Egg hatching dynamics and survival of immature stages of *Aedes aegypti* (Diptera: Culicidae) under field conditions during the cold season in Buenos Aires, Argentina.

María Sol De Majo*, Pedro Montini, Sylvia Fischer

Instituto de Ecología, Genética y Evolución de Buenos Aires, Departamento de Ecología, Genética y Evolución, CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. 4to piso, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina.

* msdemajo@ege.fcen.uba.ar

Abstract

In temperate regions, the seasonal dynamics of *Aedes aegypti* (L.) (Diptera: Culicidae) is mainly influenced by temperature. It is assumed that, during the winter season, the population remains as eggs and that the development and population growth of surviving eggs begin during the following spring. The aim of the current study was to assess the temporal dynamics of egg hatching of *Ae. aegypti* during the winter in Buenos Aires city (Argentina), and analyze the survival of immature stages and their ability to complete development. The experiments consisted in immersing eggs and studying the development of immature stages of cohorts from June and September under natural conditions. The proportion of hatched eggs was compared between weeks of immersion and related to environmental variables. Survival was compared among cohorts and the development rate was related to the mean temperature during development. The results showed that, in general, egg hatching was over 50% during the whole winter period. The proportion of hatched eggs was positively associated with environmental variables. The immature stages completed the development during the cold season, with a trend towards increased survival of late-hatching cohorts. Survival was 30% at 13.2°C and above 90% at 20°C, whereas the development time at low temperatures was 49.4 days at 13.2°C, and 17.7 days at 20°C. The high hatching and survival observed throughout the cold season suggest that the local population might be benefiting from early hatching, by anticipating pupation and emergence of the first cohorts of adults in the early spring.

Key words: unfavorable conditions, temperate region, overwintering, survival.

Introduction

Aedes (=Stegomyia) aegypti (Diptera: Culicidae) is a mosquito species of tropical origin that has a worldwide distribution, ranging from tropical to temperate regions. In tropical and subtropical regions, *Ae. aegypti* has been extensively studied because it is the main vector of several viral diseases including dengue fever, Chikungunya, Zika and yellow fever (Gubler 2004, Barrett and Higgs 2007, Vega-Rúa et al. 2014, Rodriguez-Morales 2015). In contrast, in temperate regions like Buenos Aires (Argentina), this mosquito species has been much less studied, despite the risk of transmission of dengue virus due to the arrival of infected individuals from other countries (Seijo et al. 2000, 2001), to the fact that the vector is found in abundance (Vezzani et al. 2004), and to the favorable weather conditions for the completion of the extrinsic cycle of the virus (Carbajo et al. 2001). In 2009, many endemic cases of dengue virus were recorded in Buenos Aires, in correspondence with the epidemics recorded at the national level and in several neighboring countries (Seijo 2009).

Although *Ae. aegypti* is associated with the domestic environment, its population dynamics is closely dependent on the climate regime. In temperate regions with moderate winters, such as New Orleans, Charleston, and Memphis in the USA, and Buenos Aires in Argentina, this species exhibits seasonal variations, with medium to high abundances of the active stages (immature and adult) only during part of the year (Eisen et al. 2014).

In Buenos Aires city, the population dynamics of *Ae. aegypti* is mainly modulated by temperature (Otero et al. 2006). Both oviposition activity and the presence of immature stages have been recorded only during the warm season from October to May-June (Carbajo et al. 2004, Vezzani et al. 2004). On this basis, it is assumed that, during the winter period, the hatching response and development of the active stages are restricted by the low temperatures, that the population remains as eggs, and that the development and population growth of the surviving eggs begin during the following spring. However, the results of some previous experiments have led us to question this scenario.

A study carried out by Bond et al. (1970) in a temperate zone (Mississippi, USA) showed that, in natural conditions, egg hatching occurred at low temperatures, and that mortality of larvae within the first 24 hours at daily mean temperatures below 9.5°C

reached 100%, while at daily mean temperatures between 9.5°C and 12°C partial survival was recorded.

In Buenos Aires, during a study of the winter survival of eggs under natural conditions, 4.2% of the eggs hatched during the second half of August due to a short period of increased temperature (mean temperature 15.2°C) and another 3.8% hatched during the first half of September (mean temperature 12.9°C) before the beginning of spring (Byttebier, doctoral thesis in preparation). Because the aim of the experimental design of that study was not to assess egg hatching, the results may have underestimated the proportion of eggs that may have hatched during the winter.

Among environmental variables, the temperatures during and before immersion are supposed to affect the hatching of *Ae. aegypti* eggs (Clements 1992, Vinogradova 2007). The results of a recent laboratory study with eggs from Buenos Aires have shown that more than 25% of eggs hatched with a constant immersion temperature of 12°C, and that a higher proportion of eggs hatched at 14°C and 16°C (in all cases the first larval stage remained alive during the first 24 hours) (Byttebier et al. 2014). This study also showed that eggs previously stored at warm temperatures in the laboratory and eggs previously stored under cold temperatures in natural conditions showed different predisposition to hatch, which could indicate that the temperature before immersion could influence the hatching process (Byttebier et al. 2014). Another variable that affects some Aedine mosquitoes is the photoperiod, which is involved in the induction of diapausing eggs. Along with temperature, the photoperiod influences the hatching response of several mosquito species in temperate climates (Vinogradova 2007). However, until now, the diapause phenomenon has not been documented in *Ae. aegypti* (Denlinger & Armbruster 2014).

In Buenos Aires city, the average monthly temperature during the winter months (June-August) is lower than 11.6°C, and due to daily fluctuations, average maximum temperatures reach 15.7°C in June, 15.4°C in July and 17.1°C in August (National Meteorological Service 2014). Occasionally, periods with temperature increases for several days are recorded, but it is unclear whether hatching events occur during those periods or only in late winter, or whether larvae are able to survive the thermal conditions of the post-hatching weeks. In the case that the larvae hatch but do not survive, winter hatchings could represent an additional factor of mortality of egg banks, not covered in previous studies.

Some laboratory studies have shown that the lowest constant temperature with complete development of *Ae. aegypti* is 14°C, with a survival rate of 24%, and that at lower temperatures, larvae are unable to complete their development (Bar Zeev 1958, Rueda et al. 1990, Tun Lin et al. 2000). In Taiwan, Chang et al. (2007) observed that under natural conditions with average daily temperatures of 14-15°C developmental survival was approximately 30%, whereas in Japan, Tsuda and Takagi (2001) found that with average daily temperatures below 10°C *Ae. aegypti* development could not be completed. To our knowledge, there are no studies of *Ae. aegypti* survival in the characteristic intermediate temperature range of Buenos Aires city.

The aim of the current study was to assess the temporal dynamics of egg hatching of *Ae. aegypti* during winter in Buenos Aires, and to analyze the survival of immature stages and their ability to complete development.

Materials and methods

Study area. Buenos Aires city (34° 35' S, 58° 29' W), Argentina, is characterized by a temperate climate with a pronounced thermal seasonality. The mean temperature during the cold season is 11.5°C, whereas that in spring varies around 17°C, with cool mornings and nights. The rainfall events do not present seasonality: they are recorded regularly throughout the year, but are slightly lower between June and September, with a mean of eight monthly rainfall events, with an average 8.64 mm per event (National Meteorological Service 2014).

The study site was located in Mataderos, an urban neighborhood characterized by high survival of eggs in winter (Fischer et al. 2011) and by high oviposition activity in summer (De Majo et al. 2013).

The experiments were performed in the backyard of a family residence with 20 m^2 of ceramic floor, no roof, three 9-m-high walls, and a 2.20-m-high wall with glass windows. The backyard has sun during the afternoon and flowerpots with vegetation both on the floor and walls.

Egg collection and conditioning. The eggs used in the experiments were collected with ovitraps, during four consecutive weeks from the third week of April to the second week of May in four neighborhoods within the Metropolitan Area of Buenos Aires (for more details on the collection method see De Majo et al. 2013). Ovitraps were reconditioned weekly. Paddles with less than 20 eggs were left in the traps for another week, while those with 20 or more eggs were transported to the lab and replaced in the

4

trap by new ones. All the eggs collected were assumed to correspond to *Ae. aegypti* because this is the only container breeding Aedine mosquito species in this region (Rubio et al. 2012).

The oviposition substrates were inspected under a stereoscopic microscope in the laboratory. The intact eggs were counted and the collapsed or hatched eggs were removed. Then, the substrates with eggs were fixed vertically by a clip in closed plastic containers (9.4 cm high and 6.5 cm wide) conditioned with wet filter paper and cotton to maintain air humidity. Plastic containers were stored outdoors under natural conditions and ambient temperature, at the experimental site, until the start of the experiments.

Experimental design.

Egg hatching during the cold season. The experiment consisted in immersing eggs under natural conditions during the winter, to assess the hatching dynamics. Every week from June throughout September 2013, 19 paddles with a known number of eggs (a total of approximately 1000 eggs per week) were immersed in 50-ml plastic Falcon tubes 12 cm high and 3 cm diameter, containing 40 ml of tap water that had been previously conditioned at ambient temperature.

The substrates were removed from the solution after 48 hours, and the number of live and dead larvae in each tube was counted. The substrates were transferred to the laboratory, and intact eggs were counted under a stereoscopic microscope.

The substrates with eggs were acclimated in the laboratory under controlled conditions of temperature ($24 \pm 1°C$), humidity (~ 80%) and photoperiod (LD 12:12 h) for 5 days. After this, to assess the viability of the remaining eggs, the substrates were individually subjected to a strong hatching stimulus (immersion in a 0.25 g/l yeast solution in dechlorinated water). This stimulated the hatching of most of the remaining viable eggs. After this period, the substrates were removed, and the number of hatched larvae and the number of unhatched remaining eggs were counted. Corions of the few unhatched eggs were bleached with a commercial 50% sodium hypochlorite solution to allow direct observation of the embryos. Embryos that were yellow-brown or red-brown were considered non-viable (McHaffey and Harwood 1970). Completely collapsed eggs and eggs that contained non-viable embryos were considered dead, whereas eggs that either hatched or contained viable embryos were considered alive.

Development of immature stages during the cold season. Following each event of egg immersion between June and September, larval development was studied under natural conditions. Each week, one cohort consisting of three groups (replicates) of 20 larvae hatched in natural conditions and three groups of 20 larvae hatched in the laboratory were placed in a 220-ml cylindrical container that contained 135 ml of a nutrient solution. All larvae hatched within 48 h before the beginning of the experiments. The containers were placed outdoors but protected from rainfall. The nutrient solution (37.5 mg of yeast per 1000 ml of tap water conditioned for 48 hours at ambient temperature) was changed three times a week, and each time the larvae were counted and transferred to the new solution.

The pupae were transferred to individual containers fitted with a support to facilitate the resting of the newly emerged adults. The sex of the adults and time of adult emergence were recorded.

Hourly temperature was recorded during the experiments with a meteorological station placed in situ. In addition, the meteorological data collected in situ were corroborated with the data provided by the National Meteorological Service for Villa Ortúzar Station, located at 8.16 km from the study site.

Data analyses. Mean daily temperature was calculated based on the hourly data from the meteorological station placed in the study site. Temperature data that could not be recorded during September because of malfunction of the station were estimated from hourly data from Villa Ortúzar station. To this end, the relationship between the temperature at the experimental site and that from Villa Ortúzar station was assessed with linear regressions stratified by time intervals with similar dynamics (24-hour data were divided in four strata). The equations obtained were used to estimate the missing temperature data for the corresponding time interval.

Egg hatching during the cold season. The proportion of field-hatched eggs (P_{hatch}) was calculated for each oviposition substrate by dividing the number of observed larvae by the initial number of viable eggs. The hatching response in the field was compared between weeks of immersion with a Generalized Linear Mixed Model, (GLMM) using R software, Version 3.1.2 (R Core Team 2014), accessed through a user friendly interface in Infostat software (Di Rienzo et al. 2014). An observation-level random intercept variable was included in the model to account for overdispersion (Zuur et al.

2013). The binomial family with the logit link function was selected. Multiple comparisons were performed with Fisher´s LSD test on ranks (Conover 1999).

For each immersion, the following variables were calculated: the mean temperature of 96 hours before the immersion ($T°_p$), the mean temperature of 48 hours during immersion ($T°_i$), the mean temperature of 15 days before $T°_p$ ($T°_a$), the daily numbers of hours with light or photoperiod (P).

The relationship between P_{hatch} and $T°_p$, $T°_i$, $T°_a$, and P was analyzed with a logistic regression analysis. The logistic regression was performed by the sequential method, starting with the full model, and deleting variables one by one until all variables in the model had significant contributions at a 0.05 probability level.

Development of immature stages during the cold season. For each replica of each cohort, survival during development (S) was estimated as the number of adults at the end of the experiments divided by the number of initial larvae. In addition, the mean temperature from the beginning of each cohort to the emergence of adults was calculated for each replica.

Survival was compared among cohorts with a GLMM. The factors considered were: cohort (sixteen levels) and hatching site (two levels: field or lab). The binomial family with the logit link function was selected. To assess significant differences, post-hoc comparisons among cohorts were made with Fisher´s LSD test on ranks.

The proportion of females (F) was estimated as the number of females at the end of the experiments divided by the total number of adults. The relationship between F and S was analyzed with Pearson correlation.

The relationship between survival of each replica and the mean temperature during development was analyzed with a quadratic function of the form: $S = a\ T°m^2 + b\ T°m + c$, where $T°m$ is the mean temperature during development. The relationship between the development rate (Dr = 1/Development time) under field conditions and $T°m$ was analyzed with a linear regression of the form $Dr = a + b\ T°m$.

To assess whether the hatching response intensity was related to developmental success, the relationship between P_{hatch} and S was analyzed with a Pearson correlation.

Results

Meteorological data. $T°_p$ varied from 8.77°C in July (fourth week) to 20.38°C in September (first week). $T°_i$ was generally higher than $T°_p$ until the end of August,

except in the third week of June, the fourth week of July, and the second week of August. The higher thermal amplitude (difference between maximum and minimum temperatures) was recorded from the second week of August up to the second week of September. The maximum temperatures were recorded during the third week of August and the second week of September, while the minimum temperatures were recorded during the last week of June, the fourth week of July and the fourth week of August. The photoperiod increased gradually from a minimum of 9 hours and 50 minutes at the beginning of the experiment up to 12 hours and 26 minutes at the beginning of spring (**Fig. 1**).
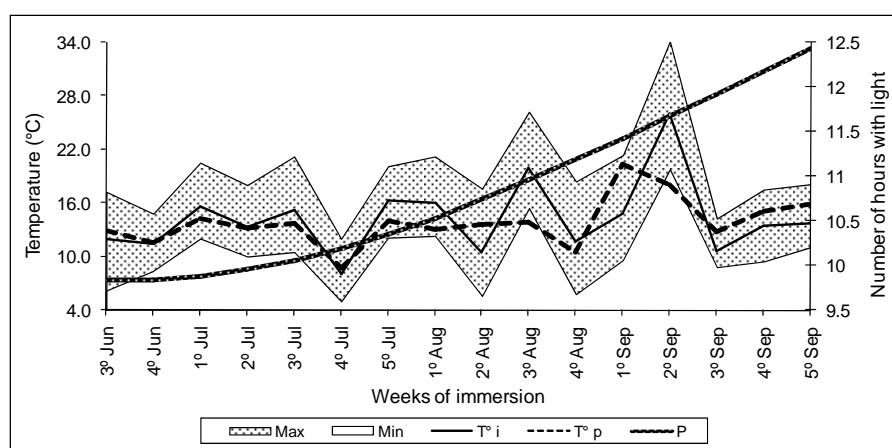

Fig. 1: Mean temperature previous to immersion ($T°_p$) and during immersion ($T°_i$), maximum and minimum temperatures during immersion (area), and number of hours with light during immersion (P) for each of the 16 cohorts studied.

Egg hatching during the cold season. Eggs of *Ae. aegypti* hatched during the whole winter period. In general, the hatching response was relatively high in June, July and August, with median values exceeding 50%, except for four weeks when the proportion was lower (48%, 12%, 5% and 22%). Furthermore, a great heterogeneity between substrates within the same week was observed during the whole study period. The proportion of dead larvae after 48 hours was less than 1% throughout the study period. In September, the median hatching response exceeded 85% (**Fig. 2**), in coincidence with the temperature increase during this month.

The GLMM showed significant differences in P_{hatch} between weeks ($p < 0.001$, n = 19). Post-hoc comparisons showed that from June through August, P_{hatch} was relatively homogeneous between weeks except on the fourth week of June, the fourth week of July, and the second week of August, when fewer eggs hatched. During September, the

highest hatching response was observed except for the third week, when hatching values were similar to those recorded in July and August.
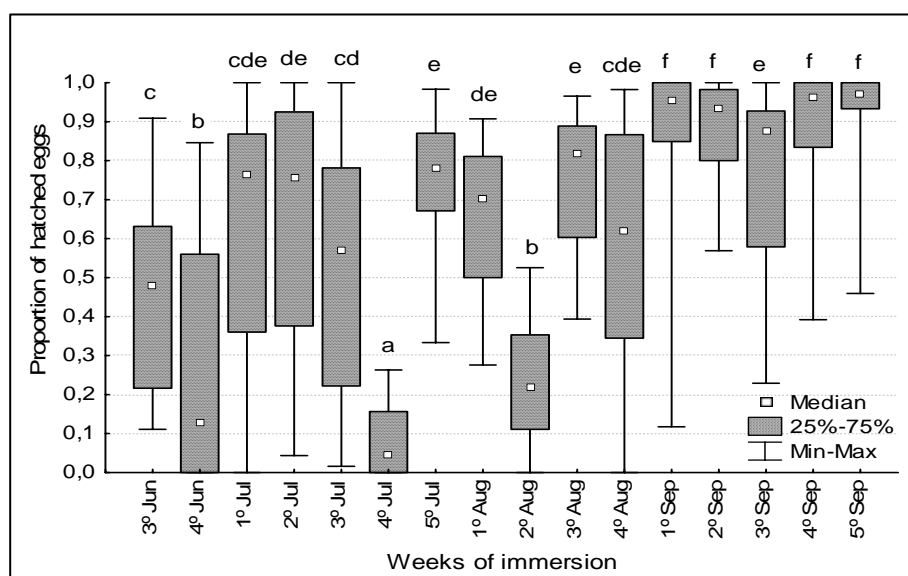
Fig. 2: Weekly proportion of hatched eggs during the cold season. The same letters indicate weeks of immersion with no significant differences.

Egg hatching and environmental variables. The proportion of remaining unhatched eggs after the field and laboratory stimuli was less than 1.5% and showed no differences between weeks except in the fourth week of June, when the proportion of remaining unhatched eggs was 4 %.

The logistic regression analysis showed a positive association between the proportion of hatched eggs and $T°_i$, $T°_p$ and P (**Table 1**), and a non-significant relationship with T_a.

**Table 1:** Logistic regression analysis of P_{hatch} during the cold season (OR: odds ratio).

Variables	OR - CI 95%	p-value
Immersion temperature	1.13 (1.12 - 1.15)	< 0.0001
Pre-immersion temperature	1.19 (1.16 - 1.21)	< 0.0001
Photoperiod	1.93 (1.83 - 2.03)	< 0.0001

OR: odds ratio; CI: confidence interval

Development of immature stages during the cold season. In all replicas of all cohorts, some individuals completed the development. However, differences in S between different cohorts were observed, with a trend towards increased survival of late-hatching cohorts.

Survival during development was significantly affected by the cohort (df = 15, p <0.001), but not by the hatching site (df = 1, p = 0.33) or interaction between both variables (df = 15, p = 0.07). Lowest S was observed in cohorts started on June and on the second and third week of July, with values less than 35%. Post hoc comparisons showed a significantly higher S for the cohorts started in the first and the fourth week of July, for which S was 51 % and 59% respectively. Furthermore, no significant differences were detected between the fifth week of July up to the second week of September, for which S ranged between 60% and 80%. In the cohorts started on the fourth and fifth weeks of September, S was significantly higher than that of most of the previous cohorts, with values exceeding 90% **(Fig. 3).**
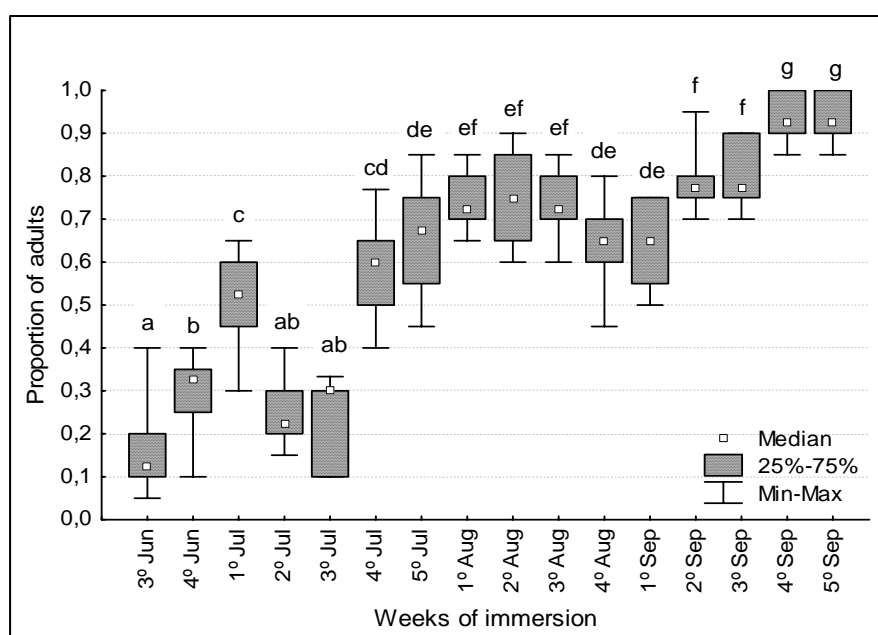

Fig. 3: Proportion of adults that emerged from eggs hatched during the cold season (winter and early spring). The same letters indicate weeks of immersion with no significant differences.

Survival is related to mean temperature by the equation: $S = -0.0182\ T°m^2 + 0.694\ T°m - 5.7031$. Within the temperature range analyzed, these results involve a gradual increase in survival from about 30% at 13.2°C to above 90% at 18 - 20°C (**Fig. 4a**).

The average development times ranged from about 49.4 days at 13.2°C to about 17.7 days at 20°C (**Fig. 4b**). The development rate under field conditions is related to the mean temperature during development by the equation: $Dr = -0.05037 + 0.00535\ T°m$.

In addition, F showed a significant and negative relationship with S (r = -0,624, df = 16, p <0.05). The proportion of females at high survival rates ranged from 0.33 to 0.59, whereas that at low survival rates ranged from 0.44 to 0.78.
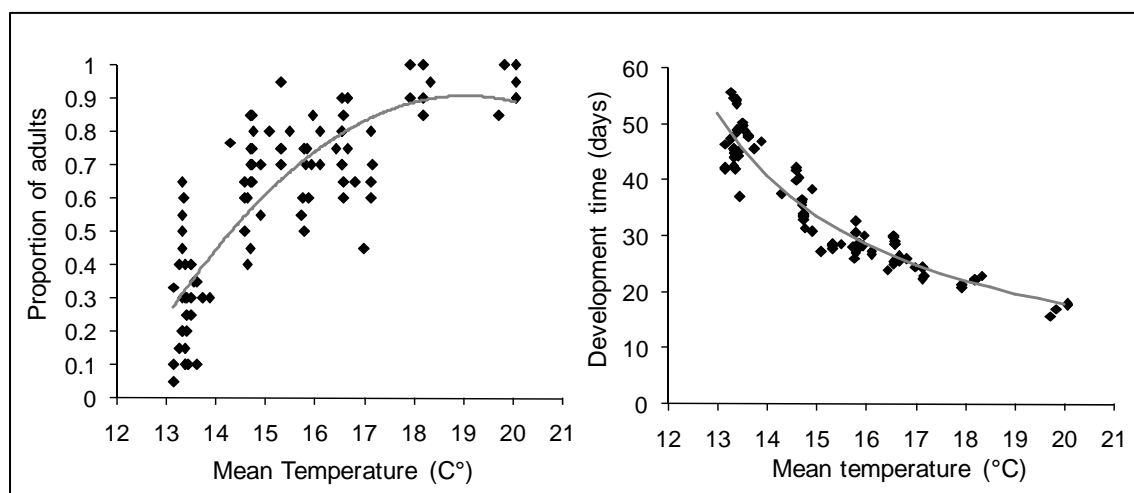
Fig. 4: **a)** Relationship between survival and mean temperature (°C). **b)** Relationship between development time (days) and mean temperature (°C) for each cohort. The line represents predicted values based on parameters obtained for the development rate.

Furthermore, the relationship between P_{hatch} and S was only marginally significant (r = 0.479, df = 16, p = 0.062). The lowest survival values were 16% and 23% with egg hatching values of 51% and 62%, whereas the highest survival was 93% with egg hatching values of 88% and 94%. The highest P_{hatch} values coincided with the highest survival and the lowest P_{hatch} value was 6.7% with 57% of survival.

Discussion

The proportion of hatched eggs with live larvae after 48 hours during the winter period suggests that a relatively high proportion of eggs immersed in water can hatch even when mean temperatures are below 12°C. Although this seems to be contrary to expectations based on results of previous studies where no free hatching at temperatures lower than 12°C was reported (Christophers 1960), other studies such as that of Bond et al. (1970) obtained results similar to those of the current study. Considering that both Bond's study and ours were conducted under natural conditions with varying

temperatures, it is possible that hatching occurs during the day when the temperature exceeds the hatching threshold. In our study, when the average temperatures during immersion were lower than 12°C, mean maximum temperatures reached 14.8°C in June, 12°C in July, 17°C and 18°C in August, and 14°C in September.

Results showed a high variability in egg hatching between weeks, with increased hatching from June to September, related to increases in the immersion temperature, in the mean temperature of 96 hours before the immersion, and in the photoperiod. These results are consistent with previous studies where the hatching rate increases when the mean temperature before and during the immersion increases (Christophers 1960, Monteiro et al. 2007, Byttebier et al. 2014).

The higher proportion of egg hatching in the second immersion in laboratory conditions and the lower proportion of hatching at lower temperatures in the field suggest that although eggs were potentially able to hatch, less favorable environmental conditions lead to an increase in the spreading of the risk, ensuring the maintenance of an egg bank for future immersion events with more favorable conditions.

In this study, we documented variable proportions of eggs hatching from June through September. Nevertheless, our results do not allow estimating the size of the egg bank remaining after the winter, since our design included only a single hatching stimulus in the field each week. Future studies should assess how successive flooding events determined by the frequency and quantity of water supply (due to rainfall or human behavior) determine cumulative hatching response during unfavorable periods.

Regarding the survival of immature stages of *Ae. aegypti*, our results indicate that a significant proportion of individuals reach the adult stage regardless of the time of hatching. The proportion of adults varied between cohorts, with survival increases for the latest cohorts, associated with the temperature increase from winter to spring in Buenos Aires city.

The higher proportion of females in cases with lower survival suggests that mortality due to unfavorable thermal conditions would act primarily on males. To our knowledge such pattern has not been reported previously, and the implications for the populations are not clear. The only marginally significant relationship between the hatching response and adult survival indicates a limited ability of eggs to anticipate favorable environmental conditions during the following weeks. One of the most reliable environmental signals to anticipate subsequent conditions is the photoperiod, since it

has a predictable pattern and, unlike temperature, is not subjected to variability (Danks 2007). Although we found a positive association between hatching and increasing photoperiod, our results do not allow concluding a causal relationship. To our knowledge, no studies have assessed the role of the photoperiod in the hatching response of *Ae. aegypti* eggs, although it has been demonstrated that this variable may affect other stages of development. For example, the photoperiod during the larval stage affects the survival of females (Costanzo et al. 2015).

The results of our study showed a survival rate higher than that observed in other studies. At 14-15ºC, a survival range of 40-80% was observed in our study, as compared to the 30% recorded in Taiwan (Chang et al., 2007). In our experiments, survival also exceeded that reported by studies at constant temperatures. For example, survival at 15°C ranged from 3% to 23% (Rueda et al., 1990; Tun Lin et al., 2000), and 24% at 14°C (Bar Zeev, 1958). In all these studies, larvae were unable to complete their development below 14ºC.

Furthermore, the development times at low temperatures in our study (40.8 days at 14°C, 33.5 days at 15°C, and 28.5 days at 16°C) were lower than those observed in previous studies at constant temperatures (60 days at 14ºC, 39.7 days at 15°C and 34.12 days at 16°C) (Bar Zeev 1958, Tun Lin et al. 2000).

It should be noted that, as in previous studies, the survival and development time for immature stages of *Ae. aegypti* at low temperatures in this work were recorded under optimal conditions of food availability. This means that our results may have overestimated the success during the larval development of *Ae. aegypti* at the low temperatures of the winter season in Buenos Aires, because the availability of food in the breeding habitats in nature may be lower than that provided in this study.

The hatching observed throughout the cold season and the high survival during development in June, July and August suggest that the winter conditions in Buenos Aires are not harsh enough to inhibit development. Moreover, the local population might be benefiting from early hatching, by anticipating pupation and emergence of the first cohorts of adults. This would allow initiating reproduction immediately after the beginning of the favorable conditions in spring. This strategy, supplemented by a higher rate of development at low temperatures, could indicate an adaptation to maximize the breeding season in temperate regions with marked seasonal temperature variations. Further studies on survival, reproduction and oviposition of adults under natural

conditions during the winter and spring would help to understand how this adaptation influences the population dynamics of *Ae. aegypti.*

Acknowledgments

References Cited

Barret, A.D., and S. Higgs. 2007. Yellow Fever: a disease that has yet to be conquered. Annu. Rev. Entomol. 52: 209–229.

Bar Zeev, M. 1958. The effect of temperature on the growth rate and survival of the immature stages of *Aedes aegypti* (L.). Bull. Entomol. Res. 49: 157–163.

Bond, H. A., J. E. Keirans, and M. F. Babbitt. 1970. Environmental influences on the viability of overwintering *Aedes aegypti* (L.) eggs. Mosq. News. 30: 528–533.

Byttebier, B., M. S. De Majo, and S. Fischer. 2014. Hatching Response of *Aedes aegypti* (Diptera: Culicidae) Eggs at Low Temperatures: Effects of Hatching Media and Storage Conditions. J. Med. Entomol. 51: 97–103.

Carbajo, A, N. Schweigmann, S. Curto, A. de Garín, and R. Bejarán. 2001. Dengue transmission risk maps of Argentina. Trop. Med. Int. Health. 6: 170-80.

Carbajo, A., S. M. Gomez, S. L. Curto, and N. Schweigmann. 2004. Variación espacio - temporal de riesgo de transmisión de dengue en la ciudad de Buenos Aires. Medicina (Buenos Aires). 64: 231–234.

Chang, L. H., E. L. Hsu, H. J. Teng, and C. Ho. M. 2007. Differential survival of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) larvae exposed to low temperatures in Taiwan. J. Med. Entomol. 44: 205–210.

Christophers, R. 1960. *Aedes aegypti* (L.). The Yellow Fever Mosquito. Cambridge Univ. Press. Cambridge. 739 pp.

Clements, A. N. 1992. The biology of mosquitoes, vol. 1. Development, nutrition and reproduction. Chapman & Hall, London, United Kingdom.

Conover, W. J. 1999. Practical Nonparametric Statistics, Third Edition. New York: John Wiley & Sons.

Costanzo, K. S., S. Schelble, K. Jerz, and M. Keenan. 2015. The effect of photoperiod on life history and blood-feeding activity in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J. Vector Ecol. 40: 164–171.

Danks, H. V. 2007. The elements of seasonal adaptations in insects. Canadian Entomologist 139(1):1-44.

Denlinger, D. L., and P.A. Armbruster. 2014. Mosquito diapause. Annu. Rev. Entomol. 59: 73–93.

De Majo, M. S., S. Fischer, M. Otero, and N. Schweigmann. 2013. Effects of thermal heterogeneity and egg mortality on differences in the population dynamics of *Aedes aegypti* (Diptera: Culicidae) over short distances in temperate Argentina. J. Med. Entomol. 50: 543–551.

Di Rienzo, J. A., F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C. W. Robledo. 2014. InfoStat version 2014. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. (http://www.infostat.com.ar).

Eisen, L., A. J. Monaghan, S. Lozano-Fuentes, D. F. Steinhoff, M. H. Hayden, and P. E. Bieringer. 2014. The Impact of Temperature on the Bionomics of *Aedes (Stegomyia) aegypti*, with Special Reference to the Cool Geographic Range Margins. J. Med. Entomol. 51: 496–516.

Fischer, S., I. S. Alem, M. S. De Majo, R. E. Campos, and N. Schweigmann. 2011. Cold season mortality and hatching behavior of *Aedes aegypti* L. (Diptera: Culicidae) eggs in Buenos Aires City, Argentina. J. Vector Ecol. 36: 94–99.

Gubler, D. J. 2004. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? Comp. Immunol. Microbiol. Infect. Dis. 27: 319–330.

McHaffey, D. G., and R. F. Harwood. 1970. Photoperiod and temperature influences on diapause in eggs of the floodwater mosquito *Aedes dorsalis* (Meigen) (Diptera: Culicidae). J. Med. Entomol. 7: 631–644.

Monteiro, L. C. C., J. R. B. De Souza, and C. M. De Albuquerque. 2007. Eclosion rate, development and survivorship of *Aedes albopictus* (Skuse) (Diptera: Culicidae) under different water temperatures. Neotrop. Entomol. 36: 966–971

National Meteorological Service (SMN). 2014.
(http://www.smn.gov.ar/serviciosclimaticos/clima/archivo/invierno.pdf).

Otero, M., H. G. Solari, and N. Schweigmann. 2006. A stochastic population dynamics model for *Aedes aegypti*: formulation and application to a city with temperate climate. Bull. Math. Biol. 68: 1945–1974.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Rodriguez-Morales, A. J. 2015. Chikungunya Virus Infection: Ecoepidemiological considerations of a new threat for Latin America. One Health Newsletter. 8: 7–9.

Rubio, A., M. I. Bellocq, and D. Vezzani. 2012. Community structure of artificial container-breeding sites (Insecta: Diptera) in relation to the urbanization level. Landsc. Urban Plan. 105: 288–295.

Rueda, L. M., K. J. Patel, R. C. Axtell, and R. E. Stinner. 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 27: 892–898.

Seijo, A., D. Curcio, G. Aviles, B. Cernigoi, B. Deodato, and S. Lloveras. 2000. Imported Dengue in Buenos Aires, Argentina. Emerg. Inf. Dis. 6: 1–3

Seijo, A., B. Cernigoi, and B. Deodato. 2001. Dengue importado del Paraguay a Buenos Aires. Estudio clínico y epidemiológico de 38 casos. Medicina (Buenos Aires). 61: 137–41

Seijo, A., Y. Romer, M. Espinosa, J. Monroig, S. Giamperetti, D. Ameri, and L. Antonelli. 2009. Brote de dengue autóctono en el área metropolitana Buenos Aires. Experiencia del hospital de Enfermedades Infecciosas F. J Muñiz. Medicina (Bs As). 69: 593–600.

Tsuda, Y., and M. Takagi. 2001. Survival and development of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) larvae under a seasonally changing environment in Nagasaki, Japan. Environ. Entomol. 30: 855–860.

Tun Lin, W., T. R. Burkot, and B. H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med. Vet. Entomol. 14: 31–37.

Vega-Rúa, A., K. Zouache, R. Girod, A. B. Failloux, and R. Lourenço-de-Oliveira. 2014. High Level of Vector Competence of *Aedes aegypti* and *Aedes albopictus* from Ten American Countries as a Crucial Factor in the Spread of Chikungunya Virus. J. Virol. 88: 6294–6306.

Vezzani, D., S. M. Velázquez, and N. Schweigmann. 2004. Seasonal pattern of abundance of *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires City, Argentina. Mem. Inst. Oswaldo Cruz. 99: 351–356.

Vinogradova, E. B. 2007. Diapause in aquatic insects, with emphasis on mosquitoes, pp 83–113. *In* V. R. Alekseev, B. T. DeStasio, and J. J. Gilbert (eds.), Diapause in aquatic invertebrates: theory and human use. Springer, Dordrecht, The Netherlands.

Zuur, A. F., J. M. Hilbe, and E. N. Ieno. 2013. A Beginner's Guide to GLM and GLMM with R, pp 253.