Impact of rearing temperature and water pH on longevity and pesticide resistance in *Aedes aegypti* (Diptera: Culicidae)

María A. López-Latorre[1], Bianca Morejón[1] & Marco Neira[1]

[1]Center for Research on Health in Latin America (CISeAL), College of Exact and Natural Sciences, Pontifical Catholic University of Ecuador, Quito, Ecuador

Corresponding author:
mvneira@puce.edu.ec

ABSTRACT

Background: *Aedes aegypti* (Diptera: Culicidae) is considered as one of the main arboviral disease vectors around the planet. Because *A. aegypti* is an ectotherm and breeds in fresh water pools, climatic conditions influence its biology, distribution, population dynamics and vectorial capacity. To investigate the potential impact of future climate change on *A. aegypti´s* biology, we studied the effect of variations in environmental temperature and the pH of breeding water on this species longevity and pesticide resistance.

Methods: Mosquitoes of both geographic (experimental) and control strains were reared under different environmental conditions (environmental temperatures of 25°, 28°, 31°C, maintained throughout the insects´ life span; water pH values of 4, 5 and 6 set at the beginning of larval development). Upon

adult emergence, we monitored the longevity and pesticide resistance for each treatment.

Results: Both environmental temperature and water pH seem to influence adult longevity and pesticide resistance in *A. aegypti*. Longevity is significantly diminished in temperatures above or below 28°C, as well as with the acidification of the initial breeding water. Similarly, pesticide resistance seems to decrease significantly in specimens reared above or below 28°C, and in specimens reared in acidic environments.

Conclusions: We found that within our temperature gradient there is an optimal rearing temperature (28°C) which maximizes the longevity of our specimens. Additionally, the acidification of the aquatic habitats of larval stages diminished the lifespan of the vector. In reference to deltamethrin resistance, acidification of the breeding water as well as non-optimal rearing temperatures (25 & 31°C) produced lower levels of resistance than those observed in the control treatments, for both temperature and water pH.

Although the definitive effects of climate change on *A. aegypti* biology and physiology in the field are hard to predict, our study contributes novel information about the biology and physiology of this vector species under controlled conditions. This information could help us understand the potential effects of putative future climate scenarios on the transmission of arboviral diseases.

KEYWORDS

Climate change, *Aedes aegypti*, vector physiology, dengue, chikungunya, Zika, yellow fever

BACKGROUND

The mosquito *Aedes aegypti* (Diptera: Culicidae) is a competent vector of four major human arbovirosis (yellow fever, dengue, chikungunya and Zika) [1] and is considered as one of the main disease vectors around the planet [2,3]. Due to *A. aegypti*'s ability to quickly adapt and colonize human settlements in a wide range of environments [3], in recent years, the diseases transmitted by this species have emerged in several new regions [4,5], expanding their range rapidly across the globe [5]. Because *A. aegypti* is an ectotherm and breeds in freshwater pools, climatic conditions, mainly temperature and precipitation [6,7], influence its biology, distribution, population dynamics and vectorial capacity [8]. The intrinsic sensitivity of *A. aegypti* to environmental conditions suggests that this species would be affected by climate change [9,10]. Thus, climate change could have a substantial impact on the epidemiological landscape of all diseases transmitted by *A. aegypti* [9–11].

Currently, world climate is in a warming phase mainly because of the accumulation of greenhouse gases in the atmosphere [12]. There are indications that the average global temperature (that is, the temperature of land and ocean surface combined) has increased by approximately 0.85°C during the period between 1880 and 2012 [13]. Furthermore, 2016 ranks as the warmest year on record [14], and for the year 2100 climate change models predict an increase that could range from 1.5°C to more than 6^{o}C, depending on future greenhouse gas emissions [13,15].

In addition to global warming there are other ways in which climate change is affecting our environment, including the acidification of water bodies across the planet. This phenomenon is a consequence of factors such as the oceanic absorption of CO_2 and acid rain, which have the effect of lowering the pH of surface water bodies [13,16]. Acid rain is defined as any form of precipitation with acidic components [17] and is the result of the interaction of atmospheric water with sulphur dioxide and nitrogen oxides, forming sulfuric and nitric acids [18], respectively. Rainwater normally exhibits a pH around 5.6 [19], whereas the pH of acid rain usually ranges from 4.2 to 4.4 [17]. Any rain that presents pH values lower than 5.6 is considered acid [18,20].

As previously stated, climate change can affect several aspects of the biology of *A. aegypti*. On the one hand, studies analyzing environmental effects in the distribution of *A. aegypti* suggest range shifts along both the latitudinal [21] and altitudinal [22,23] gradients [24]. Some of these studies also predict that if the increase in environmental temperature continues, the transmission of arboviral diseases could expand to new areas [24,25]. On the other hand, however, a recent study about the distribution of *A. aegypti* and other vector species in Ecuador [8] proposes that global warming over the next 80 years could lead to the decline of *A. aegypti* populations in several areas of the country, due to the reduction of habitats suitable for this species. This could, in turn, cause a reduction of the transmission of vector-borne diseases in the country [8].

Because temperature is an environmental factor predicted to be strongly affected by future climate change [26,27], it is worth examining the ways in

100 which it may influence the life-cycle and population dynamics of *A. aegypti* [28].
101 Former studies have proposed that the longevity of the vector is lower at high
102 temperatures [29,30], and that the highest life expectancy is reached when
103 specimens are reared at around 27°C [29,30]. Furthermore, extreme
104 temperatures (whether high or low) reduce vectorial capacity for dengue virus
105 transmission [31]. In addition, increased rearing temperatures have been
106 associated with increased sensitivity to pesticides in mosquito larvae [32].
107
108 Additionally, the deleterious effects of water acidification are well established for
109 other species of invertebrates with aquatic stages, mainly in terms of reductions
110 in abundance and species diversity [33]. Nevertheless, there is very little
111 information about the influence of water pH variation in the biology of *A.*
112 *aegypti*. It is plausible to assume that the pH of water in many (probably most)
113 mosquito breeding sites can be affected by acid rain [18]. Because
114 osmoregulation is an essential physiological process for the normal completion
115 of larval development [34], the acidification of larval environments could
116 influence the biology of the resulting adults.
117
118 Although *A. aegypti* larvae can complete their development in waters ranging
119 from pH 4 to 11 [34], it has been proposed that specimens reared at different
120 pH values do display differential characteristics. For example, one study
121 showed that the mortality rate was comparatively higher in *A. aegypti* larvae
122 reared in acidic environments (pH 4) than in larvae reared in neutral or slightly
123 alkaline environments (pH 7-8) [35], suggesting that acidic environments are
124 not optimal for the survival of this species.

In this study, we analyzed how variations in environmental conditions could affect biological parameters relevant to *A. aegypti´*s ability to transmit disease. Specifically, we evaluated the effects of temperature and breeding water pH on longevity and pesticide resistance.

MATERIALS AND METHODS

1. Mosquito rearing

Two strains of *A. aegypti* were used in our study. Our experimental strain was collected in 2015 in the city of Machala (El Oro province, Ecuador) and has been maintained in the Center for Research on Health in Latin America (CISeAL) ever since [36]. Additionally, we used the Rockefeller/UGAL strain as reference (control) for experiments on pesticide resistance, because this strain is known to lack genes associated to pesticide resistance [37].

Unless otherwise required by experimental protocols, populations were maintained in climate-controlled insectaries at standard conditions (28°C +/- 1°C temperature, 80% +/-10% relative humidity, 12 h light: 12 h darkness photoperiod). Larvae were fed finely ground fish food, and adults were fed with a 10% sucrose solution *ad libitum* [38].

2. Exposure of mosquitoes to different temperatures

Mosquitoes of both the experimental and control strains were reared in temperature-controlled environments at three different temperatures: 25°C, 28°C, and 31°C. Because 28°C is generally considered as a standard

150 temperature for *A. aegypti* rearing, this temperature was selected as our control.
151 We selected 31°C to represent the potential increase of temperature by climate
152 change, and 25°C to create a temperature gradient for the analyses.
153
154 Environments with specific temperatures were established before egg hatching,
155 and conditions were maintained throughout the insect's life cycle. Immediately
156 upon pupation specimens were sorted by sex, and only females were used
157 thereafter. Males were killed by freezing and discarded.
158
159 **2.1 Effects of temperature on longevity**
160 To determine the effect of rearing temperature on adult longevity, we
161 established a cohort of 100 virgin females of the experimental strain
162 immediately upon their emergence as adults. Mosquitoes were placed in cages
163 inside the temperature controlled environments set at the aforementioned
164 temperatures (25^{o}C, 28^{o}C, 31^{o}C) and were fed exclusively with a 10% sucrose
165 solution *ad libitum*. Daily mortality was recorded over a period of 30 days. For
166 each temperature, we conducted three replicates of the experiment, each with
167 100 virgin females.
168
169 **2.2 Effects of temperature on pesticide resistance**
170 Susceptibility to technical grade deltamethrin (TGD) was evaluated using the
171 CDC bottle bioassay method [39]. This method uses diagnostic time and dose
172 as reference points against which all results will be evaluated. The diagnostic
173 dose is defined as the concentration of insecticide that should kill 100% of

susceptible mosquitoes within the diagnostic time, which in the case of TGD has been defined as 30 minutes [39].

In order to establish an appropriate diagnostic dose for our study, we performed a calibration bioassay following the recommendations of Brogdon and Chan (2012) [38]. Briefly, glass bottles coated with different amounts (5, 2.5, 1.25, and 0.625 µg) of TGD were prepared. Susceptible mosquitoes (Rockefeller strain) were exposed to each of these TGD doses for 30 minutes (diagnostic time) and the number of dead mosquitoes was recorded after this period. Three replicates of the calibration bioassay were conducted and LOGIT statistical analysis were performed using the IBM SPSS Statistics v23 software package [40].

To perform assays aimed at evaluating pesticide resistance in our experimental groups, deltamethrin-coated glass bottles were prepared following the protocol described by Brogdon & Chan (2012) [39], using ethanol as solvent. Each bioassay consisted of four TGD diagnostic dose-coated bottles and one control bottle, coated only with diluent (ethanol). Twenty to 25 virgin female mosquitoes of the experimental strain, reared at the aforementioned temperatures, were introduced in each bottle. In parallel, identical bioassays using susceptible mosquitoes (Rockefeller strain) exposed to the same temperatures were performed as controls.

The number of dead and alive mosquitoes was recorded at 15-minute intervals for 120 minutes. Mosquitoes were considered dead when they were incapable

of flying or maintaining an upright posture on the surface of the bottle. Three replicates of the assay were conducted for each temperature group.

3. Exposure to different pH in the rearing water

In order to evaluate the effects of acidification of the rearing water, we arranged a set of three separate containers where water pH was set at 4, 5 and 6, respectively. Because the pH of normal rain water is approximately 6 [19], we used this value as our reference (control) point. Other values were selected to represent the potential acidification of water caused by climate change.

Before egg hatching, the pH of the water on each media was measured and adjusted to the specific value desired using sulphuric acid. No other pH adjustments were made during the experiment. Mosquitoes of both strains were reared in each treatment inside a climate-controlled room at standard conditions (28°C +/- 1°C temperature, 80% +/-10% relative humidity, 12 h light: 12 h darkness photoperiod). Upon pupation, individuals were sorted by sex and only females were maintained for further experimentation, while males were killed by freezing and discarded. Emerging adults were used to evaluate their longevity and pesticide resistance, as described below.

3.1 Effects of pH variation on longevity

Experiments to determine the effect of water acidification in adult longevity were set up similarly to those described in section 2.1. Briefly, we established a group of 100 virgin females of the experimental strain immediately upon their emergence as adults. Mosquitoes were placed in cages inside controlled

environments set at standard conditions (28^{o}C temperature, 80% relative humidity, 12 hours light: 12 hours darkness photoperiod) and were fed exclusively with a 10% sucrose solution *ad libitum*. Daily mortality was recorded over a period of 30 days. For each pH value, we conducted three replicates of the experiment.

3.2 Effects of pH variation on pesticide resistance

The CDC bottle bioassay method described by Brogdon & Chan (2012) [39] was carried out as described previously in section 2.2, using adult individuals emerging from the groups of larvae exposed to water with each of the different pH values throughout immature development.

4. Statistical analysis

4.1 Longevity analysis

For analyzing daily mortality, interval censored survival data was plotted and analyzed using the 'survival' function available as part of the statistical software package R [41]. A log-rank hypothesis test was used to compare the survival distributions of each treatment vs. control, and between two experimental treatments, for both temperature and water pH. Across the 30 days of tracing, comparisons were made at seven points: days 0, 5, 10, 15, 20, 25 and 30. In order to explain the differences between the survival distributions, one *p* value was obtained for each survival comparison (treatments vs. control, and between treatments).

4.2 Pesticide resistance analysis

CDC bioassay results were calculated as the average mortality percentage at the diagnostic time. The resistance status was evaluated in accordance with WHO guidelines [39] and all mosquitoes surviving the diagnostic time were considered resistant.

Using the IBM SPSS Statistics 23 software package [40], descriptive statistical analyses and a Shapiro-Wilk test were performed in order to identify the distribution of the mortality data obtained for mosquitoes exposed to each temperature and initial water pH. The variation coefficient was less than 20% in all the cases and for that reason transformation of data was not necessary (Table 1 and 5). We obtained p values > 0.05 in all the data analyzed with Shapiro-Wilk (normality test) and Levene (homogeneity of variance test), which demonstrated the normal distribution of the data (Table 1 and 5). Based on this, to determine the differences between the results, One-Way ANOVA test were performed (Table 2 and 6) with a Tukey-Kramer post-hoc test (Table 3, 4, 7 and 8).

RESULTS

1. Calibration bioassay

To establish the diagnostic dose, it is necessary to evaluate the saturation point, defined as a concentration above which the time to kill 100% of the mosquitoes remains the same even if the concentration increases [39]. After the

performance of the CDC bottle bioassay calibration, 5µg was established as the saturation point. In according with these results, the LOGIT statistical test showed that 5.4µg was the TGD concentration to kill 99% of susceptible mosquitoes (LC_{99}= 5.4µg CI= 2.6 - 8.2). Based on these results, we selected 5µg of TGD as the diagnostic dose for our pesticide resistance experiments.

2. Effects of temperature on longevity

Curves describing the average survival observed for each treatment over time are shown in figure 1. For our control group (28°C), survival probability decreases slightly over the first 25 days, reaching approximately 0.8 during this period, and approximately 0.5 by day 30. At the lowest temperature (25°C), mortality is minimal during the first 10 days, with more than 0.9 survival probability; however, survival probability decreases sharply after day 10, reaching a value of less than 0.2 by day 30. On the other side of the spectrum (31°C), survival probability decreases slowly over the first 15 days, reaching 0.8 by this point, and then dropping steeply, reaching approximately 0.2 by day 30 (fig. 1).

As previously mentioned, survival probability of the specimens was compared at days 0, 5, 10, 15, 20, 25 and 30. The Log-rank test showed significant differences ($p<0.001$) between the survival probabilities of each experimental group (25°C & 31°C) when compared with the control group (28^{o}C; figures 1A and 1B). However, when survival probabilities were compared between 31°C and 25°C, no significant differences were observed ($p= 0.8899$; figure 1C).

3. Effects of temperature on pesticide resistance

Our results show that after 30 minutes of exposure to our diagnostic dose of TGD, the average percentages of mortality in the experimental strain were 51%, 14.3% and 41.6% when reared at 31°C, 28°C and 25°C respectively (table 1 and fig. 2). For the reference strain the percentage of mortality at the diagnostic time was 100% in all temperatures.

Our One-Way ANOVA results suggest there are highly significant differences ($p<0.001$) in the mean mortalities observed among groups exposed to different temperatures (Table 2). Tukey-Kramer post-hoc testing suggested the conformation of three ranks between the data: on one hand, the mean mortality of the populations reared at control temperature (28°C) was significantly lower than the mean mortality of populations reared at both 31°C and 25°C ($p<0.001$; table 3 & 4 and figure 2). And on the other hand, mortality of populations reared at 31°C was significantly higher than the mortality of populations maintained at 25°C ($p < 0.001$; table 3 & 4 and figure 2).

4. Effects of water pH on longevity

Curves describing the average survival observed for each treatment over time are shown in figure 3. For our control group (water pH set at 6), survival probability decreases gradually over the first 15 days, reaching approximately 0.8 during this period, and approximately 0.5 after 30 days. At the most acidic water pH of our experimental gradient (pH 4), the survival probability began to decrease before day 10, and it then decreased sharply throughout the experimental period, reaching a value of less than 0.1 by day 30. At pH5,

survival probability decreased slowly until day 15, reaching 0.85 by this point; subsequently it drops steeply, reaching approximately 0.2 by day 30.

The Log-rank test comparing survival probability curves of control (pH6) with experimental groups (pH4 and 5) at 0, 5, 10, 15, 20, 25, 30 days revealed significant differences ($p<0.001$; figure 3). By comparing survival probability curves of the experimental groups with each other the Log-rank test also showed significant differences ($p<0.001$; figure 3). These results suggest that the initial breeding water pH influences substantially the longevity of adults, with more acidic pH values associated with reduced longevity.

5. Effects of pH variation on pesticide resistance

After 30 minutes exposure to deltamethrin-coated bottles, the average mortalities were 22.3%, 32.5% and 35.6% for experimental groups exposed to rearing water with pH values of 6, 5 and 4 respectively (Table 5). Mortality of the reference strain was 100% for all pH treatments at the diagnostic time.

One-Way ANOVA employed to compare the percentages of mortality observed in the populations reared at different pH revealed significant differences ($p<0.005$) in the mean mortality values among the different groups (Table 6). A Tukey-Kramer post-hoc test suggested the organization of the data in two ranks (Table 7 & 8 and figure 4), with significant differences observed only between the groups maintained at pH 6 (control) and pH 4 ($p<0.05$; table 7 & 8 and figure 4).

DISCUSSION

Organisms use a variety of physiological and behavioral strategies to face changes in temperature over space and time [42]. These strategies determine the thermal performance profile of the organisms, which in turn describe the temperature range at which each organism functions optimally, including critical temperatures [43]. Being a poikilothermic species, the biology of *A. aegypti* depends heavily on environmental conditions [44,45] to a large extent because ambient temperature directly influences the rate of biochemical reactions critical to their physiology [44,46].

All thermal adaptations depend on the distribution and investment of energy that organisms have to allocate in order to survive [42]. In *Aedes aegypti* higher temperatures are associated with faster developmental rate [29] and decreased survival [30,45]. Our results show that *A. aegypti* females reared at either 25°C or 31°C live less than those reared at 28°C (Figure 1), supporting the notion that the “optimal” temperature for this species lies around 28°C [30].

Interestingly, Mordecai et al (2017) [45] found that transmission of diseases vectored by *A. aegypti* (such as dengue, chikungunya and Zika), measured with an integral model that includes the vectorial capacity, vectorial competence, pathogen development rate, density of humans and human recovery rate, peaked at 28,5°C [45], a temperature very close to the optimal *for A. aegypti* survival. Therefore, it seems that temperatures around 28^{o}C are particularly conductive for disease transmission by *A. aegypti*.

374 Survival probability is one of the components of the formula used to estimate
375 vectorial capacity, which in turn is a concept used to describe the ability of a
376 vector to spread disease taking into account host, virus, and vector interactions
377 [31]. Therefore, the shortening of adult longevity due to sub-optimal
378 temperatures could directly influence transmission, since a reduced life span
379 could result in the death of infected vectors before they are able to transmit the
380 pathogens [31].
381
382 Another important physiological feature capable of influencing the transmission
383 of vector-borne diseases is the degree of pesticide susceptibility displayed by
384 any given insect population. In our study, mosquitoes exposed to both extremes
385 of the selected temperature gradient (25^{o}C and 31^{o}C) showed a higher
386 susceptibility to deltamethrin than those exposed to 28^{o}C (Figure 2). Since one
387 of the main physiological mechanisms associated with the detoxification of
388 pesticides in insects is the production of detoxifying enzymes [47], it seems
389 plausible that these results could reflect the effect of environmental temperature
390 on the molecular physiology of such enzymes. Insect enzymes have specific
391 temperatures in which they work optimally [39], and their activity is reduced
392 steadily as the temperature of their environments moves away from this optimal
393 temperature [48–50]. Therefore, the susceptibility to pesticides -to the extent
394 that it depends on enzymatic activity- is likely to follow a similar pattern,
395 displaying an optimum temperature which allows the insect to optimally detoxify
396 its organism and maximize its chances for survival, and increasing susceptibility
397 towards both sides of the temperature spectrum.
398

399 Polson and colleagues (2012) proposed the idea that *A. aegypti* larval
400 populations reared at higher temperatures showed increase susceptibility to
401 organophosphates [32]. Although our study evaluates the susceptibility to a
402 different kind of pesticide (a pyrethroid) in adult populations, the results we
403 obtained at 31^{o}C are compatible with those obtained by Polson and colleagues
404 [32]. Interestingly, our results for the lower temperature (25^{o}C) show a similar
405 increase in susceptibility. Unfortunately, Polson and colleagues didn´t use
406 temperatures below 25^{o}C for their experiment [32], so it is not possible to
407 establish whether susceptibility to organophosphates shows a similar pattern in
408 response to lower temperatures as the susceptibility to pyrethroids observed in
409 our study.
410
411 Regarding the effects of rearing water pH, in our study we observed a
412 significantly reduced longevity in females reared in more acidic water (pH 4, 5)
413 compared to those reared at pH 6 (fig. 3). The significance of these results
414 becomes obvious when we consider that *A. aegypti* utilizes water-filled
415 structures in human settlements for breeding [51], and rain constitutes one of
416 the main sources of water for these breeding sites; therefore, there is a strong
417 link between changes in rainwater quality and availability, and the development
418 and abundance of this vector species [51].
419
420 It has been reported that the optimal pH for the development of *A. aegypti* is
421 around 7 [34,52], although this species has the ability to develop in waters with
422 pH ranging from pH 4 to 11 [34]. As previously mentioned, the pH of rain water
423 is normally around 5.6 [19], but there is a current tendency towards its

424 acidification. Therefore, it is not unlikely that in the future we will see an
425 increasing acidification of *A. aegypti* breeding sites. Based on our results, this
426 could translate in a trend towards a continuous reduction in vector longevity,
427 which could in turn have profound implications in the context of disease
428 transmission: because the survival of adult female *A. aegypti* mosquitoes is a
429 critical component of their ability to transmit pathogens [46], a reduction in
430 longevity caused by acidification of breeding sites could conceivably translate
431 into a reduction of vector capacity, either because of a reduced rate of contact
432 between infected females and humans hosts, or because pathogens could not
433 have enough time to complete their extrinsic incubation period inside the
434 mosquitoes [53,54].
435
436 Regarding the effects of pH variation on pesticide resistance, our results show
437 that the highest mortality of mosquitoes exposed to deltamethrin was achieved
438 when they were reared in the most acidic pH (Figure 4). Although the reasons
439 for this increased susceptibility are not clear, one potential explanation is a
440 depletion of energetic reserves caused by the necessity to up-regulate
441 mechanisms responsible for ionic balance during larval development. In any
442 case, these results imply that if the aforementioned tendency towards
443 acidification of *A. aegypti* breeding sites were to be maintained, over time this
444 could have an impact on the overall susceptibility of this species to
445 deltamethrin, as well as potentially other pesticides. More research is needed to
446 fully understand the magnitude of this effect, and whether it can be generalized
447 to other vector populations.
448

449 It is important to mention that all geographic populations assayed in this study,
450 independently of their rearing conditions, showed some degree of resistance to
451 deltamethrin based on criteria defined by the WHO [39]. Our results in this area
452 are in concordance with a former study that suggested a wide distribution of
453 alleles associated to deltamethrin resistance in Ecuadorian *A. aegypti*
454 populations [36].
455
456 **CONCLUSIONS**
457
458 By determining the effects of different rearing temperatures and initial water pH
459 on longevity and pesticide resistance, our study provides valuable insights
460 regarding environmentally-induced physiological changes in *A. aegypti*.
461
462 From our studies involving environmental temperatures, we found that there
463 seems to be an “optimum” temperature (28°C) which maximizes the longevity of
464 the specimens. Temperatures above or below this optima diminished
465 substantially the lifespan of the vector. Moreover, our results suggest that
466 rearing temperature also impacts deltamethrin susceptibility, with specimens
467 reared in hotter or colder environments becoming more susceptible to this
468 pesticide. Therefore, if predictions about increasing global warming are correct,
469 it is plausible that *A. aegypti* populations in the future could display a reduced
470 lifespan and become more susceptible to pesticides than present-day
471 populations.
472

Regarding the effects of variations in rearing pH, our results suggest that the acidification of the aquatic habitats results in decreased adult survival. Furthermore, our study also suggests that there is a correlation between breeding water pH and deltamethrin susceptibility, with more acidic environments generating more susceptible adults. Taken together, these results could suggest that the expected trend towards acidification of mosquito breeding sites would result in populations where adult *A. aegypti* mosquitoes are shorter lived, and more susceptible to pesticides than current day populations. However, there are a few caveats with this prediction: on the one hand, it does not consider the effects of any future changes, such as genetic mutations in the vector population, which could also impact the life history of this species. And on the other hand, the limitations of our work do not allow us to establish whether the results presented in this report can be generalized to other *A. aegypti* populations.

The definitive effects of climate change on *A. aegypti* biology and physiology are hard to predict. With this study, we hope to provide data that contribute to our ability to better understand the effects of future environmental change in the biology of one of the main vectors of disease in our planet. Although the results presented in this study need to be confirmed and refined by further research, we hope that the trends we have outlined can one day be included as factors in predictive models to help us understand the complex and ever-changing landscape of human diseases transmitted by *A. aegypti*.

Competing interests

The authors declare no competing interests.

Authors' contributions

MAL and MN designed the experiment and wrote the manuscript. MAL conducted the experimental work, collected data and performed statistical analyzes. MN supervised all experimental work and data analysis. BM helped with statistical analysis and interpretation.

All authors have read and approved the final manuscript.

Acknowledgements

The authors would like to thank Dr. Audrey Lenhart for providing us with specimens of the Rockefeller strain. Julio César Sánchez-Otero provided helpful guidance for statistical analysis. Galo Enrique Rivera helped developing scripts in R for statistical analysis. Daniel Escobar Camacho proof-read the manuscript and provided helpful comments. Various members of CISeAL´s Medical Entomology Group contributed to the maintenance of mosquito colonies under insectary conditions.

Funding for this work included a full scholarship awarded by PUCE/CISeAL to Maria de los Ángeles López for the completion of her graduate degree, as well as PUCE´s Research Grant M13409, awarded to Marco Neira.

REFERENCES

1. Patterson J, Sammon M, Garg M: **Dengue, Zika and Chikungunya: Emerging Arboviruses in the New World.** *West J Emerg Med* 2016, 17(6):671–679.
2. Calvez E, Guillaumot L, Millet L, Marie J, Bossin H, Rama V, et al: **Genetic Diversity and Phylogeny of *Aedes aegypti*, the Main Arbovirus Vector in the Pacific.** *PLoS Negl Trop Dis* 2016, 10:e0004374.
3. Cook G, Zumla A, Manson P: **Manson's tropical diseases**. *Saunders* 2009, 1830.
4. Uyar Y: **Arboviral Infections around the World**. *J Trop Dis* 2013, 1(4): 1000e105.
5. Gubler D: **Human Arbovirus Infections Worldwide**. *Ann N Y Acad Sci* 2006, 951(1):13–24.
6. López-Latorre MA, Neira M: **Influencia del cambio climático en la biología de *Aedes aegypti* (Diptera: Culicidae) mosquito transmisor de arbovirosis humanas.** *Rev Ecuat Med Cienc Biol* 2016, 37(2):11–21.
7. Morin CW, Comrie AC, Ernst K: **Climate and Dengue Transmission: Evidence and Implications.** *Environ Health Perspect* 2013, 121(11-12):1264–1272.
8. Escobar LE, Romero-Alvarez D, Leon R, Lepe-lopez MA, Craft ME, Borbor-cordova MJ, et al: **Declining Prevalence of Disease Vectors Under Climate Change**. *Nat Sci Rep* 2016, 6:39150.
9. Campbell-Lendrum D, Manga L, Bagayoko M, Sommerfeld J: **Climate**

change and vector-borne diseases: what are the implications for public health research and policy? *Philos Trans R Soc B* 2015, 370: 20130552.

10. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA: **Climate change and vector-borne diseases: a regional analysis**. *Bull World Health Organ* 2000, 78(9):1136–1147.
11. Ogden NH, Lindsay LR: **Effects of Climate and Climate Change on Vectors and Vector-Borne Diseases: Ticks Are Different.** *Trends Parasitol* 2016, 32(8): 646-656.
12. Reiter P: **Climate change and mosquito-borne disease**. *Environ Health Perspect* 2001, 141–161.
13. IPCC in ***Climate Change 2014: Synthesis Report.*** *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Edited by Core Writing Team, Pachauri RK and Meyer LA. IPCC 2014, Geneva, Switzerland: 151.
14. NASA: **Climate Change and Global Warming** [Internet]. 2016 [cited 2017-Mar-01]. Available from: https://climate.nasa.gov/
15. Allison I, Bindoff N, Bindschadler R, Cox P, de Noblet N, England M, et al: **The Copenhagen Diagnosis.** The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia 2009, 60.
16. Gleick PH: **Climate Change, Hydrology, and Water Resources**. *Rev Geophys* 1989, 27(3): 329–344.
17. US EPA: **Acid Rain**. [Internet]. 2017 [cited 2017-Jun-5]. Available from: https://www.epa.gov/acidrain

18. Singh A, Agrawal M: **Acid rain and its ecological consequences**. *J Enviromental Biol* 2008, 29(1):15–24.
19. Liljestrand HM: **Average rainwater pH, concepts of atmospheric acidity, and buffering in open systems**. *Atmos Environ* 1985, 19(3):487–99.
20. Bandyopadhyay B, Kumar P, Biswas P: **Ammonia Catalyzed Formation of Sulfuric Acid in Troposphere : The Curious Case of a Base Promoting Acid Rain**. *J Phys Chem* 2017, 1–33.
21. Khormi HM, Kumar L: **Climate change and the potential global distribution of *Aedes aegypti*: spatial modelling using geographical information system and CLIMEX**. *Geospat Health* 2014, 8(2): 405–415.
22. Suarez MF, Nelson MJ: **Regristro de Altitud del *Aedes aegypti* en Colombia**. *Biomedica* 1981, 1(4):225.
23. Parmesan C: **Ecological and Evolutionary Responses to Recent Climate Change.** *Annu Rev Ecol Evol Syst* 2006, 37(1):637–669.
24. Hopp MJ, Foley JA: **Global-Scale Relationships Between Climate and the Dengue Fever Vector, *Aedes Aegypti***. *Clim Change* 2001, 48:441–463.
25. Hales S, de Wet N, Maindonald J, Woodward A: **Potential effect of population and climate changes on global distribution of dengue fever: an empirical model**. *Lancet* 2002, 360(9336):830–834.
26. Santer BD, Taylor KE, Wigley TML, Johns TC, Jones PD, Karoly DJ, et al: **A search for human influences on the thermal structure of the atmosphere**. *Nature* 1996, 382: 39–46.
27. Watson RT, Zinyowera MC, Moss RH, Dokken DJ: **Climate Change**

598 **1995: The IPCC Second Assessment Report Scientific-Technical**
599 **Analyses of Impacts, Adaptations, and Mitigation of Climate**
600 **Change.** *Cambridge Univ Press* 1995, 857.
601 28. Barrera R, Amador M, MacKay AJ: **Population Dynamics of *Aedes***
602 ***aegypti* and Dengue as Influenced by Weather and Human Behavior**
603 **in San Juan, Puerto Rico**. *PLoS Negl Trop Dis* 2011, 5(12):e1378.
604 29. Couret J, Dotson E, Benedict MQ: **Temperature, larval diet, and**
605 **density effects on development rate and survival of *Aedes aegypti***
606 **(Diptera: Culicidae)**. *PLoS One* 2014, 9(2):e87468.
607 30. Goindin D, Delannay C, Ramdini C, Gustave J, Fouque F: **Parity and**
608 **Longevity of *Aedes aegypti* According to Temperatures in**
609 **Controlled Conditions and Consequences on Dengue Transmission**
610 **Risks**. *PLoS One* 2015, 10(8):e0135489.
611 31. Liu-Helmersson J, Stenlund H, Wilder-Smith A, Rocklöv J: **Vectorial**
612 **capacity of *Aedes aegypti*: Effects of temperature and implications**
613 **for global dengue epidemic potential**. *PLoS One* 2014, 9(3):e89783.
614 32. Polson KA, Brogdon WG, Rawlins SC, Chadee DD: **Impact of**
615 **environmental temperatures on resistance to organophosphate**
616 **insecticides in *Aedes aegypti* from Trinidad**. *Rev Panam Salud*
617 *Pública* 2012, 32(1):1–8.
618 33. Allan J, Castillo M. **Stream Ecology**. **Structure and Function of**
619 **Running Waters**. Second edition. *Springer* 2007, 429.
620 34. Clark TM: **pH tolerances and regulatory abilities of freshwater and**
621 **euryhaline Aedine mosquito larvae**. *J Exp Biol* 2004, 207(13):2297–
622 2304.

623 35. Sequihod I: **Effects of Different pH levels on the Viability,**
624 **Metamorphosis Rate and Morphology of *Aedes* Mosquitoes**. St. Paul
625 University Dumaguete 2016, 1–27.
626 36. Aguirre A: **Determinación de la sensibilidad al insecticida**
627 **deltametrina e identificación de alelos de resistencia a piretroides**
628 **en poblaciones ecuatorianas de *Aedes aegypti***. (Bachelor thesis).
629 Universidad de las Fuerzas Armadas—ESPE, Sangolqui, Ecuador. 2016.
630 37. Kuno G: **Early History of Laboratory Breeding of *Aedes aegypti***
631 **(Diptera : Culicidae) Focusing on the Origins and use of Selected**
632 **Strains**. *J Med Entomol* 2010, 47(6):957–971.
633 38. Neira M, Lacroix R, Cáceres L, Kaiser P, Young J, Pineda L, et al:
634 **Estimation of *Aedes aegypti* (Diptera: Culicidae) population size**
635 **and adult male survival in an urban area in Panama**. *Mem Inst*
636 *Oswaldo Cruz* 2014, 109:879–86.
637 39. Brogdon W, Chan A: **Guideline for Evaluating Insecticide Resistance**
638 **in Vectors Using the CDC Bottle Bioassay**. *CDC Methods* 2012, 28.
639 40. IBM Knowledgecenter - **IBM SPSS Statistics V23.0 documentation**
640 [Internet]. 2014 [cited 2017-Jul-26]. Available from:
641 https://www.ibm.com/support/knowledgecenter/en/SSLVMB_23.0.0/spss/
642 product_landing.html.
643 41. The R Foundation. **R: The R Project for Statistical Computing**
644 [Internet]. 2017 [cited 2017 Jun 5]. Available from: https://www.r-
645 project.org/
646 42. Angilletta MJ: **Thermal adaptation : a theoretical and empirical**
647 **synthesis**. *Oxford University Press* 2009, 289.

43. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, et al: **Impacts of climate warming on terrestrial ectotherms across latitude**. *Proc Natl Acad Sci USA* 2008, 105(18):6668–6672.
44. Adamo SA, Lovett MME: **Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket Gryllus texensis**. *J Exp Biol* 2011, 214(12):1997–2004.
45. Mordecai EA, Cohen JM, Evans M V, Gudapati P, Johnson R, Lippi CA, et al: **Detecting the impact of temperature on transmission of Zika, dengue and chikungunya using mechanistic models.** *PLoS Negl Trop Dis* 2017, 11(4): e0005568.
46. Brady OJ, Johansson MA, Guerra CA, Bhatt S, Golding N, Pigott DM, et al: **Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings**. *Parasit Vectors* 2013, 6(351):1–12.
47. Dusfour I, Zorrilla P, Guidez A, Issaly J, Girod R, Guillaumot L, et al: **Deltamethrin Resistance Mechanisms in *Aedes aegypti* Populations from Three French Overseas Territories Worldwide.** *PLoS Negl Trop Dis* 2015, 9(11):1–17.
48. Daniel RM, Danson MJ, Eisenthal R: **The temperature optima of enzymes: A new perspective on an old phenomenon**. *Trends Biochem Sci* 2001, 26(4):223–225.
49. Peterson ME, Eisenthal R, Danson MJ, Spence A, Daniel RM: **A new intrinsic thermal parameter for enzymes reveals true temperature optima**. *J Biol Chem* 2004, 279(20):20717–20722.
50. Patnaik PR: **Temperature optima of enzymes: Sifting fact from**

673 **fiction**. *Enzyme Microb Technol* 2002, 31(3):198–200.
674 51. Williams CR, Mincham G, Ritchie SA, Viennet E, Harley D: **Bionomic**
675 **response of *Aedes aegypti* to two future climate change scenarios**
676 **in far north Queensland, Australia: implications for dengue**
677 **outbreaks.** *Parasit Vectors* 2014, 7(1):447.
678 52. Sequihod IM: **Effects of Different pH levels, Temperatures, and**
679 **Photoperiod on the Viability, Metamorphosis Rate and Morphology**
680 **of *Aedes* sp. Mosquitoes**. Silliman University: 3–4.
681 53. Lambrechts L, Paaijmans KP, Fansiri T, Carrington LB, Kramer LD,
682 Thomas MB, et al: **Impact of daily temperature fluctuations on**
683 **dengue virus transmission by *Aedes aegypti*.** *Proc Natl Acad Sci*
684 *USA* 2011, 108(18):7460–7465.
685 54. Carrington LB, Armijos MV, Lambrechts L, Barker CM, Scott TW: **Effects**
686 **of Fluctuating Daily Temperatures at Critical Thermal Extremes on**
687 ***Aedes aegypti* Life-History Traits**. *PLoS One* 2013, 8(3):e58824.
688
689
690
691
692
693
694
695
696
697

Figures and Tables

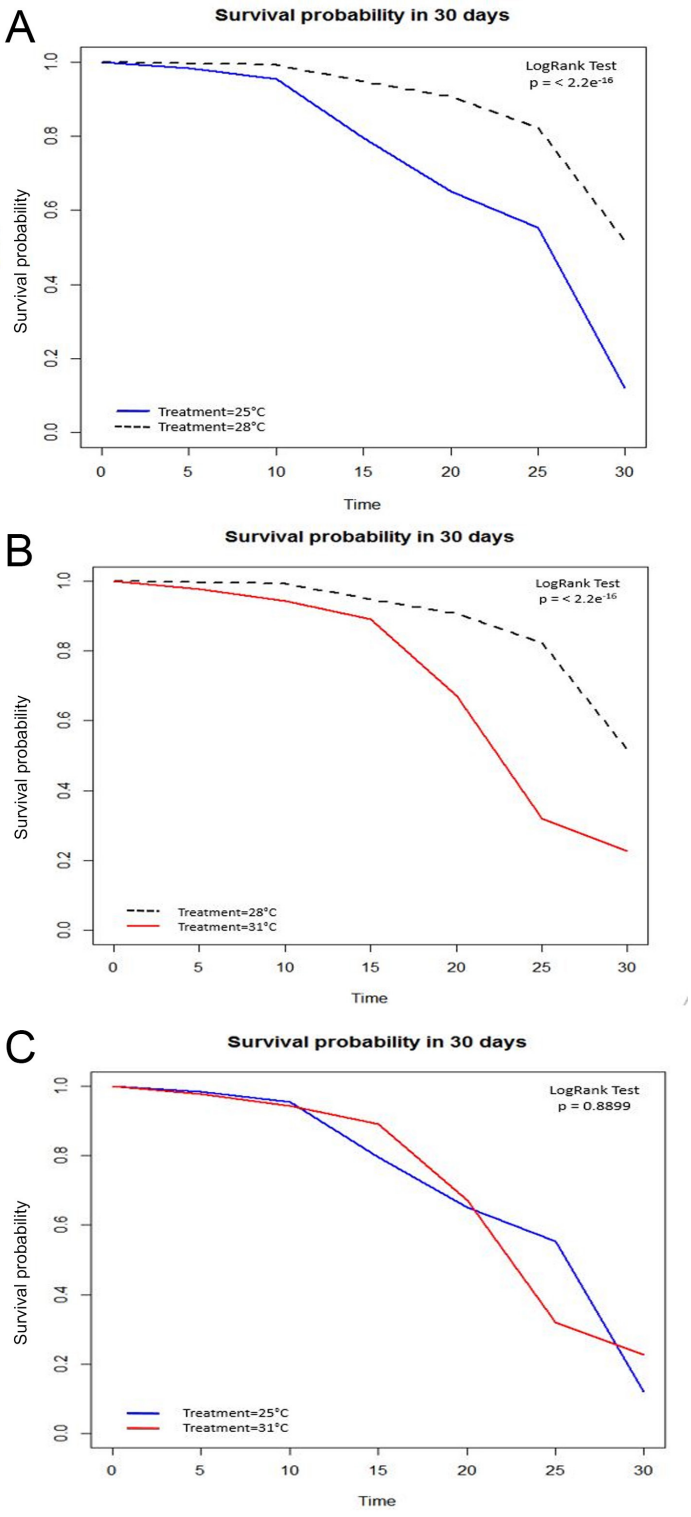

Figure 1. Survival analysis for mosquitoes reared under different temperatures. The survival probability for 30 days is shown. **A**. Survival comparison between groups maintained at 25°C and control groups (28°C). Log-rank test showed highly significant differences (p<0,001) between these two treatments. **B**. Survival comparison between groups maintained at 31°C and control groups (28°C). Log-rank test showed highly significant differences (p<0,001) between these two treatments **C.** Survival comparison of groups maintained at both experimental temperatures (25°C and 31°C). The log-rank test showed no significant differences (p>0,05) between these two treatments.

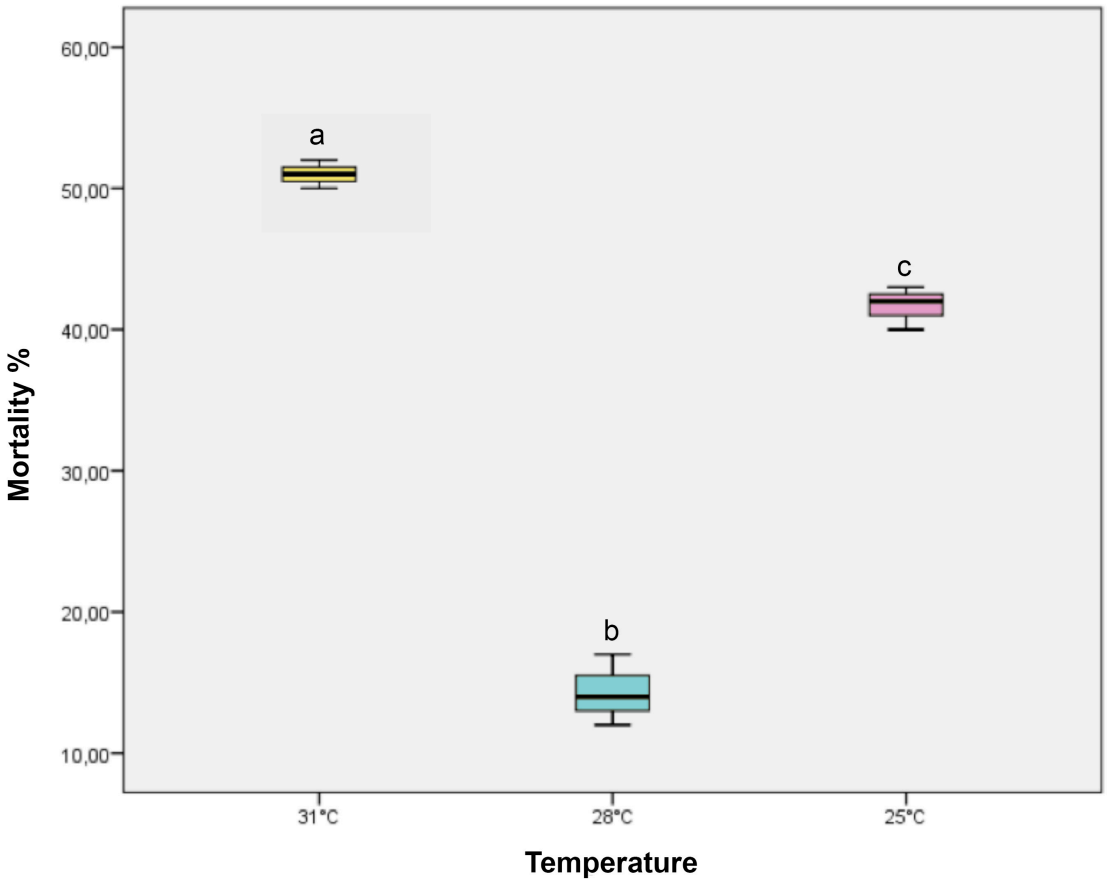

Figure 2. Distribution of the mean mortality percentage for mosquitoes of the experimental strain reared at different temperatures and exposed to deltamethrin. One-way ANOVA showed highly significant differences ($p<0,001$) between the average mortality values obtained for the three temperatures. Tukey-Kramer post-hoc testing revealed three different ranks, which are defined with letters “a”, “b”, “c”. Therefore, the mean mortality values observed in all groups are significantly different from each other. The mean mortality of the populations reared at 28°C was significantly lowest than mean mortality of populations reared at 31 and 25°C ($p=0.000$; table 3). Mortality of populations reared at 31°C was significantly higher than the mortality of populations maintained at 25°C ($p=0.002$; table 3). The black line inside the boxes represent the median and the bars standard error.

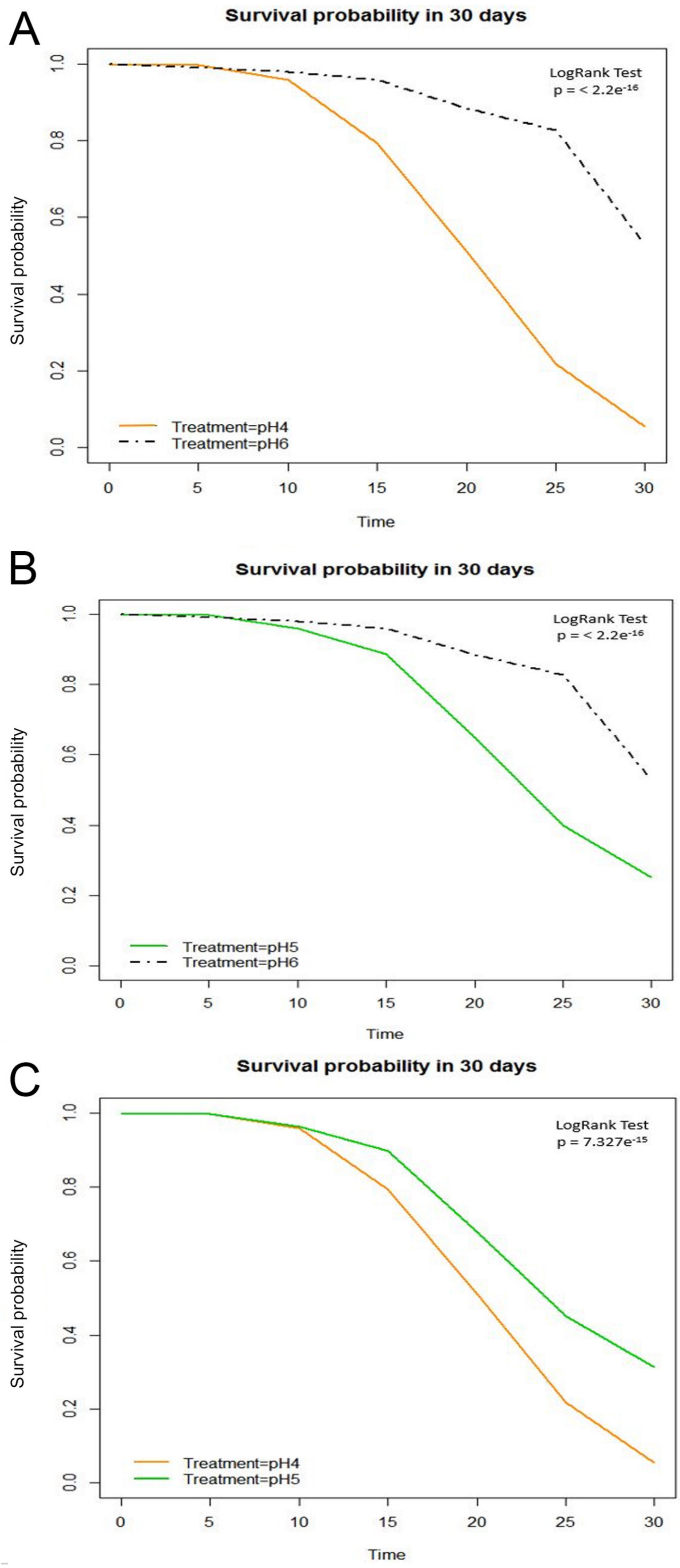

Figure 3. Survival analysis for mosquitoes reared under different initial breading pH. The survival probability for 30 days is shown. **A**. Comparison of survival between groups reared at pH4 and groups reared at pH6 (control). The log-rank test showed highly significant differences (p<0,001) between these two treatments. **B**. Comparison of survival between groups reared at pH5 and groups reared at pH6 (control). The log-rank test showed highly significant differences (p<0,001) between these two treatments **C.** Comparison of survival between groups reared at pH 4 and pH5. The log-rank test showed highly significant differences (p<0,001) between these two treatments.

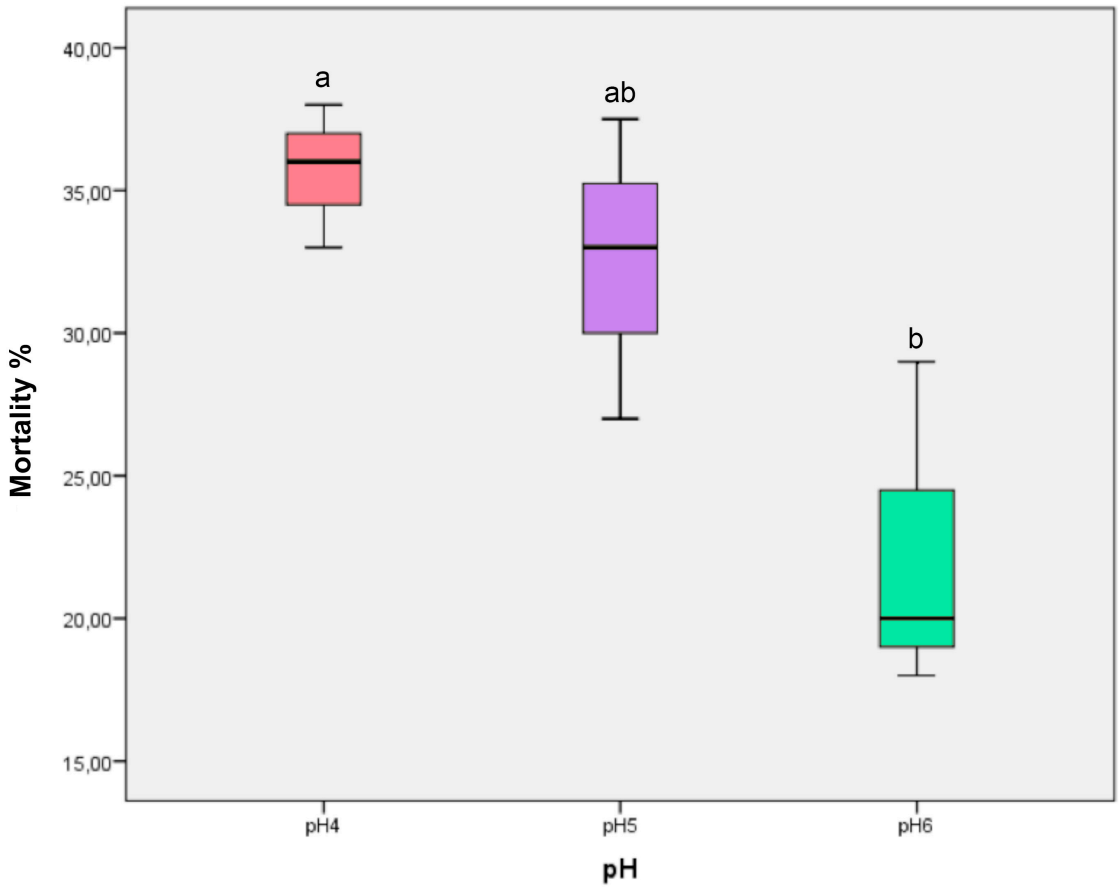

Figure 4. Deltamethrin susceptibility in mosquitoes of the experimental strain reared at different water pH. One-way ANOVA showed significant differences ($p<0,05$) between the mortality obtained for the three pH values assayed. Tukey-Kramer post-hoc testing revealed the existence of two separate ranks (shown with the letters “a” and “b”). Mortality observed in the group reared at pH 5 is not significantly different from that observed in the groups reared at pH 4 or pH 6 ($p>0.05$; table 7), and therefore this group is labeled as “ab”. Mortality in specimens reared at the most acidic pH (pH 4) is significantly higher from mortality observed in the control pH6 ($p=0.033$; table7). The black line inside the boxes represent the median and the bars the standard error.

Table 1. Descriptive statistical analysis of mortality data obtained for mosquitoes reared at different temperatures and exposed to deltamethrin.

°T	Normality Test (p-value)	Mean	95% mean confidence interval		Standar deviation	Standar Error	Variation Coefficient	Levene Homogeneity of variances (p-value)
			Lower limit	Upper limit				
31°C	1	51.00	48.52	53.48	1.00	.58	5%	0.373
28°C	0.78	14.33	8.08	20.58	2.52	1.45		
25°C	0.64	41.60	37.87	45.46	1.53	.88		
Total	0.06	35.70	22.93	48.41	16.58	5.53		

Table 2. One-way ANOVA for mortality data obtained from mosquitoes reared at different temperatures and exposed to deltamethrin.

	Sum of squares	Degrees of freedom	Cuadratic mean	Fisher test	Significance
Between grups	2178.67	2	1089.33	338.07	0.000
Into grups	19.33	6	3.22		
Total	2198.0	8			

Table 3. Tukey-Kramer post-hoc testing of mortality data obtained from mosquitoes reared at different temperatures and exposed to deltamethrin. Multiple comparisons of all the treatments are shown.

(I) Temperature	(J) Temperature	Difference of the means (I-J)	Standar error	Significance	95% Confidence Interval	
					Lower limit	Upper limit
31°C	28°C	36.67*	1.466	.000	32.17	41.16
	25°C	9.33*	1.466	.002	4.84	13.83
28°C	31°C	-36.67*	1.466	.000	-41.16	-32.17
	25°C	-27.33*	1.466	.000	-31.83	-22.84
25°C	31°C	-9.33*	1.466	.002	-13.83	-4.84
	28°C	27.33*	1.466	.000	22.84	31.83

* The difference of the means is significant.

Table 4. Ranks of Tukey-Kramer post-hoc testing of mortality data obtained from mosquitoes reared at different temperatures and exposed to deltamethrin. Means for groups in the homogeneous subsets are shown.

Temperature	N	Subsets (ranks) for alfa = 0.05		
		1	2	3
28°C	3	14.33		
25°C	3		41.67	
31°C	3			51.00

N: sample size

Table 5. Descriptive statistical analysis of the mortality in mosquitoes reared at different water pH and exposed to deltamethrin.

pH	Normality Test (p-value)	Mean	95% mean confidence interval		Standar deviation	Standar Error	Variation Coefficient	Levene Homogeneity of variances (p-value)
			Lower limit	Upper limit				
pH4	0.78	35.67	29.42	41.92	2.52	1.45	15%	0,359
pH5	0.84	32.50	19.41	45.59	5.27	3.04		
pH6	0.33	22.33	7.78	36.89	5.86	3.38		
Total	0.20	30.17	24.54	35.79	7.31	2.44		

Table 6. One-way ANOVA for the mortality data obtained for mosquitoes reared at different initial pH in the breading water, and exposed to deltamethrin.

	Sum of squares	Degrees of freedom	Cuadratic mean	Fisher test	Significance
Between grups	291.17	2	145.58	6.38	0.033
Into grups	136.83	6	22.81		
Total	428.00	8			

Table 7. Tukey-Kramer post-hoc testing of mortality data obtained from mosquitoes reared at different initial pH in the breading water, and exposed to deltamethrin. The multiple comparisons of all the treatments are shown.

(I) pH	(J) pH	Diference of the means (I-J)	Standar error	Significance	95% Confidence Interval	
					Lower limit	Upper limit
pH4	pH5	3.17	3.899	.710	-8.80	15.13
	pH6	13.33*	3.899	.033	1.37	25.30
pH5	pH4	-3.17	3.899	.710	-15.13	8.7971
	pH6	10.17	3.899	.089	-1.80	22.13
pH6	pH4	-13.33*	3.899	.033	-25.30	-1.37
	pH5	-10.17	3.899	.089	-22.13	1.80

*. The difference of the means is significant.

Table 8. Ranks of Tukey-Kramer post-hoc testing of mortality data obtained from mosquitoes reared at different initial pH in the breeding water, and exposed to deltamethrin. Means for groups in the homogeneous subsets are shown.

pH	N	Subsets (ranks) for alfa = 0.05	
		1	2
pH6	3	22.33	
pH5	3	32.50	32.50
pH4	3		35.67

N: sample size