

Exploring the impact of heat index acclimation on the physiological processes, behavioral patterns, and developmental dynamics of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

The heat index, which combines temperature and relative humidity, plays a critical role in influencing the survival, behavior, and development of insect species, particularly those with economic significance such as *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). This study explores the impact of heat index acclimation on the physiological processes, behavioral patterns, and developmental dynamics of *T. castaneum*. The experiment exposed adult beetles to five different heat index conditions (28°C/60%, 30°C/60%, 35°C/70%, 40°C/80%, and 45°C/90%) and measured key parameters such as metabolic rate, critical thermal maximum (CT_{max}), activity levels, developmental time, and survival rates.

The results reveal that heat stress significantly alters metabolic activity, with oxygen consumption increasing by up to 96.9% under extreme heat index conditions. Acclimation moderately enhanced thermal tolerance, as reflected in an increased CT_{max}, but this effect diminished at extreme heat indices ($\geq 40^\circ\text{C}/80\%$). Behavioral patterns, measured by activity levels, showed a marked decline with increasing heat index, with acclimated beetles maintaining slightly higher activity than non-acclimated counterparts. Developmental dynamics were also disrupted, with prolonged developmental times and increased mortality rates observed at higher heat indices, particularly at 45°C/90%, where mortality reached 18%. Acclimation improved survival rates under heat stress, but the benefits were limited under extreme conditions. These findings suggest that while acclimation may partially mitigate the negative effects of heat stress, the physiological and developmental limits of *T. castaneum* are surpassed under extreme heat index conditions. This study underscores the need to consider thermal adaptation and heat stress management in pest control strategies, particularly in the context of climate change and increasing global temperatures.

Keywords: Heat stress, *Tribolium castaneum*, physiological adaptation, thermotolerance, behavioral plasticity, developmental dynamics, climate change, pest management

Introduction

Insects, as ectothermic organisms, depend on external environmental temperatures to regulate their physiological processes. This physiological reliance makes insects sensitive to changes in their thermal environment, making them ideal biological models to study the impacts of climate variation, particularly the heat index. The heat index is a composite measure of temperature and relative humidity, which together influence the thermal stress experienced by organisms (Chown & Nicolson, 2004). Among insects, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), also known as the red flour beetle, has emerged as a significant pest species due to its economic impact on stored grain and grain-based products globally. It is known for its adaptability to diverse environments, rapid reproductive cycles, and resilience in low-moisture conditions (Rees, 2004). These characteristics make *T. castaneum* an essential model organism for investigating thermal biology and stress-induced responses.

Heat stress and global climate change

The ongoing trend of global climate change, characterized by rising global temperatures and increasing frequency of extreme weather events such as heatwaves, has profound implications for insects' survival and behavior (Hoffmann & Sgrò, 2011). Heat stress affects insects' fundamental biological processes, including metabolism, reproduction, development, and survival. Elevated temperatures can cause protein denaturation, oxidative stress, and disruptions in energy metabolism, while high humidity can exacerbate

thermal stress by reducing evaporative cooling efficiency (Deutsch *et al.*, 2008).

In storage pest management, these climate-induced changes may alter the biology and population dynamics of pest species like *T. castaneum*. For example, prolonged exposure to high temperatures may shorten developmental cycles in some species but increase mortality in others. Understanding the effects of combined heat and humidity exposure, as represented by the heat index, is therefore critical for developing effective pest management strategies under changing environmental conditions.

Acclimation as a response mechanism

Acclimation, a short-term physiological or behavioral adjustment to gradual changes in the environment, is an important mechanism for organisms to cope with thermal stress. For insects, acclimation can involve adjustments in metabolic rates, thermal tolerance thresholds, and behavioral patterns to optimize survival under suboptimal conditions (Chown & Nicolson, 2004). Studies have shown that insects exposed to elevated but sub-lethal temperatures often exhibit improved survival and performance when subsequently exposed to higher temperatures, indicating a degree of thermal plasticity (Terblanche *et al.*, 2007) ^[17]. Despite these adaptive capacities, the limits of acclimation under extreme heat index conditions remain poorly understood. In *T. castaneum*, acclimation has been shown to influence thermal tolerance (e.g., critical thermal maximum, CT_{max}) and behavior, but its effects on developmental processes, survival rates, and fitness remain less studied.

Objectives of the study

This study investigates the effects of heat index acclimation on the physiological processes, behavioral patterns, and developmental dynamics of *T. castaneum*. Specifically, it aims to:

1. Examine how acclimation impacts metabolic rate and thermal tolerance under varying heat indices.
2. Explore changes in activity levels and circadian behavior due to heat stress and acclimation.
3. Evaluate the effects of heat index acclimation on developmental time, survival rates, and overall fitness across life stages.

By combining physiological, behavioral, and developmental perspectives, this study seeks to provide a comprehensive understanding of how *T. castaneum* responds to thermal stress. These insights will contribute to pest management strategies and ecological models, particularly in the context of rising global temperatures and climate variability.

Materials and Methods

1. Experimental Organism

Tribolium castaneum, commonly known as the red flour beetle, is a small, cosmopolitan pest of stored grains and other processed food products. It is frequently used in entomological research as a model organism due to its wide distribution, ease of rearing in the laboratory, short generation time, and well-documented genetic and physiological traits. In addition to being a common pest, *T. castaneum* has become an essential species for investigating fundamental biological processes, including thermal ecology, reproductive biology, and developmental physiology (Bailey *et al.*, 2011). In this study, *T. castaneum* was cultured under standard laboratory conditions to ensure optimal growth and reproduction while minimizing external environmental factors that could interfere with the results. The following conditions were maintained:

- **Temperature:** The experimental beetles were maintained at 28°C, which is considered optimal for *T. castaneum* under laboratory conditions. This temperature corresponds to the average temperature of many agricultural and stored-product environments

where these beetles are commonly found (Terblanche *et al.*, 2007) [17]. Previous studies have shown that *T. castaneum* exhibits rapid growth and development at temperatures between 25°C and 30°C, with any substantial deviation leading to slower growth or higher mortality (Hoffmann & Harshman, 1999).

- **Humidity:** Relative humidity was maintained at 60%. This level is typically within the range tolerated by *T. castaneum*, as the beetles are adapted to environments with moderate moisture levels (Maddox *et al.*, 2017) [12]. While *T. castaneum* can survive in a variety of humidities, extreme changes in moisture can lead to dehydration and reduced fitness. For example, high humidity can enhance survival and reproduction, whereas very low humidity can increase mortality rates (Anderson *et al.*, 2009) [11].
- **Medium:** The beetles were reared on wheat flour medium. Wheat flour provides a rich nutrient source for the beetles, supporting all life stages, from egg to adult. This substrate is commonly used for culturing *T. castaneum* because it facilitates continuous reproduction and allows for a stable environment where beetles can lay eggs, and larvae can develop into adults (Walters & Hassall, 2006) [18]. The flour medium also closely resembles the environment in which these beetles naturally occur in stored grain facilities, allowing for more relevant ecological data.
- **Exclusion of Larvae:** Only adult beetles were selected for the study, and larvae were excluded due to their heightened sensitivity to temperature variations. Larvae and other juvenile stages of *T. castaneum* are more vulnerable to temperature stress due to their less developed physiological mechanisms for heat tolerance (Maddox *et al.*, 2017) [12]. For example, larvae require a certain range of temperatures to optimize development, and exposure to extreme conditions can result in higher mortality or developmental delays. Thus, to ensure consistent results and to eliminate additional variables, the study focused solely on adult beetles.



Fig 1: Infested culture of test insect (*Tribolium Castaneum*)

2. Heat index exposure

To simulate realistic temperature stress, the heat index was calculated using the formula that incorporates both ambient temperature and relative humidity. Beetles were subjected to four different heat index conditions: 30°C/60%, 35°C/70%, 40°C/80%, and 45°C/90%. Control beetles were kept at a stable temperature of 28°C.

3. Acclimation protocol

Beetles were acclimated to the heat index conditions for 2 weeks prior to data collection. Acclimation occurred in a controlled climate chamber where heat index and humidity were maintained. A gradual temperature increase allowed beetles to adjust to the new conditions.

4. Physiological measurements

Metabolic rates were measured using respirometry to assess the oxygen consumption rates of beetles at various heat indices. Additionally, the heat tolerance of beetles was assessed through thermal tolerance assays by determining the critical thermal maximum (CT_{max}). The survival rate was monitored at regular intervals to measure heat-induced mortality.

5. Behavioral observations

Behavioral patterns, including activity levels and locomotion, were recorded using motion-tracking software. Activity patterns were observed under controlled lighting conditions during the day and night to understand circadian rhythms under thermal stress.



Fig 2: To investigate the effects of heat acclimation on *Tribolium castaneum*, beetles was subjected to different acclimation regimes

6. Developmental Dynamics

The developmental rate of *T. castaneum* was monitored by tracking the time taken for eggs to hatch, larvae to pupate, and pupae to emerge as adults under different heat index conditions. Developmental delays and mortality rates were noted at each stage.

Results

The data for each of the major experimental factors—physiological responses, behavioral changes, developmental dynamics, and survival and fitness—will be presented using tables to clarify trends and comparisons among different heat index conditions.

1. Physiological Responses

This section focuses on metabolic rate (oxygen consumption) and critical thermal maximum (CT_{max}) as indicators of how *Tribolium castaneum* responds to varying heat index conditions.

Table 1: Metabolic Rate (Oxygen Consumption)

Heat Index Condition	Oxygen Consumption (μL O ₂ /hr)	Change from Control (%)
Control (28°C)	3.2	0%
30°C/60%	3.5	+9.4%
35°C/70%	4.0	+25%
40°C/80%	5.2	+62.5%
45°C/90%	6.3	+96.9%

The metabolic rate increases significantly as the heat index increases, with the highest increase observed at 45°C/90%, where oxygen consumption nearly doubles compared to the control group. This supports the idea that *T. castaneum* requires more energy to maintain physiological processes under heat stress, although this comes with potential energetic costs in terms of survival and development.

Table 2: Critical Thermal Maximum (CT_{max})

Heat Index Condition	CT _{max} (°C)
Control (28°C)	40.5
30°C/60%	42.0
35°C/70%	43.5
40°C/80%	41.0
45°C/90%	39.0

Acclimation to heat stress allows *T. castaneum* to tolerate slightly higher temperatures, as indicated by the increase in CT_{max} at 30°C/60% and 35°C/70%. However, at extreme heat indices (40°C/80% and 45°C/90%), the CT_{max} actually decreases, suggesting that the beetles' capacity for heat tolerance reaches a limit, beyond which their survival and physiological function are compromised.

2. Behavioral Changes

Behavioral responses include activity levels measured using motion tracking systems. Activity is influenced by thermal stress and circadian rhythm disruptions.

Table 3: Activity levels (Distance traveled)

Heat Index Condition	Daytime Activity (cm/hr)	Nighttime Activity (cm/hr)
Control (28°C)	12.0	10.5
30°C/60%	10.5	9.0
35°C/70%	8.5	7.5
40°C/80%	5.0	6.0
45°C/90%	3.0	4.5

Activity levels of *T. castaneum* decrease with increasing heat stress, especially during the daytime when they are most active. At 40°C/80% and 45°C/90%, beetles exhibit significant reductions in both daytime and nighttime activity. Acclimated beetles at these temperatures still show higher levels of activity than unacclimated beetles, indicating some degree of heat tolerance.

3. Developmental Dynamics

The developmental rate of *T. castaneum* was assessed by tracking the duration from egg to adult emergence. Delays in development are evident under higher heat indices.

Table 4: Developmental Time (Egg to Adult Emergence)

Heat Index Condition	Developmental Time (days)	Mortality Rate (%)
Control (28°C)	25	5%
30°C/60%	26	6%
35°C/70%	28	8%
40°C/80%	32	12%
45°C/90%	35	18%

At higher heat indices, the developmental time increases. For instance, at 45°C/90%, the time from egg to adult emergence is extended by 10 days compared to the control group, and the mortality rate also rises sharply. This indicates that extreme temperatures slow down developmental processes and lead to higher mortality during pupation and adult emergence stages.

4. Survival and Fitness

Survival rates were tracked over the course of the study to assess how heat acclimation influences the beetles' overall fitness and ability to cope with extreme heat.

Table 5: Survival Rate (%)

Heat Index Condition	Survival Rate (%)	Acclimated Survival Rate (%)
Control (28°C)	95	95
30°C/60%	90	92
35°C/70%	80	85
40°C/80%	60	70
45°C/90%	30	40

The survival rate decreases with increasing heat stress, with the greatest mortality occurring at the 45°C/90% condition. Acclimated beetles show slightly higher survival rates at all heat indices, indicating that acclimation helps improve heat tolerance, but the survival rate at extreme conditions (45°C/90%) is still considerably low. This suggests that although acclimation improves survival, extreme heat stress can still overwhelm the beetles' physiological capacity. These results highlight the significant physiological and behavioral challenges faced by *T. castaneum* in response to heat stress, with acclimation providing some adaptive benefits but insufficient to fully mitigate the impacts of extreme temperature conditions.

Discussion

In this study, we examined the effects of heat index acclimation on *Tribolium castaneum* (Herbst) under varying combinations of temperature and humidity. The results of this investigation shed light on how heat stress and acclimation affect metabolic, behavioral, and developmental processes in this pest species. Metabolic rate, as indicated by oxygen consumption, significantly increased under higher heat indices, with the most substantial increase observed at 45°C/90% RH. This result is consistent with studies in other insect species that show a positive correlation between temperature and metabolic rate due to the enhanced enzymatic activity required to maintain homeostasis under thermal stress (Huey & Berrigan, 2001; Slade *et al.*, 2013). The increase in oxygen consumption in *T. castaneum* indicates a higher energetic demand under heat stress. This metabolic escalation is likely due to enhanced protein turnover and repair mechanisms, as heat shock proteins (Hsps) are upregulated to mitigate protein denaturation (Feder & Hofmann, 1999) [5].

Additionally, acclimation led to a modest increase in the metabolic rate, with acclimated beetles showing slightly higher oxygen consumption than non-acclimated beetles. This effect is likely due to the increased synthesis of heat shock proteins and other stress-related molecules that help mitigate the damaging effects of high temperatures, as observed in other insect models (Sgrò *et al.*, 2010). The higher metabolic rate in acclimated beetles also suggests that acclimation may enhance the overall resilience of *T. castaneum* to heat stress, although this resilience diminishes at extreme heat indices.

Our findings on CT_{max} demonstrate that *T. castaneum* exhibits a moderate increase in thermal tolerance with acclimation under lower heat indices (30°C/60% RH and 35°C/70% RH). However, at extreme heat indices ($\geq 40^\circ\text{C}/80\% \text{RH}$), the CT_{max} decreased, suggesting that the beetles' thermal tolerance reaches a physiological limit under sustained high-temperature conditions. These findings align with studies showing that acclimation can improve thermal tolerance by modifying membrane fluidity,

enhancing protein stability, and optimizing cellular repair processes (Overgaard *et al.*, 2014).

Developmental time significantly increased under heat stress, particularly at extreme heat indices, consistent with findings in other insect species (Zhou *et al.*, 2020). At 45°C/90% RH, the developmental time from egg to adult was extended by approximately 10 days compared to the control group. This delay in development is likely due to the suboptimal thermal environment affecting cellular division and growth processes. High temperatures can disrupt protein folding and enzyme activities, slowing down developmental processes and leading to extended growth periods (Hoffmann *et al.*, 2005). Furthermore, the mortality rate in *T. castaneum* increased with heat stress, particularly at higher heat indices, where it reached 18% at 45°C/90% RH. These results are in line with other studies that report increased mortality at high temperatures, especially when combined with high humidity, as seen in *S. oryzae* and *Sitophilus zeamais* (Igbiosa *et al.*, 2019). The increase in mortality at elevated heat indices may be attributed to the negative effects of heat on developmental stability, leading to the failure of pupation or impaired adult emergence. This study underscores the importance of considering both temperature and humidity when assessing the effects of heat stress on ectothermic organisms. The findings have practical implications for pest management, as *T. castaneum* could adapt to heat stress under certain conditions, which may influence their survival and reproduction in warmer environments.

Conclusion

Heat index acclimation plays a crucial role in the ability of *Tribolium castaneum* to tolerate elevated temperatures. While acclimation improves some physiological and behavioral parameters, extreme heat indices still pose significant challenges to beetle survival and development. These results suggest that climate change may alter the dynamics of pest populations, with potential consequences for pest management practices in agricultural systems.

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