Ae. albopictus* would not be sufficient to extend its bounded distribution. We believe that these findings represent a novel contribution to current knowledge about the ecophysiology of *Ae. albopictus* and *Ae. aegypti*, two species with great epidemiological relevance in this subtropical region.

Key words. adaptation, development, distribution, dormancy, mortality.

Introduction

Aedes albopictus and *Aedes aegypti* are the main vectors of dengue, yellow fever, Zika and other arboviruses of great impact

on human health (Christophers, 1960; Soper, 1967; Rezza, 2012; Ferreira-de-Brito *et al.*, 2016; Amraoui *et al.*, 2018). Unlike *Ae. aegypti*, in South America *Ae. albopictus* remains bounded to subtropical areas (Schweiggmann *et al.*, 2004;

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Lizuain *et al.*, 2019) and to date, the causes that prevent the species expansion to southern locations remain unclear.

Environmental factors can affect life-history traits in insects, e.g. development time, survival, fecundity, etc. (Bale, 2002; Chown & Nicolson, 2004; Schowalter, 2011). In the case of mosquitoes, particularly for *Ae. aegypti* and *Ae. albopictus*, temperature represents one of the most frequently studied factors regarding its effects on life cycle parameters (Rueda *et al.*, 1990; Alto & Juliano, 2001; Delatte *et al.*, 2009; Brady *et al.*, 2013; De Majo *et al.*, 2016). Overall, development time is inversely affected by temperature and thermal thresholds (Rueda *et al.*, 1990; Tun-Lin *et al.*, 2000; Farjana *et al.*, 2012; Couret *et al.*, 2014). Survival also is influenced by temperature conditions. The optimal survival temperatures (Tun-Lin *et al.*, 2000; De Majo *et al.*, 2019) usually differing from those associated with higher development rates, suggesting a critical relationship between temperature and the life cycle (Bayoh & Lindsay, 2004). Although photoperiod tends to be overlooked when analysing environmental conditions, it represents another critical factor that may influence the life history and survival of mosquitoes (Costanzo *et al.*, 2015). Day length, which is closely related with temperature (Vinogradova, 2007; Diniz *et al.*, 2017), provides information about seasonal changes that mosquitoes receive and process, triggering changes in development, growth and behaviour, or inducing diapause (Yee *et al.*, 2012; Lacour *et al.*, 2014; Armbruster, 2016). Several studies have established the relationship between the short day length and the beginning of the dormancy process (Kostál, 2006) and other effects of day length have been observed on development time, survival and other life-history traits (Yee *et al.*, 2012; Costanzo *et al.*, 2015; Ukubuiwe *et al.*, 2018).

Dormancy is defined as a physiological state of suspended development or suppressed metabolic activity in an organism (Diniz *et al.*, 2017). Different types of dormancy, such as diapause and quiescence have been described for *Ae. albopictus* and *Ae. aegypti*, respectively. These physiological traits usually occur at different stages of the life cycle (Bradshaw & Lounibos, 1972; Vinogradova, 2007) and can be triggered by specific environmental signals or conditions (Kostál, 2006). Photoperiod for temperate culicids and relative humidity for tropical culicids are diapause stimuli that allow them to survive under unfavourable conditions (Bradshaw & Lounibos, 1972; Vinogradova, 2007). The study of these types of dormancy is, therefore, crucial for understanding the geographical distribution of these species and their current boundaries.

Worldwide, *Ae. albopictus* can be found in both tropical and temperate regions. This wide distribution can be explained by the existence of two natural lineages with variable ability to perform diapause (Lacour *et al.*, 2014).

In order to survive sub-zero temperatures during the winter in temperate areas, *Ae. albopictus* can develop different adaptations such as photoperiodic sensitivity, cold-hardiness and diapause (Hawley *et al.*, 1987). In the United States, *Ae. albopictus* was first detected in southern and warmer areas. Since then, it expanded its range northward and eastward to temperate regions (Moore, 1999). The occurrence of diapause responses detected shortly after the introduction of the species suggested that North American populations of *Ae. albopictus* might have originated from temperate Japan (Hawley *et al.*, 1987). In the case of

Florida (U.S.A.), the species entered the state from the temperate north in 1986 and it expanded to the south, colonizing tropical areas (Moore & Mitchell, 1997). Although it was first claimed that the ability to generate diapause was lost in Florida populations after they had adapted to subtropical conditions (Craig, 1993), subsequent studies showed that all populations could express this trait after exposure to short-day lengths (Lounibos *et al.*, 2003).

In South America, *Ae. albopictus* populations have dispersed from tropical regions towards higher latitudes with subtropical and temperate climates (Lounibos, 2002). Previous studies using mitochondrial haplotypes suggested that South American populations of *Ae. albopictus* have originated from tropical regions (Birungi & Munstermann, 2002). Indeed, the absence of diapause in most *Ae. albopictus* specimens from Brazil supports a possible tropical origin for these populations (Lounibos, 2002). However, two *Ae. albopictus* populations from southern Brazil were able to generate diapause in a small but significant percentage of eggs (Lounibos *et al.*, 2003). Selection for diapause expression has therefore undertaken opposite directions in North and South America, allowing adaptive evolution from temperate to subtropical regions in the United States and from tropical to temperate areas in Brazil (Lounibos, 2002). In this case, the diapause response was directly associated with latitudinal and temperature conditions. *Aedes albopictus* females from northern and warmer locations such as Rio de Janeiro and Sao Paulo states did not respond to diapause-inducing photoperiods. On the other hand, a slight percentage of eggs laid by females from southern and temperate locations such as Santa Catarina state (26.9 and 27.7°S latitude) entered diapause in response to short day lengths (Lounibos *et al.*, 2003).

Aedes aegypti populations can prolong egg viability, generating a state of embryonic quiescence (Diniz *et al.*, 2017). This strategy allows eggs to survive lower temperatures during the winter and has contributed to the expansion of the species from subtropical to temperate regions in Argentina (Vezzani & Carbajo, 2008; Zanotti *et al.*, 2015). A recent study suggested that local *Ae. aegypti* specimens from the temperate city of Buenos Aires can inhibit egg hatching when parents were exposed to short-day photoperiods (Fischer *et al.*, 2019).

To date, the ability of *Ae. albopictus* populations from Argentina to generate any dormancy status (diapause or quiescence) has not been addressed. Intense movement between its current distribution range and southern areas would allow passive transportation of immature stages to higher latitudes. However, its distribution range remains bounded to a small area in the northeastern province of Misiones for reasons that have not been discerned. Analysing the effects of environmental conditions, resources and interactions with other species are crucial to determine barriers for geographic distribution and expansion. In addition, given recent findings suggesting that *Ae. aegypti* populations from temperate Argentina can reduce egg hatching response when parents are exposed to short-day lengths, it is highly relevant to evaluate this trait in other Argentinian populations.

The aim of this work was to assess the ability of *Ae. albopictus* and *Ae. aegypti* from subtropical Northeastern Argentina to undergo dormancy under different photoperiod and temperature

conditions. In addition, we aimed to determine the effect of these conditions on development time and mortality for both species.

Material and methods

Experimental colony and egg collection

Immature stages of *Ae. albopictus* and *Ae. aegypti* were collected from 40 households in the rural village of Colonia Aurora (27°28'29"S, 54°31'28"W) (Misiones, Argentina) during January 2019. The study area was chosen because it is one of the few localities in the region where *Ae. albopictus* is established and coexists with *Ae. aegypti* (Lizuain *et al.*, 2019).

The experiments were carried out in the insectarium of the Instituto Nacional de Medicina Tropical (INMeT) located in Puerto Iguazú City (Misiones Province) where *Ae. albopictus* is already located to avoid accidental dispersal.

Larval and pupal samples were reared under controlled laboratory conditions ($27 \pm 2.4^\circ\text{C}$, 75% RH and photoperiod 14 light : 10 dark) in acrylic containers (5 cm in height \times 10 cm in diameter) with 100 mL of distilled water. Larval samples were fed a 10% suspension of powdered baker's yeast. Once the pupae were obtained, they were separated individually in small containers until adult emergence. Species determination was performed under a stereoscopic microscope and specimens were placed in different cages (30 cm \times 30 cm \times 30 cm) in order to generate the founding colonies. Adult mosquitoes were fed *ad libitum* with a 10% sugar solution.

Three days after mating each colony was offered a blood meal and sites for oviposition. Blood, obtained from the volunteers of our research team under medical supervision, was provided using an artificial feeder constructed using a 50 mL Falcon tube with 5 mL of heparinized blood (5000 IU/mL) at 37°C with the tube opening covered with a pig gut membrane. The device was placed upside down on the tulle fabric roof of the cages in order to allow females to feed. The oviposition sites consisted of cylindrical acrylic containers (5 cm in height \times 4 cm in diameter) internally covered with cardboard and filled with 2 mL of distilled water. Cardboards with eggs were removed daily and observed under a stereoscopic microscope. Viable eggs were stored under laboratory conditions ($27 \pm 2.4^\circ\text{C}$, 75% RH and photoperiod 14 light : 10 dark).

Photoperiod and temperature treatment

All collected egg batches were flooded with distilled water to obtain first instars for each species. Larval samples were randomly placed in groups of five individuals per cylindrical acrylic container (5 cm in height \times 4 cm in diameter) with 20 mL of distilled water (experimental unit). Twenty experimental units (800 first instar) were randomly selected per treatment, which consisted of factorial design for both species with two temperatures and two different photoperiods. We selected temperatures and LD lengths that resembled natural conditions observed in the study area during the summer: long photoperiods of 16 light : 8 dark (LD) and $27 \pm 2.2^\circ\text{C}$. Summer conditions were determined by effective daylight hours in the area, from morning to evening

twilight. For winter conditions, we applied the inverse ratio of light/dark hours that was used for summer conditions in order to force the response to that stimuli in both species: short photoperiods of 8 light : 16 dark (SD) and $16 \pm 2.3^\circ\text{C}$. Larval specimens were placed in Styrofoam incubators (40 cm \times 40 cm \times 100 cm) equipped with a water cooling system and thermostats to achieve the selected temperature. Illumination was provided by 36-watt bulbs regulated by electronic timers for each photoperiodic condition. Each experimental unit was supplied with 1 mL of powdered baker's yeast suspension (0.5 g/100 mL) every 48 h until reaching the pupal stage.

Once specimens reach the pupal stage, they were separated within the same incubator in individual containers and covered until emergence. Once emerged, adults were then transferred to 0.005 m³ tulle fabric cages placed inside the incubators and were fed with a 10% sugar solution *ad libitum*. The cages were inspected daily through an acrylic window and after mating was observed, they were offered a daily blood meal and sites for oviposition as previously described. Eggs were removed daily, counted and then stored for 48 h under laboratory conditions ($27 \pm 2.4^\circ\text{C}$, 75% RH and day-lengths of 14 light : 10 dark) to ensure the complete development of the embryos.

Larval hatching for each treatment was examined simultaneously. Egg cardboards were placed in plastic trays and flooded with distilled water at room temperature and 2 mL of 10% yeast suspension to induce hatching. Twelve hours later, the number of first instars was recorded for each treatment. Unhatched eggs were separated, stored for 48 h and flooded one more time as previously described. Finally, eggs that did not hatch during either flooding were bleached with a 50% solution of commercial sodium hypochlorite to evaluate the presence or absence of embryos by direct observation (Fischer *et al.*, 2019). Creamy-white embryos with visible eyes, abdominal segmentation and a hatching spine were considered alive (Farnesi *et al.*, 2009). Hatching percentage was calculated for each treatment as the number of first instars divided by the total number of viable eggs (with embryo alive).

In order to study the development time and mortality of each species under different conditions, larval specimens in each container were examined daily for moulting (determined by the presence of exuviae) and the number of larvae of each instar was registered. Mortality was only assessed at the end of the experiment. Development time was defined as the number of days from the first instar to adult.

Data analysis

Development time was analysed with general linear models (GLM), by maximum likelihood method, using R Version 3.4.0 (R Core Team, 2017), in interface with the Infostat software (Di Rienzo *et al.*, 2017). When necessary, the Fisher LSD test was used as an *a posteriori* test (Kuehl, 2001). For mortality analysis, all individuals of the same treatment were grouped and homogeneity tests (Chi-square tests) were performed to compare between treatments and between sex ratios (Zar, 1996). Then, with GLM and *a posteriori* tests (Fisher LSD test), the number of dead individuals per container was analysed as a response

Table 1. Hatching response proportion in relation to factor levels photoperiod and temperature in *Aedes aegypti* and *Aedes albopictus* larvae.

Factor	Fixed factor	Hatching response	P-value
Photoperiod	AB – 27 °C	LD: 0.99 (82) vs. SD: 0.80 (205)	<0.0001
	AE – 27 °C	LD: 0.97 (251) vs. SD: 0.85 (380)	<0.0001
Species	LD – 27 °C	AB: 0.99 (82) vs. AE: 0.97 (251)	0.4617
	SD – 27 °C	AB: 0.80 (205) vs. AE: 0.85 (380)	0.1056
Temperature	AE – LD	27 °C: 0.97 (251) vs. 16 °C: 0.80 (114)	<0.0001

In brackets, the number of flooded eggs is indicated. Fisher's exact test to compare proportions.

AB, *Aedes albopictus*; AE, *Aedes aegypti*; LD, long day (16 L : 8 D); SD, short day (8 L : 16 D).

variable for each treatment. The final means of each treatment were divided by five to express them as a percentage of mortality.

To evaluate the effect of the treatment on larval hatching response, a Chi-square Test of Homogeneity was first carried out taking the total number of hatched larvae over the total flooded live eggs (Zar, 1996). Then, to compare between levels within a factor (e.g. temperature), an exact Fischer test was performed (Kuehl, 2001), fixing the other two factors (i.e. species and photoperiod). Alphas were set at 0.05 in all statistical tests.

Results

Hatchability

Although 37 and 54 specimens (among females and males) of *Ae. aegypti* and *Ae. albopictus*, respectively, reached adult stages, no eggs were obtained from individuals reared under 16 °C and SD photoperiod.

The homogeneity test indicated that the hatching response was dependent on the type of treatment (chi-square test: $\chi^2 = 56.07$; GL = 5; P -value <0.0001).

For both species, the hatching response for larvae obtained from individuals reared in SD lengths was lower with respect to larvae reared under LD lengths at 27 °C. The difference in hatching between SD and LD conditions was 19% for *Ae. albopictus* and 12% for *Ae. aegypti* (Table 1).

For LD lengths, hatching response was higher for the first flooding when compared to the second (average approx., 1st: 82% vs. 2nd: 10%) for both rearing temperatures. Conversely, for SD length, the hatching response was higher during the second flooding with respect to the first (average approx., 1st: 22% vs. 2nd: 60%, Fig. 1). No significant differences between species were observed at 27 °C, for any photoperiod (Table 1). Because only 38 eggs were obtained for *Ae. albopictus* at 16 °C and LD photoperiod, it was not possible to perform a comparative analysis with *Ae. aegypti* under these conditions.

For LD lengths the hatching response in *Ae. aegypti* was higher at 27 °C (97%) than at 16 °C (80%) (Table 1). For *Ae. albopictus* we could not analyse differences between rearing temperatures due to the low number of eggs obtained at 16 °C ($n = 38$).

Development time

At 16 °C, development time for *Ae. albopictus* was slower under SD (28 ± 2.5 d) than under LD (22.8 ± 2.3 d) conditions

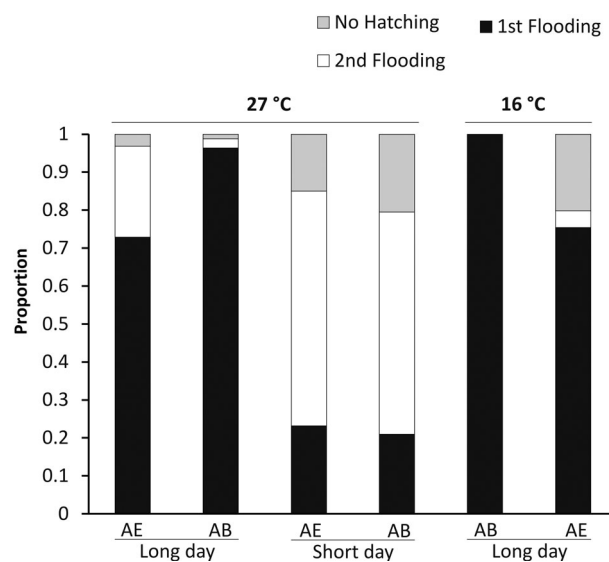


Fig. 1. Total proportion of hatching in the first and second flooding event and larvae not hatched for each treatment of photoperiod and rearing temperature. AE, *Aedes aegypti*; AB, *Aedes albopictus*.

(Fig. 2). Under higher temperatures (27 °C) a similar pattern was observed: development time was significantly longer under SD lengths (9.9 ± 1.8 d) with respect to development time under LD conditions (8.2 ± 0.5 d) (Fig. 1). At 16 °C, *Ae. aegypti* showed slower development times under SD conditions (23.9 ± 2.9 d) with respect to LD lengths (20.6 ± 3.5 d). At 27 °C, the same trend was observed for this species with 10.3 ± 1.7 d for SD and 7.8 ± 1.5 d for LD lengths (Fig. 2).

In *Ae. albopictus*, GLM analysis of development times showed significant interaction between photoperiod and temperature (F -value = 10.11; $df = 1$; P -value = 0.0023). Therefore, the treatments were compared with *a posteriori* test (Fisher LSD test) that globally showed significant differences. For both temperatures, SD extended the development time with respect to LD (LD) (Table 2), with a difference of almost 5 days at 16 °C and approximately 2 days at 27 °C.

In *Ae. aegypti*, GLM analysis of development times showed no interaction between photoperiod and temperature. The main effects were significant (Table 3), with shorter development times under LD (12.8 ± 6.8 d) than under SD (16.7 ± 7.3 d).

For both species, development times at 27 °C were shorter than at 16 °C (Fig. 2, Tables 2 and 3).

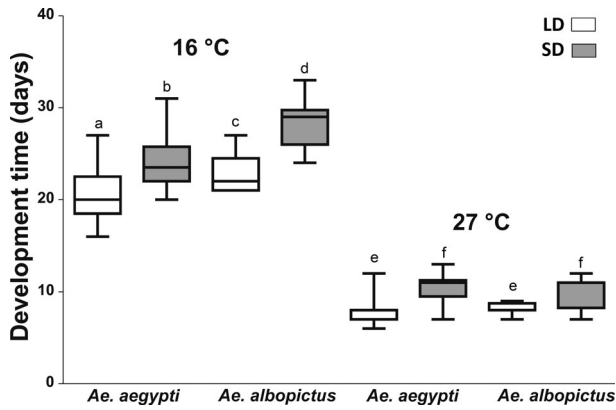


Fig. 2. Total development time for *Aedes albopictus* and *Aedes aegypti* reared under the short day (SD) and long day (LD) and two temperature conditions, 16 and 27 °C. Different letters above the box-plot indicate significant differences.

Table 2. Development time (days) of *Aedes albopictus* by photoperiod and temperature.

Treatment	n	Mean	SE	
Photoperiod × temperature				
SD-16	20	28.00	0.61	A
LD-16	20	22.75	0.86	B
SD-27	20	9.85	0.30	C
LD-27	20	8.20	0.30	D

Rearing temperature: 16 °C (16) and 27 °C (27). Fisher LSD test to compare the means, different letters indicate significant differences ($P < 0.05$).

SD, short day; SE, standard error; LD, long day treatment; n, replicate number.

Table 3. Development time (days) of *Aedes aegypti* for temperature, photoperiod and their interactions.

Factor	df	F-value	P-value
(Intercept)	1	2174.14	<0.0001
Photoperiod	1	46.43	<0.0001
Temperature	1	422.61	<0.0001
Photoperiod × temperature	1	0.33	0.5701

df, degree freedom; GLM, general linear model.

Mortality

Mortality for both species and photoperiods was higher at 16 °C (46–87%) when compared with specimens at 27 °C (8–35%). Chi-Square Test for Homogeneity showed significant differences in the mortality between treatments ($\chi^2 = 196.6$; GL = 7; P -value < 0.0001) whereas the sex ratio was similar for all treatments ($\chi^2 = 7.56$; GL = 7; P -value = 0.3733) (Fig. 3).

For *Ae. albopictus*, the GLM showed an interaction between photoperiod and temperature (F -value = 25.5; df = 1; P -value < 0.0001). Therefore, the means of treatments were analysed using *a posteriori* contrasts tests (Table 4). At 27 °C, percentage of mortality under SD condition, 17% (0.85/5),

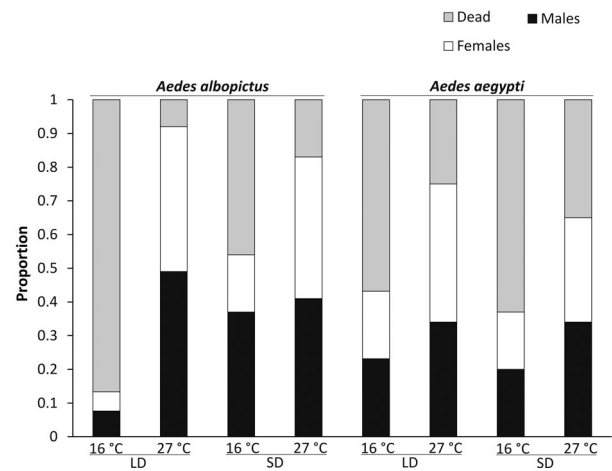


Fig. 3. Mortality proportion and emerged adults (males and females) pooled by treatment. Rearing temperature: 16 °C and 27 °C. SD, short day; LD, long day.

Table 4. *Aedes albopictus*, mean dead individuals per container (mortality) by photoperiod and temperature.

Treatment	n	Mean (%)	SE	
Photoperiod × temperature				
LD-16	20	4.33 (86.6)	0.24	A
SD-16	20	2.30 (46)	0.25	B
SD-27	20	0.85 (17)	0.25	C
LD-27	20	0.40 (8)	0.25	C

Rearing temperature: 16 °C (16) and 27 °C (27). (%): mortality percentage calculated as the mean number of deaths divided by 5 initial individuals × 100. Fisher LSD test to compare the means, different letters indicate significant differences ($P < 0.05$).

LD, long day treatment; n, replicate number; SD, short day; SE, standard error.

Table 5. Analysis of mortality of *Aedes aegypti* for photoperiod and temperature and their interactions.

Factor	df	F-value	P-value
(Intercept)	1	214.63	<0.0001
Photoperiod	1	1.92	0.1695
Temperature	1	23.88	<0.0001
Photoperiod × temperature	1	0.10	0.7544

GLM, general linear model; df, degree freedom.

showed no significant differences ($P > 0.05$) with respect to LD conditions, 8% (0.4/5). In contrast, at 16 °C mortality was significantly higher under LD than under SD conditions: 87% (4.33/5) vs. 46% (2.3/5), respectively (Table 4). In addition, for both photoperiods mortality was higher at 16 °C when compared to 27 °C (Table 4).

In *Ae. aegypti*, the interaction between photoperiod and temperature was not significant. The temperature was a determining factor (Table 5), showing higher mortality at 16 °C (3/5; 60%) with respect to 27 °C (1.5/5; 30%).

Discussion

In this work, we evaluated for the first time, the effect of different photoperiod and temperature conditions on dormancy induction, development time and mortality of immature stages, in *Ae. albopictus* and *Ae. aegypti* from Northeastern Argentina.

Hatchability and dormancy status

Hatching response in *Ae. albopictus* under SD (8 light : 16 dark) and LD (16 light : 8 dark) lengths showed a difference of 19%. This is consistent with a previous study using *Ae. albopictus* populations from similar latitudes in Brazil, where minimum differences (3.4%) between hatching response under SD (10 light : 14 dark) and LD (14 light : 10 dark) stimuli were found (Lounibos *et al.*, 2003). Greater percentage differences found in our study could be explained because in our experiments, we used more contrasting photoperiod conditions than the ones employed by Lounibos *et al.* (Lounibos *et al.*, 2003). This could suggest a possible direct relationship between dormancy intensity (measured as hatching response) and day length (Pumpuni *et al.*, 1992), although different photoperiodic conditions should be tested.

In the case of *Ae. albopictus* it has been shown that diapause is well developed for populations of temperate origin and less likely for those of tropical origin (Lounibos *et al.*, 2003). Furthermore, the critical photoperiod for diapause induction and intensity varies with latitude and may be influenced by temperature and larval rearing condition (e.g. diet) (Pumpuni *et al.*, 1992; Yee *et al.*, 2012). A tropical origin has been proposed for Brazilian populations of *Ae. albopictus* (Birungi & Munstermann, 2002). Although we cannot assume that *Ae. albopictus* from Northeastern Argentina share a common origin with Brazilian populations, an independent introduction from temperate populations into this area seems unlikely. Our results support the tropical origin of Argentinean specimens given that dormancy was much less extensive than in North American populations. Future studies involving genetic samples should be performed in order to confirm this hypothesis. It remains unclear whether diapause has arisen *de novo* in Brazilian (or Argentinian) *Ae. albopictus* from non-diapausing tropical ancestors (Lounibos *et al.*, 2003).

The process of diapause involves a metabolic energetic cost (Kostál, 2006; Denlinger & Armbruster, 2014), therefore, requiring a strong selective pressure to acquire it, whereas the reversal or loss of this physiological trait would be less restricted. Therefore, it seems more likely that the trait has been retained in *Ae. albopictus* populations with a tropical origin, expressing it at low intensities, than for it to reemerge after being lost.

For *Ae. albopictus* a crucial question remains whether environmental conditions such as temperature and photoperiod are responsible for its bounded distribution in Argentina. Here, we have demonstrated that local populations are able to induce dormancy in eggs, which could allow them to survive in temperate areas, although we cannot assume that this response would be automatically triggered under such temperature and photoperiodic conditions. Although in Germany it has been observed that

cold hardiness of *Ae. albopictus* eggs are dependent on the geographic origin of the strain, individuals from a warmer climate had a lower tolerance against low temperatures than those from cooler climates, and all were able to survive ordinary winters in the area (Tippelt *et al.*, 2019). Therefore, even for *Ae. albopictus* populations of tropical origin, such as our population from Northeastern Argentina, the temperature may not act as a limiting factor for expanding its distribution to temperate areas.

Other environmental conditions not evaluated in this study such as relative humidity could also play an important role in restricting the species distribution. This environmental factor is closely linked to the induction of diapause or quiescence and consequently the hatching response of larvae (Judson, 1960). For *Aedes*, resistance to desiccation as eggs plays an important role in survival. For example, *Aedes* species inhabiting forests have been shown to be less resistant to changes in humidity with respect to urban species (Diniz *et al.*, 2017). In subtropical Argentina, *Ae. albopictus* has been recorded in greater abundance in rural villages with abundant vegetation when compared to urban areas (Lizuain *et al.*, 2019). It is possible that the humidity associated with these landscapes and their effect on egg desiccation could play an important role in the limited distribution of the species (Sota & Mogi, 1992; Charlwood *et al.*, 2000). Resistance to egg desiccation in *Ae. albopictus* could be lower in specimens of tropical origin such as Brazilian (Lounibos *et al.*, 2003) or specimens from Northeastern Argentina with respect to temperate specimens. Intraspecific differences in egg resistance to desiccation also were observed in *Ae. riversi* and *Ae. flavopictus*, in which strains from subtropical regions showed lower viability than strains from temperate regions (Sota & Mogi, 1992). This would limit not only their ability to colonize a non-native habitat with unfavourable humidity conditions but also their ability to survive long-distance transport in the event of passive dispersal (Juliano & Lounibos, 2005). Passive transport of eggs and immature stages from Northeastern Argentina to southern locations has been proposed for *Ae. aegypti* (Rondan Dueñas *et al.*, 2009). Although we cannot discard this possibility for *Ae. albopictus*, a lower abundance of this species in large urban centres in the area could also reduce its flow via human-mediated transport to southern areas. Future studies with Argentinean *Ae. albopictus* populations should introduce relative humidity within evaluated experimental conditions.

Surprisingly, *Ae. aegypti* from Northeastern Argentina showed a dormancy intensity similar to that of local *Ae. albopictus*, suggesting that both species can respond to seasonal changes in the amount of daylight hours. Previous studies suggested that *Ae. aegypti* performs a prolonged quiescence during unfavourable periods but not diapause (Denlinger & Armbruster, 2014; Diniz *et al.*, 2017). However, a decrease in hatching response due to SD photoperiod stimulation in *Ae. aegypti* eggs from a temperate area in Argentina (Buenos Aires) has recently been reported, suggesting that the species could develop a diapausic state (Fischer *et al.*, 2019). The subtropical population evaluated in this research has shown a decrease in hatching percentage from 97% to 85% in response to changes in the photoperiod. If this presumption of diapause is confirmed by future genetic-molecular studies, our results could suggest that these *Ae. aegypti* populations are also able to induce diapause in their

eggs. According to our results and those obtained by Fischer *et al.* (2019), *Ae. aegypti* could be able to develop a potential diapause in a low percentage of eggs and only have quiescence as in tropical regions (Oliva *et al.*, 2018).

Regardless of the temperature, a higher hatching response was observed during the second flooding in eggs from mothers reared under SD conditions for both species. An inverse result was observed for eggs obtained from mothers reared under LD conditions. In other words, there would be necessary conditioning (Horsfall, 1956) for individuals exposed to longer periods of darkness. Regardless of dormancy, this response could represent a strategy to avoid hatching during the first flood stimulus under occasionally favourable thermal conditions during the winter. Delayed hatching patterns are usually bet-hedging strategies that allow species to avoid elevated mortality risks (Khatchikian *et al.*, 2009, 2010). Photoperiod conditions would ensure that eggs do not hatch during unfavourable periods, first decreasing the hatching response and then requiring a second flood as an effective hatching stimulus. It has been shown that day length is a very precise indicator of seasonality, especially in temperate climates (Armbruster, 2016).

For *Ae. aegypti* reared under LD photoperiod, the percentage of hatching was higher at 27 °C (97%) than at 16 °C (80%), indicating that temperature also plays an important role as a hatching signal (Kostál, 2006; Diniz *et al.*, 2017). Variation of seasonal environmental factors (photoperiod and temperature) would have a wide effect on development, survival and reproduction for this species as has been already reported for *Ae. albopictus* (Armbruster, 2016).

It is important to point out that under experimental conditions of SD photoperiod and cold temperature (16 °C), emulating an unfavourable winter season, neither species was able to complete its life cycle. In the case of *Ae. aegypti*, the low tolerance of adults to low temperatures implies that the greatest activity of flight, bite and reproduction occurs during the non-winter period, at least for temperate regions (Vezzani *et al.*, 2004).

Development time

Development times were prolonged for both species under SD length when compared to LD conditions, regardless of the rearing temperature. The delay in development, particularly observed for the first cohort, suggests the susceptibility of final stages to photoperiodic conditions, which could represent an indicator for potential diapause in these populations (Diniz *et al.*, 2017). Our results showed that the differences between development times under different photoperiods were more pronounced under lower temperatures. Low temperatures tend to delay the development and consequently increase the exposure to SD lengths during the photosensitive stage, therefore, incrementing the incidence of diapause (Denlinger & Armbruster, 2014). On the other hand, adults reared at 16 °C tended to be larger than those reared at 27 °C (data not shown) as was previously reported for *Ae. aegypti* and other mosquito species (Rueda *et al.*, 1990; Loetti *et al.*, 2011; Garzón & Schweigmann, 2015). Larger adults resulting from a prolonged

time of development might be more successful in tolerating lower winter temperatures in case of emergence during this season (De Majo *et al.*, 2019). In addition, a developmental delay in the pre-diapause phase, e.g. immature stages, could stimulate the storage of energy reserves (Diniz *et al.*, 2017), resulting in larger adults with greater resources for the nutrition of diapausic eggs (Kostál, 2006; Costanzo *et al.*, 2015).

As expected, there was a dependence between the development time and the rearing temperature for both species (Tun-Lin *et al.*, 2000). A shorter development time for *Ae. aegypti* at 16 °C, with respect to *Ae. albopictus* could be a favourable feature for the temperate climate, allowing expansion to higher latitudes (Vezzani & Carbajo, 2008; Zanotti *et al.*, 2015) while *Ae. albopictus* maintains a bounded distribution within subtropical Northeastern Argentina (Schweigmann *et al.*, 2004; Lizuain *et al.*, 2019).

At 27 °C, no differences were detected between *Ae. aegypti* and *Ae. albopictus*, with development times of approximately 8 days for both species. This would allow them to reach early population peaks in this subtropical region due to high population growth rates (Alto & Juliano, 2001).

Mortality

For both species and photoperiodic conditions, mortality was higher at 16 °C relative to 27 °C, indicating that temperature is a critical variable for survival. It has been reported that the mortality of *Ae. aegypti* immatures increases sharply from 20 to 15 °C (Rueda *et al.*, 1990), although this relationship depends on specific adaptations (Ciota *et al.*, 2014).

Aedes albopictus reared at 27 °C showed no differences in mortality under different photoperiods. A fast development time (8 days approximately) at warm thermal conditions due to the commonly observed relation between development and rearing temperature (Rueda *et al.*, 1990; Tun-Lin *et al.*, 2000) could minimize the effect of the photoperiod. On the other hand, in *Ae. albopictus* reared at 16 °C, the LD length had a negative effect on survival with respect to SD conditions, whereas there was no effect of photoperiod on *Ae. aegypti* mortality. Similar results have been found in previous studies at 25 °C (Costanzo *et al.*, 2015). This suggests that, in *Ae. albopictus*, the rearing temperature could condition the photoperiod effect on survival, whereas *Ae. aegypti* is not affected by day length.

Conclusion

In conclusion, *Ae. albopictus* and *Ae. aegypti* from a subtropical region in Argentina seems to be able to respond to photoperiod stimuli during the development of immature stages. The minimum intensity in dormancy observed in subtropical *Ae. albopictus* with respect to temperate populations from the northern hemisphere could confirm the tropical origin of South American populations. Dormancy in *Ae. aegypti* could allow avoidance of unfavourable environmental conditions in temperate regions whereas in *Ae. albopictus* the same feature would not be sufficient for this species to colonize temperate regions. Other

environmental factors such as humidity may also play an important role in *Ae. albopictus* bounded distribution in Northeastern Argentina.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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