

# Photoperiod affects female life history traits in temperate populations of *Aedes aegypti* from South America

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In many insects, short photoperiods induce females to lay diapausing eggs, which are associated with a reduction in female fecundity, and/or with an increase in their growth rate, allowing for a larger size at adulthood. The effects of short photoperiods may also differ among populations, depending on the different selection pressures imposed by the winter conditions. Thus, this study aimed to experimentally assess the effects of short photoperiods on *Aedes aegypti*, an invasive mosquito species that also vectors several viral diseases. We compared life history parameters of the females reared from the first larval instar under contrasting photoperiods, in two populations from the temperate region of Argentina, one with a milder winter (BA) and the other with a harsher winter (SB). The results showed a significant increase in the time to oviposition, a trend to a larger size of females (significant only for SB) and higher inhibition of egg hatching (more pronounced in SB), in response to short photoperiods. No differences in fecundity were detected among treatments. The different reaction norms of the populations in female body size and egg hatching response suggest an adaptation to local conditions, which might favour a further expansion of *A. aegypti* towards colder climates.

ADDITIONAL KEYWORDS: Argentina – body size – diapause – fecundity – hatching response – photoperiod – temperate climate – yellow fever mosquito.

## INTRODUCTION

Insects have various mechanisms that favour the persistence of populations during adverse periods. One of the most common adaptations to seasonal climatic variability is the response to shortening photoperiods, which anticipates unfavourable conditions (Tauber & Tauber, 1981). In some mosquito species of the genera *Aedes* and *Psorophora*, the most common response to short photoperiods is the laying of diapausing eggs, which are inhibited from hatching even when environmental conditions are favourable (Denlinger & Armbruster, 2014). Diapausing eggs are larger (Linley & Craig, 1994, Lacour *et al.*, 2014), contain more lipids (Reynolds *et al.*, 2012), and

have a higher tolerance to extreme environmental conditions such as low humidity (Sota & Mogi, 1992; Urbanski *et al.*, 2010) or low temperature (Thomas *et al.*, 2012). These characteristics deserve scientific attention, since they could favour inter-annual persistence in regions with harsher winters, and expansion of the species distribution, which might be accompanied by the introduction of arboviral diseases to new areas.

Based on the assumption that a female has a fixed amount of resources to invest in reproduction, there should be a trade-off in the investment per individual offspring (i.e. egg size) and the number of offspring. Empirical studies indicate that this is the case in different Arthropods of the suborder Cladocera and of the orders Isopoda, Orthoptera, Heteroptera, Lepidoptera, Coleoptera and Diptera (reviewed in Fox & Czesak, 2000). According to this ‘offspring size–offspring number trade-off’ hypothesis, the larger size

†We are deeply saddened by the passing of Raúl E. Campos.

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and higher lipid content of diapausing eggs is predicted to be associated with a lower fecundity. Studies that have assessed this issue in mosquitoes are scarce and contradictory, since this hypothesis has been supported for *Aedes atropalpus* (Kalpage & Brust, 1974) but not for *Aedes albopictus* (Costanzo *et al.*, 2015).

In addition, the number of eggs a female lays is known to be related to its body size (Honek, 1993). Insects subjected to short photoperiods are predicted to shorten the development time and thus ensure emergence of the adult before the climate becomes too harsh for reproduction and survival (Nylin & Gotthard, 1998). In mosquitoes, it is not clear how these shorter development times relate to body size. In *A. albopictus*, different studies have reported either smaller (Yee *et al.*, 2012) or larger (Costanzo *et al.*, 2015) females reared under short photoperiods, as compared to those reared under long photoperiods, which might be related to differences in the experimental protocols.

The effects of short photoperiods may differ among populations, since the importance of the photoperiod might vary depending on the harshness of winter conditions. Several studies have shown that *A. albopictus* and *Wyeomyia smithii* populations from different latitudes differ in the incidence of diapause (Denlinger & Armbruster, 2014). In contrast, for *A. albopictus*, other studies have not found an effect of the geographical origin on the phenotypic plasticity in body size (Yee *et al.*, 2012).

*Aedes aegypti* is a widespread species native to tropical and subtropical regions. In recent decades the species has expanded its distribution towards temperate/cold regions of both the northern hemisphere (Kraemer *et al.*, 2019) and the southern hemisphere (Diez *et al.*, 2014; Zanotti *et al.*, 2015; Carrizo Páez *et al.*, 2016; Rubio *et al.*, 2020) of the American continent. In Argentina, the expansion of *A. aegypti* towards colder regions provides a unique opportunity to study the adaptation to new environments of this major disease vector.

Recent studies on the temperate climate population of *A. aegypti* in Argentina (Buenos Aires city) have shown eco-physiological changes in response to short photoperiods, which are consistent with egg diapause (Fischer *et al.*, 2019). The observed responses to short photoperiods experienced by the parental generation include the inhibition of egg hatching (Fischer *et al.*, 2019), and increases in the lipid content and size of the eggs (Mensch *et al.*, 2021). The effects of the short photoperiods also extend to the females born from these eggs, since they have been found to have shorter development times and be smaller than those from long parental photoperiods (Loetti *et al.*, 2021). Such responses to photoperiod had not been reported previously for *A. aegypti*, and the findings in Argentinean populations defy the accepted paradigm

that diapause is not possible in this species (Denlinger & Armbruster, 2014). Given that Buenos Aires city has relatively mild winters and that a certain proportion of larvae can complete development (De Majo *et al.*, 2017), more pronounced effects should be expected in localities with harsher winters (i.e. with stronger selection pressures).

On the other hand, studies on the effects of short photoperiods on the generation experiencing them are scarce for *A. aegypti*. For a tropical population from Miami (USA), a short photoperiod was associated with a higher predisposition to blood-feed and higher female longevity, but no effects on development time, size or fecundity were detected (Costanzo *et al.*, 2015). In addition, for a subtropical population from Argentina (Colonia Aurora, Misiones province), short photoperiods caused longer immature development times (Garzón *et al.*, 2021). However, no equivalent studies have yet been performed in populations from the temperate region of Argentina.

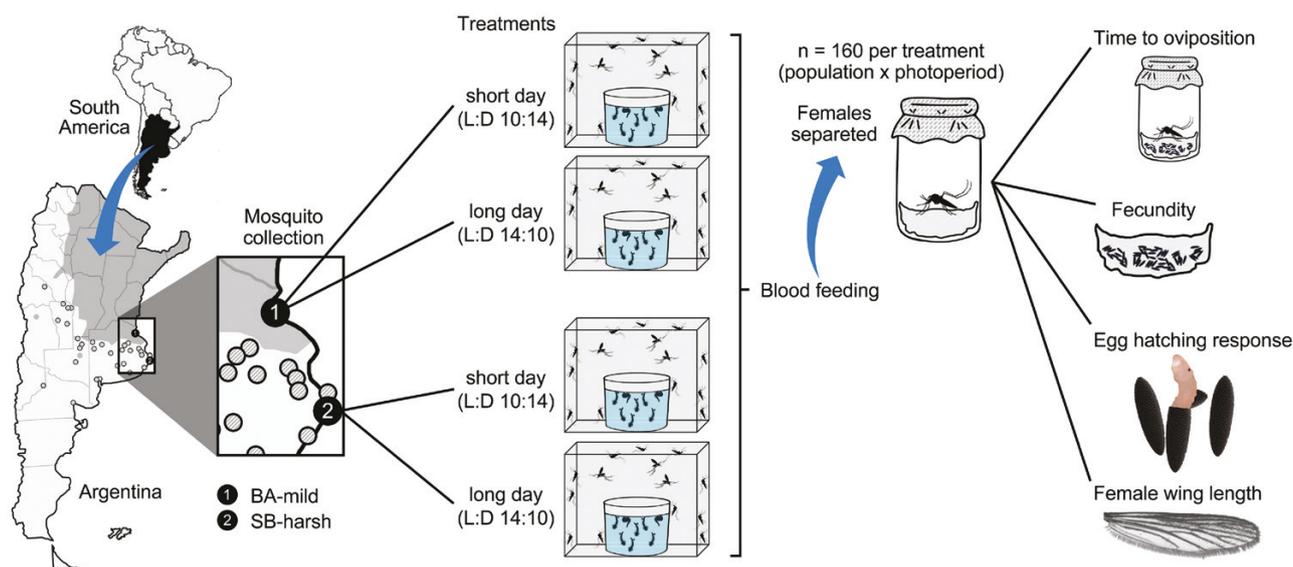
Thus, the aim of the present study was to analyse the effects of photoperiod on reproductive parameters of the female generation experiencing it, and on the ability to induce diapause in their eggs, comparing two localities with different winter harshness in the temperate region of Argentina. The hypotheses we attempted to assess were: (1) females reared under short photoperiods will take longer to lay their eggs, and either be larger or have lower fecundity than those reared under long photoperiods, to compensate for the higher energetic investment in diapausing eggs; and (2) the population from the locality with harsher winter conditions will exhibit larger differences between photoperiods in the traits analysed than the population from the locality with milder winter conditions.

## MATERIALS AND METHODS

### ORIGIN OF THE POPULATIONS STUDIED

Two populations of *A. aegypti* were studied: one collected from Buenos Aires city (BA-mild) and the other from San Bernardo (SB-harsh), both located in the temperate region of Argentina. Climate data were obtained from the National Meteorological Service of Argentina (2020). The available information comprises 30 years (1981–2010) for BA and 10 years (2001–2010) for SB.

BA-mild (34°36'13.26"S, 58°22'53.61"W) is located in north-east Buenos Aires province, on the coast of the Río de la Plata River (Fig. 1). The presence of *A. aegypti* has been recorded in BA for at least 27 years, since 1995 (Junín *et al.*, 1995). The city is part of the urban agglomeration called the Buenos Aires Metropolitan Area, which has ~13 million inhabitants in an area of 2680 km<sup>2</sup> (INDEC, 2010). The climate is temperate humid, with an average



**Figure 1.** Schematic drawing of the experimental design, with a detail of the current distribution of *Aedes aegypti* in Argentina, showing the recent expansion to colder regions in the south and south-east of the country. On the map of Argentina, grey areas represent the distribution up to 2001, and the circles with a black border represent places where this species was detected recently.

annual temperature of 18.2 °C, an absolute minimum temperature of -2.1 °C, yearly accumulated rainfall of 1237 mm and average annual humidity of 72%. Monthly average temperatures close to or lower than 12 °C extend from June to July, and thus the unfavourable period for the development of immature mosquitoes lasts about 2 months, and during this period the average temperature is 11.7 °C.

SB-harsh (36°41'10.92"S, 56°40'45.11"W) is located in south-east Buenos Aires province, on the coast of the Atlantic Ocean, where the presence of *A. aegypti* has been recorded for at least 8 years, since 2014 (Zanotti *et al.*, 2015). The location of SB is near to the limit of the southern distribution of *A. aegypti* in Argentina (Fig. 1). It is a small city of 4.3 km<sup>2</sup>, with 8133 permanent inhabitants (INDEC, 2010), whose population increases during summer because it is a tourist town. The climate is temperate oceanic, with an average annual temperature of 15.2 °C, an absolute minimum temperature of -7 °C, cumulative rainfall of 1061 mm and average annual humidity of 85%. Average monthly temperatures below 12 °C extend from June to September, and thus the period considered unfavourable for development of immature mosquitoes lasts ~4 months, and during this period, the average temperature is 10.6 °C.

#### SOURCE OF *Aedes Aegypti* EGGS

The eggs used to start the experimental colonies of BA were collected from late summer to mid-autumn

(March 15 to May 15) of 2019 with ovitraps distributed throughout the city. By contrast, the eggs for the SB colonies were laid in the laboratory by individuals collected from the field. In this case, larvae were collected in early February of 2019 from car tires, and maintained in cages at the laboratory up to the emergence of adults. Adult females were then blood-fed to obtain the eggs, which were laid between February 15 and May 15. Thus, the eggs used to start the experimental colonies were the F0 and F1 generations from individuals collected in the field in BA and SB respectively.

All eggs from both localities were stored in the dark at 18 °C, under saturation humidity, until the start of the experimental colonies.

#### EXPERIMENTAL DESIGN

A schematic drawing of the experimental design is shown in Figure 1. For the experiment, two colonies were initiated for each locality and kept at 21 °C throughout the experiment, a temperature similar to that of previous studies on *A. aegypti* (Fischer *et al.*, 2019). The colonies were maintained under different photoperiods: one with a short day (SD) of 10:14 h (light:dark) and the other with a long day (LD) of 14:10 h (light:dark). These photoperiods represent the natural conditions corresponding to the autumn–winter and spring–summer transitions of both localities [i.e. in BA-mild and SB-harsh the shortest days (sunrise to sunset) last 9:50 and 9:39 h:min respectively, and the longest days last 14:10 and 14:21 h:min respectively.

The four colonies (two localities – BA and SB – by two photoperiods – SD and LD) were started simultaneously, at the end of May 2019, from ~3000 eggs from each locality. The eggs were submerged in water containing yeast, and the next day all the larvae of each locality were separated into two groups with the same number of larvae, and assigned to the photoperiod treatment, which was maintained throughout the development of the individuals. The larvae were reared in trays (52 × 37 × 7 cm), one for each colony, each containing 6 L of dechlorinated water and powdered baker's yeast as food. The total amount of yeast supplied per tray was 1.5 g, incorporated in three doses of 0.5 g, the first immediately after placing the larvae in each photoperiod (day 1), the second when they reached the fourth larval instar and the third when the first pupae were recorded. Each colony was checked to confirm pupation simultaneity (Supporting Information, Table S1), and the pupae were transferred to rearing cages, where the adults subsequently emerged.

The females of each colony were blood-fed, by providing them access to a restrained guinea pig (*Cavia porcellus* Linnaeus) for 2 h, on two occasions, with a 3-day interval. Immediately after the second intake, the engorged females were separated and transferred to individual jars 6 cm high × 3 cm in diameter, conditioned with wet paper for laying the eggs and a raisin as a sugar source. For each treatment, 160 females were separated and maintained at the same rearing photoperiod until eggs were laid. The females were inspected 7, 10, 12 and 14 days after the first blood intake, and the time of detection of the first eggs was recorded for each individual. The period of time considered was enough for the females to start laying eggs after completing the gonadotrophic cycle at the study temperature (Carrington *et al.*, 2013), even for those that had only ingested blood on the second occasion.

Once oviposition was complete, the females were separated, killed by freezing, and individually stored in labelled Eppendorf tubes. The two wings of each female were subsequently removed, placed on a slide, and measured from the basal notch to the distal end excluding the marginal scales, to the nearest 0.001 mm, using a stereomicroscope (Leica EZ4 HD equipped with an integrated digital camera).

The papers with the eggs were kept in the same laying conditions for a minimum of 6 days, to ensure the development and maturation of the embryos (Farnesi *et al.*, 2009). As an indicator of fecundity, the eggs from each batch were counted, and then differentiated and classified into: intact, hatched and collapsed eggs during the first 6–12 days after oviposition. For each batch, between ten and 37 eggs were randomly chosen, separated and stored

in individual Petri dishes (one per batch), under saturated humidity until they were immersed to study their hatching response.

Three consecutive immersions were performed, on days 6, 11 and 18, after the last eggs were collected, all under a 12:12 h (light:dark) photoperiod. The first two immersions were performed at 21 °C in a solution of 46.8 mg yeast/L, representing a moderate hatching stimulus. The third immersion was performed at 25 °C in a solution of 250 mg yeast/L, representing a strong stimulus to induce hatching as much as possible (Byttebier *et al.*, 2014). For each immersion, the papers containing the eggs from each replicate were placed in a tube with 10 mL of the solution. After 24 h, the papers with the eggs were removed from the water, and the larvae present in each tube were counted. Between immersions, the papers with the remaining eggs were kept in the original Petri dishes on dry absorbent paper to reduce humidity. Finally, the remaining eggs (intact eggs that had not hatched) were bleached with sodium hypochlorite to evaluate the viability of the embryos. Creamy white embryos with visible hatching spines and eyes were considered viable (Farnesi *et al.*, 2009), while those without these characteristics were considered non-viable.

#### DATA ANALYSES

The proportion of females that laid eggs in each treatment (compared to the 160 initially separated in each treatment) was compared using a  $\chi^2$  test for multiple independent proportions, followed by  $\chi^2$  tests between the groups that showed the greatest differences, and between treatments within each group (Fleiss *et al.*, 2003).

For each female, the time (in days) elapsed between the first blood offer and the first egg detection (time to oviposition) was calculated. For the analysis of wing length, the average size of the right and left wing was calculated for each female as an indicator of body size (Van Handel & Day, 1989). To estimate the fecundity of each female, all the eggs laid by each female were considered. For each of these variables (time to oviposition, wing length and fecundity), comparisons between the treatments were made using generalized linear models (GLMs), using the normal distribution with the identity link function. The photoperiod (SD and LD), the population of origin (BA and SB) and the interaction between them were included as independent variables. In cases where significant differences were detected, *post hoc* comparisons were made using Fisher's least significant difference (LSD) test on ranks (Conover, 1999).

The status of the eggs in each treatment was compared through two variables: the proportion of spontaneously hatched eggs and the proportion of collapsed eggs.

The hatching response of the eggs after immersion was calculated as the cumulative number of observed larvae after the three immersions divided by the initial number of viable eggs. The number of viable eggs was calculated as the sum of the larvae observed in the three immersions plus the number of viable embryos. The proportion of spontaneously hatched eggs, the proportion of collapsed eggs and the hatching response in each treatment were compared by GLMs using the quasi-binomial distribution and the logit link function. The quasi-binomial distribution was used because the results of a preliminary analysis with the binomial distribution showed over-dispersion of the data. All GLM analyses were performed with the R package v.3.6.2 (R Core Team, 2019), accessed through a user-friendly interface in Infostat Software (Di Rienzo *et al.*, 2019).

## RESULTS

Not all the 160 females initially separated for each treatment laid eggs (Table 1; Supporting Information, Table S2). The proportion of females that laid eggs showed differences between at least one pair of treatments ( $\chi^2 = 10.48$ , d.f. = 3,  $P < 0.05$ ). The comparison between groups showed that the proportion of females that laid eggs was significantly higher for the BA-SD treatment ( $\chi^2 = 9.76$ , d.f. = 1,  $P < 0.005$ ), but not among the remaining treatments ( $\chi^2 = 0.73$ , d.f. = 2,  $P > 0.05$ ).

Oviposition was recorded between less than 7 days and 14 days from the first blood offer for all treatments. The time to oviposition (Fig. 2; Supporting Information, Table S3) was significantly affected by the photoperiod (GLM:  $F_{1,352} = 30.6$ ,  $P < 0.001$ ), but not by the population of origin ( $F_{1,352} = 0.24$ ,  $P = 0.62$ ) or by the interaction between the population and the photoperiod ( $F_{1,352} = 0.03$ ,  $P = 0.85$ ).

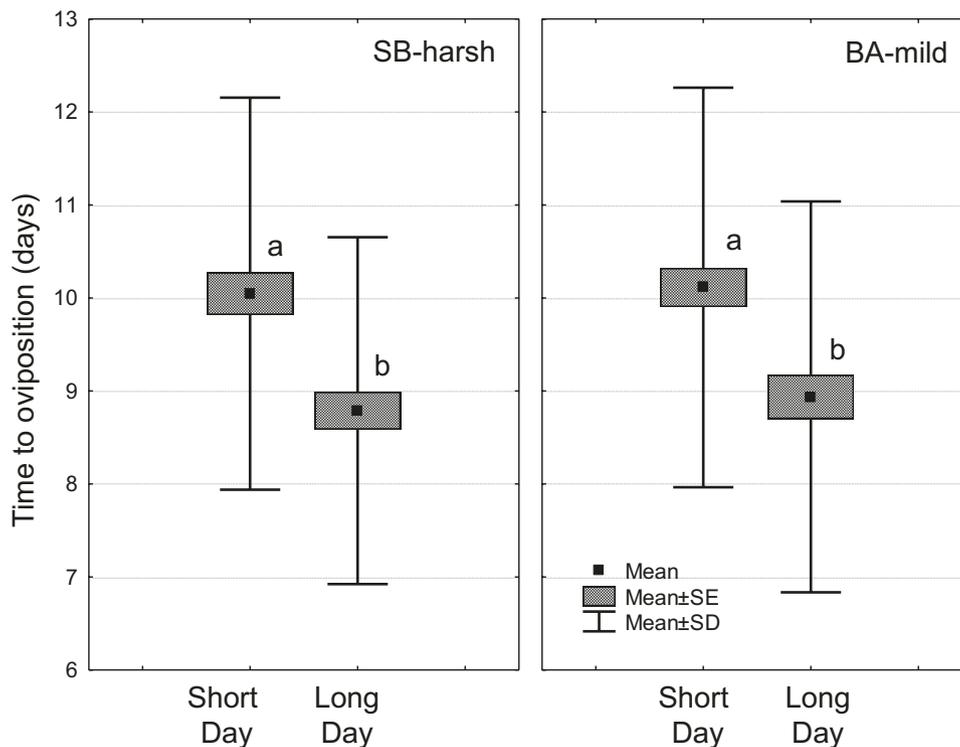
Fecundity showed variability among females, with values of less than 15 to more than 90 eggs per female in each treatment (Supporting Information, Table S3). The fecundity of the females was not affected by the photoperiod (GLM:  $F_{1,352} = 0.28$ ,  $P = 0.60$ ), by the population of origin ( $F_{1,352} = 0.10$ ,  $P = 0.75$ ), or by the interaction between the population and the photoperiod ( $F_{1,352} = 2.47$ ,  $P = 0.12$ ).

Wing length was variable within and between the population  $\times$  photoperiod combinations (Fig. 3; Supporting Information, Table S4). Wing length was significantly affected by the photoperiod (GLM:  $F_{1,309} = 24.14$ ,  $P < 0.001$ ), by the population of origin ( $F_{1,309} = 16.36$ ,  $P < 0.001$ ), and by the interaction between the population and the photoperiod ( $F_{1,309} = 6.17$ ,  $P < 0.05$ ). Although LD females tended to be smaller than SD females and SB females tended to be smaller than those of BA within the same photoperiod, only the SB females reared with LD were significantly different from the other three groups (Fisher's LSD test  $P < 0.05$ ), among which no differences were detected.

Regarding the eggs, some spontaneously hatched on the paper where they had been stored, without being submerged in water (Table 1; Supporting Information, Table S3). The GLM showed an effect of the photoperiod ( $F_{1,352} = 14.99$ ,  $P < 0.001$ ) on the proportion of spontaneously hatched eggs, but not of the population of origin ( $F_{1,352} = 0.53$ ,  $P = 0.47$ ) or the interaction between the photoperiod and the population ( $F_{1,352} = 0.08$ ,  $P = 0.77$ ). The proportion of spontaneously hatched eggs was significantly lower for the SD than for LD treatments (Fisher's LSD test:  $P < 0.05$ ). A small proportion of collapsed eggs were also recorded during the first 2 weeks after oviposition. The GLM showed a significant effect of the population of origin ( $F_{1,352} = 5.16$ ,  $P < 0.05$ ) on the proportion of collapsed eggs, but not of the photoperiod ( $F_{1,352} = 3.41$ ,

**Table 1.** Attributes measured for the females and eggs for each population studied (BA-mild: Buenos Aires, SB-harsh: San Bernardo) and photoperiod (short day, long day). Different letters within each variable (row) indicate significant differences

	BA-mild short day	SB-harsh short day	BA-mild long day	SB-harsh long day
Number (proportion) of females that laid eggs	106 (0.66) <sup>a</sup>	86 (0.54) <sup>b</sup>	79 (0.49) <sup>b</sup>	85 (0.53) <sup>b</sup>
Time to egg detection (mean $\pm$ SE)	10.11 $\pm$ 0.2 <sup>a</sup>	10.05 $\pm$ 0.22 <sup>a</sup>	8.94 $\pm$ 0.23 <sup>b</sup>	8.79 $\pm$ 0.22 <sup>b</sup>
Fecundity per female (mean $\pm$ SE)	56.7 $\pm$ 2.2 <sup>a</sup>	61.3 $\pm$ 2.5 <sup>a</sup>	59.2 $\pm$ 2.6 <sup>a</sup>	56.2 $\pm$ 2.5 <sup>a</sup>
Female wing length (mean $\pm$ SE)	2.864 $\pm$ 0.01 <sup>a</sup>	2.846 $\pm$ 0.011 <sup>a</sup>	2.836 $\pm$ 0.012 <sup>a</sup>	2.762 $\pm$ 0.012 <sup>b</sup>
Total number of eggs	6006	5274	4679	4773
Total number of intact eggs	5985	5162	3994	4242
Total number of spontaneously hatched eggs	5 <sup>a</sup>	1 <sup>a</sup>	610 <sup>b</sup>	350 <sup>b</sup>
Total number of collapsed eggs	16 <sup>a</sup>	111 <sup>b</sup>	75 <sup>a</sup>	181 <sup>b</sup>
Egg hatching response (mean $\pm$ SE)	0.633 $\pm$ 0.027 <sup>b</sup>	0.496 $\pm$ 0.029 <sup>c</sup>	0.929 $\pm$ 0.017 <sup>a</sup>	0.946 $\pm$ 0.014 <sup>a</sup>



**Figure 2.** Days elapsed (mean, SE and SD) between the first blood offer and the first egg detection for each *Aedes aegypti* population (BA-mild: Buenos Aires, SB-harsh: San Bernardo) and photoperiod (short day, long day). Different letters indicate significant differences.

$P = 0.066$ ) or the interaction between the population and the photoperiod ( $F_{1,352} = 0.85$ ,  $P = 0.36$ ). The proportion of collapsed eggs was significantly lower for BA than for SB (Fisher's LSD test:  $P < 0.05$ ).

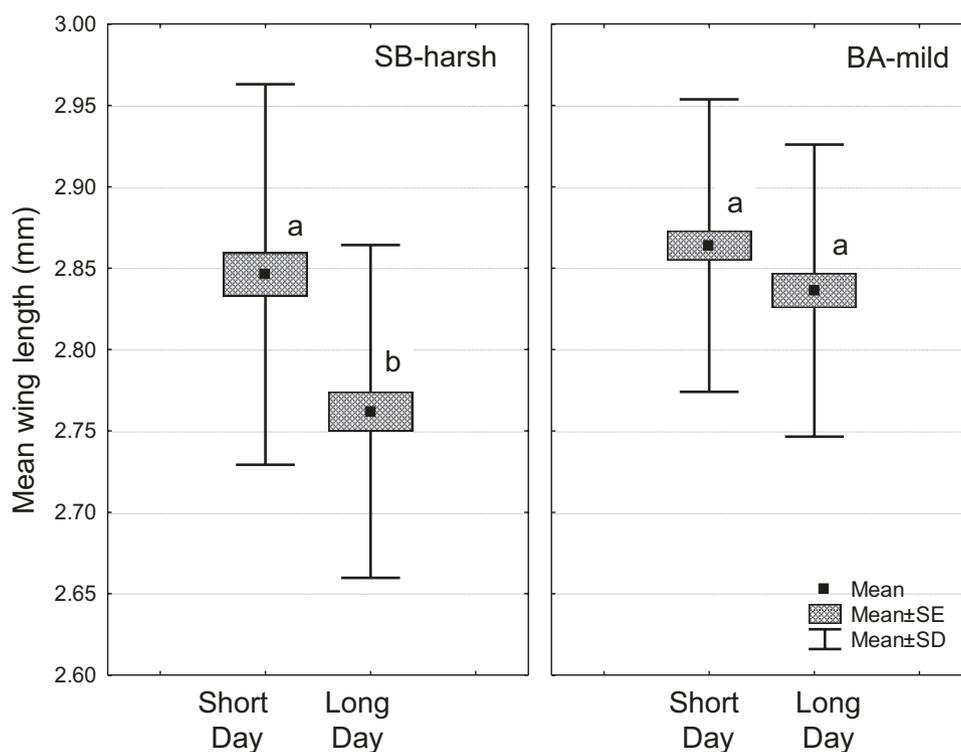
A total of 8047 viable eggs were used for the immersions, 1217 (15.1%) of which hatched during the first immersion, 245 (3.0%) during the second and 4221 (52.4%) during the third. The GLM showed an effect of the photoperiod ( $F_{1,337} = 141.2$ ,  $P < 0.001$ ) on the proportion of hatched eggs accumulated in the three immersions, and of the interaction between the photoperiod and the population ( $F_{1,337} = 4.3$ ,  $P = 0.04$ ), but not of the population of origin ( $F_{1,337} = 0.43$ ,  $P = 0.51$ ). The proportion of hatched eggs in the LD treatments did not differ significantly between populations (BA = 0.93, SB = 0.95), but was significantly higher than that of hatched eggs in the SD treatments (Fisher's LSD test:  $P < 0.05$ ). Within the SD treatments, hatching was significantly higher in BA (0.63) than in SB (0.50) (Fisher's LSD test:  $P < 0.05$ ) (Fig. 4; Supporting Information, Table S5).

## DISCUSSION

The results of our study show effects of the photoperiod on some traits related to *A. aegypti* female reproduction,

but not on others. Our results confirm the ability of females raised under a short photoperiod to lay eggs inhibited from hatching, and show for the first time the effects on some reproductive traits of the females, such as time to oviposition and body size. Our results also show that the populations analysed have different reaction norms in response to the photoperiod, both in female body size and in the hatching response of eggs. These findings are important because the populations studied are near to the distribution limit of this species, and might reflect a process of adaptation to colder climates.

According to the 'offspring size–offspring number trade-off' hypothesis, it was expected that females reared under short photoperiods would have lower fecundity than females reared under long photoperiods. However, as no differences in individual fecundity were detected between the photoperiod treatments, this hypothesis was not supported by our results for the temperate populations of *A. aegypti* analysed. This might be explained by the larger size of SD females in our study, which might have compensated for the costs of laying larger eggs, as observed in other studies (Fox & Czesak, 2000). However, the proportion of females that laid eggs was higher in the BA-SD treatment. This might be related either to differences in blood feeding or to differences in the success in



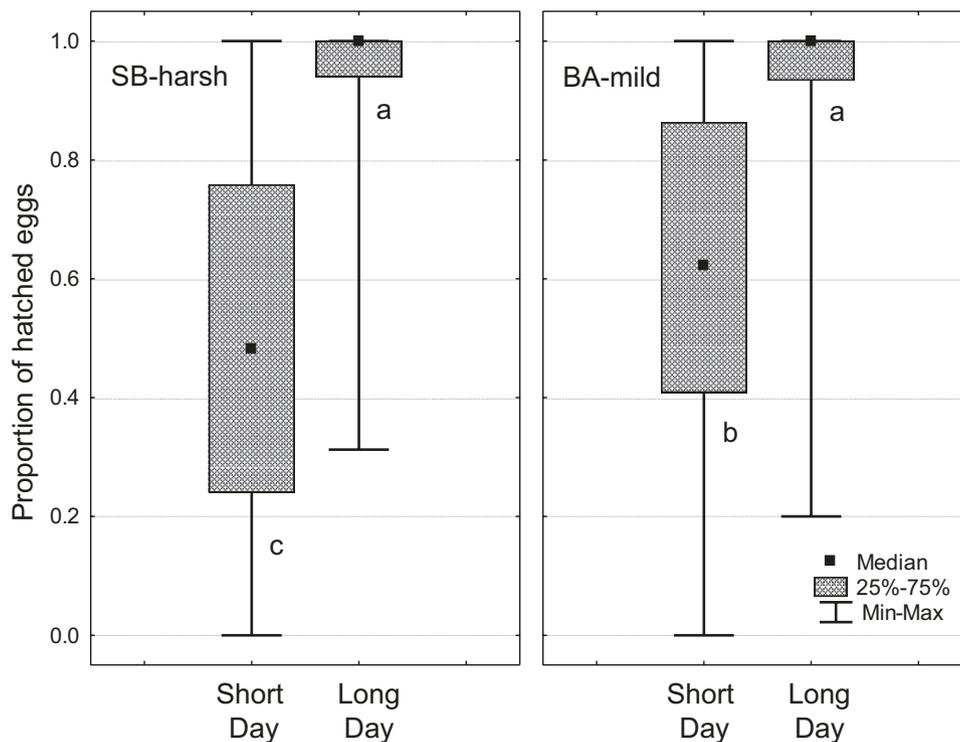
**Figure 3.** Wing length (mean, SE and SD) for *Aedes aegypti* females of each population studied (BA-mild: Buenos Aires, SB-harsh: San Bernardo) and photoperiod (short day, long day). Different letters indicate significant differences.

completing maturation of the eggs. Considering that no differences in fecundity were observed between the two photoperiods, the most likely explanation is that they had differences in their predisposition to feed, in accordance with the results of previous studies in a tropical population from Miami (USA), where the females raised under a short photoperiod had a higher predisposition to blood-feed (Costanzo *et al.*, 2015).

In contrast to fecundity, larger females and a delay in oviposition in response to the short photoperiod were observed. Considering that we monitored the simultaneity of the pupation process among treatments during the experiment, the larger size of females might indicate a higher growth rate in the short photoperiods, without a noticeable shortening of the development time. This is in contrast to the response to short photoperiods most frequently observed, where a shorter development time is observed (Nylín & Gotthard, 1998), with a reduction in body size in some taxa (Gotthard, 2004; Kutcherov *et al.*, 2011; Sniegula *et al.*, 2014) but not in others (Leimar, 1996; Reznik *et al.*, 2015). Future studies should assess whether the larger size of the females raised under a short photoperiod is associated with a higher nutritional content of recently emerged adults, as observed for the mosquito *Culex quinquefasciatus* (Ukubuiwe *et al.*, 2018), or to a lower nutritional status at emergence, later compensated with a higher

feeding rate, as observed in the coleopteran *Harmonia axyridis* (Reznik *et al.*, 2015). However, considering the delay in the time to oviposition observed in the present study in females raised under short day photoperiods, the second option is likely, with females from short day photoperiods attaining a larger size, but obtaining the nutritional reserves to lay their eggs through post-emergence feeding.

Differences in the response of female size to photoperiod were observed between the two populations analysed. Within the long photoperiod treatment, the larger size of females from the population with a milder climate (BA) is consistent with the common assumption that larger adults will be favoured by longer warm seasons (Telfer & Hassall, 1999), and with observations from different insect taxa (Blankenhorn & Fairbairn, 1995; Schutze & Clarke, 2008). This pattern, called 'the converse to Bergmann's rule', might be related to the time constraint for development in the southern population (SB), where fast development and a smaller body size might be of advantage to complete as many life cycles as possible during the warm season, before the onset of unfavourable conditions (Mousseau, 1997). In the populations studied here, this 'converse to Bergmann's rule' was observed under the long photoperiod but not under the short one. The larger change in size between photoperiods in the population from the harsher



**Figure 4.** Proportion of *Aedes aegypti* hatched eggs (median, quartiles and range) for the egg batches of each population (BA-mild: Buenos Aires, SB-harsh: San Bernardo) and photoperiod (short day, long day). Different letters indicate significant differences.

climate (SB) might indicate greater sensitivity to the conditions anticipating the end of the favourable season in this population, which might be a consequence of a stronger selection force. However, these patterns need to be confirmed in further studies analysing *A. aegypti* populations along a larger climate gradient.

As expected, short photoperiods also affected the eggs, and the inhibition of hatching of eggs from short photoperiods confirms the findings of a recent study for the BA population (Fischer *et al.*, 2019), and extends them for another temperate *A. aegypti* population, supporting the occurrence of egg diapause in these populations, as previously suggested based on the egg lipid content (Mensch *et al.*, 2021). To confirm that true diapause is occurring in these populations, further research should investigate other ecological and physiological aspects of short-photoperiod eggs and compare them with long-photoperiod eggs. Some of the characteristics of diapausing eggs in other mosquito species (e.g. *A. albopictus*) include their increased tolerance to stressful conditions (e.g. low temperature or humidity), and the differential expression of genes, especially those involved in lipid metabolism (Denlinger & Armbruster, 2014).

The stronger hatching inhibition in the eggs from the population from the harsher climate (SB) might be related to stronger selection occurring in colder localities, according to the expected trends. The lack of differences

between the populations studied under long photoperiods indicates that, under appropriate favourable conditions, none of the populations is inhibited from hatching. An unexpected result was the higher proportion of collapsed eggs for the SB population, regardless of the photoperiod. It is not clear whether this is related to the experimental methods (e.g. the use of F1 and F0 generations for SB and BA respectively), or to differences between populations related to the environmental conditions (in particular humidity) at the localities of origin, i.e. SB has higher humidity than BA. These aspects should be investigated in future studies.

In conclusion, the results of our study provide novel information regarding the response to photoperiod of the populations from temperate Argentina, and suggest the potential for further expansion of *A. aegypti* towards colder climates, and emphasize the need for continued surveillance and monitoring of this mosquito species in the temperate region of Argentina, especially under current climate warming.

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#### DATA AVAILABILITY

The supporting data underlying the work are available in the Supporting Information.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Table S1.** Observations of the number of pupae and adults that confirm simultaneous development.

**Table S2.** Number of females within each treatment for which egg-laying was detected on a given day.

**Table S3.** Total number of eggs and number of intact, hatched and collapsed eggs.

**Table S4.** Average wing length (left and right wing) for each female.

**Table S5.** Hatching response for each replicate (number of larvae observed from the number of viable eggs).