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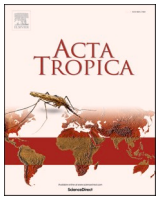

Adaptation and carry over effects of extreme sporadic heat stress in *Culex* mosquitoes

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ABSTRACT

Mosquitoes, as temperature-sensitive ectothermic vectors, exhibit temperature-dependence. This study investigates *Culex pipiens pallens (Cx. pallens)* responses to abrupt temperature increases and their implications on mosquito physiology.

First instar larvae (24hr post hatching) and newly enclosed adults (24hr post emergence) were separately exposed to heat shock regimes of 33 °C, 37 °C, and 42 °C for 3 days alongside a control temperature of 27 °C. Results showed that mortality was triggered at 42 °C within a day. Adult male mosquitoes were less tolerant to all temperatures than larvae and adult females ($p < 0.05$). Exposing larvae to constant temperatures for 3 days significantly decreased larvae's development time, growth rate and adult emergence ($p < 0.05$). Reproductive fitness was significantly reduced ($p < 0.05$) in males emerging from larvae exposed to 37 °C. Life table parameters showed significant increased mortality rate, kill power and decreased life expectancy at the embryonic stage ($p < 0.05$). Furthermore, heatwaves deactivated the Transient receptor protein ankyrin 1 at 37 °C ($p < 0.05$) in larvae but not adults. Calmodium, Heat shock protein 90, and small heat shock protein expression were significantly decreased in larvae at 37 °C ($p < 0.05$) as compared to larvae raised at 33 °C and 27 °C.

In conclusion, we classified the heat waves into three categories: adaptable (33 °C), critical (37 °C), and fatal (42 °C). Prolonged exposure of *Culex pallens* larvae to extreme heat affects the male reproductive output. These findings may serve as an important reference for forecasting vector and pest dynamics and used to tailor mosquito prevention and control measures.

1. Introduction

Mosquitoes are ectotherms who depend on the ambient temperature to regulate their physiological functions. Thus, it's not surprising that extreme climatic conditions are already promoting the emergence and re-emergence of mosquito-borne diseases (VBDs) by altering the life cycles of pathogens and vectors, expanding their geographical distributions, and changing the dynamics of disease transmission (Rocklöv and Dubrow, 2020).

Increased atmospheric temperatures and moisture have been found to influence mosquito physiological processes and behaviors yet they solely depend on these physiological mechanisms of heat tolerance and thermoregulation and on dispersal behaviour to find cooler sites, and appropriate microclimates to regulate their body temperature (González-Tokman et al., 2020). In female mosquitoes, high temperatures have a detrimental effect on fertility and fecundity (Ezeakacha and Yee, 2019), yet several studies report that heat exposure can affect the insect's male reproductive organs (Ana Caroline P. Gandara and Daniela Drummond-Barbosa, 2023; Grandela et al., 2024). Hence, the stimulus for post-mating effects of males may differ under a changing climatic scenario.

It is worth noting that environmental conditions experienced during

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