

Life-history Traits of Two Medically Important Insects *Culex quinquefasciatus* Say and *Musca domestica* L. Influenced by Temperature and Humidity

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Abstract

Such vital life-history traits as incubation periods, immature durations and total egg-to-adult developmental periods of two medically important dipteran vector species viz., *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Musca domestica* L. (Diptera: Muscidae) were exposed to environmental chambers at 25°-32 °C and 60-95% RH to evaluate the impacts of the abiotic factors on growth and development of the insects. Results showed that highly significant decreases in the incubation, larval, pupal and total developmental periods were induced in both the species of insects ($P < 0,001$) by the increase in temperature by 8° C. In accordance with the temperature rise, however as expected, RH had a synchronous effect on all the life-history traits of the experimental insects. It was remarkable to note that *C. quinquefasciatus* and *M. domestica* responded differently to the changes in temperature and RH. Further analyses of the experimental data revealed that highly significant but negative correlations (r values between -0.68 and -0.98; $P < 0.001$ each at 18 df) existed between the developmental parameters and the abiotic factors under study. The relevance of the present findings to the SIT (sterile insect technique)-based control programme as well as the effects of climate change on the vector competence in the field have been discussed.

Keywords: *Culex quinquefasciatus*, *Musca domestica*, life-history traits, incubation, larval and pupal periods, egg-to-adult development, temperature, relative humidity, climate change.

Introduction

In several reports the succession of immature development, survival and adult distribution of dipteran insects was found to be affected and influenced by such abiotic environmental factors as

temperature and relative humidity (RH). Thus, the development of immature gall midge *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae) (Gillespie *et al.*, 2000), survival of larvae of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) (Tomberlin *et al.*, 2009; Holmes *et al.*, 2012), fecundity, fertility, larval development, adult reproduction and survival of *Aedes aegypti* L. (Diptera: Culicidae) (Costa *et al.*, 2010; Carrington *et al.*, 2013; Marinho *et al.*, 2016), development time, immature and adult survival, and mosquito size in *Culex* species complex (Ciota *et al.*, 2014), development and survival of immature stages of *Bactrocera* fruit flies (Danjuma *et al.*, 2014), development of the sheep blowfly *Lucilia cuprina* (Bansode *et al.*, 2016), and fecundity and survival of the tsetse fly strains *Glossina palpalis gambiensis* (Pagabeleguem *et al.*, 2016) have been investigated.

Dipteran insects such as mosquitoes and house flies are of much public health importance in both urban and rural areas in developing and populous countries like Bangladesh. The common housefly *Musca domestica*, which breeds in garbage, moist and dirty places, is most notorious since it is reported to transmit lethal diseases such as diarrhoea, cholera, dysentery, typhoid and shigellosis in the country (Farak *et al.*, 2013; Parvez *et al.*, 2016). *Culex quinquefasciatus*, on the other hand, is the most prevalent mosquito species in Bangladesh, where it transmits such dreadful diseases as elephantiasis and filarial infections (Ahmed *et al.*, 1988; Khan *et al.*, 2014; Alam *et al.*, 2015; Irish *et al.*, 2016).

Temperature and RH are vital abiotic variables that influence the rate of immature development, adult emergence and longevity in various arthropods species (Bansode *et al.*, 2016; Pagabeleguem *et al.*, 2016). It is generally well-established that increase in

temperature and RH induce significant variation in both adult and immature stage characteristics of insects including larval growth rates, development time, body size, fecundity and longevity (Loetti *et al.*, 2011; Ciota *et al.*, 2014). Based on an updated literature survey, it is hypothesized that the changes in temperature and RH will affect and/or favour reproduction, development, dispersal and geographical expansion of dipteran insects (Marinho *et al.*, 2016). In this study we aimed at: (1) investigating the impacts of variable temperatures and RH on such vital life-history traits as incubation, larval, pupal and egg-to-adult developmental periods of mosquitoes and house flies; and (2) comparing the effects of environmental factors on the life-cycle completion of these two insects under laboratory conditions. Findings of the study would help us understand how climate change could affect the biology, ecology and the risk of disease transmission by these vector species which, in turn, could contribute to a more precise interpretation of the field data.

Materials and Methods

The experiments were carried out during July 2015 and June 2016 in the Ecology, Biodiversity and Conservation Laboratory, Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh. Brief protocol and the experimental design are described in the following paragraphs.

Colonization of adult mosquitoes

The laboratory colonization technique for mosquitoes was adopted from Ferdousi & Islam (2006), the brief protocol is as follows: Using soft paint brushes, egg rafts of *C. quinquefasciatus* were collected from the stagnant water of several drains and ditches of the RU Campus, and transferred to the laboratory for mass rearing and colonization. The collected egg-rafts were released into 500 mL beakers provided with pond water. After hatching, the larvae were fed with toast biscuits and yeast in a ratio of 3:1 on each alternate day until pupation. Then the pupae were sieved through a strainer and transferred into beakers with clean pond water without food, and the beakers were placed in adult rearing cages of 35cm × 28cm × 33cm wooden frame construction covered on three sides by fine metallic mesh and nylon screening. The bottom of the cage was made of hard board, and the

fourth side was made up of plywood with a large square opening covered with a piece of fine-mesh cloth. Through the circular opening, beakers for oviposition, pupae for emergence, food for adults and other necessary materials were taken in and out, without letting mosquitoes to escape. This opening was tied with a piece of cord when not in use. The emerged adults were offered 10% glucose solution soaked in cotton pads on 9-cm diameter Petri dishes for the first 2-3 pre-oviposition days. Blood meals from tender and constrained chickens were provided to the females. Egg-rafts were collected in beakers provided with pond water. Thus a colony of *C. quinquefasciatus* was set up and inbred for two generations to eliminate natural or deleterious mutations, if any, which might have been incorporated in their genome.

Estimation of life-history traits in mosquitoes

Freshly laid egg-rafts, each containing about 80-150 eggs, were collected, transferred into larval food medium, and then transferred into an environmental chamber maintained initially at 25°, 28°, 30°, 32°, 35°, 38° and 40° C, and corresponding 60–95% RH and with a photoperiod of 12:12 (L:D). Owing to failure to complete the life-cycle and alarmingly increased mortality rates, however, data from such higher temperatures as 35°, 38° and 40° C were abandoned. The vital life-history traits such as incubation, larval, pupal and egg-to-adult (*i.e.* total) developmental periods in hours were therefore recorded at 25°-32 °C. Ten replicates were maintained for each developmental period.

Colonization of adult house flies

To produce a consistent quality of house flies at an economical cost, the methods described by Morgan *et al.* (1981) and Islam & Aktar (2013) were adopted with little modifications as follows: Adults of the local variety of *M. domestica* was collected from the poultry and fish markets at Binodpur, located adjacent to the Rajshahi University (RU) Campus. Small pieces of rope were hanged from the ceiling of the poultry and fish shops where adult flies accumulated at night and they were caught using polythene bags. Soon after catching, the flies were provided with milk soaked in sterilized cotton pads and then transported to the Laboratory of Ecology, Biodiversity and Conservation, Department of Zoology, RU, for colonization in 50cm × 30cm ×

200cm wooden cages with nylon nets. The doors of the cages were provided with wooden gates and pieces of muslin cloth for easy handling of flies during experiment. The food for the larvae and adult flies was prepared with 9 g powdered milk, 5 g fresh baker’s yeast dissolved in 100 mL distilled water. The adults were provided with food in 9-cm diameter Petri dishes containing cotton wool soaked in prepared food medium mentioned above. The cotton wools in the Petri dishes were changed every 24 hour as the medium tended to dehydrate and produce an unpleasant odour.

Estimation of life-history traits in house flies

Adult females laid in several batches of 75 to 150 eggs over a period of three to four days. The eggs were then collected, transferred into the larval culture medium, and then transferred into an environmental chamber maintained at an identical condition as that of mosquitoes mentioned above. Similar to mosquitoes, the incubation, larval, pupal and egg-to-adult developmental periods of the house flies were recorded in hours. Each developmental period for the insects also had 10 replicates.

Statistical analyses

Statistical analyses were performed using a statistical package SPSS version 16.0 for Windows. The effects of temperature and RH on the incubation, larval, pupal and egg-to-adult developmental periods of the insects were analyzed with one-way analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) tests, where P-values of ≤ 0.05 were regarded as statistically significant (Steel & Torrie, 1984). The influence of abiotic factors on these mean life-history traits were estimated using the Student’s 2-tailed independent sample *t*-tests, while coefficient of correlation (*r*) values and regression lines between temperature and RH ranges and the traits were estimated (Costa *et al.* 2010).

Results

Life-history traits in mosquitoes

The mean \pm SD incubation periods of the experimental mosquitoes were found to decrease gradually from 43.10 ± 1.85 hrs, 37.30 ± 0.95 hrs, 35.60 ± 1.58 hrs to 32.00 ± 1.56 hrs, at 25° , 28° , 30°

and 32° C, respectively (Fig. 1a). The corresponding RH values were $66.00 \pm 3.16\%$, $64.60 \pm 3.20\%$, $84.50 \pm 3.03\%$ and $89.70 \pm 4.35\%$, respectively (Table 1). The larval period also declined from 149.20 ± 2.30 hrs, 140.30 ± 2.00 hrs, 132.30 ± 2.11 hrs to 129.10 ± 2.56 hrs, respectively at 25° - 32° C (Fig. 1b). The pupal period, however, varied from 37.00 ± 1.05 hrs, 35.00 ± 1.33 hrs, 32.80 ± 1.40 hrs to 31.90 ± 0.57 hrs, respectively at the temperature range under study (Fig. 1c). The total egg-to-adult developmental period of *C. quinquefasciatus*, therefore, ranged from 229.30 ± 3.23 hrs, 212.60 ± 2.07 hrs, 199.70 ± 3.20 hrs to 193.00 ± 3.09 hrs, respectively at 25° , 28° , 30° and 32° C (Fig. 1d). Results therefore demonstrate that highly significant decrease in the incubation ($F_{3, 36} = 105.60$; $P < 0.001$), larval ($F_{3, 36} = 166.65$; $P < 0.001$), pupal ($F_{3, 36} = 40.59$; $P < 0.001$) and total ($F_{3, 36} = 296.35$; $P < 0.001$) developmental periods were induced by the increase of temperature of 32° - $25^\circ = 8^\circ$ C.

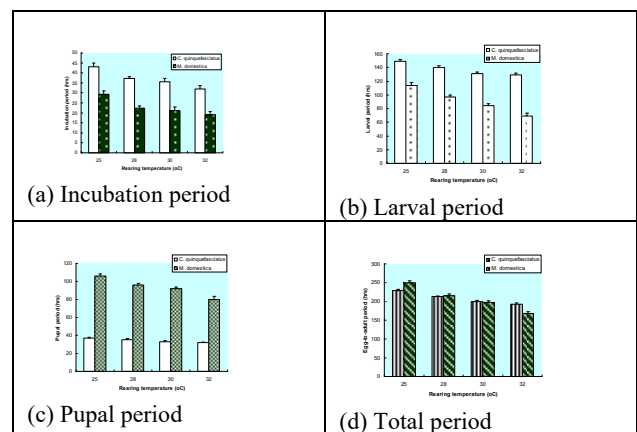


Fig. 1 Variations in life-history traits of *C. quinquefasciatus* and *M. domestica* reared in environmental chambers at 25° - 32° C

Table 1 Life-history traits of *C. quinquefasciatus* and *M. domestica* reared in environmental chambers at 60-95% RH

RH (%)	IP (hrs)	LP (hrs)	PP (hrs)	TP (hrs)
<i>Culex</i>				
66.00	43.10	149.20	37.00	229.30

±3.16	±1.85 ^a	±2.30 ^a	±1.05 ^a	±3.23 ^a
64.60	37.30	140.30	35.00	212.60
±3.20	±0.95 ^b	±2.00 ^b	±1.33 ^a	±2.07 ^b
84.50	35.60	131.30	32.80	199.70
±3.03	±1.58 ^c	±2.11 ^c	±1.40 ^b	±3.20 ^c
89.70	32.00	129.10	31.90	193.00
±4.35	±1.56 ^d	±2.56 ^c	±0.57 ^b	±3.09 ^d
<i>Musca</i>				
65.50	29.40	113.50	106.20	249.10
±3.03	±1.58 ^a	±4.53 ^a	±2.10 ^a	±6.06 ^a
63.20	22.40	96.70	96.20	215.30
±2.44	±1.17 ^b	±3.37 ^b	±1.55 ^b	±4.17 ^b
84.50	21.10	84.40	92.10	197.60
±3.03	±1.91 ^b	±2.91 ^c	±1.66 ^c	±4.45 ^c
92.10	19.00	69.30	80.00	168.30
±1.66	±1.63 ^c	±4.22 ^d	±3.65 ^d	±4.74 ^d

All values are mean ±SD; superscripts in dissimilar letters in each column and species differ significantly by LSD at P<0.05; RH= relative humidity; IP=incubation period; LP=larval period; PP=pupal period; TP=total period.

Life-history traits in house flies

In comparison to the life-history traits of the mosquitoes described above, the mean ±SD incubation periods of the experimental house flies decreased from 29.40±1.58 hrs, 22.40±1.17 hrs, 21.10±1.91 hrs and 19.00±1.63 hrs, respectively at 25°, 28°, 30° and 32° C (Fig. 1a). The corresponding RH values during the periods were 65.50±3.03%, 63.20±2.44%, 84.50±3.03% to 92.10±1.66%, respectively (Table 1). The larval period of the insects also declined from 113.50±4.53 hrs, 96.70±3.37 hrs, 84.40±2.91 hrs to 69.30±4.22 hrs, respectively at 25°-32° C (Fig. 1b). The pupal period also varied from 106.20±2.10 hrs, 96.20±1.5, 92.10±1.66 to 80.00±3.65 hrs, respectively (Fig. 1c). So, the total egg-to-adult developmental period of *M. domestica* fluctuated from 249.10±6.06 hrs, 215.30±4.17 hrs, 197.60±4.45 hrs to 168.30±4.74 hrs, respectively at 25°, 28°, 30° and 32° C (Fig. 1d). The present data therefore indicate that highly significant decline in the incubation ($F_{3, 36} = 79.73$; $P < 0.001$), larval ($F_{3, 36} = 241.60$; $P < 0.001$), pupal ($F_{3, 36} = 205.37$; $P < 0.001$) and egg-to-adult ($F_{3, 36} = 473.92$; $P < 0.001$) developmental periods were induced by the increase of 8° C under study.

Differences in response between two vector species

Although *C. quinquefasciatus* and *M. domestica* were raised in identical temperatures that ranged between 25° and 32° C, there existed quite significant

differences in the incubation ($t = 14.72$ at 78 df; $P < 0.001$), larval ($t = 15.65$ at 78 df; $P < 0.001$) and pupal ($t = 37.42$ at 78 df; $P < 0.001$) periods of the two vector species, although the total egg-to-adult developmental period did not vary statistically ($t = 0.20$ at 78 df; $P = 0.84$). These results suggest that the mosquitoes and house flies would respond differently in the field temperature and RH conditions.

Correlation between abiotic factors and life-history traits in mosquitoes

Statistically highly significant correlation coefficient (r) values ($P < 0.001$ at 18 df each) between the temperature range and the incubation, larval, pupal and egg-to-adult developmental periods were -0.94, -0.95, -0.87 and -0.97, respectively at 25°, 28°, 30° and 32° C. The corresponding regression lines presented in Fig. 2 indicate that increasing temperature, as expected, declined the life-history traits in *C. quinquefasciatus*.

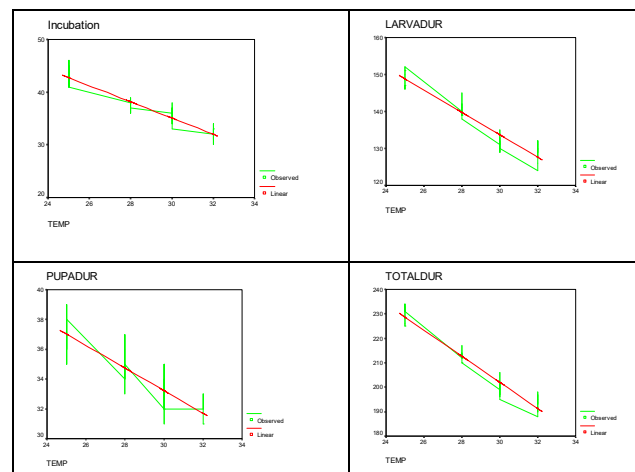


Fig. 2 Regression lines showing the negative impacts of temperature rising on various life-history traits in *C. quinquefasciatus*

Similar to temperature effects, however, RH also was found to have significantly negative impacts ($P < 0.001$ at 18 df each) on all the life-history traits in the mosquitoes under investigation. Thus, the correlation coefficient values of -0.75, -0.83, -0.81 and -0.84 were calculated for the incubation, larval, pupal and total developmental periods, respectively.

Correlation between abiotic factors and life-history traits in house flies

The estimated correlation coefficient (r) values between 25°, 28°, 30° and 32° C and the incubation, larval, pupal and egg-to-adult developmental periods were -0.90, -0.97, -0.95 and -0.98, showing highly significant associations between the abiotic factor and the life-history parameters ($P < 0.001$ at 18 df each). The corresponding regression lines shown in Fig. 3 suggest that increasing temperature results in the decrease in the life-history traits in *M. domestica*.

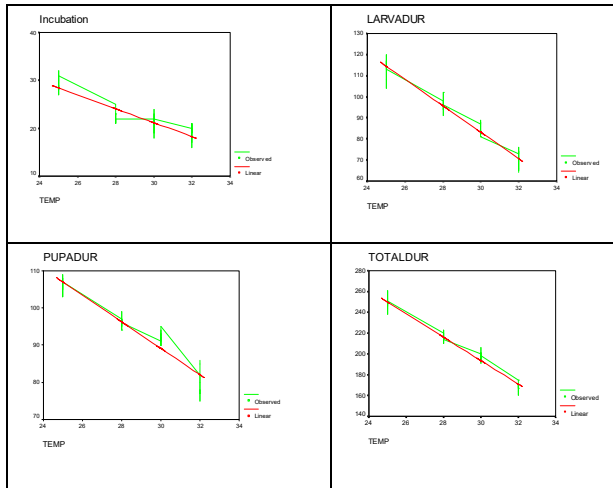


Fig 3 Regression lines showing the negative impacts of the RH on various life-history traits in *M. domestica*

In synchrony with the temperature effects, RH also induced significantly negative effects on all the life-history traits in the experimental house flies ($P < 0.001$ at 18 df each). The correlation coefficient values of -0.68, -0.88, -0.81 and -0.85 were worked out for the incubation, larval, pupal and total developmental periods, respectively in *M. domestica*.

Discussion

In the present investigation, it has clearly been demonstrated that rise in both temperature and RH, within survival limits of the insects, enhances developmental periods in the two dipteran species. It also appeared from the correlation estimates that temperature perhaps had more drastic effects than the RH counterpart. Moreover, the differences in egg-to-adult development between *C. quinquefasciatus* and *M. domestica* varied significantly for the incubation, larval and pupal periods, suggesting that the two species would respond differently to any change in climatic conditions under field situations.

In low RH environments, water loss through the egg and pupal membranes can be detrimental to the survivorship of holometabolous insects such as mosquitoes and house flies, resulting in desiccation (Wigglesworth, 1984). Gillespie *et al.* (2000) noticed that at 20°C, developmental time was significantly shorter at 96% RH than at 84% RH in the predatory gall midge, *Feltiella acarisuga* (Vallot). In earlier reports Kamimura *et al.* (2002) observed that larval rearing temperatures in *A. aegypti* and *A. albopictus* can have a major impact on disease transmission by affecting body size, development time and production. Temperature, humidity, food type and nutrient have been reported to affect larval growth in blow fly *Lucilia sericata* (Clark *et al.*, 2006). The effect of temperature on the development of mosquitoes was studied and a higher rate of development was observed in comparison to susceptible strain of *C. quinquefasciatus* (Swain *et al.*, 2008). The survival and development rates of both *Anopheles gambiae* and *A. arabiensis* larvae were dependent on water temperature, though there were clear differences in the responses of the species to each temperature regime (Kirby & Lindsay, 2008).

Subsequently, Tomberlin *et al.* (2009) observed that temperature differences of even 3°C produce significant fitness tradeoffs for males and females, influencing life-history attributes in the black soldier fly, *Hermetia illucens* L. and temperatures over 35 °C are likely to induce a negative effect on several aspects of *Aedes aegypti* mosquito biology including oviposition, egg-fertility and survival (Costa *et al.*, 2010). Mohammed & Chadee (2011) reported that the shorter development time at the higher temperatures generally resulted in significantly smaller adults in *Ae. aegypti*. However, In agreement with Hewitt (2011), the present data showed that *M. domestica* was unable to complete its life-cycle at temperatures higher than 32° C through mortality of larval instars. Holmes *et al.* (2012) reported that in black soldier flies *H. illucens* egg-hatch and adult emergence success increased with increasing RH, while development time decreased with rising RH. At low temperature (16° C), pupation in *Aedes aegypti* is delayed, whereas at high temperatures (35°-37° C) the growth rate of the mosquito population is significantly increased (Carrington *et al.*, 2013). All these findings corroborate to the present results obtained for *C. quinquefasciatus* and *M. domestica*.

Climatic changes forecasted in the coming years are likely to result in substantial alterations to the distributions and populations of vectors of arthropod-borne pathogens (Ciota *et al.*, 2014). Characterization of the effect of temperature shifts on the life history traits of specific vectors is therefore needed to define more accurately how such changes could impact the epidemiological patterns of vector-borne disease. Accordingly, Ciota *et al.* (2014) determined the effect of temperature ranges of 16, 20, 24, 28, and 32° C on development time, immature survival, adult survival, mosquito size, blood feeding, and fecundity of both field and colonized populations of *Culex pipiens* L., *Culex quinquefasciatus* Say, and *Culex restuans* Theobald where the results demonstrated that temperature significantly affects all of these traits and higher temperature significantly increased mortality. Danjuma *et al.* (2014), who compared development and survival of immature stages of two fruit fly species *Bactrocera dorsalis* and *B. papayae* at six constant temperatures of 15□, 20□, 25□, 27□, 30□ and 35 □C, 70±5% RH, and a photoperiod of 12:12 (L:D), reported that a strong and positive linear relationship existed between temperature and developmental rate of immature stages of both fruit flies. The present results fit well with the above findings.

In the sheep blow fly *Lucilia cuprina*, on the other hand, Bansode *et al.* (2016) showed that development of the fly was slow at lower temperature like 20 □C in which the body weight was greatest, but at the high temperatures (30□-40 □C), developmental rate was rapid but slight mortality was observed. In an attempt to study the life-cycle of the mosquito *Aedes aegypti* in detail, Marinho *et al.* (2016) used six constant temperatures *viz.*, 16°, 22°, 28°, 33°, 36° and 39° C, where they observed that increasing temperature caused rapid population growth. While Pagabeleguem *et al.* (2016) investigated the survival and fecundity of three strains of the tsetse fly *Glossina palpalis gambiensis* at 25°, 30°, 35°, 40°, 50° and 60 °C, and at 60-75 % RH, in which a temperature of about 32 °C was the limit for survival for all strains and a RH ranging from 40% to 76% had no effect on fecundity at 25°-26 °C. In concordance with these previous studies on important dipteran species, results presented here demonstrate highly significant negative correlations between

temperatures and RH and the vital life-history traits like egg-to-adult developmental periods, indicating that increasing abiotic factors like temperature and RH could generally lead to a more rapid proliferation of *Culex* and *Musca* populations. Moreover, temperatures of 25°-32° C and RH of 60%-95% were both found to induce significantly negative impacts on the egg-to-adult duration, demonstrating that rising mean temperatures and RH would benefit the vector species by shortening their life-history traits.

Conclusions

This study can provide important SIT-based control strategies for the disease vector species like mosquitoes and house flies. In addition, the present data could be useful in predicting dispersal of these dipteran insects because earlier studies revealed that temperature and RH influence the flight performance of virgin female *Ae. aegypti* (Rowley & Graham, 1968) and increase in these couple of abiotic factors can affect vector population biology and, consequently, the disease transmission by them (Costa *et al.*, 2010). Moreover, our results could be helpful in estimating values for numerous life-history traits compared to more natural field conditions, which may turn reduce the accuracy of population dynamics modeling and downstream applications for vector surveillance and disease prevention (Carrington *et al.*, 2013). To sum up, since climatic changes forecasted in the coming years are likely to result in substantial alterations to the distributions and populations of vectors of arthropod-borne pathogens (Ciota *et al.*, 2014), the present findings might also be beneficial in predicting how a warming climate could impact the distribution, abundance and vectorial capacity of important disease vectors like *C. quinquefasciatus* and *M. domestica*.

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