



Research paper

Analysis of the transcription of genes encoding heat shock proteins (hsp) in *Aedes aegypti* Linnaeus, 1762 (Diptera: Culicidae), maintained under climatic conditions provided by the IPCC (Intergovernmental Panel On Climate Change) for the year 2100

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ABSTRACT

Human actions intensify the greenhouse effect, aggravating climate changes in the Amazon and elsewhere in the world. The Intergovernmental Panel on Climate Change (IPCC) foresees a global increase of up to 4.5 °C and 850 ppm CO₂ (above current levels) by 2100. This will impact the biology of the *Aedes aegypti* mosquito, vector of Dengue, Zika, urban Yellow Fever and Chikungunya. Heat shock proteins are associated with adaptations to anthropic environments and the interaction of some viruses with the vector. The transcription of the *hsp26*, *hsp83* and *hsc70* genes of an *A. aegypti* population, maintained for more than forty-eight generations, in the Current, Intermediate and Extreme climatic scenario predicted by the IPCC was evaluated with qPCR. In females, highest levels of *hsp26*, *hsp83* and *hsc70* expression occurred in the Intermediate scenario, while in males, levels were high only for *hsp26* gene in Current and Extreme scenarios. Expression of *hsp83* and *hsc70* genes in males was low under all climatic scenarios, while in the Extreme scenario females had lower expression than in the Current scenario. The data suggest compensatory or adaptive processes acting on heat shock proteins, which can lead to changes in the mosquito's biology, altering vectorial competence.

1. Introduction

Since the last decades of the twentieth century, climate change has occupied a prominent place in the scientific and in the world, as the temperature and greenhouse gases (GHG) increased, when compared to the pre-industrial era averages (Derksen et al., 2017; Tedesco et al., 2013), resulting in the warming of the Earth's surface by around 0.6 °C, with projections for increments in the range of 1.4 °C to 5.8 °C by the year 2100 (IPCC, 2014a). The increase in average temperature is associated with anthropic actions such as the emission of greenhouse gases GHG and aerosols, deforestation followed by fires and the formation of urban heat islands (Hamilton et al., 2015; IPCC, 2014a).

Due to its extensive area, the Amazon region shows heterogeneous profiles of climate change, when compared to global patterns

(Guimberteau et al., 2017; IPCC, 2013). Theoretic models estimate that if CO₂ increase the temperature rises from 1.5 to 4.4 °C, a feasible scenario is one with extreme climate events such as short periods of intense rain, followed by a prolonged drought and a decrease in evapotranspiration from 2.2 to 11%. These events associated with the advancing arc of deforestation towards the northeast of the state will lead to a reduction in the forest between 7 and 34%, accelerating the change of vegetation from tropical rain forest to the formation of fields or deserts (Chou et al., 2014; Ingham et al., 2019; Sorribas et al., 2016).

The increase in the release of carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and fluorinated gases have contributed, since the pre-industrial era, to an intensification of the greenhouse effect and its associated environmental effects in the Amazon region (Griggs and Nogueir, 2002; IPCC, 2014b). Due to these changes, the

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Intergovernmental Panel on Climate Change (IPCC), has predicted three probable climatic scenarios for 2100: mild (RCP 2.6); intermediate (RCP 6.0) and extreme (RCP 8.5) (Griggs and Noguier, 2002; IPCC, 2013).

Such climate change-mediated modifications in different environments may favor the reproduction of *Aedes aegypti* which is currently one of the most important vectors of medical health in the world (Carvalho and Moreira, 2017). In addition to being the vector of important arboviruses, including dengue, urban yellow fever, Zika and chikungunya fever, *A. aegypti* is highly anthropophilic (Kraemer et al., 2015). The species is considered cosmopolitan, with a wide geographical distribution, being present throughout the globe's tropical and sub-tropical areas (Glasser and Gomes, 2002; Kraemer et al., 2015; Kubota et al., 2003).

Climate change can significantly influence the growth rate and longevity of immature and adult *A. aegypti* (Chaves et al., 2014; Mohammed and Chadee, 2011), changing fertility and blood feeding rates, because warmer and wetter environments influence the biological cycle of the species, accelerating development (Benoit et al., 2011; Couret et al., 2014; Farjana and Tuno, 2013). As a consequence, the incidence of arbovirolosis transmitted by this vector tends to increase, due to the favorable conditions for the reproduction and survival of this mosquito under new climatic regimes (Chaves et al., 2014; Lima-Camara, 2016).

In addition to temperature, high CO₂ levels in natural and artificial environments can influence vectoring behavior in this species, as mosquitoes use, among other gases, the exhaled CO₂ in the environment to guide it to a blood-supplying host, and humans are the preferred host for this species (Majeed et al., 2014; Smith et al., 2013). Because it is associated with biochemical stimuli emitted by the host to detect the mosquito to its host, fluctuation in the atmospheric concentration of CO₂ can alter the sensory capacity of the mosquito (de Ázara et al., 2013; Raji et al., 2019). Thus, changes in the concentration of atmospheric CO₂ may influence the frequency and scale epidemics in the future.

Adaptations of mosquitoes to climate change include heat shock proteins (*Hsp*) (Couret et al., 2014; Zhao et al., 2010). This group of proteins act as response factors to some aspects of cellular stress, including oxidative stress, heat, cold and cellular respiration (He et al., 2014; Kumar et al., 2017; Vogt et al., 2019). *Hsps* are related to protein stability and synthesis, directing cellular repairs, assisting in protein folding and in conducting mature proteins to their target site (Gross et al., 2009; Sivan et al., 2017). They have an important role in mosquito survival, reducing cell damage by cooperating with the ubiquitin/proteasome system in the degradation of malformed proteins (Esser et al., 2004; Gross et al., 2009; Sivan et al., 2017; Zhao et al., 2010).

In *A. aegypti*, *Hsp26* is a small heat shock protein associated to acclimatization and adaptation to thermal shock (Zhao et al., 2010, 2009). In *Drosophila melanogaster* this protein is associated with stress factors and survival at high temperatures (Sørensen et al., 2019). Also in *D. melanogaster*, *Hsp83* interacts with Juvenile Hormone (JH) promoters and receptors responsible for cell growth and division in the larval phases of this species (He et al., 2014; Muturi et al., 2012, 2011). In the genus *Aedes*, JH also acts in the maturation of early phases, regulating immune responses and diapause (Batz et al., 2019; Kim et al., 2020). *Heat shock cognate 70 (Hsc70)* belongs to the *Hsp70* family, and has constitutive expression, being correlated with hormones, protein folding and transcription factors maturation (Zhang et al., 2019), binding to improperly folded proteins, and tagging them for degradation (Sun et al., 2019). In addition, this protein is associated with cellular homeostasis during the infection of mosquito cells by the Chikungunya virus (Ghosh et al., 2017; Kang et al., 2008).

The genetic and biochemical mechanisms involved in the responses to climatic acclimatization of *A. aegypti* are still not well understood. However, *Hsp* emerges as the key piece in molecular processes that guarantee the survival of this species when experimenting environmental, climatic and cellular changes, in the future. (Sørensen et al., 2019; Zhao et al., 2009). Accordingly, study of *hsp* expression patterns

may give insights into their behavior during climatic stress and fortunately, to predict the potential responses of *A. aegypti* to the different climatic scenarios that, eventually, planet Earth will face, including the set of predictive modeled climate changes by the IPCC, until the end of the XXIst century.

2. Materials and methods

2.1. *Aedes aegypti* colonies and microcosms

The *A. aegypti* strains used in this study were obtained from the permanent colony established in the Laboratory of Malaria and Dengue (LMD) of the National Institute for Research in the Amazon (INPA). The sampling of wild mosquitoes occurred in the six administrative zones of Manaus city, Amazonas State, Brazil, between 2010 and 2011, using an oviposition trap (ovitrams). Ovitrams is a matte black container (500 ml), with Duratree reeds (3 cm × 12 cm) vertically fixed and filled with 10% grass infusion, an attractive solution (Fay and Eliason, 1966; Reiter et al., 1991). At the LMD/INPA the reed were dried at room temperature, and the eggs were counted under 40× magnification using a Zeiss Stemi 2000-C microscope. Subsequently, the reed were placed in basins containing chlorine-free water to hatch the larvae. The larvae were fed with cat food (Whiskas®) and rodents (Teklad®), ground and mixed in a 1:1 ratio. The adults were identified by the taxonomic key (Consoli and de Oliveira, 1998; Forattini, 2002), and used to originate the first colony, called MAO.

Mosquitos from MAO colony started new populations in rooms of 25 m² each, in which climatic scenarios predicted by the IPCC for 2100 are simulated. All variables such as temperature, CO₂ concentration, humidity and photoperiod are controlled by computers. These rooms are called environment room, or microcosm. Microcosm 1 (control room) reproduces the current and local atmospheric conditions of Manaus city, with 12L:12D photoperiod (twelve hours of light and twelve hours of darkness). All increments in temperature and CO₂ at the following microcosm were based in the values recorded in the microcosm 1. Microcosm 2 simulates the mild scenario (RCP 2.6), with an increase 1.5 °C in ambient temperature and an increment of 200 ppm of CO₂. Microcosm 3 simulates the intermediate scenario (RCP 6.0), with an increase of 2.5 °C in ambient temperature and an increment of 400 ppm of CO₂. At last, microcosm 4 simulates the extreme scenario (RCP 8.5), with an increase of 4.5 °C in ambient temperature and an increment of 850 ppm of CO₂. Measurements of CO₂ concentration, room temperature and relative humidity are recorded automatically at two-minute intervals. The microcosms belong to a permanent program of climate simulation of the Laboratory of Ecophysiology and Molecular Evolution – LEEM/INPA.

2.2. Maintenance of *Aedes aegypti* colonies in the microcosm

To maintain the colonies viable in each microcosm, routinely, strips of filter paper containing *A. aegypti* eggs are placed in screened polyethylene basins, containing 500 ml of chlorine-free water. After hatching up to 200 larvae are kept in each basin. The larvae are fed daily with 0.5 g of cat food (Whiskas®) and rodents (Teklad®), ground and mixed in a 1:1 ratio. The pupae are collected and transferred to 50 ml plastic cups, containing chlorine-free water and transferred to screened cardboard cages (18 cm in diameter and 17 cm in height) for adult emergence. Males and females are fed with cotton soaked in 10% sucrose solution. Additionally, the females received blood feeding, for 30 min, in hamster, *Mesocricetus auratus*, anesthetized following the LMD breeding protocol for *A. aegypti* (Pinheiro and Tadei, 2002).

All experimental procedures were carried out in accordance with the guidelines of the CONCEA Brazilian Guide for the Production, Maintenance or Use of Animals for Scientific Teaching or Research Activities, under the authorization of the INPA Ethics Committee (protocol n°: 033/2018). Tests with genetic material were authorized and were

registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN), under permit no ABBBD90.

2.3. Preparation of experimental groups

The climate simulation experiments were conducted at the LEEM/INPA, from 2017 to 2018. The RNA preparation and cDNA library construction were carried out at the LMD/INPA and quantitative Real Time PCR (qPCR) was conducted at the Laboratory of Biotechnology established in the Faculty of Agricultural Sciences (FCA) of the Federal University of Amazonas (UFAM).

Specimens were sampling from October 2017 to November 2018, in microcosms 1, 3 and 4. Microcosm 2 was removed from the analysis after verification of failure in environmental parameters. The total RNA was prepared from the following generations: microcosm 1 (48th, 51st and 55th generation); microcosm 3 (49th, 52nd and 56th generation), and microcosm 4 (67th, 70th and 74th generation). The sampling occurred four days after blood feeding of females and during this period males and females continued to be fed as previously described. Thirty males and thirty females adults were randomly collected in triplicate for each studied generation.

2.4. Extraction of total RNA and cDNA synthesis

Thirty males and thirty females from microcosm 1, 3 and 4 were immediately frozen in triplicate in liquid nitrogen (N₂) and transferred to 2 ml microtubes pre-prepared with 1 ml of TRIzol™ (Invitrogen by Thermo Fischer Scientific), then homogenized by pipette up and down. The total RNA preparation followed the manufacturer's recommendations, in an RNase-free environment. RNA samples were quantified using Qubit 2.0 fluorometer. RNA integrity was evaluated in 1% agarose gel stained with ethidium bromide 5 µg/ml, diluted in TBE-RNase free buffer. The total RNA was treated with DNase I (Invitrogen™ by Thermo Fischer Scientific), and the cDNA libraries were constructed with the High-Capacity DNA Reverse transcription kit (Applied Biosystems™ by Thermo Fischer Scientific), following the manufacturer's recommendations.

2.5. Real time quantitative PCR (qPCR)

All qPCR reactions were performed using QuantStudio 6 (Applied Biosystems™). The assays were performed in triplicate, and qPCR reactions were prepared using the Power SYBR™ Green PCR Master Mix reagent (Applied Biosystems™). qPCR reactions were adjusted to a final volume of 10 µl containing 1× Power SYBR™ Green Master Mix, 0.10 µM of each primer, 100 ng of cDNA and ultrapure water q.s.p. The

cycling conditions were as follows: pre-run at 50 °C for 2 min and at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. The post-run melting curve analysis were carried out from 60 °C to 95 °C with 0.05 °C/s.

The primers targeting the *hsp26*, *hsp83*, *hsc70* genes and reference genes for normalization (actin, α -tubulin, *RPS17*, *RPL32* and *eEF1a*) (Table 1), were modified from previous works (Dzaki et al., 2017; Zhao et al., 2010), using the PrimerQuest tool and analyzed using the Oligo Analyzer tool (IDT Integrated DNA Technologies). (See Table 1.)

The primers were designed using mRNA reference sequences (Table 1). The primer efficiency were evaluated from titration curves, prioritizing concentrations with lower Ct and higher ΔR_n , and absence of dimers in the melting curve analysis. The levels of relative expression were determined using the $\Delta\Delta C_t$ method, using, for normalization, the average Ct from multiple endogenous controls (Livak and Schmittgen, 2001).

2.6. Statistical analysis

Normality (Kolmogorov-Smirnov) and equality of variance (Brown-Forsythe) tests were performed. Two-way ANOVA analysis of variance was used to compare the two variables studied: sex and climatic scenarios for each protein, with a paired post-hoc multiple comparison test multiple comparison (Holm-Sidak) with a significance level of $p < 0.05$. Tests were expressed as means, with standard deviations. Statistical analyzes were performed using SigmaStat v3.5 software, with graphs made in SigmaPlot v.11.0 software.

3. Results

The fluctuations in the CO₂ concentration, ambient temperature and relative humidity from natural seasonality of Manaus city, including the nictemeral variations of the external environment, were accurately mirrored in all microcosms monitored, when considering the relative increment for each IPCC scenario (Fig. 1).

The *hsp26* gene was differentially expressed for: (1) the sex variable ($p = 0.002$) (F = 16,060; gl 1), in this test, the data from the three scenarios were pooled into two groups according to sex; (2) the scenario variable ($p = 0.006$) (F = 8130; gl 2), in this test the data from males and females from each scenario were grouped (simulating the population expression profile for each scenario). There was also a differential expression in the test of interactions between the variables sex and climatic scenarios ($p < 0.001$) (F = 30,680; gl 12). The paired analysis for the scenario variable has not demonstrated significant differences between the current and RCP 6.0 scenarios ($p > 0.05$), however the expression of *hsp26* gene in the RCP 8.5 scenario was almost the double of current and RCP 6.0 scenarios ($p = 0.007$) (Fig. 2A).

Table 1
Sequences of qPCR primers for *Aedes aegypti* heat shock and reference genes.

| Gene | ID | Primer sequences | Amplicons |
|-------------------|--------------|--|-----------|
| <i>hsp26</i> | LOC5577707 | F: GACCATTCTGTTCAAGTATT R: TTGCTTGTTCCTTCGTTT | 196 pb |
| <i>hsp83</i> | LOC5565405 | F: AGAGAAGAAGGACAAGAAGAA R: GTTGGTGAGCGATTGTAG | 145 pb |
| <i>hsc70</i> | LOC110678734 | F: AACGGTATCCTGAATGTGA R: TGGTTCCTTCTGCTTCT | 160 pb |
| actin | LOC5574526 | F: CTGTATGCCAACACAGTATTAT R: CGATCCAGACGGAGTATT | 148 pb |
| α -tubulin | LOC5577489 | F: ATTACGGCAAGAAATCCAAG R: ACGGCAGATGTCATAGATAG | 164 pb |
| <i>RPS17</i> | LOC5564226 | F: AAGGTCATTATTGAGAAATACTACA R: ACGAAACCAGCGATCTT | 122 pb |
| <i>RPL32</i> | LOC5577996 | F: CGTAAGCCGAAAGGTATTG R: TTGTGGACCAGGAACCT | 137 pb |
| <i>eEF1a</i> | LOC23687516 | F: GTGTTGGACAACTGAAGG R: GGTAATCATGTTCTTGATGAAATC | 138 pb |

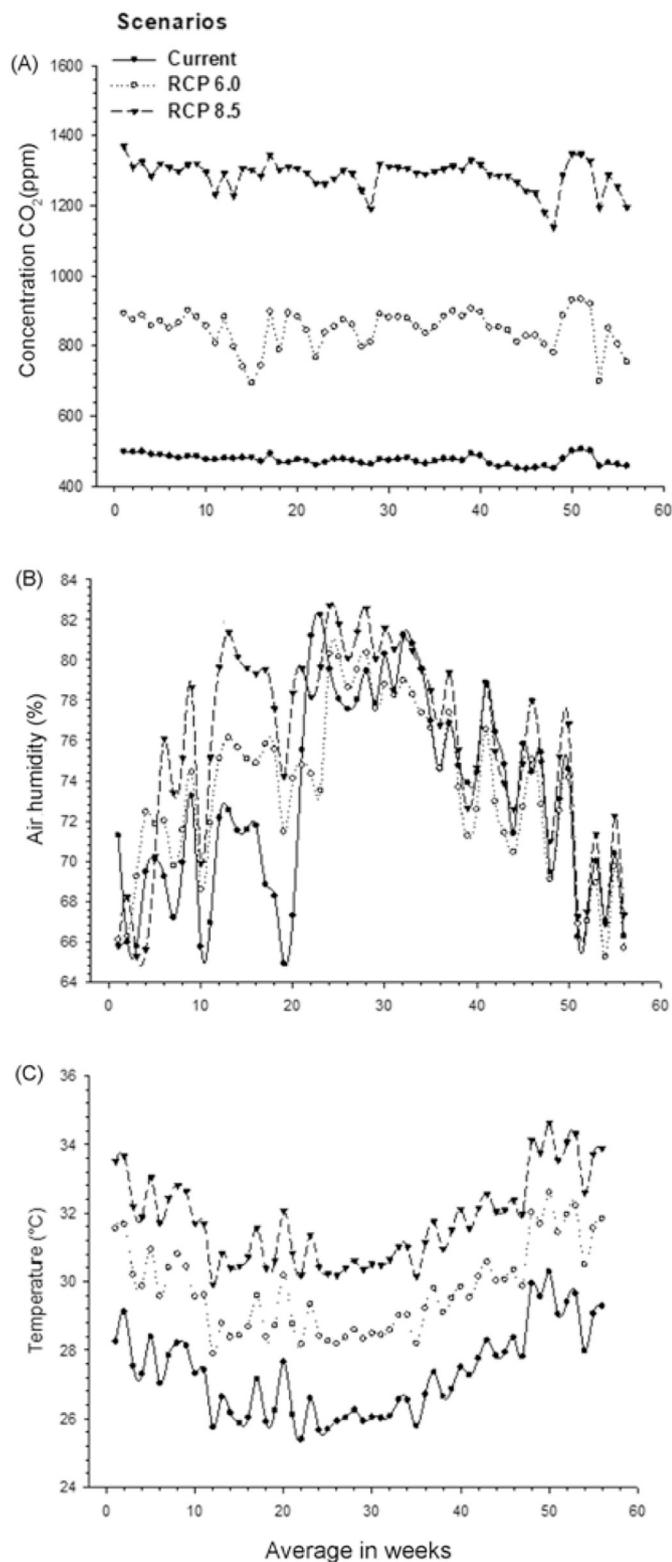


Fig. 1. Weekly average of daily values of CO₂ concentration in ppm (A), temperature in °C (B), and atmospheric humidity in % (C) of the environmental chambers, recorded from October 2017 to November 2018.

The *Hsp26* gene was also differentially expressed between males and females all evaluated climatic environments, being 4.3 and 3.2 times greater for males in the current scenario ($p < 0.001$) and in the RCP 8.5 scenario ($p < 0.001$), respectively, and 3.9 times greater for females in the RCP 6.0 scenario ($p = 0.002$). Comparisons only among males

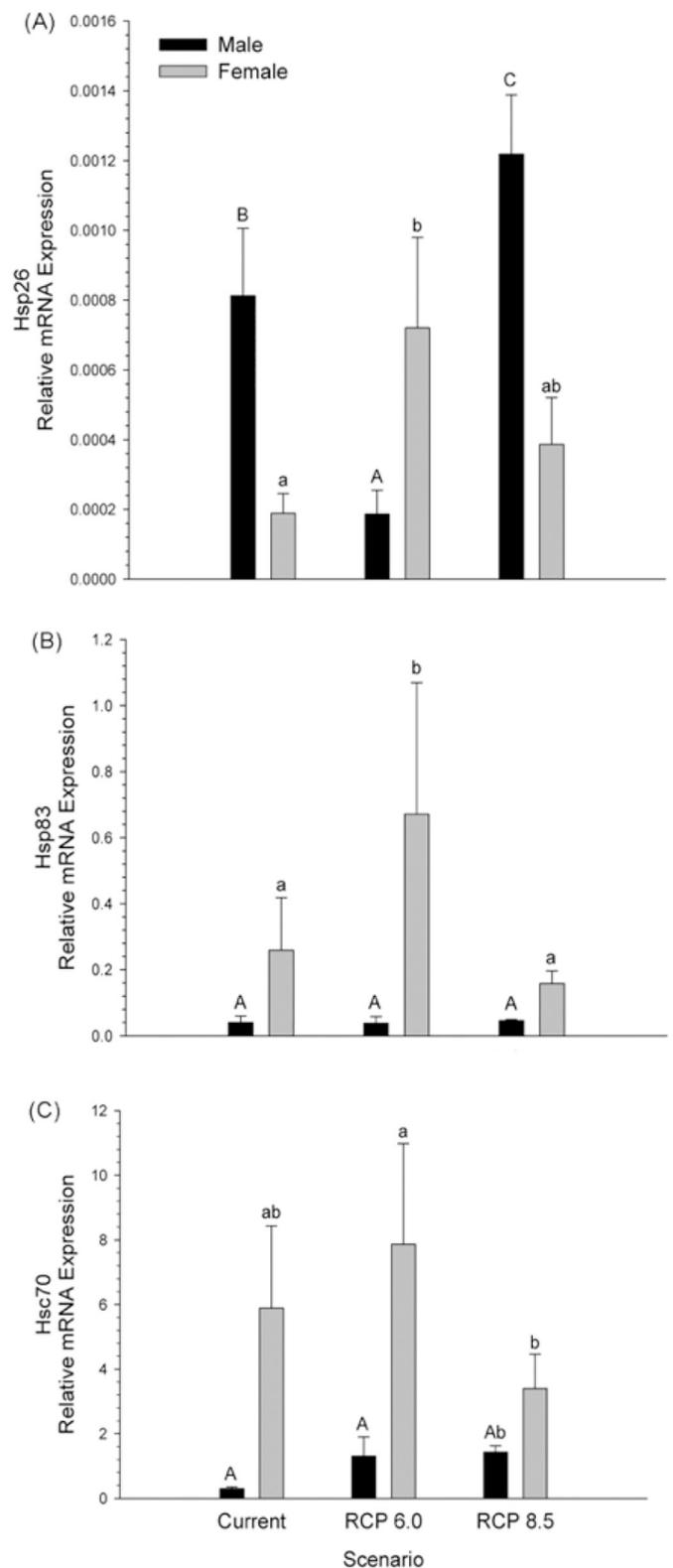


Fig. 2. Relative expression of the *hsp26* (A), *hsp83* (B) and *hsc70* (C) genes of adult *Aedes aegypti* males and females, maintained under the microcosm climatic chamber conditions. Equal capital letters (males) and lower-case letters (females) indicate absence of significant statistical differences. For the analysis of variance, two-way ANOVA and Holm-Sidak post-hoc test significance values were $p < 0.05$.

showed that the expression of the *hsp26* gene in the current scenario was 4.4 times higher in relation to RCP 6.0 ($p = 0.001$), however 1.5 times lower in relation to RCP 8.5 ($p = 0.010$), and as a consequence, 6.5 times lower in RCP 6.0 when compared to the RCP 8.5 scenario ($p < 0.001$). Among the groups of females, the differences observed between RCP 8 and current scenario or RCP 6 were not significant ($p > 0.05$), however in contrast to males, the gene was 3.8 times greater expressed ($p = 0.002$) in the RCP 6 when compared to the current scenario (Fig. 2A).

The *hsp83* gene was differentially expressed for the sex variable ($p = 0.002$) ($F = 14.899$; gl 1), in this test, the data from the three scenarios were pooled into two groups according to sex, and this difference was mainly influenced by the results from RCP 6.0 scenario where the levels of expression were 17.7 times higher in females ($p < 0.001$) (Fig. 02-B). However, there was no statistically significant difference in the gene transcription at population-level (regardless of gender) between the three climatic scenarios and neither significant interaction between the two variables (sexes and climatic scenarios) evaluated ($p > 0.05$).

There was no significant difference for *hsp83* gene expression of *A. aegypti* males in any of the studied scenarios ($p > 0.05$). However, for females there were significant differences between: (1) current and RCP 6.0 scenarios, being 2.6 times smaller ($p = 0.014$) in the current scenario; and also (2) RCP 6.0 and RCP 8.5, being 4.2 times greater ($p = 0.004$) in the RCP 6.0 scenario (Fig. 2B).

The *hsc70* gene expression showed significant differences ($p < 0.001$) ($F = 33.802$; gl 1) between males and females in the global analysis (combination of the three scenarios). However, there was no significant difference among the climatic scenarios, and neither significant interaction between the two variables (sexes and climatic scenarios) evaluated ($p > 0.05$).

There were no significant differences in *hsc70* expression among males from the three climatic scenarios studied ($p > 0.05$) (Fig. 2C). However, among females the gene was differentially expressed between RCP 6.0 and RCP 8.5, being 2.3 times greater ($p = 0.008$) in the RCP 6.0 scenario. There were no significant differences for all other possible comparisons between the studied scenarios ($p > 0.05$). Finally, the comparison for males and females from each climatic scenario, showed that the expression levels were 19.6 times greater for females from the current scenario ($p = 0.002$) and 6 times from RCP 6.0 ($p < 0.001$), and there was no significant difference for RCP 8.5 scenario ($p > 0.05$).

4. Discussion

Heat shock proteins are a group of proteins widely-conserved in eukaryotes and are related to several physiological responses associated with intracellular stress which, in mosquitoes, facilitate adaptation to anthropic environments (Pan et al., 2018; Zhao et al., 2009). A key function of Hsp is the ability to maintain the stability of the various protein systems related to cellular and transcriptional metabolisms. This provides *A. aegypti* with the capacity to survive a variety of environmental stressors. Such changes may have an influence on the vector biology, as well as on the metabolism of infecting viruses and their various interactions with intracellular mechanisms (Kang et al., 2008).

The small heat shock proteins, a group that includes *hsp26*, are essential for the cell function in several ways, such as to assist the correct folding of target proteins or by avoiding the aggregation of non-native proteins. Thus, they present high expression when stress negatively impacts the cellular homeostasis (Moutaoufik et al., 2017; Sharma et al., 2020).

Hsp26 is ubiquitously spread in eukaryotes and, despite having a similar profile of responses to stress, they show different levels of expression among species (Sørensen et al., 2016). In an acclimatization test, *Drosophila subobscura* and *D. melanogaster* showed different responses to the heat stress. *D. subobscura* expression levels just doubled, while those of *D. melanogaster* increased by up to 100 times (Sørensen et al., 2019). In thermal shock tests with *A. aegypti* females, *hsp26* expression increased 39 times when exposed for 1 h at 42 °C, compared

to the control (23 °C for 1 h) (Zhao et al., 2009). Other species from the Order Diptera show similar behavior. During females acclimatization test of *Megaselia scalaris* (a fly species found in anthropic areas following such disturbances as burning) under thermal stress from 37 to 45 °C, expression levels were 10 times greater than those at the control temperature (25 °C), and the expression values remained relatively constant, while thermal stress was maintained. (Malewski et al., 2015). Under heat stress, *hsp26* prevents the agglomeration of the malate dehydrogenase (MDH), a critical enzyme to cellular respiration, delaying its inactivation (Huang et al., 2018; Ungelenk et al., 2016; Ungelenk, 2015).

In this study, the *hsp26* gene showed a similar expression profile for both males and females from *A. aegypti* colonies living under the stress of the current and extreme climate scenario (RCP 8.5). In these two scenarios the gene is more expressed in males and, although it is not statistically significant, it is also possible to observe that there is a tendency to increase expression for both sexes in the most extreme scenario (RCP 8.5). This tendency is confirmed at population level, i. e., grouping the data from males and females, because there is an unequivocal response to extreme scenario. In the natural environment, the climate changes are perceived and answered at the population level and not separately for each sex, thus, an increase in *hsp26* expression, observed in mosquitoes exposed to the RCP 8.5 scenario, might be related to a cellular response to preserve energy metabolism in adverse conditions, such as high temperature and CO₂ concentration (Huang et al., 2018; Ungelenk et al., 2016).

For each scenario, there were clear differences in the expression pattern of the *hsp26* genes between males and females, and in the RCP 6.0 scenario, an intriguing inversion in its expression profile was observed, once only in this scenario it is more expressed in females. The graphic analysis suggests a pattern of expression inversely proportional between males and females. Despite being subjected to the same environmental conditions, it is not possible to affirm that sexes are affected by stress in a similar way, it is necessary to consider the biological characteristics of their life cycle, thus future efforts might help understand whether other variables such as blood feeding or oviposition stress contribute to modulation of *hsp26* expression in *A. aegypti*.

Hsp83 has a protective activity against extreme temperatures, for both low and high, and may also impact on the mosquito's larvae life time and immune response (Muturi et al., 2012). Low levels, or suppression of *hsp83* expression, has direct effects on individual biology, decreasing life span, number of offspring, fertility, as well as embryogenesis and juvenile hormone of other invertebrate species (Li et al., 2019; Will et al., 2017). In other species of the order Diptera, such as *M. scalaris* and the genus *Drosophila*, thermo-tolerance is accompanied by increasing *hsp83* expression at high temperatures (Helms Cahan et al., 2017; Zhao and Jones, 2012). Additionally, relationships between the expression of this gene and the processes of diapause, sleep and wakefulness has also been documented in invertebrates (King and MacRae, 2015; Malewski et al., 2015; Rodrigues et al., 2018).

The data presented in this study show, at population level, a similar pattern of *hsp83* gene expression between current and RCP 8.5 scenarios. Although the RCP 8.5 scenario was the most extreme climatic scenario used, the significant increase of expressions was observed only for RCP 6.0. This tendency was influenced by females *hsp83* gene expression, once the pattern for males kept unaltered throughout the scenarios. This findings suggest that for males, *hsp83* is not principal trigger at least against height temperature. The metabolic network of the small heat shock proteins is still poorly understood in *A. aegypti* and explain the behavior of *hsp83* expression for females from RCP 6.0 scenario is not an easy task. However, it is known that generally Hsps bind to misfolding proteins, and have an ATP-dependent action associated with *hsp70*, a *hsc70* cognate protein. These heat shock proteins form a complex with the substrate, signaling other heat shock proteins in cellular proteostasis (Lei et al., 2017; Mogk and Bukau, 2017; Ungelenk et al., 2016). Expression levels may vary depending on the timing and type of cell

stress, in addition, in the long term, might occur a selection of mosquitoes for a suite of different environmental pressures, which impact both the life-history of mosquito populations and their competence as a vector (Graham et al., 2012; Muturi et al., 2011). Thus future efforts can test the whether multiple triggers are activated depending on the level of suffered stress, in this sense, *hsp83* might be important for mild change in the current temperature and other chaperones are expressed if this limit is overcome.

A similar pattern of *hsp83* expression was observed for *hsc70*. At population level, the response for current and RCP 8.5 scenarios were similar and lower than quantified in the RCP 6.0. As tendency, the graphical analysis suggests a slight increase in the expression for males, however not statistically significant. It was expected an increase in the expression of this gene in population living at extreme conditions (RCP), however the expression tended to be higher in the RCP 6.0 scenario at population level and also for females. The lower expression observed in the RCP 8.5 scenario might indicate a possible adaptive metabolic pathways of *A. aegypti* to prolonged exposure to extreme environment (Zhao et al., 2010), in addition could result in a natural defense of the species against Chikungunya virus (CHIKV) infection. The relationship of *hsc70* with viral infection was already related for o'nyong-nyong virus (ONNV) in *Anopheles gambiae* and has also been reported for C6/36 cells treated with *anti-hsc70* antibodies; these cells were capable to inhibit the adsorption and fusion of Chikungunya virus (CHIKV) while untreated C6/36 cells suffered viral infection (Ghosh et al., 2017; Sim et al., 2007). In the current study, low *hsc70* expression levels were observed in female *A. aegypti* in the RCP 8.5 scenario, which suggest a potential adaptive advantage against infection by Chikungunya in future.

The maintenance of specimens in colonies for long periods is documented as one of the sources of genetic variance between laboratory and field populations, being able to fix or delete population characteristics through evolutionary events (Gloria-Soria et al., 2019, 2016; Sterken et al., 2015). However, when the specimen is submitted to specific living conditions, there is a fitness, at the population and genetic level, in order to guarantee the species survival in the new environments (Beserra et al., 2009; Forattini et al., 1997; Kuno, 2010; da Silva et al., 2006). Thus, adaptive mechanisms are expected for populations of *A. aegypti* maintained under the climatic conditions defined by IPCC. These adaptive mechanisms at genomic level might also be associated to the expression levels of heat shock proteins observed.

In this study, the simulated climate changes in the microcosms had significant effects on the expression of heat shock genes related to cell stress. The differential expression of the heat shock genes (*hsp26*, *hsp83* and *hsc70*) for males and females living in different scenarios suggests alterations in their cellular metabolism such as mitochondria activity, resistance to desiccation, detoxification and osmotic stability (Jayasundara, 2017; López-Uribe et al., 2014; Mao et al., 2019), possibly as consequence to the continuous and long-term exposure (more than forty and eight generations) to the simulated climate scenarios. Such changes may potentially affect the biological cycle, pathogen/vector relationship and the permanence of *A. aegypti* in a perspective future climate scenario.

The increase in ambient CO₂ and its fluctuation in the capacity of *A. aegypti* to locate its host it was already reported (Majeed et al., 2017, 2014). Groups of *A. aegypti* exposed to environments with CO₂ concentrations between 600 and 1200 ppm CO₂ are capable to locate their host for reduced blood feeding when compared to groups exposed to concentrations up to 400 ppm CO₂ (Majeed et al., 2017, 2014). The increase in the rate of environmental CO₂, as well as the odors exhaled by the host, are also documented as factors capable of influencing the behavior of other mosquito species, such as *Aedes albopictus*, *Aedes triseriatus*, *Anopheles coluzzii*, *Anopheles gambiae*, *Culex quinquefasciatus*, *Culex coronator* and *Culex nigripalpus* (Majeed et al., 2017; Smith et al., 2013). However, the relationship between heat shock proteins and host odors/CO₂ sensory mechanism of *A. aegypti* (and other mosquito species), is still unclear. Thus Further studies are needed to evaluate the

contribution of the CO₂ variable for the patterns of *hsp26*, *hsp83* and *hsc70* gene expressions of *A. aegypti* living in the simulated IPCC conditions.

Together, the results found in this study suggest the climatic events predicted by the IPCC have a positive influence in the expression profile of heat shock proteins *hsp26*, *hsp83* and *hsc70*. Females showed the highest levels of expression for *hsp83* and *hsc70* genes in all evaluated scenarios and among these RCP 6.0 seems to triggers the chaperon response. The data suggest different compensatory (still unknown) or adaptive processes for male and females involving heat shock proteins, especially for *hsp26*, which can lead to changes in the biology of the mosquito, reflecting on the various aspects of their life cycle, survival of males and females, and vector competence. Future studies are needed to better estimate the behavior of *A. aegypti* in future climatic conditions and, therefore, the relationship with man and it is vectorial competence.

Author contributions

NASCIMENTO NETO, J. F. Worked on all stages of the study and on manuscript preparation; MOTA, A. J. Responsible for overseeing of all stages related to molecular biology techniques: experimental design, RNA extraction, qPCR tests and manuscript review; ROQUE, R. A. Statistical analysis; CALDAS, W. H. qPCR analysis; TADEI, W. T. Responsible for monitoring all study aspects related to the vector in the environmental chambers.

Declaration of Competing Interest

All authors declare that they have no conflict of interest in this study.

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